mechanism by which isoflavones can inhibit breast cancer development. Genistein has effects on signal transduction in vitro and in vivo (Dalu et al., 1998). In vitro genistein inhibits tyrosine protein kinase (Akiyama et al., 1987; Ogawara et al., 1986), MAP kinase (Frey and Singletary, 2003), and DNA topoisomerase (Constantinou and Huberman, 1995), enzymes which influence cell growth and development. These effects account for why this isoflavone can inhibit the growth of a wide range of both hormone-dependent and independent cancer cells (Constantinou et al., 1990; Ford, 2002; Kim et al., 1998; Upadhyay et al., 2001; Weber et al., 1999). For these reasons even classifying isoflavones as phyto-SERMS is probably an incomplete characterization.

Soya, isoflavones, and cancer: animal and epidemologic studies

Several different models have been used to study the relationship between diet and mammary cancer in animals. These include the use of chemical carcinogens such as dimethylbenz[a]anthracene (indirect acting) and N-methyl-N-nitrosourea (direct acting) to induce tumors and the implantation of breast cancer cells into the mammary pad or subcutaneously injected into the skin. The results from these studies are somewhat inconsistent but generally show the substitution of soya protein for casein (the reference animal protein used in rodent diets) and the addition of isolated isoflavones to a standard laboratory diet reduces chemically induced mammary tumor multiplicity (the number of tumors per rat) and tumor weight and volume by 25–50% although incidence (percentage of animals with tumors) is less often affected (Baggott et al., 1990; Barnes et al., 1990; Gotoh et al., 1998a; Hakkak et al., 2000; Hawrylewicz et al., 1991; Ohta et al., 2000; Zhou et al., 2004). Several studies have also noted increases in tumor latency (average time to first tumor) (Jin and MacDonald, 2002) and a decreased rate of metastasis (Hawrylewicz et al., 1995). Thus, overall, the animal data are supportive of soya intake reducing breast cancer risk.

In 1991, Lee et al published a case control study from Singapore which found that soya intake dramatically decreased breast cancer risk (Lee et al., 1991). This study helped fuel interest in the anticancer effects of soya. A few years later Yuan et al also published a Chinese case control study showing soya intake was associated with a reduced breast cancer risk (Yuan et al., 1995). Often overlooked however, was that both studies found soya intake was protective only against premenopausal breast cancer. And in fact, overall, in contrast to the animal data, and despite the low breast cancer mortality rates in Asia, there is relatively little epidemiologic evidence from case-control and prospective studies supporting the hypothesis that adult soya intake reduces postmenopausal breast cancer

risk (Peeters et al., 2002; Trock et al., 2000). Although the epidemiologic data are often cited as evidence that soya intake is protective against breast cancer generally, statements to this effect refer to the markedly lower rate of breast cancer in Japan compared to the West. Obviously, these kinds of gross ecological comparisons have limited ability to provide any insight regarding the effects of soya since there are so many lifestyle differences between Western and Eastern cultures that might be involved in the etiology of breast cancer.

Soya intake and biomarkers of cancer risk: clinical findings

The lack of epidemiologic support significantly weakens the hypothesis that soya intake is protective against breast cancer. Obviously, favorable animal findings by themselves are insufficient to validate a hypothesis. Unfortunately, for obvious reasons the ability to conduct randomized clinical trials utilizing cancer development or even the survival of breast cancer patients as endpoints is extremely limited. Consequently, much emphasis is placed on the results of short-term trials examining the effects of soya on biomarkers of breast cancer. There are many such markers but there is much debate about the relative merits of each.

In this regard, Kurzer from University of Minnesota noted in a review paper that although no studies found statistically significant effects, seven of eight reported a trend toward an increase in menstrual cycle length (Kurzer, 2002). Longer cycles are associated with a reduced risk of breast cancer (Whelan et al., 1994). This is thought to be because during the luteal phase, the second half of the menstrual cycle, breast cell replication is occurring at a much faster rate than during the follicular phase, the first two weeks of the cycle (Pike et al., 1993). Generally, faster cell replication increases risk of cells being transformed into cancer cells since there is less time for cellular machinery to repair damage to DNA. Longer cycles usually have an extended follicular phase relative to the luteal phase. Dietary measures that result in longer cycles typically but not always increase the follicular phase.

Several studies have found a decrease in urinary estrogens and increased ratios of urinary 2-(OH) to 16alpha-(OH) and 2-(OH) to 4-(OH) estrogens, findings suggestive of a protective effect (Kurzer, 2002). Estrogen is metabolized through two different pathways, the 16-hydroxylation pathway produces metabolites that are very estrogenic and possibly carcinogenic whereas the 2-hydroxylation produces metabolites with opposite properties. Studies that have examined the effects of soya intake on serum estrogen levels have led to inconsistent findings with some studies showing decreases

whereas others have reported no changes and even increases (Kurzer, 2002). Furthermore, two pilot studies suggested that soya could actually have an estrogen-like effect on breast tissue and by implication possibly increase breast cancer risk (Hargreaves et al., 1999; Petrakis et al., 1996). However, the design of both studies limits the contribution of these findings to understanding of the relationship between breast cancer risk and soya intake. One of these studies had no control group (Petrakis et al., 1996)and the other was conducted for only two weeks (Hargreaves et al., 1999).

Recently, two groups (Atkinson et al., 2002; Maskarinec et al., 2003) examined the effect of isoflavone supplements on breast tissue density although one of these is thus far published only as an abstract (Atkinson et al., 2002). Although there are certainly no perfect nor universally accepted biomarkers of cancer risk, mammographic density appears to have considerable potential as a noninvasive means by which to assess the efficacy of dietary modifications on breast cancer risk (Boyd et al., 2001a; Boyd et al., 2001b; Vachon et al., 2000). Interventions that decrease density are thought to decrease breast cancer risk whereas interventions that increase density, such as conventional hormone replacement therapy, increase risk (Greendale et al., 1999; Lundstrom et al., 2001). One of the supplement studies, which involved 34 premenopausal women who consumed 76 mg (aglycone weight) of isoflavones for one year found no effect of isoflavones on tissue density (Maskarinec et al., 2003). However, considerable time elapsed between the time supplementation stopped and the final mammogram was taken. In the other study, there were no effects overall in perimenopausal and postmenopausal women, but in women 56–65 y old breast tissue density significantly decreased (Atkinson et al., 2002). The latter finding is suggestive of a protective effect but should be viewed as a very preliminary finding.

Early soya intake and adult breast cancer risk

Overall, when viewing the data in its entirety, a reasonable conclusion is that the evidence that *adult* soya intake reduces breast cancer risk is equivocal. Certainly, there is an assortment of mechanisms by which soya can reduce breast cancer risk. But at this point it is not possible to conclude that adult soya intake is protective. Nevertheless, there are emerging data suggests that soya likely contributes to the low breast cancer mortality rates in Asian countries but primarily as a result of consumption during the early part of life.

Adult lifestyle does influence breast cancer risk; for example, adulthood obesity (Morimoto et al., 2002) and alcohol consumption (Singletary and Gapstur, 2001) increase risk. However, identifying dietary connections to breast cancer risk has proven difficult. In part, this may be because of the

pronounced effects that early life influences have on breast cancer development later in life. For example, slower fetal growth rates (McCormack et al., 2003), early pregnancy (Chie et al., 2000), parity (2002), and breast feeding (2002), are all protective against breast cancer. Differences in these types of factors likely contribute significantly to the large variation in breast cancer rates among countries. Early pregnancy is thought to reduce risk as a result of the high estrogen levels during this period of life stimulating differentiation of the breast tissue (Guzman et al., 1999). According to Russo and colleagues, differentiation of the breast determines susceptibility to carcinogenesis (Russo et al., 2001). Interestingly, research suggests soya exposure when young may mimic the effects of early pregnancy on breast cancer risk.

The first research to draw attention to the possible protective effects of early soya consumption was conducted by Lamartiniere and colleagues from the University of Alabama. They have consistently shown that exposing rats to genistein through the oral or intravenous route for just brief periods during the perinatal and prepubertal periods reduces chemically induced mammary tumor multiplicity by approximately 50% (Lamartiniere et al., 2000). Exposure to genistein causes breast tissue differentiation and reduces the number of terminal end buds, the anatomical structure within the rodent mammary gland that is the likely site of tumor development (Lamartiniere et al., 2000; Rowlands et al., 2002). Consistent with the animal studies are the results from two case control studies, one conducted in Shanghai and the other in the United States but that involved women of Asian ethnicity. In the former study, soya consumption (cutoff for the fourth quartile, >11.2 g soya protein/d) during the teenage years reduced risk of developing breast cancer by approximately 50% (Shu et al., 2001). Soya consumption during adulthood had no bearing on these findings. In the latter study, soya consumption throughout life reduced risk by 35% whereas soya consumption only during adulthood was not protective (Wu et al., 2002).

Is soya contraindicated for some women?

Concerns have arisen that soya intake might increase breast cancer risk in women at high risk of developing this disease or increase the likelihood of breast cancer patients developing a secondary tumor (Bouker and Hilakivi-Clarke, 2000). In fact, several health organizations of have issued cautionary statements to this effect. The basis for this concern is the estrogen-like effects of soya isoflavones. This is a very complex and controversial subject to be certain. Recently, Messina and Loprinzi reviewed the relevant literature and concluded that soya was not contraindicated for high-risk women or

breast cancer patients (Messina and Loprinzi, 2001). There conclusion was based in part on epidemiologic data showing that whereas the use of estrogen plus a progestin markedly increase breast cancer risk, estrogen use alone does not (Pritchard, 2002). Experimental data also indicate that the combination hormones is much more carcinogenic than estrogen alone (Messina and Loprinzi, 2001). Consistent with these findings are the results from the Women's Health Initiative (WHI); in this trial breast cancer risk was significantly increased but thus far there has been no increase noted in the estrogen only arm of this trial (Writing Group for the Women's Health Initiative Investigators, 2002). These observations are important because soya has no progesterone activity (Zava et al., 1998). Findings from a recent animal study by Zhou et al are also quite reassuring about soy not stimulating the growth of estrogen receptor positive breast tumors (Zhou et al., 2004).

There is also controversy about the use of soya by women taking tamoxifen. A recent study in mice found that genistein markedly inhibited the efficacy of this drug (Ju et al., 2002). Although this issue remains unresolved three animal experiments have found that soya and isoflavones act either additively (Constantinou et al., 2001) or synergistically to enhance the efficacy of tamoxifen (Gotoh et al., 1998b). Certainly, it is too early to draw conclusions, but the available evidence suggests there is more likely to be a beneficial than harmful interaction between tamoxifen and soya. Of course, breast cancer patients need to consult with their oncologist when making dietary decisions.

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Soy intake and breast cancer risk: a review of the epidemiologic data

S.Yamamoto[1]

Abstract

So far, 14 epidemiological studies have been conducted for Asian populations and western populations to evaluate the association between soy or isoflavone consumption and breast cancer risk. Among them, 1 prospective cohort studies showed an inverse association, but 4 did not, and 9 retrospective case-control studies showed inconsistent results. We compared the detail and discuss possible reasons of inconsistency of the results.

Breast cancer incidence is higher in western countries than in Asian countries. In addition, migrant populations from Asia to the United States are known to have increased incidence of breast cancer (Parkin 1997). As for Japan, breast cancer mortality is higher in the metropolitan areas, but shows steadily increasing trends for all the prefectures (Statistics and Information Department 1990). This evidence suggests that the difference in life style between the western countries and Asia, in particular, the difference in eating habits, may be associated with the difference in incidence of breast cancer. Among the food items, attention has been paid to soybean products since they are widely consumed in Asia but rarely consumed in western countries (at least, until quite recently). One report gives a 700-fold difference in consumption between Japan and the United States (Yamamoto et al. 2001). In addition, isoflavones, plant estrogens found mainly in soybean products, have been shown to have anticarcinogenic effects on hormone-related cancers in experimental studies, perhaps because of the similarity of the isoflavone structure to that of estrogens. Other possible mechanisms for anticarcinogenic effects are also suggested by experimental studies (Adlercreutz and Mazur 1997; Bingham et al. 1998; Adlercreutz et al. 1995; Messina et al. 1994). Contrary to them, epidemiological studies did not provide consistent evidence for an association between isoflavone intake and breast cancer incidence.

To date, 14 studies have evaluated associations between soy or isoflavone consumption and breast cancer risk (Table 1). Among studies that reported

[1] PhD, Cancer Information and Epidemiology Division National Cancer Center Research Institute 5-1-1, Tsukiji, Chuo-ku, Tokyo, Japan, Phone: +81-3-3542-2511 ext. 2632 Fax: +81-3248-1553, E-mail: siyamamo@ncc.go.jp

results for premenopausal women, four case-control studies conducted among Singapore Chinese (Lee et al. 1992), Japanese (Hirose et al. 1995), Asian American (Wu et al. 1996), and Chinese (Dai et al. 2001) populations found statistically significant inverse associations between soy consumption and breast cancer risk but two prospective study conducted among American (Horn-Ross et al. 2002) and Japanese (Yamamoto et al. 2003) and four case-control studies conducted among Chinese (Yuan et al. 1995), American and Canadian (Witte et al. 1997), non-Asian American (Horn-Ross et al. 2001), and Asian American (Wu et al. 2002) populations found no such association. Among studies that reported results for postmenopausal women, two case-control studies conducted among Chinese (Dai et al. 2001) and Asian American (Wu et al. 2002) populations and one prospective Japanese study (Yamamoto et al. 2003) found statistically significant inverse associations but two prospective American studies (Horn-Ross et al. 2002; Greenstein et al. 1996) and five case-control studies conducted among Singapore Chinese (Lee et al. 1992), Japanese (Hirose et al. 1995), Chinese (Yuan et al. 1995), Asian American (Wu et al. 1996), and non-Asian American (Horn-Ross et al. 2001) populations found no such associations. Two other prospective studies and one case-control study conducted among Japanese populations (Hirayama 1990; Key et al. 1999; Hirohata et al. 1985) reported combined results for pre- and post-menopausal women and found no statistically significant association between soy consumption and breast cancer risk. The results were not consistent even in the same study design, geographic location, and menopausal status.

If we assume the association between soy intake and breast cancer risk exits, possible reasons of the lack of associations observed in the previous studies are considered to be the result of recall bias in case-control studies, errors in exposure measurements, or small exposure variation in Western subjects. Especially, association between disease and exposure can be detected easier when exposure has wide variability. Indeed, median isoflavone intake among the Japanese study participants (Yamamoto et al. 2003) was seven times higher than that among Chinese in Singapore, 15 times higher than that among U.S. non-Asian (African-American, Latina, and White) women, and 700 times higher than that among U.S. Caucasians (Seow et al. 1998; Horn-Ross et al. 2000; Strom et al. 1999). Two other Japanese prospective studies, which did not show the association, may also have high level but small variation in soy intake because soy intake was uniformly high when the cohorts were established in 1960's and 1970's (Hirayama 1990; Key et al. 1999).

Table 1. Summary of Epidemiological Studies Investigating Dietary Soybean-Products and Breast Cancer (modified Yamamoto et al. 2003)

Author, Publication Year	Population	Number of subjects	Exposure Comparison category	Menopausal status	Relative risk (95% confidence intervals)
Cohort study					
Hirayama 1990	Japanese	241 cases 2,140,369 person-years	Miso soup Daily vs. Non-daily	Combined	0.85 (0.68 to 1.06)
Greenstein et al. 1996	American	1,018 cases 34,388 women	Soy or tofu: Consumers vs. nonconsumers	Post	0.76 (0.50 to 1.18)
Key et al. 1999	Japanese	427cases 488,989 person-years	Miso soup ?5 vs. ?1 times/week	Combined	**0.87 (0.68 to 1.12)**
Horn-Ross et al. 2002	American	711 cases 222,249 person-years	Genistein Fifth vs. first quintile	Combined*	1.0 (0.7 to 1.3)
Yamamoto et al. 2003	Japanese	179 cases 209,354 person-years	Genistein Fourth vs. first quartile	Pre Post	0.66 (0.25 to 1.7) 0.32 (0.14 to 0.71)
Case-control study					
Hirohata et al. 1985	Japanese	212 cases 212 controls	Fat from soybean products Mean intake	Combined	Not significant
Lee et al. 1992	Singapore Chinese	200 cases 420 controls	Total soya products ?55.0 vs. <20.3 g/day	Pre Post	0.4 (0.2 to 0.9) 1.1 (0.5 to 2.3)
Hirose et al. 1995	Japanese	1,186 cases 23,163 controls	Tofu >3 vs. ?3 times/week	Pre Post	0.81 (0.65 to 0.99) 1.17 (0.92 to 1.49)
Yuan et al. 1995	Chinese	834 cases 834 controls	Soy protein Per 18 g/day	Combined*	1.0 (0.7 to 1.4)
Wu et al. 1996	Asian-American	597 cases 966 controls	Tofu Per 1 time/week	Pre Post	0.84 (0.70 to 0.99) 0.86 (0.66 to 1.13)
Witte et al.	American and Canadian	140 cases	Tofu or soybean:	Pre	0.5 (0.2 to 1.1)

* Results were not substantially different when analyzed separatedy by menopausal status

If we assume the association does not exist, several possibilities are considered as the observed associations. One is of course a chance. Another possibility is the failure of controlling potential confounding factors. Low breast cancer incidence may be associated with other Asian dietary habits such as eating more rice, pickles, vegetables, fish, and less bread and butter, other Asian life style factors, and genetic predispositions. It still remains the possibility of residual confounding even if they are controlled for in the multivariate models.

In order to obtain more confirmative evidence from epidemiological studies, it is necessary to conduct studies with more cases, studies clarifying the difference in effects among various soybean products, and studies investigating breast cancer and biomarkers of soybean intake such as serum isoflavones. In addition, intervention studies for human should be conducted to clarify the mechanism of action for soybean ingredients. It may be effective to conduct such studies for high risk population of breast cancer with low soybean intake, to investigate efficacy and safety.

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Early exposure to genistein reduces chemically-induced mammary cancer

C.A. Lamartiniere[1]

Abstract

Genistein, the primary isoflavone of soy, has been investigated as a chemopreventive agent against breast cancer in an animal model. Sprague-Dawley rats were fed AIN-76A diet ± 25 mg and 250 mg genistein/kg diet. DMBA was administered by gavage at day 50 postpartum to induce mammary tumors. Mammary cancer chemoprevention was demonstrated after prepubertal and combined prepubertal and adult genistein treatments but not after prenatal- or adult-only treatments, demonstrating that timing of exposure to genistein is important for mammary cancer chemoprevention. The cellular mechanism of action was found to be mammary gland and cell differentiation, as shown by whole-mount analysis and beta-casein expression. An imprinting effect was shown for epidermal growth factor receptor expression in mammary terminal end buds.

Abbreviations

DMBA, dimethylbenz[a]anthracene; EGF, epidermal growth factor.

Introduction

We have hypothesized that exposure to hormonally-active nutritional chemicals during early windows of development plays a key role for cancer prevention. This is consistent with the reports that Asian women consuming a diet high in soy products have a low incidence of breast cancer (Lee et al., 1991; Ziegler et al., 1993; Shu et al. 2001) yet, when Asians immigrate to the United States and adopt a Western diet they lose this protection. Soy-based diets are high in phytochemicals and quantitative results indicate that isoflavone, phytoestrogens are normal constituents of human urine from subjects consuming large amounts of soy products (Adlercreutz et al., 1991).

The predominant isoflavone phytoestrogen found in soy is genistein. Genistein is a planar molecule with an aromatic A-ring, has a second oxygen atom 11.5 Å from the one in the A ring, and has a molecular weight similar

1 Department of Pharmacology and Toxicology, UAB Comprehensive Cancer Center, University of Alabama at Birmingham, Birmingham, AL 35294 USA, Corresponding author: Coral.Lamartiniere@ccc.uab.edu

to those of the steroidal estrogens. It has estrogenic properties in receptor binding assays, cell culture, and uterine weight assays (Makela et al., 1994). Genistein inhibits topoisomerase II (Okura et al., 1988), epidermal growth factor-induced expression of c-fos (Tripathi et al. 1992), and tyrosine kinases (Akiyama, et al., 1987). Genistein exhibits antioxidant properties (Wei et al., 1995) and has been reported to induce cell differentiation (Watanabe, et al., 1991).

Results and dissussion

To investigate the potential of perinatal genistein exposure to protect against chemically induced mammary cancer, female Sprague-Dawley rats were fed 0, 25, and 250 mg genistein/kg AIN-76A diet starting 2 wk before breeding (Fritz et al., 1998; Lamartiniere et al., 2000). The dietary concentrations were chosen because they yield serum genistein concentrations in rats similar to blood genistein concentrations in men and women eating a diet high in soy (Adlercreutz et al., 1993). After parturition, dams and offspring were fed the same diets until time of weaning (day 21). From that time onward, all female offspring from the three treatment groups were fed AIN-76A diet only. At day 50 postpartum, 80 mg DMBA/kg body weight was administered by gavage to induce mammary tumors. Animals were palpated for tumors and necropsied at 180 d after DMBA treatment or when tumors developed to 2.5 cm in diameter. Control animals (zero genistein, DMBA) developed approximately 9 tumors whereas dietary genistein suppressed DMBA-induced mammary tumor development in a dose-dependent manner. Rats exposed to 25 and 250 mg genistein/kg AIN-76A diets had 7.1 and 4.3 mammary tumors, respectively (Figure 1). This dietary genistein chemoprevention study is consistent with our previous work demonstrating that injections of pharmacologic doses of genistein during the neonatal and prepubertal periods suppressed chemically-induced mammary tumor development (Lamartiniere et al., 1995; Murrill et al., 1996).

We have also addressed the possibility that genistein exposure, via the dam during the prenatal period only, might alter the female offspring's susceptibility for mammary cancer. One group of female rats was fed 250 mg genistein/kg AIN-76A diet during breeding and pregnancy. At parturition, the dams and offspring were switched to AIN-76A diet without genistein supplement. The control group for this experiment was females fed AIN-76A diet from time of breeding throughout the experiment. Dietary exposure to genistein, merely during the prenatal period, neither increased mammary carcinogenesis nor conferred protection against DMBA-induced mammary cancer.

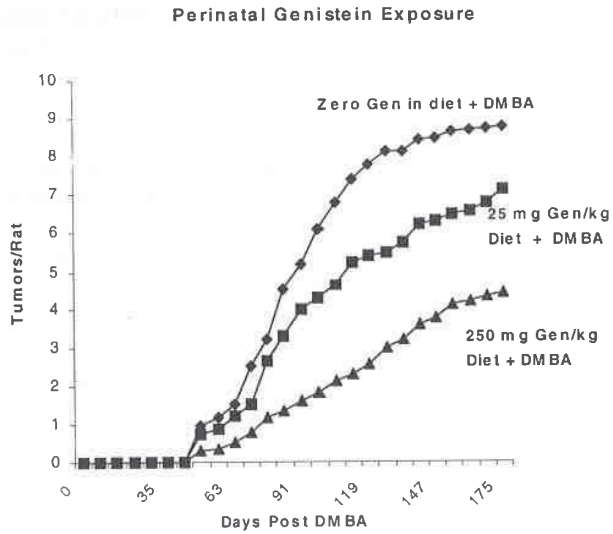

Figure 1. Ontogeny of palpable mammary tumors in female Sprague-Dawley CD rats exposed perinatally to genistein in the diet. Starting at time of breeding, dams were fed 0, 25, and 250 mg genistein/kg AIN-76A diet. After weaning, the offspring were fed AIN-76A diet only. On day 50 postpartum, female offspring were treated with 80 mg DMBA/kg body weight. (Modified from Fritz et al., 1998). Permission requested from Oxford University Press.

Since breast cancer has been demonstrated to be estrogen dependent, we have been concerned that genistein, a phytoestrogen, may contribute to mammary cancer development. Rats were fed AIN-76A diet ± 250 mg genistein/kg diet at three time periods, and all females were treated intragastrically with 80 mg DMBA/kg body weight. Rats exposed to the control diet, AIN-76A only, from birth until the end of the experiment had the highest average number of tumors (9.0 tumors/rat) (Lamartiniere et al., 2002). Rats exposed to genistein from 1 to 21 d postpartum only developed 4.5 tumors. Animals exposed to genistein from d 1 to 21 and d 100 to 180 developed the fewest number of tumors (2.8 tumors/rat). The latter genistein feeding was initiated 50 d after the DMBA treatment, the time of onset of palpable mammary tumors. This demonstrated that genistein fed to adult rats previously exposed prepubertally to genistein provided these animals with additional protection against mammary cancer. Prepubertal genistein exposure appears to permanently affect the animal or mammary gland in a way that determines how that animal later responds to the same or similar

chemical stimuli. In this case, genistein fed during the prepubertal period programmed future (adult) genistein response against mammary cancer susceptibility.

In Table 1, we summarize the relation among dietary genistein, timing of exposure, and chemically induced mammary cancer in rats (Lamartiniere et al., 2002). Limiting exposure to dietary genistein to the prenatal or adult periods does not predispose or protect against mammary cancer. In contrast, exposure to dietary genistein during the prepubertal and prepubertal-plus-adult periods protected against chemically induced mammary cancer in rats. An epidemiological report using the Shanghai Cancer Registry has shown an inverse relationship (50%) between adolescent (13-15 y) soyfood intake and breast cancer incidence later in life (Shu et al. 2001).

Table 1. Dietary Genistein, Timing of Exposure and Mammary Cancer Chemoprevention

Exposure period	Tumor Multiplicity*
No genistein	8.9
Prenatal genistein	8.8
Adult genistein (after tumors)	8.2
Prepubertal genistein	4.3
Prepubertal and adult genistein	2.8

Diets contained ± 250 mg genistein/kg AIN-76A. *All rats were treated with 80 mg DMBA/kg body weight at Day 50 postpartum.

Analysis of mammary gland morphology in rats treated with genistein revealed that its cellular mechanism of action is enhancement of mammary gland differentiation (Fritz et al., 1998; Lamartiniere et al., 2000). We demonstrated that genistein administered to prepubertal rats reduced the number of terminal end buds and increased the number of lobules. Mammary terminal end buds are terminal ductal structures found primarily in young animals (and humans) and contain many undifferentiated epithelial cells (Russo and Russo, 1978). Terminal end buds are the most susceptible structures to chemical carcinogens; lobules are the terminal ductal structures most differentiated and least susceptible to chemical carcinogens. Further evidence that genistein enhances differentiation was obtained by measuring beta casein in mammary glands. Beta-casein is a milk protein and biomarker of mature mammary glands and differentiated cells. Using Western blot analysis, we found that prepubertal genistein treatment increased beta-casein expression in mammary glands of prepubertal and adult rats

(Lamartiniere et al., 2002). In the adult rats beta-casein was measured 30 d after genistein treatment.

Because genistein has been reported to inhibit protein tyrosine kinases *in vitro* (Akiyama et al., 1987), we investigated the potential of genistein to regulate the epidermal growth factor (EGF) receptor in vivo. In 21-d-old rats treated prepubertally with genistein, we found increased EGF receptor expression in mammary terminal end buds (Brown et al. 1998). Not only was this finding contrary to the *in vitro* reports, but this was surprising because we expected a chemopreventive agent to down-regulate the expression of this growth factor signaling pathway. However, when we extended our studies to 50-d-old rats treated prepubertally with genistein, we observed that EGF receptor expression was down-regulated in terminal end buds of these animals. We interpret this to mean that early in postnatal life, genistein initially up-regulated the EGF-signaling pathway to enhance mammary gland development that resulted in early mammary gland differentiation. Reduced EGF signaling and decreased cell proliferation at 50 d when the DMBA was given is associated with reduced susceptibility to chemical carcinogenesis (Fritz et al., 1998; Lamartiniere et al., 2000). Developmental modifications by a hormonally-active chemical that result in altered biochemical or behavioral responses later in life has been defined as imprinting (Gorski, 1974; Lamartiniere et al., 1982). We speculate that down-regulated EGF receptor signaling in mammary terminal end buds at the time of carcinogen exposure plays a role in reduced mammary cancer development.

Summary

We conclude that for genistein to protect against breast cancer, initial exposure must occur during the early sensitive period of mammary gland development, that is, neonatal through prepubertal periods. We have demonstrated that prepubertal and prepubertal-plus-adult genistein exposures protect against chemically induced mammary cancer in rats. The cellular mechanism of action of genistein is to enhance mammary cell differentiation (Fritz et al., 1998; Lamartiniere et al., 2000). One identified biochemical mechanism is short-term and direct up-regulation of the EGF-signaling pathway that plays a role in cell differentiation (Brown et al., 1998). Paradoxically, this results in the epithelial cells of the mammary terminal end buds of adult animals having reduced EGF receptor expression. EGF signaling has been associated with cell proliferation, hence we believe that down-regulated EGF receptor in adults contributes to the genistein chemoprevention. In reference to genistein in adults conferring additional protection when given to rats previously exposed to genistein compared

with genistein-naive animals, we speculate that the early developmental effects have altered the molecular blueprint from which mammary cells respond to cancer initiators and promoters. Our laboratory data are consistent with the epidemiological report showing an inverse relationship between adolescent soyfood intake and breast cancer incidence later in life (Shu et al., 2001). We conclude that the most sensitive period for mammary cancer chemoprevention in the rat is the prepubertal period and in the human is probably the adolescent period.

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Soy and isoflavone intake inhibits experimentally induced prostate cancer

J. R. Zhou[1]

Abstract

The objectives of our research are to evaluate the effects of soy bioactive components on the prevention and treatment of prostate cancer in experimental animal models and to determine the underlying cellular and molecular mechanisms of action. In the first animal experiment, we have utilized an orthotopic implant model to characterize the *in vivo* response of androgen-sensitive LNCaP prostate tumors to three well-characterized soy dietary supplements: isoflavone depleted soy protein, soy phytochemical concentrate (SPC) and genistin. In male SCID mice orthotopically implanted with the androgen sensitive human prostate cell line LNCaP, dietary supplements of soy protein, genistin and SPC reduced primary tumor weight by 42% ($P=0.07$), 57% ($P<0.05$) and 70% ($P<0.005$) respectively. All three soy supplements significantly increased tumor apoptosis and decrease microvessel density, with no significant change in tumor proliferation. Each supplement produced a distinct serum androgen response, with genistin producing the greatest decrease in total serum testosterone and dihydrotestosterone (DHT) ($P<0.05$) and the greatest increase in testosterone to DHT ratio ($P<0.05$) and soy protein the greatest decrease in bioactive androgen ($P<0.05$). Only SPC significantly inhibited metastases to lymph nodes and lungs, and only SPC produced a significant increase in tumor p53 expression.

We have also determined the effects of SPC and tea combinations on the prostate cancer by using the same orthotopic prostate tumor model. SPC, black tea and green tea were compared with respect to tumorigenicity rate, primary tumor growth, tumor proliferation index and microvessel density, serum androgen level and metastases to lymph nodes. SPC, black tea and green tea significantly reduced tumorigenicity. SPC and black tea also significantly reduced final tumor weights. Green tea did not reduce final tumor weight although it tended elevate ($p=0.14$) the serum DHT concentration. The combination of SPC and black tea synergistically inhibited prostate tumorigenicity, final tumor weight and metastases to lymph nodes *in vivo*. The combination of SPC and green tea synergistically inhibited final tumor weight and metastasis and significantly reduced serum levels of both testosterone and DHT *in vivo*. Combined inhibition of tumor progression was associated with reduced tumor cell proliferation and tumor angiogenesis. These results suggest that the anti-prostate cancer activity of dietary soy protein, soy phytochemicals and genistin utilize different molecular pathways, and that combination of dietary soy bioactive components with tea may provide a more effective regimen in prostate cancer prevention. In addition, we have demonstrated that this animal model can be used in the design of dietary strategies for prostate cancer prevention and therapy.

[1] Ph.D. Assistant Professor of Surgery, Harvard Medical School, Director, Nutrition/Metabolism Laboratory, Beth Israel Deaconess Medical Center, Boston, MA, USA

Introduction

Prostate cancer (CaP) is the most frequently diagnosed invasive cancer and the second leading cause of cancer related death in U.S. men. Epidemiological studies indicate that the rate of clinically indolent CaP is similar worldwide (Yatani et al., 1989), while the rate of clinically significant and metastatic CaP is significantly lower in Asia than that in Western populations (Messina et al., 1994; Parkin and Muir, 1992). It has been proposed that these differences are largely due to environmental influences such as diet and nutrition, rather than genetic variations (Boyle et al., 1993; Mandel and Schuman, 1980; Morton et al., 1996), since Asian men who adopt a Western lifestyle show an increased incidence of clinically significant disease. Among dietary factors, a greater consumption of soybean products in the traditional Asian diet has been suggested to contribute to reduced risks of prostate cancer progression and mortality (Hebert et al., 1998; Messina et al., 1994).

Several epidemiological studies have been conducted to determine the association of soy and CaP risk, and it was found that increased intake soy products or soy bioactive components was significantly associated with a reduced CaP risk (Jacobsen et al., 1998; Kolonel et al., 2000; Strom et al., 1999; Villeneuve et al., 1999) and/or mortality (Hebert et al., 1998). In the recent years, more animal studies using different types of animal models for CaP have been conducted to identify the bioactive components in soybeans, to evaluate the efficacy of the components, and to elucidate the mechanisms of action. In DMBA-induced prostate carcinogenesis model, genistein and daidzein significantly inhibited multiplicity of the ventral prostate carcinoma (Kato et al., 2000), and a soy isoflavone mixture significantly reduced tumor incidence (Onozawa et al., 1999). In MNU-induced prostate carcinogenesis model, soy protein isolate (SPI) reduced the incidence of prostate tumor (Pollard et al., 2000), and genistein reduced the incidence of invasive prostate adenocarcinoma (Wang et al., 2002).

The transgenic TRAMP model was also used, and the study showed that genistein significantly inhibited the development of poorly differentiated prostate adenocarcinoma in a dose-dependent manner (Mentor-Marcel et al., 2001).

Transplantable prostate tumor models have been widely applied to determine the effects of soy components on the growth and progression of prostate tumors. Defatted soy flours significantly reduced incidence and growth of Dunning R3327 prostate tumors in a rat model (Landstrom et al., 1998). On the other hand, SPI stimulated growth of an androgen-independent prostate tumor in rats (Cohen et al., 2003). Dietary SPI (Bylund et al., 2000), or SPI with soy isoflavones in low-fat diet (Aronson et al., 1999) significantly inhibited the growth of androgen-sensitive human prostate LNCaP tumors. By using subcutaneous tumor model, we found that SPI, SPC, or SPI plus SPC significantly inhibited tumor growth (Zhou et al., 1999). Soybean protease inhibitors, Bowman-Birk inhibitor (BBI), or a BBI concentrate (BBIC), also significantly inhibited the growth of LNCaP tumor (Wan et al., 1999).

While no animal model provides all of the characteristics of the human disease, androgen regulated growth and the potential for metastasis are two clinically important characteristics that are captured in orthotopic implant models utilizing the SCID mouse and the LNCaP human prostate cancer cell line (Sato et al., 1997). In addition, this model allows us to use serum prostate specific antigen (PSA) as a surrogate marker of tumor growth in response to defined soy based dietary interventions. We have conducted a series of animal studies by applying this orthotopic SCID-LNCaP model to evaluate the effectiveness of soy supplements or the combination of soy supplements with tea as a preventive intervention. In addition, we have determined a series of biomarkers that were associated with tumor growth inhibitory effects of soy supplements. The detailed results are reported as follows.

Animal study 1: the effect of soy supplements on orthotopic growth and metastasis of androgen-sensitive prostate tumors (Zhou et al., 2002)

We first evaluated the effects of three different dietary soybean components on prostate cancer growth and metastasis: isoflavone depleted soy protein, soy phytochemical concentrate (SPC) and genistin. Isoflavone-depleted soy protein isolate (soy protein supplement) contains more than 90% protein, less than 4% fat, less than 5% ash, less than 6.5% moisture and 0.01% soy isoflavones as aglycone equivalents. SPC contains 51.9% soy isoflavones by weight (50.8% genistein aglycone equivalents, 40.5% daidzein aglycone equivalents, and 8.7% glycitein aglycone equivalents, other phytochemicals not quantified) and genistin (the glycoside form of

soy isoflavone genistein, the proposed major bioactive ingredient in soy). Genistin was purified from SPC and contains 100% soy isoflavones by weight (90.1% genistein aglycone equivalents, 9.1% daidzein aglycone equivalents and 0.8% glycitein aglycone equivalents). All supplements were provided by Archer Daniels Midland Co. (Decatur, IL).

Animal study

After one-week of acclimation and adaptation to the AIN-76A diet, male SCID mice were randomized into four groups (in each group, n=8) and fed one of the following experimental diets for two weeks: (i) AIN-76 as the control diet; (ii) AIN-76 with isoflavone-depleted soy protein in place of casein, 20% by weight, providing 20 mg total isoflavone aglycone equivalents/kg diet; (iii) AIN-76 with SPC at 0.5% of the diet, providing 1700 mg total isoflavone aglycone equivalents (812 mg genistein aglycone equivalents, 648 mg daidzein aglycone equivalents, and 139mg glycitein aglycone equivalents)/kg; and (iv) AIN-76 with genistin at 0.14% of the diet, providing 812 mg genistein aglycone equivalents/kg, the same amount of genistein equivalents as that in the diet (iii). Mice were then inoculated intraprostatically with LNCaP human prostate cancer cells and continued on experimental diets. Body weight and food intake were measured twice weekly. At 4 weeks and 8 weeks after tumor cell inoculation, phlebotomy was performed by accessing the retro-orbital venous plexus to obtain 100 mõL blood from each animal. Serum PSA level was measured by ELISA assay to estimate the tumor-take rate and tumor size. The experiment was finished when the average tumor weight in the control animals reached 2-5% of the body weight (10 weeks after tumor cell inoculation). The mice were sacrificed; primary tumors were excised and weighed. A tumor slice from each primary tumor tissue was carefully dissected and fixed in 10% buffer-neutralized formalin, paraffin-embedded, and sectioned at 4mõm thickness for histology and immunohistochemistry. Lymph nodes and lungs were harvested, fixed, paraffin-embedded and H&E stained for metastases quantification. Blood samples were collected.

Biomarker determinations

Apoptotic cells were determined by a terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling (TUNEL) assay using the ApopTag *in situ* Apoptosis Detection System (Oncor Inc., Gaithersburg, MD). Proliferating cell nuclear antigen (PCNA) was determined by immunohistochemical staining to quantify proliferation index. Microvessel density (MVD) was used as a marker for tumor angiogenesis and quantified by immunohistochemical staining of Factor VIII. Automated immunohistochemistry followed by image analysis was applied to quantify

the effects of dietary soy treatments on the expression of AR, p53, p21/wafl, VEGF and bFGF. The serum level of PSA was determined by ELISA. Serum levels of testosterone and DHT were measured by enzyme immunoassay.

Results

The experimental dietary treatments did not significantly alter food intake or final body weight. Final tumor weights in mice treated with soy protein, genistin and SPC were reduced by 42% ($P=0.07$), 57% ($P<0.05$) and 70% ($P<0.005$) respectively, compared with that of the control mice treated with AIN-76 control diet. In parallel, serum levels of PSA in mice treated with soy protein, genistin and SPC were reduced by 14% ($P=0.59$), 53% ($P<0.05$) and 59% ($P<0.005$) respectively, compared with that of the control group. In addition to its inhibitory effect on primary tumor size, the dietary supplement SPC inhibited tumor metastasis both to the lymph nodes and to the lungs. Dietary SPC significantly reduced the multiplicity of lymph node metastases by 82% ($P<0.05$), and the multiplicity of lung metastases by 91% ($P<0.05$) when compared with the control. Dietary SPC also reduced the incidences of LNCaP cell metastasis to lymph nodes by 67% ($P<0.05$) and to lungs by 50% ($P>0.05$), compared with the control. In contrast, soy protein and genistin treatments did not significantly ($P>0.05$) reduce either the incidence or the multiplicity of lymph node or lung metastases (data not shown).

Apoptosis rates of primary tumors in mice treated with soy protein, genistin, and SPC were significantly increased by 56% ($P<0.05$), 64% ($P<0.01$), and 83% ($P<0.005$) respectively, compared with that of the control. The soy dietary treatments did not significantly alter the percentage of p53-positive tumor cells *in vivo*. In contrast, dietary SPC significantly increased the intensity of p53 protein expression by 47% ($P<0.05$) compared with that of the control. None of the dietary treatments significantly altered either the percentage of p21/wafl-positive tumor cells or the intensity of p21/wafl expression in tumor cells *in vivo*.

The MVD of primary tumors derived from mice treated with soy protein, genistin or SPC diets were reduced by 58% ($P<0.01$), 58% ($P<0.05$), and 56% ($P<0.05$) respectively, compared with that of the control. bFGF staining intensities in primary tumors from mice treated with soy protein, genistin, and SPC were reduced by 10.3% ($P<0.05$), 10.6% ($P<0.05$) and 8.6% ($P=0.08$), respectively, compared with that of the control. The above dietary treatments did not significantly reduce the VEGF intensity of the primary tumors.

Soy protein, genistin, and SPC did not significantly alter either the circulating levels of total testosterone or tumor AR expression. Interestingly, genistin treatment substantially reduced serum levels of DHT by 95% ($P<0.05$), compared with the control group. Statistical analysis also indicated that serum levels of total testosterone and DHT in the genistin group were significantly lower than that in the SPC group ($P<0.05$). Since SPC diet contained the same dietary level of genistein equivalents as that in the genistin diet, these results suggest that while genistin is a potent soy component that down-regulates serum androgen levels, SPC may contain other soy phytochemical components that can counteract the androgen-lowering effect of genistin *in vivo*. Serum total testosterone to DHT ratio in genistin treated mice was 7 fold higher than that in the control group ($P<0.05$), suggesting that dietary genistin may also significantly inhibit conversion of testosterone to DHT *in vivo*.

Proliferation rates of primary tumors in mice treated with the above diets were not significantly reduced.

Summary

Our results demonstrate that genistin and SPC supplements significantly inhibit tumor weight of orthotopic LNCaP prostate tumor. However, only SPC significantly inhibits metastasis of these LNCaP tumors to lymph nodes and lungs. Moreover, analysis of a series of serum and tissue tumor biomarkers indicates that these three soy components have mechanistically distinct activities as anti-prostate cancer agents.

Animal study 2

The Effect of Soy and Tea Combination on Orthotopic Growth and Metastasis of Androgen-Sensitive LNCaP Prostate Tumors (Zhou et al., 2003)

The chemopreventive properties of the Asian diet may result from interactions among several components that potentiate the activities of any single constituent. In Asia, where intake of soy products and tea consumption are very high, aggressive prostate cancer is significantly less prevalent in Asian men. The objective of the present study was to identify possible synergistic effects between soy and tea components on prostate tumor progression in a mouse model of orthotopic androgen-sensitive human prostate cancer. SPC was used as the source of the soybean phytochemical supplement. Both green tea and black tea infusions were prepared by extracting tea leaves with boiling water (100 °C) for 10 min twice. For animal studies, the tea infusions (15g tea leaves/L water) were freshly prepared every Monday, Wednesday, and Friday, and were used as the

sole source of drinking fluid for tea-treated mice throughout the study. There were six experimental groups: (i) Control: AIN-93M; (ii) SPC: AIN-93M with 5.0 g/kg SPC; (iii) Black tea: AIN-93 with black tea infusion (15 g tea leaves/L water) in place of drinking water; (iv) Green tea: AIN-93 with green tea infusion (15 g tea leaves/L water) in place of drinking water; (v) SPC/Black tea: AIN-93 with 5.0 g/kg SPC and with black tea infusion in place of drinking water; or (vi) SPC/Green tea: AIN-93 with 5.0 g/kg SPC and with green tea infusion in place of drinking water. The experimental procedures and biomarkers determined were the same as described in the Animal Study 1.

Results

Mice treated with SPC, black tea, green tea, the SPC/black tea combination, and the SPC/green tea combination had significantly reduced tumorigenicity rates of 43.8%, 55.0%, 42.9%, 18.8% and 35.7% respectively, compared with the control (87.5%). SPC/black tea combination had a synergistic inhibitory effect on tumorigenicity.

Compared with the control (50%), dietary SPC (31.3%) or tea treatment (21.4% for black tea and 42.9% for green tea) alone did not reduce the incidence of tumor metastasis to lymph nodes. However, mice treated with the SPC/black tea (6.3%) or SPC/green tea (14.3%) combination had a significantly lower incidence of lymph node metastases. The results suggest that that both the SPC/black tea and SPC/green tea combinations synergistically inhibited prostate tumor metastasis to lymph nodes.

Compared with the control, SPC, black tea, SPC/black tea combination, and SPC/green tea combination significantly reduced the final tumor weight by 63.3% ($P<0.05$), 76.7% ($P<0.05$), 93.0% ($P<0.0001$) and 88.0% ($P<0.005$) respectively, but green tea did not reduce final tumor weight (22%, $P=0.11$). In parallel, serum levels of PSA, a marker that is secreted by LNCaP cells and reflects tumor size, in mice in the experimental groups other than the green tea group were significantly reduced. Comparisons of expected and observed values suggest that SPC combined with black tea or green tea synergistically reduced final tumor weight and serum PSA concentration.

Compared with the control, proliferation indices of primary tumors in all experimental groups were significantly reduced by 44%-75%. Similar to the proliferation index, the MVD of primary tumors in all treated groups were significantly reduced by 56%-69% compared with the control. The treatments did not affect the tumor cell apoptotic index.

Mice treated with black tea tended to have a greater serum testosterone concentration (34.4%, $P=0.50$) and had a 72% lower DHT concentration than controls ($P<0.05$), suggesting that black tea may contain components that inhibit the activity of 5aδ-reductase, an enzyme that converts testosterone to the more bioactive DHT. Green tea tended to increase serum testosterone and DHT levels by 73.8% ($P=0.14$) and 194% ($P=0.076$), respectively. The combination of SPC and green tea reduced serum levels of DHT ($P<0.05$).

Summary

Our studies suggest that combination of bioactive components in the Asian diet may be more relevant to explain the cancer-prevention activity of the Asian diet.

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Soy isoflavones in the treatment of prostate cancer

O.Kucuk[1]

Abstract

Epidemiological studies suggest an inverse association between soy intake and prostate cancer risk. We have previously shown that soy isoflavones induce apoptosis and inhibit growth of both androgen-sensitive and androgen-independent prostate cancer cell lines. We conducted a pilot study in patients with advanced prostate cancer to determine the clinical efficacy of soy isoflavones in this population. Patients with prostate cancer were enrolled on the study if they had either newly diagnosed and untreated disease under watchful waiting with rising PSA or had increasing serum PSA following local therapy (Groups I-II) or had failed hormone therapy (Group III). WE administered 100 mg of soy isoflavone (NovasoyÒ) orally twice daily for three to six months. Forty-one patients were enrolled (22 in groups I-II and 19 in group III). Median PSA level was 13.3 ng/ml. Thirty-nine patients were assessed for response. Soy isoflavones were given for a median of 5.5 (range 0.8-6) months. Although there were no decreases in PSA, stabilization of the PSA occurred in 83% of patients in hormone-sensitive (group II) and 35% of hormone-refractory (group III) patients. There was a decrease in the velocity of serum PSA in the whole group ($p=0.01$) with the velocity decreasing from 14% to 6% in Group II ($p=0.21$) and from 31% to 9% in Group III ($p=0.05$) following the soy isoflavones. Serum genistein and daidzein levels increased during supplementation from 0.11mM to 0.65 mM ($p=0.00002$) and from 0.11 mðM to 0.51 mðM ($p=0.00001$), respectively. These data suggest that soy isoflavones may benefit patients with prostate cancer.

Introduction

Epidemiologic studies have shown an inverse association between soy consumption and prostate cancer risk (1-4). Isoflavones have been suggested as the principal chemical constituents responsible for the potential preventive effect of soy against prostate cancer (5). In some Asian countries with high soy consumption, the incidence of latent and small prostate carcinomas

[1] Division of Hematology and Oncology, Wayne State University and the Barbara Ann Karmanos Cancer Institute, Detroit, Michigan, USA

is the same as in Western countries, while the mortality from clinically diagnosed prostate cancer is lower (6), suggesting that soy isoflavones may also inhibit the progression of prostate cancer. A variety of possible mechanisms have been proposed for the activity of soy isoflavones in prostate cancer, which include estrogen-like effects (7), prevention of oxidative DNA damage (8,9), reduction in cancer cell proliferation (10), inhibition of angiogenesis (11), modulation of steroid-metabolizing enzymes (12), tyrosine kinase (13) and topoisomerase II (14), and effects on signal transduction molecules (15).

We have previously observed that soy isoflavone genistein inhibits prostate cancer cell growth in culture in a dose-dependent manner accompanied by a G2/M cell cycle arrest (15). Genistein induced inhibition of cell growth was associated with down-regulation of cyclin B, up-regulation of the $p21^{WAF1}$ and induction of apoptosis. We have also observed that oxidative stress (TNF-ad or H_2O_2) induced activation of transcription factor NF-kB can be abrogated by genistein in prostate cancer cell lines (16) and by soy isoflavone supplementation in normal human volunteers (17). These data provided the rationale for investigating the *in vivo* effects of genistein in patients with prostate cancer.

Methods

Patients had to have histologically proven prostate cancer with a rising PSA and one of the following criteria: (1) untreated disease in patients electing watchful waiting which is deemed appropriate by the managing physician (Group1); (2) patients whose only indication of relapse was rising PSA after having had radical prostatectomy or radiation therapy (Group II); or (3) patients relapsing after having received hormone therapy, i.e. those with androgen-independent PSA progression with or without metastasis following hormonal therapy and anti-androgen withdrawal (4 weeks for flutamide and 6 weeks for bicalutamide) when applicable (Group III). Rising PSA was defined as three successive levels at least 2 weeks apart with a rising trend or PSA $\geq$ 10 ng/ml at two successive evaluations at least 2 weeks apart. Patients had to have a life expectancy of more than 12 weeks and a performance status of 0-3 by Southwest Oncology Group (SWOG) criteria (18).

Treatment plan

Tablets containing 50 mg soy isoflavones (Novasoy®) were provided by Archer Daniels Midland Company, Decatur, Illinois. Each tablet contains 50 mg of total soy isoflavones at a ratio of 1.1:1:0.2 for genistein:daidzein:glycitein, thus each 50 mg tablet provides roughly 25

mg of genistein. The isoflavones are present in both glucosylated, i.e. conjugated (40%) and aglycone, i.e. unconjugated (60%) forms. Other contents of the tablets were soya saponins (30-40%), protein (9%), sugars (<1%), fat (<1%) and other minor constituents. Patients had to take two 50 mg Novasoy® tablets twice daily (it could be taken in one dose or two divided doses with meals).

Complete PSA response was defined as normalization of PSA (< 4 ng/ml, except for patients with history of radical prostatectomy where normalization = < 0.4 ng/ml) sustained for 3 successive determinations, a minimum 2 weeks apart. Partial PSA response was defined as $\geq$ 50% reduction of PSA sustained for at least 3 successive determinations (each minimum 2 weeks apart). Stable PSA was defined as any PSA change that does not qualify for complete (CR) or partial response (PR) or progressive disease. PSA progression was defined as two PSA values at least 2 weeks apart with > 50% increase over the minimum PSA level observed during the study.

Total serum isoflavones were measured using minor modifications of the validated procedure previously published (20). Off-line solid phase extraction of total serum isoflavones was performed following enzymatic deconjugation (Isolute ENV+, 25 mg, Jones Chromatography, Lakewood, CO) followed by quantification of genistein and daidzein using isotope dilution LC-ES/MS with d4-genistein and d3-daidzein internal standards. The method detection limit was approximately 0.02 mdM and the inter- and intra-assay precision and accuracy was ±5-10%. Quality control procedures included concurrent analysis of isoflavone-fortified human serum and blank serum.

Linear mixed-effects modeling was used to test the hypothesis that soy supplementation reduces the rate of PSA rise in patients with prostate cancer. Analysis was stratified by the prior treatment received. PSA measurements within one year prior to intervention were analyzed as baseline levels. Only the PSA measurements within the maximum treatment time of six-month study period were analyzed as post-intervention data. Patients in our study had different numbers of repeat PSA levels measured and all patients did not have their PSA levels measured at precise intervals. Mixed effects models provide a useful alternative to classical multivariate regression techniques for modeling such data. Our model included random patient-specific intercept and slope to account for the natural heterogeneity in the population. This heterogeneity would be expected due to uncontrolled factors affecting the natural course of prostate cancer in our patient population. The fixed effects part of our model included time-effect, effect of the soy isoflavone intervention and time ´ intervention interaction. Logarithmic

transformation of the outcome variable was considered to achieve a better model fit. For the covariance model, we fitted several structures, and the model for final inference was selected based on Akaike's Information Criterion (AIC) and Schwarz' Bayesian Criterion (BIC). All analyses were performed using PROC MIXED in SAS. Baseline measures of genistein, daidzein, testosterone, IGF-1, IGFBP-3, and 5-OH-mdU were compared to post-intervention values using paired t-test.

Results

From January to July of 1999, forty-one patients with prostate cancer and rising PSA were enrolled. Patient characteristics are shown in Table 1. The median age was 73 years, and the median PSA was 13.3 ng/ml. Of the 41 patients, four were in Group I, 18 were in Group II and 19 were in Group III. Among group III patients, 14 had metastatic disease and 5 had non-metastatic (i.e. no radiologic findings of metastases) androgen-independent prostate cancer. All group III patients had castrate range testosterone.

Soy isoflavone supplementation was given on study with a median duration of 5.5 months (range: 0.8-6 months). If subjects had stable disease and wished to continue supplementation beyond the six months required by the protocol they were allowed to do so. However, for the purpose of the efficacy analysis, PSA data collected on these patients beyond the 6 months on protocol were not used so as not to bias results. Patients with disease progression by PSA or clinical criteria were taken off the study and placed on other treatments. Overall 26 patients received between 3 and 6 months of supplementation, 7 patients received more than 6 months of supplementation (off study), and 8 patients received less than 3 months of supplementation. The mean post-intervention serum levels of total genistein and daidzein increased by approximately five-six fold from the pre-intervention level ($p<0.001$).

Two patients from Group III were not assessable for response. Data from a total of 39 patients were analyzed. The median number of pre-intervention PSA measurements per patient was 4 (range 2-5) and the median number of post-intervention measurements per patient was 5 (range 1-6). Overall, there were no complete or partial PSA responses (as defined in the Methods section) in any of the groups. However, stable PSA (as defined in Methods) was observed in 3 of the 4 (75%) patients in Group I, 15 of the 18 (83%) patients in Group II and 6 of the 17 (35%) patients in Group III. Because of the latter observation we then evaluated the rate of rise in PSA. Overall there was a decrease in the rate of PSA rise, which was 20% prior to intervention and 6% during the isoflavone supplementation ($p=0.01$). For

group II (androgen-dependent, non-metastatic, PSA-relapse) the rate of PSA rise decreased from 14% velocity to 6% velocity ($p=0.21$) and for group III (androgen-independent, i.e. hormone-refractory PSA rise) the rate of rise (i.e. velocity) decreased from 31% to 9% ($p=0.05$) following intervention. Separate analysis was not feasible for group I due to the small number of patients.

Discussion

We have previously reported data suggesting growth inhibitory effects of soy isoflavones in androgen-sensitive and -insensitive prostate cancer cell lines (15,23). In this pilot clinical trial we sought evidence of clinical activity of soy isoflavone supplementation in patients with prostate cancer. Since soy isoflavones are found in the human diet and have no known significant toxicity, they are attractive compounds in patients with advanced prostate cancer, who may not be candidates for standard therapy because of their old age, indolent disease and/or co-morbid conditions. Our results confirmed that soy isoflavones are safe, well accepted and well tolerated by prostate cancer patients. Although no sustained PSA declines qualifying for a PR or CR (see Methods section for a description of PR and CR) were observed, a significant number of patients had stable PSA levels (83% and 35% of patients in groups II even prolong survival.

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Status of traditional fermented soy food products: a future trend

S. Maneepun and M.Yunchalad[1]

Abstract

Traditional fermented soy food products have become part of daily diet in Asian countries. The fermented process has been studied to improve quality, taste, flavor, aroma and nutritional values to expand the market to other continents. Both salted and non-salted soybean fermentation products were identified to eliminate wild microorganisms to improve quality and safety. The well-known salted soybean fermentation products are soy sauce, soy paste and fermented soy curd. Presently, soy sauce is the most recognized product in global market, which quality standards and safety have been under consideration from member countries of FAO/WHO Codex Commission. The non-salted soybean fermentation products have traditionally been produced and consumed in various parts of Asian continent, which the phenomenal practices found the uniqueness of similarities. Most products obtained from natural fermentation and became a cheap source of high protein-rich food in local diet. Several investigations were also found predominant microbial activities produced polypeptides and amino acids from soybean protein during fermentation. The products have the improvement of nutritional quality, which has been promoted for health benefit. However, there are some products still consuming in the regions where they originated. Only natto in Japan and tempe in Indonesia have gained popularity nationwide. The others, chongkuk-jang in Korea, dou-chi in China, kinema in Nepal, Bhutan and northeastern Himalayan regions of India and thau-nao in northern Thailand have still been assessed microbial diversity association with traditional fermentation practices in producing specific taste and flavor for their consumers. The future research and development should be able to standardize a fermentation process to have widely acceptable products.

Keywords

fermented soy food products, salted, non salted, soy sauce, soy paste, fermented soy curd, natto, tempe, chongkuk-jang, dou-chi, kinema, thau-nao

[1] Institute of Food Research and Product Development Kasetsart University, Bangkok, Thailand, e-mail: usmp@ku.ac.th

Introduction

Several traditional fermented soy food products have widely consumed and become palatability of daily diet in Asian countries for centuries. The scientific studies of soy foods have continuously been viewed the numerous health benefits resulting a dramatic increase the consumption. Individual traditional fermented process has been identified specific microorganisms, which involve in producing flavors, aroma, texture and nutritional value, and also considerable to improve quality and safety of the final products. The fermentation process is classified into 2 categories; salted and non-salted soybean fermented products. These fermented products from both processes have been studied the conversion of chemical and physical properties of soybean component to savory, meat-flavored sauces and pastes (Shurtleff and Aoyagi, 1980). The products from salted fermentation are mainly used for condiments, seasoning agents, and flavor enhances, while the non-salted fermentation is used for preparing dishes and snack foods as shown in Table 1.

Salted soybean fermentation products: The common practices of traditional salted soybean fermentation start from cleaning, soaking in water overnight and drain, cooking with a small amount of water, mashing and then forming into ball. Soybean balls are covered with rice straw and placed under the ceiling of warm room for 1-2 months. During this periods, *Aspergillus spp* molds grow on the surface and bacteria, especially *Bacillus spp.,* grow inside of the balls (Lee, 2001). The molds covered soybean balls are then mixed with brine solution and allow digesting for a year or longer. Enzymes from the mold digest proteins, lipids and carbohydrates yielding savory, amino acid/peptide flavors were identified and then the controlled fermentation process also developed. However, the manufacturing process of fermentation from individual country has modified base on traditional taste, flavor and aroma to foods that consumers prefer. Some countries have been developed their fermentation process far from traditional methods.

Fermented soy sauce: fermented soy sauce was developed and become the most

recognized product in the global market. The characteristics of soy sauce have ranges of color from bright reddish-brown to dark-brown liquid, with a salty taste, unique sharp flavor and aroma. Quality criteria is examined by organoleptic evaluation, content of nitrogen, alcohol, soluble solids other than sodium chloride and color. Superior-quality of soy sauce has to obtain from the action of microorganism only(Fukushima, 1989). The manufacturing process consists of 3 major processes: fermentation starter, brine fermentation and refining.

Table1. Traditional fermented soy food products

Names and main consumers	Substrate form	Type of fermentation	Main uses
1. Salted fermentation			
1.1Soy sauce (worldwide)	whole	Mold + bacteria +yeast	All purpose seasoning agents for cooking
1.2 Soy paste (*SEA Countries, Japan, Korea, China)	whole	Mold + Bacteria + yeast	Flavoring agents
1.3 Fermented soy curd (*SEA countries, Japan, Korea, China)	Soy-curd	Mold + Bacteria + yeast	Relish, ingredient for Cooking with meat and vegetables
1 **Non-salted fermentation**			
Natto (Japan)	Whole	Bacteria	Main dishes and seasoning Main dishes and snack foods
Tempe (Indonesia)	Whole	Mold	Main dishes and seasoning Main dishes and seasoning
Thau-nao (Thailand)	Whole	Bacteria	Main dishes and seasoning Main dishes
Chongkuk-jang (Korea)	Whole	Bacteria	
Dou-chi (China)	Whole	Mold or Bacteria	
Kinema (Nepal, Bhutan and Northern India)	Whole	Bacteria	

*Southeast Asia (SEA) Countries consist of Cambodia, Indonesia, Laos, Malaysia, Mynmar, Philippines, Singapore, Thailand and Vietnam

The development of fermentation starter from pure mold culture was transferred to fermented soy sauce factory to improve the quality products. The process is called soy sauce *koji* in Japan and Southeast Asian countries. The ratio of cereal grains, i.e. wheat flour, broken rice, corn flour etc, and soybeans showed significantly different characteristics in amino acid contents, myriad flavor and color. In Korea has also developed starter called *meju* balls from cooked soybean with small rice flour on the surface and incubated till mold fully grown, dried and packing in polyethylene film bag for home made soy sauce.

Brine fermentation is the second stage of soy sauce making by transferring *koji* in salt solution of 22-25.5 (w/v) and keeping for 4-6 months. The fermentation activities of halophlic lactic acid bacteria, *Bacillus spp,* and salt tolerant yeast, *Saccharomyces rouxi* and *Pediococcus halphilus,* produced amino acids and low molecular weight peptides, and starch converted to simple sugars, lactic acid, alcohol, and carbon dioxide. The

techniques to control specific microorganisms during brine fermentation were established by adding pure cultures to produce desirable quality soy sauce.

The refining process is the final stage, the ripening mash is filtering with hydraulic press machine and pasteurizing liquid part by heat. The heating process has the important roles in developing large numbers of flavor compounds, color, improving clarity, destroying yeast and inactivating most of the enzyme (Fukushima, 1985). After clarifying, the clear soy sauce is bottled and packed.

The proposed draft FAO/WHO codex standard was submitted for committee meeting in September 2002. The description for product definition is; " Naturally brewed soy sauce is the product obtained by: (a) *Aspergillus oryzae* and/or *A. sojae* as a starter are cultured in either soybean or soybean and cereal grains; bacteria and/or molds and/or yeast are culture in either soybean or soybean and cereal grains, (b) the mixture of the product obtained by (a) and salt water is fermented and aged for not less than 90 days below its temperature of 40 °C and for short-term brew soy sauce the aging is less than 90 days." The quality factors include;" (a) Total nitrogen not less than 0.7% (w/v); provided that the product derived wheat not less than 80% of total of soybeans and cereal grains weight shall have the total nitrogen of not less than 0.4%(w/v), (b) Soluble solids contents, exclusive of added salted not less than 6%(w/v)." The draft is at step 3 of codex procedure and still inviting member countries for more comments.

Fermented soy paste: Fermented soy paste products were processed slightly difference and consumed as seasoning in SEA countries and over 90% used as soup in Japan called *miso*. The production has 2 stages, fermentation starter (koji) and brine fermentation with 3-4 months aging. The microorganism involved in fermentation starter, *Aspergillus oryzae, Bacillus spp*, and *Saccharomyces spp, Pediococcus halophilus* during brine fermentation. Semi-solid paste, light brown to dark brown, salty taste with specific flavor and aroma produced after aging for 3-4 months (Maneepun, 2000). Since the process of both soy sauce and soy paste is similar, solid part after liquid separation was added to increase the solid content in soy paste in some factory for second grade soy paste. The soy paste has been improved especially reduced salt content, lighter color and aroma to extend the consumption to western foods in salad dressing and barbecue sauce. Future research and development to increase the utilization as ingredients in foods should be emphasized.

Fermented soy curd: Fermented soy curd is a cream cheese-type product with mild flavor and a fine texture and nutritionally rich with good protein,

which is made from soybean curd and the action of mold culture. The production of enzymes and pigments obtained from incubated rice with starter inoculums of *Monascus spp* producing red-*koji* and *Aspergillus oryzae* producing yellow-*koji.* Red-*koji* has better taste, aroma, and color than yellow-*koji* to food(Yasuda, 2003). There are 3 stages for fermented soy curd processing; preparing tofu, molding and brine. The development of soy bean curd after applying pure culture of appropriate mold with incubation at 32°C for 7 days. The process follows with soaking in brine and aging for 4-5 months to develop flavor and aroma. Yellow color product is consumed directly as relish and red color is for cooking.

Non salted soybean fermentation products: These traditional products obtained from natural fermentation of cooked soybean with mold or fungus. The whole soybeans are normally used in the fermentation process, therefor these fermented soybean food products were studied their nutritional values and promoted for consumption since 1950 (Murata et al, 1990). The product became a cheap source of high protein-rich food in local diet. Microorganisms involved in the fermentation process have been identified and developed quality and safety of products to processing industry especially tempe in Indonesia and natto in Japan. The main of microorganisms in non-salted soybean fermentation process is shown in Table 2

Table 2. Main microorganisms in non- salted soybean fermentation process

Products	Main microorganisms in fermentation process
Natto	*Bacillus subtillus* var. natto
Tempe	*Rhizopus oryzae, R. oligosporus, Mucor indicus*
Thau-nao	*Bacillus subtillis*
Chongkuk-jang	*Bacillus subtillis*
Dou-chi	*Mucor racemosus, Mucor spp, Aspergillus spp*
Kinema	*Bacillus subtillis*

All products after 3-4 days fermentation the cooked soybeans were covered with a sticky mucous, colorless gum and strong smell. The mucous substance is peptido-polysccharides producing from *Bacillus subtillis,* which recently studied discovered the health beneficial physiological function to body. This short fermentation process produced strong proteolytic enzymes and partially hydrolyzed protein that have an effect to suppress the incidence of high blood pressure (Lee, 2001). The individual product has different development and mainly is consumed where it was originated and practiced. Fresh product in paste has only 2-3 day shelf life or more depending on hygienic practices. Dried product in chunks or chips can be kept for year.

The production of natto product was fully developed at industrial level by using pure culture,, *Bacillus natto* , with controlling temperature chamber. Retail package was also available for consumers selection (Dangsupa, 1994). Tempe from Indonesia was identified 50 molds isolated, *R. oryzae, R. oligosporus* and *M. indicus,* contributed 78 % of total molds during fermentation (Samson et al. 1987). The product has gained the popularity nationwide and also promoted the consumption in several dishes and as food ingredients. The product was furthermore developed to produce meat-like texture as vegetarian meat substitute (Steinkraus, 2003). Thau-nao from Thailand has improved by selecting microorganisms and introducing mixed cultures of *Bacillus sp.* B_4, *Klebsiella sp*. KB_2 and *Candida sp*. KB_1 to enhance more soluble protein, to increase vitamin B_{12} content and to reduce strong smell respectively (Tangtitjaroenkun et al.,2003). The product is still investigating to improve nutritional values to promote nationwide consumption.

Chongkuk-jang in Korea is not popular among young generation due to strong fermented smell. The product is known as an instant military food in the old day. Spicy seasonings, ginger, garlic and pepper, were introduced to cover fermented smell. Nutritional values were studied to promote the consumption. Dou-chi from China was investigated microbial organisms involved in fermentation process, which has practiced differently in the country. Black and yellow soybeans were used to produce the products with slightly different fermentation processes. Microbial organisms were mainly, *M. recemosus, Mucor spp.* and *Aspergillus spp.*, which are still using traditional process (Li and Li, 2000). Kinema from northeastern India, Bhutan and Nepal was assessed microorganisms associated with fermentation process in producing taste, flavor and aroma. The product is still consuming where it is originated (Tamang, 2000).

Conclusions

Salted soybean fermentation products were fully developed and introduced the production to industry. Pure culture, controlling temperature chamber and processing equipment was designed to produce the products with consistency quality and safety. The FAO/WHO Codex Commission for soy sauce product was drafted for international trade. Some non-salted soybean fermentation products need furthermore development especially, thua-noa from Thailand, chongkuk-jang from Korea, dou-chi from China and Kinema from northeastern India, Bhutan and Nepal to nationwide consumption. Natto from Japan and tempe from Indonesia should be promoted the consumption worldwide.

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Depart. of Microbiology, Fac. of Sci., Kasetsart U., Bangkok, Thailand

Tempeh as a functional food

M.J.R.Nout[1] and J.L.Kiers[2]

Abstract

Tempeh is a fungal fermented soybean food originating from Indonesia but with increasing international interest. It is produced by a process of dehulling, soaking, cooking, and fermenting soybeans. The fungal enzyme activity causes significant decomposition of polymeric components and modifocation of soybean flavonoids. As a result, tempeh has a number of proven health benefits including strongly improved digestibility, protection against diarrhoea and chronic degenerative diseases. Tempeh is an interesting food-grade ingredient for formulated functional foods.

Introduction: tempeh fermentation

Tempeh or in Indonesian spelling "tempe", is a collective name for a sliceable mass of pre-cooked fungal fermented beans, cereals or some other food processing by-products bound together by the mycelium of a living mould (mostly *Rhizopus* spp.). Yellow-seeded soybeans are the most common and preferred raw material to make tempeh (Nout, Rombouts, 1990; Steinkraus, 1995). Figure 1 shows a cross section of soybean tempeh, as sold in the Netherlands.

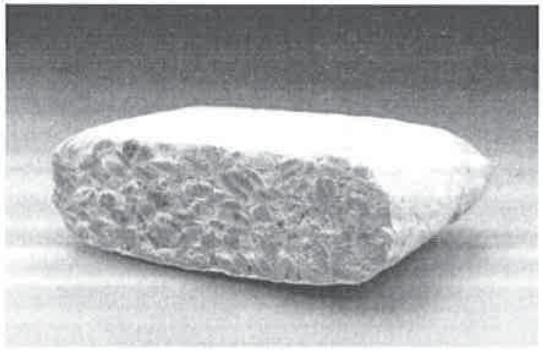

Figure 1. Cross section of tempeh showing the fungal mycelium penetrating the mass of soybeans.

[1] Laboratory of Food Microbiology, Wageningen University, P.O.Box, 6700 EV Wageningen, The Netherlands, (rob.nout@wur.nl)
[2] Friesland Nutrition Research, Friesland Coberco Dairy Foods,P.O. Box 226, 8901 MA Leeuwarden, The Netherlands

The process of tempeh manufacture is shown in Figure 2 and involves dehulling , soaking in water, boiling in fresh water, inoculation with fermentation starter, and solid-state fermentation of beds of inoculated beans. After incubation periods of typically 2 days at 30°C, fresh tempeh can be harvested and processed into meal components, snacks, or dehydrated to obtain powdered protein enrichment.

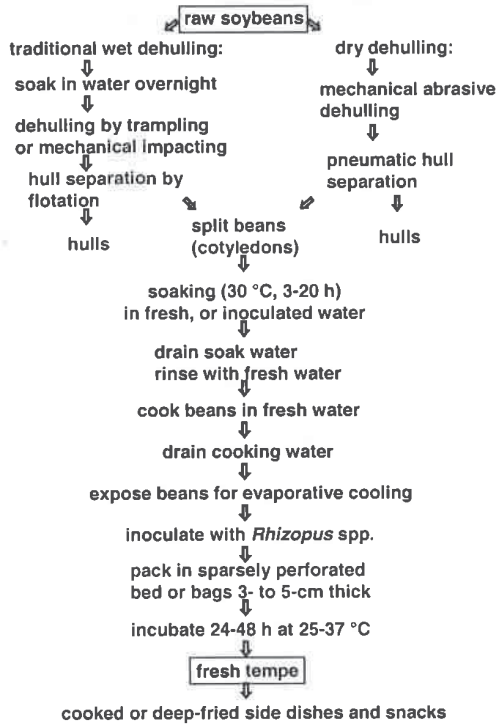

Figure 2. Simplified process diagram of tempeh manufacture.

A wide variety of microorganisms is involved in the fermentation. During the soaking stage, bacterial activity is fuelled by the water-soluble matter leaching from the beans. During the solid-state fermentation, moulds (especially *Rhizopus oligosporus, R. oryzae*, and *Mucor indicus*) are responsible for texture, flavour but most importantly, for the enzyme activities that are expressed. Important enzymes include carbohydrases (Sarrette et al., 1992) degrading fiber, proteases (Baumann, Bisping, 1995) and lipases (Ruiz-Teran, Owens, 1996). As a result of these enzymatic activities, the cooked beans undergo sugnificant biochemical modifications

which improve the taste and flavour, as well as the functional properties of the product.

Functional properties of tempeh

History of use
Tempeh has evolved as a traditional meat alternative in Indonesia. It was locally known for its easy digestibility, and anecdotal evidence says that many prisoners of war survived the internment period due to tempeh as this was one of the few foods they could tolerate. During the 1960ies, tempeh became global and became favorite with vegetarians. Nowadays, increasing numbers of non-vegetarian consumers include it in the diet for the purpose of variation and to reduce the number of meat-days. Local experience in Indonesia shows that addition of tempeh to the diet of (young) diarrhoea patients shortens the recovery period (Sudigbia, 1999).

Pre-digestion of nutrients
The enzymic degradation of polymeric soybean comounds into smaller-sized water-soluble products increases the level of water-soluble dry matter in tempeh 3- to 4-fold. This can be demonstrated very well using in-vitro digestion techniques using artificial saliva, stomach buffer and pancreatic juice (Kiers et al., 2000a). In-vivo trials with rats and piglets show evidence of increased protein digestibility, protein efficiency ratio and net protein utilization (Tchango, 1995), and higher uptake of total solutes (Kiers et al., 2000b).

Antimicrobial effects
The antibacterial effects of tempeh appear to be rather divers: no inhibtition of enterotoxic *Escherichia coli* was observed (Kiers et al., 2002), whereas in different settings broad-spectrum antimicrobial effects against *B.cereus*, *E.coli*, *B.subtilis*, *Proteus vulgaris*, *S.aureus* and *Salmonella typhimurium* were reported (Li et al., 1993).

Protection against diarrhoea
Recent work shows that several tempeh extracts were able to inhibit adhesion of *E. coli* to piglet small intestinal brush border membranes and might have a protective effect against *E. coli* infection (Kiers et al., 2002). In rabbits and piglets, diarrhoea caused by E.coli was reduced by tempeh. These findings correlate with a protective effect against fluid losses in small intestinal segment perfusion experiments (Kiers et al., 2000b). Tempeh appeared to contain a high-molecular-weight fraction (> 5kDa) that protected against fluid losses induced by ETEC. Tempeh can be very useful as a nutritional supplement in oral rehydration therapy, and in case of (postweaning) diarrhoea and accelerating

the recovery of young animals and young children, who are most at risk for enterotoxic diarrhoea and malnutrition.

Intestinal growth and proliferation
Weaning is often associated with marked histological and biochemical changes of the small intestine, causing decreased digestive and absorptive capacity and contribute to post-weaning diarrhoea. Biopsies from the small intestinal mucosa showed improved repair after intestinal inflammation as a result of tempeh supplementation (Sudigbia, 1999). In a trial with piglets, no indication of beneficial effects of tempeh on maintaining or quickly restoring villous height in piglets after weaning was observed.

Anti-oxidative properties of fermented soybeans
Soybeans contain natural antioxidants. It is interesting to note that fermented soybeans foods do not lose their anti-oxidative properties, but in contrast show increased anti-oxidative capacity (Berghofer et al., 1998). The 4 important aglycones in tempeh are genistein, daidzein, and glycitein, and factor 2 (6,7,4'-trihydroxyisoflavone) (Hoppe et al., 1997). Another anti-oxidative substance in tempeh was identified as 3-hydroxyanthranilic acid (HAA); this was not detected in unfermented beans (Esaki et al., 1996). In human trials it was shown that isoflavones formed during the tempeh fermentation are available to the human body.

Chronic degenerative diseases
Besides the role of antioxidants in protecting the food against oxidative stresses, antioxidants in soybean (tempeh) are of interest with respect to their protective role against oxidative stress known to be involved in the pathogenesis of various chronic degenerative diseases such as cancer, coronary diseases, osteoporosis and menopausal symptoms. Soybean protein has been known for many years to have a hypocholesterolaemic effect. It is therefore not surprising that tempeh has also been found to lower blood cholesterol levels (Guermani et al., 1993) and may therefore be of benefit as a protective agent against cardiovascular disease. In a number of clinical intervention trials, total cholesterol and low-density lipoprotein cholesterol were significantly reduced in subjects treated with tempeh, whereas HDL cholesterol was raised (Karyadi, Lukito, 1996; Brata-Arbai, 1997; Brata-Arbai, 1999). It was demonstrated that tempeh, especially its glucolipids, inhibits the proliferation of tumour cells in mice (Kiriakidis et al., 1997; Jha et al., 1997b). Indonesians are known as the largest soybean-consumers, especially in the form of tempeh and tofu (locally called tahu), in the South-East Asian countries. Epidemiological studies relating to tempeh consumption and the prevalence of cancer, particularly in Indonesia, have not yet been conducted.

Novel applications

In addition to its traditional use in oriental and western cuisine, tempeh can be processed into powdered form for convenient use in formulated foods and feeds. Its functional properties will be of interest in the areas of diarrhoea management, nutritional recovery of comprimized patients, health foods, as well as in the specialized feeds such as weaning formula for piglets.

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Kinema, as a functional food in the Indian Himalayas

J. P.Tamang[1]

Abstract

Kinema is a traditional fermented soybean food, which is sticky, slightly alkaline with typical flavour produced by bacterial fermentation. *Kinema* is a low-cost, high plant protein food in the local diet, eaten as side-dish curry. The possible hypothesis of origin of *kinema* has been discussed. *Bacillus subtilis* is the functional microorganism in *kinema* fermentation. On a protein cost per kilogram basis, *kinema* is the cheapest source of plant protein than animal and dairy products. Total amino acids, free amino acids and mineral contents are increased during *kinema* fermentation, and subsequently enriched the nutritional value of the product. *Kinema* is projected as a functional soybean-food in the Indian Himalayas. Selected strain of *Bacillus subtilis* is used as pulverized starter for upgradation of *kinema* production.

Introduction

Soybean has been traditionally used to prepare fermented and non-fermented foods in the Eastern Himalayan regions of Nepal, India and Bhutan for centuries. Soybean is a summer leguminous crop grown under rainfed conditions in upland terraces as a sole crop as well as mix-crop with rice and maize up to an altitude of 1500 m. Indigenous local varieties of soybeans [*Glycine max* (L.) Merrill] 'yellow cultivar' and 'dark brown cultivar' are sown in May to June, and are harvested in November. Most of the soybeans produced in these regions are consumed locally (Tamang, 1996). *Kinema* is a soybean-based fermented food, which is sticky, slightly alkaline product with typical flavour produced by bacterial fermentation.

Origin of *kinema*

Though there is no historical document on origin of *kinema*, it is certain that the Limboo (one of the major ethnic communities of Nepali) started production and consumption of this fermented flavoursome soybean food. The word *kinema* has been derived from '*kinambaa*' of the Limboo language, "*ki*" means fermented and "*nambaa*" means flavour (Tamang, 2001). A

1 Food Microbiology Laboratory, Department of Botany, Sikkim Government College, Gangtok, Sikkim 737 102, INDIA, E-mail: jyoti_tamang@hotmail.com

kingdom of 'Limbuwan' (presently the districts of Nepal viz. Therathum, Taplejung, Panchthar, Dhankuta, Ilam) had been established by the Limboo earlier than 7th century and they ruled independently till unification of Nepal in the 18th century (Subba, 1999). Unification of Nepal, existence of mixed society of multiethnic communities and migration of people from one place to another might have resulted in diversification of *kinema* making and eating to other related Nepali communities such as Rai, Tamang, Gurung, Mangar, Sunwar, etc. Other mountain ethnic communities in the Eastern Himalayas have now shared delicacy of *kinema*. The Lepcha calls it *satlyangser* and the Bhutia calls it *bari* in Sikkim. In Meghalaya, the Khasia calls *turangbai*; in Manipur, the similar product is known as *hawaijar*, in Mizoram, the Mizo calls it *bekanthu* and in Nagaland, the Naga calls it *aakhuni*. In Myanmar, it is commonly called *pe-poke*. Probably, *kinema* diversification had route from eastern Nepal. Some food anthropologists believed that the present day traditional Asian fermented soybean foods had originated from *douchi*, the oldest type of fermented soybean in southern China around 300 BC during the Han dynasty (Bo, 1984). Yoshida (1993) believed that during the Han dynasty, *douchi* was spread northward to the northern part of China, westward to eastern part of Nepal, southward to Indonesia and eastward to Japan. The nature of the products showed more similarities, however, the method of preparation and mode of consumption vary from place to place. Hara *et al.* (1995) reported that plasmid of *Bacillus subtilis* (*natto*) strain (microorganism in *natto*) resembles that of *Bacillus subtilis*, the dominant microorganisms of other Asian fermented sticky soybean foods such as *thua-nao* and *kinema*. Tamang *et al.* (2002) confirmed similarity among *Bacillus subtilis* of fermented soybean foods of Asia on the basis of phylogenetic analysis by determining the 16s RNA sequencing. This proved that they might have originated from the same stock. *Douchi* may be the source of origin of *kinema* and other fermented foods, but I believe that unless and until we have the scientific basis, probably on the basis of powerful tool of molecular identification of the functional microorganism in these products, we will not be able to trace the antiquity of *kinema*. Moreover, food anthropologists should also trace the antiquity of practice of soybean cultivation in Nepal and adjoining regions in the eastern Himalayas, and consequently preparation of *kinema*. If we look at the history of the Kirat (to which Limboo belongs), the Kirat race is believed to have come to their present abode in "Limbhuwan" in Nepal in between 600 to 100 BC (Subba, 1999). The appearance of *douchi* during Han dynasty in China around 300 BC might be correlated in regards to *kinema* origin. Whether *kinema* appear first and diversified to other similar products or vice versa, is a big challenge to food anthropologists, historians and food microbiologists. *Kinema* is, no doubt, consumed as one of the oldest cultural foods in Asia.

Hypothetically, it can also be said that origin of fermented sticky soybean is just an accident. Perhaps, some cooked soybeans were left after the meal, next morning; viscous stringy thread with typical flavoured product was seen. People might have liked the 'new' soybean product with appealing flavour and texture, instead of monotonous cooked beans. This could probably be the origin of *kinema* and other similar soybean products, and subsequently developed by the innovation and demand of local people depending on agro-climatic conditions. Antiquity of *kinema*, however, is yet to be explored.

Preparation of *kinema*

During preparation of *kinema*, yellow variety of small-sized (~ 6 mm) soybeans are soaked overnight in water and cooked by boiling until they are soft. Excess water is discarded and soybean seeds are cracked lightly by a wooden pestle (locally called *muslo*) in a wooden mortar (locally called *okhli*) to split the cotyledons, probably to accelerate the fermentation and increase the surface area for aerobic spore-forming bacteria. About 1 % of fresh firewood ash is added to soybeans to maintain the alkaline condition of the product. Grits are placed in a bamboo basket lined with locally grown fresh fern {*Glaphylopteriolopsis erubescens* (Well ex. Hook.) Ching} fronds, and covered in a jute-bag. At this stage the beans are still hot (50-60° C). Grits of beans inside the basket are dimensioned to ensure adequate aerobic condition. A package is made by folding the ferns and sack-cloths in closed position and left to ferment naturally at ambient temperatures (25-40° C) for 1-2 days above earthen-oven kitchen. In eastern Nepal, dark-brown varieties of soybean are used to make *kinema*. Instead of fern leaves, fig (*Ficus* spp.) and banana leaves are used as wrapping materials. Other methods remain same. Completion of fermentation is indicated by appearance of white viscous mass and typical *kinema* flavour with slight ammonia odour. Shelf-life of fresh *kinema* is 2-3 days during summer and a maximum of one week in winter without refrigeration. Dried *kinema* is stored for several months at room temperature. Preparation of *kinema* varies from place to place and is still restricted to household level. It is interesting to note the mountain women using their indigenous knowledge of food production prepare *kinema*. This unique indigenous knowledge of *kinema*-making has been protected as hereditary right and passes from mother to daughter, mostly among the Limboo women. *Kinema* is eaten as curry with boiled rice. Fresh *kinema* is fried in vegetable oil, and chopped onions, tomatoes and turmeric powder are added and fried for 2 min. Then salt and sliced green chilies are added and fried for 3-5 min. A little water is added to make thick gravy, and cooked for 5-7 min. *Kinema* curry is ready for serving with boiled rice (Tamang and Tamang, 1998). Dried *kinema* is sometimes mixed with leafy vegetable to make mixed curry.

Economy

Kinema production is done at individual or household level by rural women. Some people are economically dependent on this product. *Kinema* is sold in local markets. It is sold in amount equivalent to weights of 150-200 g packages of *kinema*, usually packed in *nevara* (fig plant) leaves and tied loosely by straw. The 100 g of *kinema* cost about Indian Rupees 5/-. Daily 3-5 kg of *kinema* is sold by each seller in market who produces *kinema* for commercial purpose. About 40 % of profit can be made out of selling *kinema*. The 60 % expense includes cost of raw soybeans, fuel for cooking, transportation from village to town, etc. This little profit they spend on children's education and procuring of essential commodities not locally available, and also on domestic expenses. This trade has been protected as hereditary right and passes from mother to daughter (Tamang, 2000a). *Kinema* production is an income-generation for some families. Though there is a good market for *kinema*, and some village women are involving in this income generation, *kinema* processing has not been included in loan-scheme of public sector banks or financial institutions, neither incorporated in the rural development programme and small-cottage industry scheme of the government.

Microorganisms and nutritional value

Bacillus subtilis, Enterococcus faecium, and few contaminant yeasts such as *Candida parapsilosis* and *Geotrichum candidum* are present in *kinema* (Sarkar *et al.*, 1994). However, *Bacillus subtilis* is the dominant functional microorganism in *kinema* fermentation (Sarkar and Tamang, 1994; Tamang and Nikkuni, 1996; Sarkar *et al.*, 2002). It is observed that rich microbial diversity in various sources particularly soybean, equipment and leaves as wrapping materials harness indigenous microbiota for spontaneous fermentation of *kinema* (Tamang, 2000b). Practice of not cleaning up the mortar and pestle, and using fresh leaves as wrapping materials by rural people, significantly correlate their indigenous knowledge of 'microbiology' to preserve and supplement microorganisms for spontaneous fermentation of *kinema* without using starter cultures (Tamang, 2003). The traditional way of preparation and its culinary use in curries is safe for consumption (Nout *et al.*, 1998).

During *kinema* production, soya-proteins, which have been denatured by cooking process, are hydrolyzed by proteolytic enzymes produced by *Bacillus subtilis* into peptides and amino acids enhancing digestibility (Tamang and Nikkuni, 1996). Remarkable increase in water-soluble nitrogen and trichloroacetic acid (TCA)-soluble nitrogen contents were observed during kinema fermentation (Sarkar and Tamang, 1995). Total amino acids, free

amino acids and mineral contents (Table 1) were increased during *kinema* fermentation, and subsequently enriched the nutritional value of the product (Tamang, 1995; Nikkuni *et al.*, 1995). *Kinema* is highly nutritious food containing (per 100 g dry matter) Protein 48 g, Fat 17 g, Carbohydrate 28 g and Calorie 2 MJ (Tamang, 1992). *Kinema* is projected as a low-cost functional food in the local diet.

Table 1. Nutritive value of *kinema*

Parameters	Raw soybean	*Kinema*
Protein (g on dry matter)	47.1	47.7
Fat (g on dry matter)	22.1	17.0
Carbohydrate (g on dry matter)	25.8	28.1
Ash (g on dry matter)	5.0	7.2
Fe (mg/100 g)	8.7	17.7
Mn (mg/100 g)	2.7	5.4
Zn (mg/100 g)	3.8	4.5
Na (mg/100 g)	1.7	27.7
Ca (mg/100 g)	186.0	432.0
Total amino acids (mg/100 g)	43654	46218
Free amino acids (mg/100 g)	472	5129
Water soluble nitrogen (% of TN)	15.4	62.0
Formol nitrogen (% of TN)	1.5	7.0
Energy (MJ/100 g dry matter)	2.1	2.0

Pulverized starter for *kinema* production

The traditional preparation of *kinema* varies in quality. Strains of *Bacillus subtilis* KK-2:B10 (deposited at Microbial Type Culture Collection, Chandigarh 2756) and GK-2:B10 (deposited at Microbial Type Culture Collection, Chandigarh 2757), previously isolated from naturally fermented *kinema*, were selected as starters for laboratory-scale under optimized conditions (Tamang and Nikkuni, 1996). The quality of *kinema* was maintained with pure culture fermentation by *Bacillus subtilis* KK-2:B10 at 40° C for 20 hour followed by maturation at 5° C for 1 day (Tamang and Nikkuni, 1998).

Use of ready-made pulverized starter appears more appropriate in *kinema* production at household level (Tamang, 1999). This will foresee the commercialization of low-cost, high plant protein food, with diversification of recipe acceptable to consumers. Patent has been filed to Indian Patent Office.

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Recent progress in flavor research of miso with emphasis on 4-hydroxyfuranones

Y. Hayashida[1]

Abstract

4-Hydroxyfuranones, HDMF and HEMF are flavor-active compounds which have a caramel-like odor and have been identified as important compounds in the miso fiavor. Recently, some reports on the physical effects of these furanones involving anti-carcinogenesis were appeared on journal, then these furanones has been industrially remarked from the aspect not only of aroma quality improvement, but also of enhancing the third function, physical effect, of food. However, the formation mechanisms of HDMF and HEMF in miso production had been uncertain, so scientific research on this matter was required in order to control the concentrations of these furanones in this food. As a result of our research project, author clarified that intermediates of the Maillard reaction be converted to HDMF and HEMF by yeast fermentation, and suggested a hypothesis pathway for biological formation of these furanones in yeast cell. After then, based on the suggested mechanism, a technique was developed to increase HEMF concentration in industrial miso production.

Importance of 4-hydroxyfuranones

The sweet aroma which comes from the cooking oven would be attractive and probably stimulate human's appetite. The aroma is mainly formed by the Maillard reaction which starts from heating sugars and amino acids followed by a complex series of chemical reactions. The reaction gives a brown color to a baked food materials and, in same time, produces a number of chemicals including volatile compounds. Although many of the routes and intermediates are still uncertain, it is well known that two furanones, 4-hydroxy-5-methyl-3(2H)-furanone (HMF, shown the chemical structure in Fig. 1) and 4-Hydroxy-2,5-dimethyl-3(2H)-furanone (HDMF, in Fig. 1) can arise in this way (Hodge, Mills & Fisher, 1972; Nursten &O'Relly, 1983). HDMF is flavor-active compound which has a caramel-like odor and the aroma threshold is very low at a value of 0.16 mg/L in water (Huber,1992). The furanone has been identified as a compound largely contributing to form the sweet aroma in a wide range of cooked foods,

1 Dept. Bio-Chemistry and Food Science, KUMAMOTO INDUSTRIAL RESEARCH INSTITUTE, Higahshi-machi 3-11-38, Kumamoto, Post Code 862-0901, Japan, E-mail:HAYASIDA@KMT-IRI.GO.JP

beef broth (Tonsbeek, Plancken & van den Weerdhof, 1968), breadcrust (Schieberle, 1990), popcorn (Buttery, Ling & Stern, 1997), tomato paste (Buttery, Takeoka & Ling, 1995) and cooked clam (Sekiwa, Kubota & Kobayashi, 1997). HMF has a meaty or beefy odor, but the aroma threshold is relatively high at a value of 8.3 mg/L in water (Huber, 1992), so the importance in flavor is probably fairly restricted.

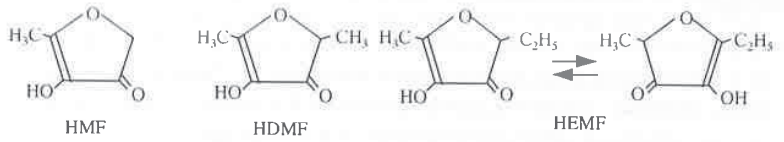

Figure 1. Chemical structures of 4-hydroxyfurnaones

As the Maillard reaction progresses in a moderate condition at around room temperature even though the rate would be slow, two furanones above can be produced in fermented food which take a long time incubation in their production. In addition, another furanone, 4-hydroxy-2(or 5)-ethyl-5(or 2)-methyl-3(2H)-furanone (HEMF, in Fig. 1) has been isolated only from fermented food among all kinds of foodstuff. HEMF has a potent caramel-like odor and the aroma threshold is approximately ten times lower than that of HDMF at a value of 0.02 mg/L in water (Huber, 1992). The furanone was firstly identified in shoyu flavor as the special compound of Japanese style soy sauce in 1970s (Nunomura et al., 1979), after then, has been found as a important flavor compound in other fermented foods, beer (Sakuma et al, 1996), wine (Kotseridis et al., 2000), cheese (Preininger & Grosch, 1994) and miso (Hayashida, Nishimura & Slaughter, 1998a). In the assessment on the flavor of thirteen miso samples accepted for the Kumamoto Prefectural Miso Competition in 1995, author identified the three furanones at concentration range of 0.9 to 5.9 ppm for HMF, 0.2-4.4 ppm for HDMF and 0 (not detectable) to 23 ppm for HEMF in the miso samples, and found significant positive relationships between the aroma quality and the concentrations of HDMF and HEMF in perceived quality ($P < 0.05$), not of HMF of which concentrations were all lower than its aroma threshold (Hayashida, Nishimura & Slaughter, 1998a).

In addition to the importance in flavor, recently, the physical effects of these furanones, involving the anti-carcinogenesis caused by their potent anti-oxidation activity, have been reported in several papers. Nagahara et al. (1992), Pariza (1994) and Kataoka et al. (1997) tested the effect of shoyu and furanones on the occurrence of forestomach neoplasia in rats induced by direct application of benzo[a]pyrene and found a reduction both in the incidence of neoplasms and number per affected animals. With the

HDMF and HEMF, a significant effect was seen at 4 mg/kg body mass. At 12 mg/kg, the incidence had fallen to approximately 65 % from a control value of 90 % and the neoplasms per affected animal decreased from 4.4 to 1.2. HMF was slightly less effective. Taking into account the known concentrations, it seems unlikely that the furanones will exert a significant anti-cancer influence by themselves unless we require very much lower levels for effect than do rats. However, this group of compounds would be expected to contribute to the overall protective compounds ingested in the foods (Slaughter, 1999).

Formation mechanism of HDMF and HEMF in miso fermentation

Given the clear significance in flavor and expectation for the physical effect, the manner in which HDMF and HEMF are produced is obviously important from both theoretical and practical points of view.

For understanding the manner, a series of experiments were firstly carried out to determine the time changes in HDMF and HEMF concentrations through out the course of miso fermentation (Hayashida, Nishimura & Slaughter, 1998a). In the samples, HDMF was spontaneously formed during the incubation of cooked soy bean and koji, with the additional quantities produced by yeast fermentation. However no HEMF was found in incubated miso and was only detected in the yeast fermented miso. The results indicated that the biosynthetic route for both furanone compounds is important in miso, and HEMF can only be formed in this way. The results also suggested that the precursors of both furanones existed at a low level in the initial miso sample but the concentrations increased during incubation. The precursors did not increase without the addition of soy beans and both furanones were produced at high concentrations by the yeast fermentation in the colored miso as a result of fermentation or incubation at fairly high temperature. Soy beans are the main source of amino acids which are a essential part of the Maillard reaction, which caused the coloring of miso. Taking into account these facts, the suggestion arises that the Maillard reaction in miso is responsible for the formation of the precursors of HDMF and HEMF. It is consistent with the idea that the color of malt extract correlates directly with the furanone formation after yeast fermentation (Hayashida & Slaughter, 1997).

This suggestion is confirmed by the simple media fermentation experiments (Hayashida, Nishimura & Slaughter, 1998b). The fermentations in simple media based on Koji extract showed that HDMF was produced by heating the medium with a hexose sugar and that the concentration was increased by yeast fermentation. In addition, fermentation of medium which had

been heated with a pentose sugar resulted in high concentration of HEMF. The experimentation with the simple media based on YPD medium (Hayashida et al., 1999a) also showed HDMF was produced by heating an amino acid with a hexose sugar, and that the concentration of the furanone was increased by yeast fermentation. Again, HEMF was produced only by yeast fermentation in the medium in which a heated solution of a pentose sugar and amino acid had been added. The formation of HDMF and HEMF was not seen by yeast fermentation in the media containing either an added reducing sugar or a amino acid alone or when an unheated solution of a amino acid and a sugar was used. It is well known that the Maillard reaction has two stages; Amadri compounds which are formed from amino acids and sugars, are degraded to a number of small chemicals (first stage) which then combine to form the Meranoisine polymer(second stage). It is suggested that the precursor of both furanones could be intermediates in first stage of the Mailard reaction. In both Miso production and simple medium fermentation, HEMF produced by yeast fermentation increased with the incubation period and heating time increase. However, the levels of the furanone decreased when the pentose sugar, a potential substrate of the Maillard reaction, became depleted although the colors of sample darkened throughout the incubation or heating period (Hayashida et. al. 1999b; Harada et al., 2000).

Blank & Fay (1996) suggested a formation mechanism of both furanones through the Maillard reaction. In this suggested mechanism, the furanone are derived from 1-deoxydiketose, and intermediate in the first stage of the Maillard reaction. On heating with amino acids, sugars give rise first to Amadori compounds which lead to C6-1-deoxydiketose from hexoses and C5-1-deoxydiketose from pentoses. The C6 compound can then cyclise to give HDMF directly. Similar reaction of the C5 compound results in HMF production, but with addition of a C2 fragment, HEMF is formed. Due to their structural similarity to sugars the 1-deoxydiketose could be taken up into the yeast cells by the normal sugar transporters, and the final stages of furanone synthesis completed within the cell. Acetaldehyde and acetyl CoA are produced in the yeast cell and may be a source of C2 fragment donor. Considering this, author suggested a hypothetical pathway for the biological formation of the HDMF and HEMF in yeast cells in Fig. 2 (Hayashida, Nishimura & Slaughter, 1998b).

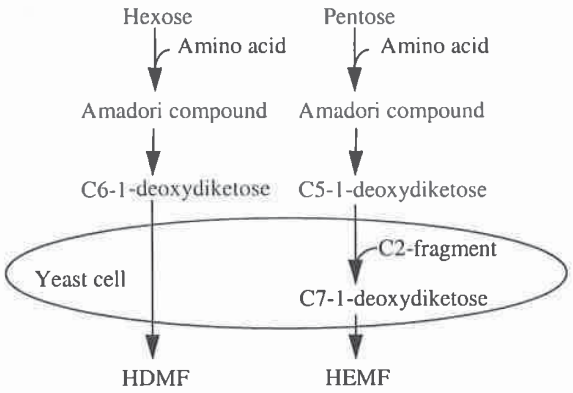

Figura 2. Hypothetical formation pathway of HDMF and HEMF from hexose and pentose sugars while fermentation by yeast

Some workers have suggested that the furanones are formed from sugar phosphates. Sasaki et al. (1991) reported that HEMF was produced by yeast fermentation in simple media based on koji extract with added ribulose-5-phosphate, the combination of xylulose-5-phosphate and ribose-5-phosphate, and also sedoheptulose-7-phosphate, and suggested that HEMF was formed via the pentose-phosphate cycle. Hecquet et al. (1996) reported that HDMF was produced by yeast fermentation in simple medium based on koji extract with added fructose-1,6-bisphosphate. Roscher et al. (1998) reported that HDMF in strawberries could be derived from glucose-6-phosphate, fructose and fructose-1,6-bisphosphate on the basis of radiotracer experiments. However, it is puzzling how the yeast could absorb the sugar phosphates when the cell membrane is impermeable to charged compounds and there is no known specific uptake system. Alternatively, as suggested by the mechanism present by author, the sugar phosphates may react with the amino acids in the medium, during incubation, and form 1-deoxydiketose which is subsequently taken up and converted to the furanone compounds by the yeast as the precursor.

Enhancement of 4-hydroxyfuranone in industrial miso production

Considering the suggested furanone formation mechanism mentioned above, it is likely that Tenchi Kaeshi, a traditional process during which the miso is thoroughly mixed and transferred to a second pot can be used to enhance the levels of HDMF and HEMF in industrially produced miso. This was confirmed by pilot scale miso production (Hayashida, Nishimura & Slaughter,1999). It was previously thought that the Tench-Kaeshi process

was performed in order to produce a homogenous miso product by mixing the miso in middle of its production. However, it is likely that this process also activates the yeast fermentation and enhances the production of the furanones from their precursor previously formed during the incubation stage. The same stimulatory effect of preincubation before yeast inoculation was found in a model fermentation of shoyu production (Hayashida, Nishimura & Slaughter, 1997).

The suggested mechanism for furanone formation may be applied to a wide range of fermented foods and plants, and the industrial techniques of furanone enhancement could be developed by exploitation of this mechanism in these areas. In Kumamoto prefecture, Japan, some miso companies started the inoculation of cultivated yeast and used the techniques shown above. This has resulted in the improvement of their product's quality due to an enhancement of the furanones. The results have been evaluated both from the academic and practical aspects, and author have been awarded the technology prize in 1999 from Brewing Society of Japan and MacFarlane Medal, highly commended, from Heriot-Watt University, UK in 2000.

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Fermented soybean foods of China and the global market

L. Li[1]

Abstract

In China there are many types of fermented soyfoods, but only parts of them have evolved to a biotechnological state and are carried out on a large commercial scale. In this paper, the Chinese fermented soyfoods are discussed with respect to their preparation methods, nutrition composition, health benefits, consumers awareness, and the future outlook on the market in the world.

Introduction

Most fermented soybean foods are known to have originated in China, and then gradually introduced or spread into Korea, Japan and other Asian countries. After centuries preparation, considerable modification and adaptation in processes have been made to suit specific environmental conditions and cultural practices (Hesseltine and Wang, 1980).

Although such traditional foods are increasingly popular in most of the Asian countries today, they are almost limited to the Orient. Considering that fermented soybean foods are nutritious and highly digestible, it is clear that they have played an important nutritional role for the Oriental population in the past and will continue to popular in the world in the future.

Manufacturing profess, nutrition composition and health benefits

Douchi

Douchi is made by fermenting soaked and boiled whole yellow soybeans or black soybeans with microorganism, usually it is consumed as seasoning (Zhang and Liu, 2000). As the first traditional fermented soy food to be described in written recordsýÿit originated in China before the Han dynasty (206 B.C.). In *BenCaoGangMu* (Chinese Materia Medica), which written by Li Shizhen at Min dynasty (1368-1644 A.D.), recorded some healthy function of douchi, such as enhance the appetite, promote digestion, induce sweat and recover from fatigue, smooth the mood and avoid asthma, and etc (Catharin et al., 1999).

1 College of Food Science & Nutritional Engineering, China Agriculture University, Beijing, China, lit@mail.cau.edu.cn

According to the fermentation microorganism, douchi has three varieties: Aspergillus type, Mucor type, and bacteria type. Among them, Aspergillus type is the earliest and the commonest type, but the output of Mucor type is the biggest.

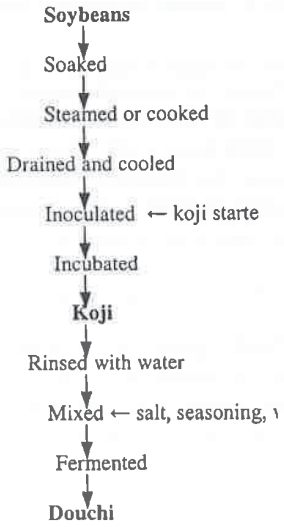

Figure1. The schematic diagram for production of Aspergullius douchi

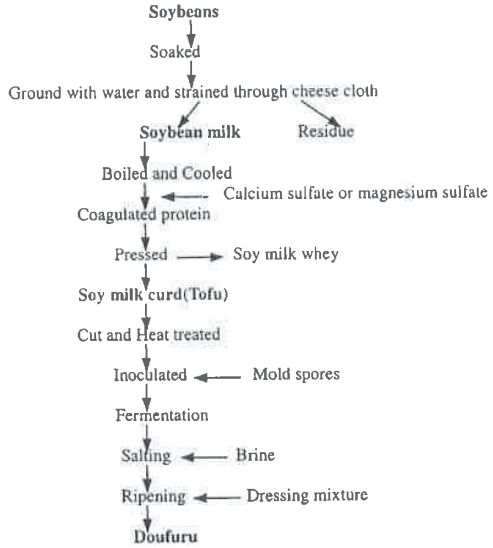

Figure 2. The schematic diagram for production of mould-type doufuru

Table 1. Organic acid content of different douchi products mg/100gdouchi

Type	Product	Acetic acid	L-pyroglutamic acid	Lactic acid
	Tianmashan koji	8.7	3.61	9.41
Aspergillus-type	Tianmashan douchi	38.94	7.98	88.89
	Yipinxing koji	5.87	1.1	2.94
	Yipinxing douchi	19.24	5.1	80.82
Mucor-type	Yongchuan	3.4	5.1	12.89
	Black soybean	1.51	4.22	1.8

The protein, fat and carbohydrate contents and various vitamin content demonstrate that douchi is a nutrient food (Table 2 which showed that douchi is a nutrient food.

Table 2. Nutritional components in douchi (100g douchi)

components	content	components	content
Water(g)	35-50	Calcium(mg)	184
Protein(g)	20	phosphorus (mg)	198
Fat(g)	7.1	iron(mg)	5.5
Carbohydrate(g)	21.4	Vitamin?1 (mg)	0.13
Total acid (g)	1.5	Vitamin?2 (mg)	0.23
Formal nitrogen(g)	0.7-1.0	Nicotinic acid(mg)	3.2

A water-solube extract of douchi (Touchi, TE) was found to exert a strong inhibitory activity against rat intestinal á-glucosidase. Type-2 diabetic patients ingested 0.3g of TE before every meal for 6 months, blood glucose and HbA1c levels gradually decreased, the effect is significant. It demonstrate an antihyperglycemic effect and may have potential use in the management of patients with non-insulin-dependent diabetic mellitus (Fujita and Yamagami, 2001). A fibrinolytic enzyme (subtilisin DFE) was purified from the culture broth supernatant of B. amyloliquefaciens DC-4, a strain isolated from douchi, and displayed thermophilic, hydrophilic and strong fibrinolytic activity (Peng et al., 2003). The water extract of douchi has a high ACE inhibitory activity, and the activity is increased evidently when digested by gastrointestinal proteases.

Doufuru (fermented tofu)

Doufuru is a soft cheese type product made from soy milk curd (tofu) by the action of microorganisms. It has a long history and written records

date back to the Wei Dynasty (220-265 AD) in China. Doufuru has been widely consumed in a similar way as cheese by Chinese people (Wang and Du, 1998), mainly as an appetizer and a side dish.

In China, mainly there are three types according to the processing and the microorganism involved in the fermentation: mould fermented doufuru, bacteria fermented doufuru, and enzymatically ripened doufuru. The process of making doufuru was considered a natural phenomenon until a microorganism, *Mucor* doufuru, was isolated by Wei (1928). It is believed to be responsible for doufuru fermentation. The schematic diagram for production of mold-type doufuru is showed in Fig.2.In spite of their differences in color and flavor, most types of doufuru have a similar proximate composition. The data of Table 3 tell us that doufuru is a nutritional food.

Table 3. Composition of commercial doufuru (100g doufuru)

components	content	components	content
Water(g)	56.3	Calcium(mg)	231.6
Protein(g)	15.6	phosphorus (mg)	301.0
Fat(g)	10.1	iron(mg)	7.5
Carbohydrate(g)	7.1	Zinc (mg)	6.89
Crude fiber(g)	0.1	Vitamin?1 (mg)	0.04
Ash (g)	1.12	Vitamin?2 (mg)	0.13
Cholesterol(g)	Not detected	Vitamin?12 (mg)	1.77
Caloric (KJ)	703.4	Nicotinic acid(mg)	0.5

The V_{B2} content in red-doufuru is 0.42mgýÿ0.78mg/100g, and in grey-doufuru, it is up to 9.8mgýÿ18.8mg/100g, only lower than that in animal livers. Moreover, doufuru contain V_{B1} 0.04mgýÿ0.09mg/100g, nicotinic acid 0.5mgýÿ1.10mg/100g. Mineral are abundant in doufuru, especially for calcium, iron and zinc(Yasuda et al., 1994.). Asians prefer the doufuru, not only for its desired texture, but also for its calcium content.

The soluble protein of final product is up to 18～20% (dry base), and the methyl nitrogen is above 0.5% (wt%). the molecular weight of peptides in 4 weeks and 5 weeks aged doufuru were lower than 31 × 103 and 14 × 103 Da, respectively(Li et al., 2001). The insoluble peptides in doufuru have the effect that reducing the cholesterol level in blood serum. Moreover, peptides with the capacity of bonding cholic acid were found in water-insoluble fraction of doufuru. On the other hand, peptides with ACE inhibitory activities were

found in water-soluble fraction of doufuru (Iwami et al.,1986.). Isoflavone are existed as aglycon in doufuruýÿthe content of daidzein and genistein are about 250ìg/g and 288ìg/g, respectively.

Doujiang (fermented soy paste)

Fermented soy paste is a light yellow to dark brown thick paste. Chinese jiang is believed to be the oldest form of fermented soy paste. According to the ancient Chinese book — Analects of Confucius, it has a history of more than 3000 years (SIBS, 1998).

The process of soy paste making is very similar to that of soy sauce preparation. The main steps include preparation of starter—qu or koji, mixing the starter with salt and water, and fermentation.

Consumers awareness on healthy ability

Because of the discovery on nutrition and functionality of the Chinese fermented Soyfoods, in recent years, consumers awareness increases quickly. Fig.3 showed the consumers awareness on healthy ability of fermented soyfoods in china.

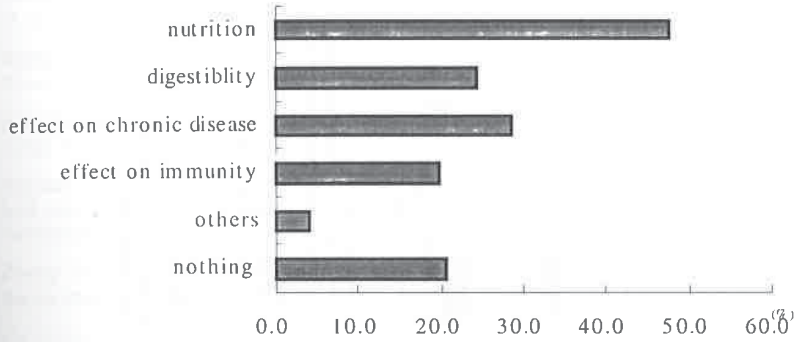

Figure 3. Consumers awareness on healty ability of fermented soyfoods in China

Effect on global soybean consumption

In global market, the place that has most potential is traditional fermented soybean food. Fig.4 and Fig.5 showed consumption of doujiang and doufuru in China respectively. Analyzing from the view of the consumption demand of the Chinese fermented soyfoods, there are about 10000 thousand tons scarcity in the need of Soybean material in China.

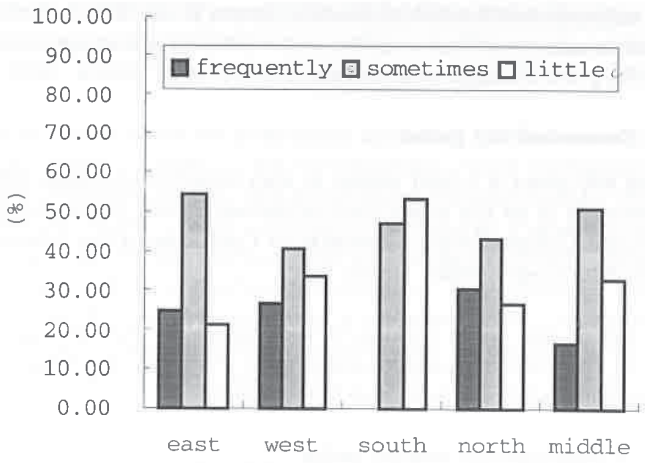

Figure 4. Consumption of Doujiang in China

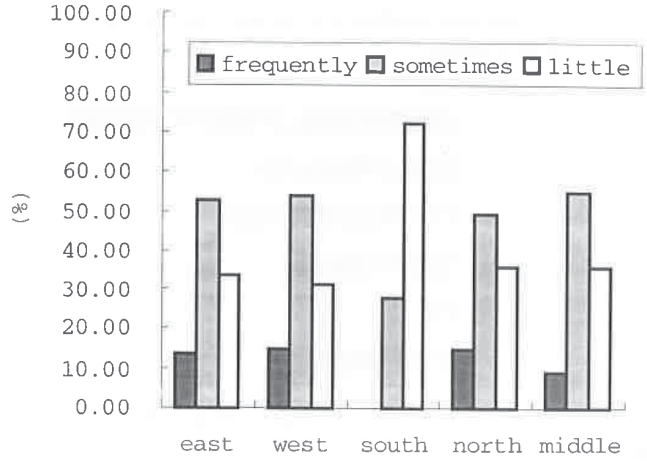

Figure 5. Consumption of Doufuru in China

However, to be accepted by the consumers in the world, the manufacturer of traditional fermented soyfoods must overcome some difficulties. Firstly, the exploitation on traditional products is limited by old traditional mode, and there lack safe and convenient traditional soybean product fit for different consumers. Secondly, the lack of propagandize induce that the consumer of new generation has less cognition to the soybean products. Thirdly, low level of automation, the big lag of quality standards, and the

sanitation situation is not good. If the consumers have a scientific cognition to soybean's value of nutrition, and they could buy convenient, health, delicious and cheap Soybean food in the market, the quantity of consumption will increase largely.

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Biosynthesis and utilization of gamma-polyglutamic acid in natto, soybeans fermented by *Bacillus subtilis*

Y. Tahara[1]

Gamma-polyglutamic acid (PGA), an amino acid polymer that consists of D- and L-glutamic acid polymerized through gamma-glutamyl bonds, is a main constituent of the sticky material in natto, a Japanese traditional food made from soybeans that have been steamed and then fermented by *Bacillus subtilis*. Since PGA is a water-soluble and biodegradable polymer, its utilization in the fields of food, cosmetics, or medicine has been proposed, and biodegradable fibers and hydrogels have been made from PGA. In the meeting, I mention the characterization of the enzymes involved in PGA biosynthesis and degradation. The genes required for PGA production were cloned from *B. subtilis* IFO16449, a strain isolated from natto. There were four open reading frames in the cloned 4.2-kb DNA fragment, and they were almost identical to those in *ywsC* and *ywtABC* genes of *B. subtlis* 168. Northern blot analysis showed that the four genes constitute an operon. Three genes, *ywsC*, *ywtA* and *ywtB*, were disrupted to determine which gene plays a central role in PGA biosynthesis. No PGA was produced in *ywsC* and *ywtA*-defective strains, indicating that both of the genes are essential for PGA production. To clarify the function of the YwsC protein, histidine-tagged YwsC was produced in the *ywsC*-defective strain and purified from the lysozyme-treated lysate of the transformant by Ni-NTA affinity chromatography. Western blot analysis revealed that the YwsC protein consists of two subunits, 44-K and 33-K proteins, which are encoded by overlapping in the *ywsC* gene. ^{14}C-Labeled PGA was synthesized by the purified proteins from ^{14}C-L-glutamate in the presence of ATP and $MnCl_2$, through an acyl-phosphate intermediate, indicating that the *ywsC* gene encodes PGA synthetase [EC 6.3.2], a crucial enzyme in PGA biosynthesis (Urushibata, Tokuyama, and Tahara, 2002, J. Bacteriol., 184: 337-343). The *ywtD* gene, which codes for an enzyme that degrades PGA, was cloned from the same strain. The gene is located immediately downstream of the *ywsC* and *ywtABC*, and it showed partial similarity to genes coding for DL-endopeptidase, a peptidoglycan-degrading enzyme. The *ywtD* gene was inserted into pET15b, and the recombinant plasmid was then transformed into *E. coli*. Histidine-tagged YwtD was purified from sonicated cells of the transformant. The purified YwtD degraded PGA to yield two hydrolyzed

1 Dept. of Applied Biological Chemistry, Faculty of Agriculture, Shizuoka University, Shizuoka, 422-8529, Japan, acytaha1@agr.shizuoka.ac.jp

products: One is a high-molecular-mass product (490 kDa with nearly 100% L-glutamic acid) and the other is an 11-kDa product (with D- and L-glutamic acid in a 80:20 ratio). This finding and results of enzymatic analysis of the two products with carboxypeptidase G suggest that YwtD is a novel enzyme cleaving the gamma-glutamyl bond only between D- and L-glutamic acid of PGA, and it may be called gamma-DL-glutamyl hydrolase (Suzuki and Tahara, 2003, J. Bacteriol., 185: 2379-1282).

Soybean oil based composites

S. Z. Erhan and Z. Liu[1]

Abstract

Unites States agriculture produces over 15 billion pounds of soybean oil annually, and frequently carry-over exceeds 1 billion pounds. Only 3% of soybean oil produced is used in industrial applications. Development of economically feasible new industrial products from soybean oil is highly desirable. In this study, we prepared soybean oil based composites by the solid freeform fabrication (SFF) method. Epoxidized soybean oil (ESO) is solidified with a gelling agent and composites are formed by fiber reinforcement. Glass, carbon, and mineral fibers are used in the formulations. The type of fiber and degree of fiber alignments affect the properties of the composites. In addition, the effects of curing agents, curing temperature, fiber combination, and fiber loading on mechanical properties of composites and dynamic analysis are studied and reported.

Introduction

Soy-based polymers could support global sustainability and provide an alternative to synthetic polymers for many manufacturing applications (1). The solid freeform fabrication method (SFF) is a method of making shapes without molds. It is best known in its stereolithography forms as a method of rapid prototyping. In stereolithography, a laser photopolymerizes successive thin layers of monomer to build up a solid object. The reactive extrusion free-forming technique was developed by Calvert and co-workers (2). In this system, a liquid or particle slurry is dispensed by syringe into a two-dimensional (2-D) pattern. A motorized table is mounted on an x-y drive. In the format used in this project, a stepper-motor driven syringe is mounted above the table, with both controlled from a computer. The process is analogous to the use of a pen plotter for writing a 2-D pattern (Figure 1). The shape to be produced may be derived from a 3D CAD program or from standard drawing packages.

1 USDA/ARS, National Center for Agricultural Utilization Research, 1815 N. University St., Peoria, Illinois

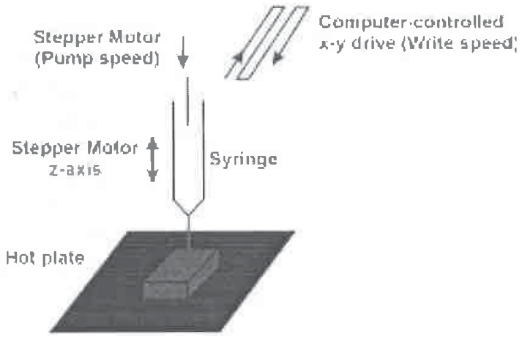

Figure 1. Sketch of the extrusion freeform fabrication apparatus.

Epoxy-containing soybean oil used as raw materials for the synthesis of new polymers suitable for liquid molding processes have been reported by Wool and co-workers (3, 4). We have prepared soy-based polymers ranging from soft rubbers to hard plastics (5, 6). In this paper, the development of epoxidized soybean oil-based composites with soft rubber properties by embedding strong fibers (e.g., glass and carbon) and mineral fibers (calcium sulfate microfiber and Wollastonite mineral fiber) are reported using the SFF method.

Results and discussion

Effect of curing temperature

Effect of curing temperature on the mechanical properties of composite filled with Franklin fiber® H-45 is presented in Table I. Young's modulus increases as the curing temperature increases because at lower temperature, the epoxy groups are mainly consumed by primary amine, but secondary amine reaction is relatively small. Padma and co-workers (7) reported an intensity increase in the secondary amine peaks by Fourier transform infrared (FTIR), compared with primary amine peaks. At higher temperature, both primary and secondary amine reacted with epoxy groups. As the reaction proceeds further, the epoxy-hydroxyl etherification reaction dominated the reaction. With increased cross-linking degree, the composites get better mechanical properties.

Table I

Effect of Curing Temperature		
Temperature (°C)	Tensile Strength (MPa)	Tensile Modulus (MPa)
130	0.72	2.18
140	1.02	4.30
150	1.52	7.20
160	1.40	8.81

Conditions: ESO, 56 wt %; Franklin fiber, 15.6 wt %; Jeffamine EDR-148, 37 g/100 g ESO; fumed silica, 13 g/100 g ESO.

Effect of different types of fibers

Four different kinds of fibers were used in the composite formulations. With fiber concentration at 12 vol. %, the mechanical properties of the composite were determined, as shown in Table II. Among different fiber types, glass fiber and carbon fiber show better reinforcing effects than the mineral fibers, Franklin Fiber® H-45, and Wollastonite fiber, Fillex® 17-AF1. Because E glass fiber has a tensile strength of 3 GPa and a modulus that approaches 100 GPa, it exhibits a large contribution to the strength and rigidity of the reinforced composites.

Table II

Tensile Test Results of Composites with Different Types of Fibers		
Fiber	Tensile Strength (MPa)	Tensile Modulus (MPa)
E-glass fiber	1.50	12.2
Carbon fiber	1.45	9.3
Franklin Fiber® H-45	1.43	9.1
Fillex® 17-AF1	1.30	9.0

Conditions: fiber, 12 vol %; Jeffamine EDR-148, 37 g/100 g ESO; fumed silica, 13 g/100 g ESO.

Effect of curing agent

The Young's modulus date for a composite consisting of ESO (52 wt %), Franklin fiber (22 wt %), and curing agent (37 g/100 g ESO) are shown in Table III. When Jeffamine D-230 is used as curing agent, the composite has lower physical properties. This result is due to the reactivity of the amine groups in the Jeffamine D-230 (polyoxyproplenediamine), where the amine groups are located on secondary carbon atoms at the ends of an aliphatic polyether chain.

In contrast, the amine groups of Jeffamine EDR-148 (triethyleneglycoldiamine) are located on primary carbon atoms at the ends

of an aliphatic polyether chain. The product is an unhindered diamine that is miscible in a wide variety of solvents and much more reactive than Jeffamine D-230. Jeffamine T-403 (polyoxypropylenetriamine) is a trifunctional primary amine. Its amine groups are located on secondary carbon atoms at the ends of aliphatic polyether chains. The reactivity of amines located on secondary carbon atoms is weaker than that of amines located on primary carbon atoms. Compared with the Jeffamine curing agent, TETA and DETA have better physical properties (Table III). The reason for this difference is that there are two primary amine groups located on primary carbon atoms at the ends of an aliphatic polyimine chain in TETA and DETA.

At the same time, there is one secondary amine group in DETA and two secondary amine groups in TETA. Those secondary amine groups also take part in crosslinking reaction and formulate a network structure of polymer matrix. A network structure of polymer matrix provides strong mechanical properties of composites.

Table III

Effect of Curing Agent			
Curing Agent	Tensile Strength (MPa)	Tensile Modulus (MPa)	Epoxy/Amine H
Jeffamine D230	0.11	1.01	1:1.47
Jeffamine T403	1.25	7.5	1:1.26
Jeffamine EDR 148	1.43	8.00	1:2.28
DETA	6.29	231.39	1:4.10
TETA	8.29	301.60	1:2.10

Conditions: ESO 52 wt %; Franklin fiber, 22 wt %; curing agent, 37 g/100 g ESO; fumed silica, 13 g/100 g ESO.

Influence of fiber orientation on young's modulus

The strength and moduli of fiber-reinforced composites depend on fiber volume fraction, aspect ratio, and orientation. Direction of reinforcement is critical in determining the mechanical properties of a composite. Strength as well as stiffness follows the direction of the reinforcement. To explore the effect of orientation on the properties of composities, test bars were made by writing at varying angles relative to the axis of the test bars. The effect of this orientation on elastic modulus is shown in Table IV. Modulus is doubled when the fiber axis is parallel to the writing direction compared with the modulus with an axis across writing direction.

Table IV

Influence of Fiber Orientation on Tensile Modulus		
Angle of Writing Direction of Axis to Tension, degrees	Tensile Strength (MPa)	Tensile Modulus (MPa)
0	1.51	7.20
30	1.49	7.05
45	1.27	4.71
60	0.89	4.26
90	0.88	4.18

Conditions: ESO 56 wt %; Franklin fiber, 15.6 wt %; curing agent, 37 g/100g ESO; fumed silica, 13 g/100g ESO.

Effect of fiber combination

The combination of glass or carbon fiber with mineral fiber in a thermoset matrix has recently been investigated by Peng (8). Experimental results suggest that combining different fiber types into testing bars tends to get higher flexural modulus compared with single-type fiber-epoxy composites. Here, we studied the fiber combination such as short milled glass fibers (1/32 in.) and Franklin Fiber® H-45, as well as short carbon fibers and Franklin Fiber® H-45. The results of these experiments, shown in Table V, clearly demonstrate that moduli of the composites increase by combining two types of fiber rather than using one type of fiber. These experimental results confirm the theory that combining different fiber types in testing parts gives higher flexural modulus compared with single-type fiber-ESO composites. By utilizing the packing concepts, the use of reinforcement permits the economics of fiber combination system to be calculated for wide ranges of fiber loading and resin and fiber cost.

Table V

Effect of Fiber Combination		
Fiber Combination	Tensile Strength (MPa)	Tensile Modulus (MPa)
Glass fiber (44 wt %) and Franklin fiber H-45 (7 wt %)	18.21	794.81
Carbon fiber (20 wt %) and Franklin fiber H-45 (8.5 wt %)	11.30	528.68

Conditions: curing agent, 37 g/100 g ESO; fumed silica, 13 g/100 g ESO.

Effect of fiber loading

The Young's modulus of composite reinforced with Franklin Fiber® H-45 as a function of fiber loading is shown in Figure 2. Jeffamine EDR-148 (37 g/100 g ESO) and fumed silica (13 g/100 g ESO) were used in the formulation. The increase in fiber content leads to an increase in the tensile modulus at least up to 23 wt %, which subsequently levels off at loadings beyond this point. Higher fiber loading makes it difficult to evenly mix with the polymer matrix. The poor dispersion of the fibers and reduced interfacial adhesion to the polymer matrix explains this reduction. In the case of fiber-reinforced composites, it is well known that there exists a critical aspect ratio at which the mechanical properties of the composites are maximized. This critical aspect ratio depends on the volume fraction of the fiber and also on the ratio of the modulus of fiber to the matrix modulus (9). In our system, increasing the volume fraction of the fiber results in slurry which will not flow through the needle, therefore data for higher Franklin fiber content is not available.

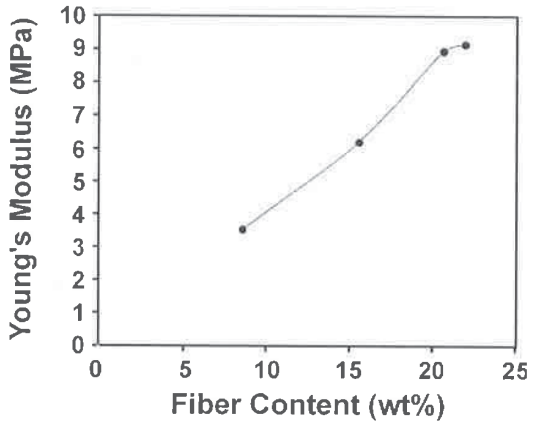

Figure 2. Modulus of Franklin Fiber® H-45-filled composites versus fiber loading.

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Soybean oil-fuel, hydraulic fluid and engine oil research

J. M. Perez[1]

Abstract

Research studies of soybean oils at The Pennsylvania State University include bio-diesel fuel, hydraulic fluids and engine oils. Laboratory and engine tests of bio-diesel fuel have shown advantages in emission reduction and lubricity. Research on hydraulic fluids and engine oils using vegetable oil base stocks focused on improving the performance characteristics of soybean and other vegetable oils to meet or exceed the performance of petroleum based oils. Several soybean oils including normally refined, genetically modified and chemically modified oils are used to demonstrate improvements in oxidation stability based on the degree and type of unsaturation. The difference in the rates of oxidation and the formation of volatile products depends on the type of acid moieties present in these triglyceride esters. Differences in fluid volatility depend on both evaporation and fragments due to oxidative decomposition of the molecules. Oxidation stability is described by the rate of formation of deposits in a thin-film oxidation bench test. Both the total number of double bonds and the type of acid moieties present in the molecule affect the rate of oxidation and the formation of deposits.

Introduction

Increased interest in environmentally friendly fluids resulted in considerable research effort on the use of vegetable oil as an alternative fuel and renewable lubricant base stocks. Research in the Penn State Tribology Group has focused on both the development of biodiesel fuels and quality lubricants utilizing vegetable oils [1-7]. The advantages of using vegetable oils as lubricants are well known – biodegradability, renewable resource, and lubricity. Also, well known are the weak link properties - oxidation stability, low temperature characteristic and cost relative to petroleum basestocks. This presentation will review the progress made in resolving these issues. Research on biodiesel fuel also will be discussed.

[1] The Pennsylvania State University, University Park, PA 16802

Experimental.

Test Fluids. The test fluids include a normal soybean oil (NSB), a genetically modified soybean oil (HOSB), an epoxidized soybean oil (ESB) and a trimethyl propane (TMP) ester and two partially hydrogenated soybean oils. The TMP ester is a commercial product prepared using oleic acid as the acid moiety. The composition of these oils are found in Table 1. This series of oils contain various amounts of unsaturation ranging from an oleic acid content of 0 to 100 percent and various amounts of linoleic and linolenlic acid content. A total unsaturation factor was determined from the composition and olefinic bonds per 100 molecules.

Test Methods. The oxidation and fluid volatility tests in this study were conducted using the thin-film Penn State Micro-oxidation test. The test unit, described in reference [8] enables rapid evaluation of oxidation and the rate of formation of deposits using micro-samples of 20 – 40 ul of test fluid. Reactions can be followed by using gel permeation chromatography (GPC) or Fourier Transfer Infra-red Spectroscopy (FTIR).

Friction and wear characteristics of the test fluids are determined in the four-ball wear test, using a 30-minute sequential test[9]. The chemical structure of the base fluids is a factor in the performance of additives and the base fluid in controlling wear[6,7].

Table 1. Fluid Composition and Total Unsaturation

Fluid Designation	C18:0	C18:1	C18:2	C18:3	Total C=C Per 100 Molecules
A (TMP)	0	100	0	0	100
B (NSB)	16	23	53	7	150
C (ESB)	100	0	0	0	0
D (HOSB)	11	83	3	2	95
E (SB-44)	15	44	37	2.5	126
F (SB-66)	13	66	20	1	109

Discussion of experimental results

Fuel Studies. Production of low sulfur diesel fuels in the U.S. are environmentally driven. Increased wear and durability are concerns. Addition of biodiesel fuel improves friction and wear [10]. Biodiesel also significantly reduces particulate emissions [11]. However, biodiesel fuel does compete for the soy oil with other value added products. The energy security implications of using biodiesel fuels reported by McCormick [12] point out a gap between supply and demand of biodiesel, Figure 1.

Use of biodiesel has increased and is projected to exceed 2.6 billion gallons by 2016. Only 35 million gallons was projected for use in 2002. Currently, there are only about 150 million gallons of soybean oil produced. This means that there is an insufficient quantity of vegetable oil available in the U.S. to satisfy the current use as a B20 diesel fuel. The need is to develop strategies that will satisfy the need, even at the B20 level.

An approach to satisfy lubricity needs in low sulfur fuels is to use additives developed from biodiesel. One additive that is effective in reducing friction and wear is based on oxidized bio-diesel [10]. This process resulted in improved lubricity at a two percent level of biodiesel. A second approach is to utilize Fisher-Tropsch produced from biomass in combination with biodiesel.

Regarding the economics, the cost of biodiesel exceeds that of diesel fuel but as usage increases, the cost comes down. The cost of B20 in the U.S. has dropped from over $3 per gallon to about $1.50 per gallon in the past 5 years as volumes have gone from essentially zero to about 35 million gallons in 2002[12].

Lubricant Studies. The lubricant research at Penn State has included studies of greases, metal working fluids, hydraulic fluids and engine oils with the major effort on the latter two, hydraulic fluids and engine oils. For success as a hydraulic fluid or engine oil, the final fluid must meet a number of requirements, many of which are similar. Of these, the critical areas are oxidation stability and low temperature properties of the oils. A number of vegetable oils including corn, soy, canola, rapeseed, palm, and various chemical and genetically modified oils have been considered in our studies as lubricant base stocks.

The research described in this presentation involves the several soybean oils and evaluates the effect of unsaturation on the performance of the oils in laboratory bench tests.

Volatility Studies. Oxidation affects the volatility of the fluid. Vegetable oils have an advantage over many fluids in that they have low rates of evaporation. Evaporation is low due to the low vapor pressure of the large triglyceride molecules. However, oxidation can result in degradation of the side chains resulting in an increase in volatiles. The fluid loss is increased as oxidation produces low molecular weight products. The ESB exhibits the lowest loss in the sequence of tests at 175°C., Figure 2 The TMP and the genetically modified HOSB both of which are high in 18:1 side chains are similar in their volatility loss. The NSB is intermediate suggesting not only the presence of the double bonds but their location is important. The

oxidation attack on the NSB results in smaller volatile degradation fragments due to the location of the double bonds. This results in a lower loss due to volatiles.

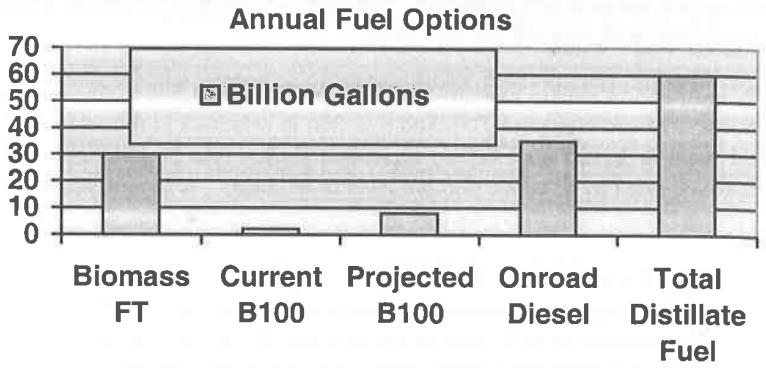

Figure 1. Supply and demand of current and alternative diesel fuels (Source: Ref. 12)

The thermal-oxidative products of the four fluids significantly increase the volatility loss. Less than one third of the indicated loss due to oxidation is the result of evaporation.. This was confirmed by comparing the volatile loss obtained under an inert atmosphere (evaporation loss) with the loss obtained under an oxidative atmosphere, Figure 2.

Partial hydrogenation of the soybean oil does not result in a significant change in volatility. The presence of multiple double bonds appears to dominate the oxidation. This is better demonstrated by the deposit formation studies.

Oxidation. In lubricating oils, a major concern is the formation of deposits resulting from high molecular weight oxidation products. Oxidation is a complex series of reactions resulting in deposit formation, Figure 3 The rate of oxidation of vegetable oils is directly related to the type of hydrogen atoms adjacent to the double bonds. A structure related "order of battle" is the double allylic bonds are more reactive that the allylic bonds and the allylic bonds are significantly more reactive than the hydrogen bonds found in saturated hydrocarbon molecules. The relative reaction rates of oxidation of stearic, oleic, linoleic, and linolenic side chains are estimated to be 1:10:100: 400. Validation of the effect of the chemical structure of the fluid on the rate of oxidation and the effect of test environment such as materials and coatings were studied [13,14].

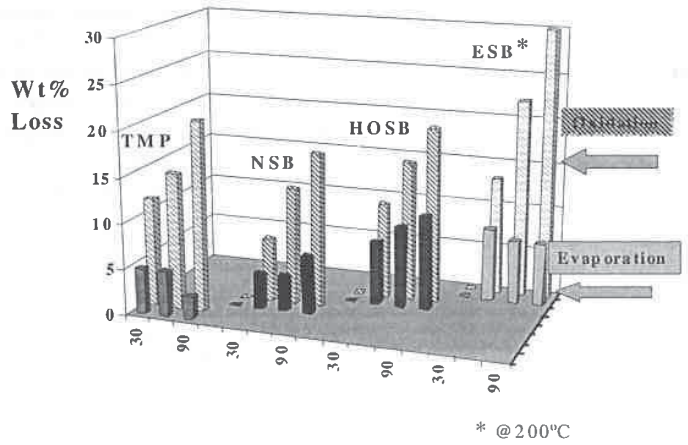

Figure 2. Evaporation vs oxidative volatiles

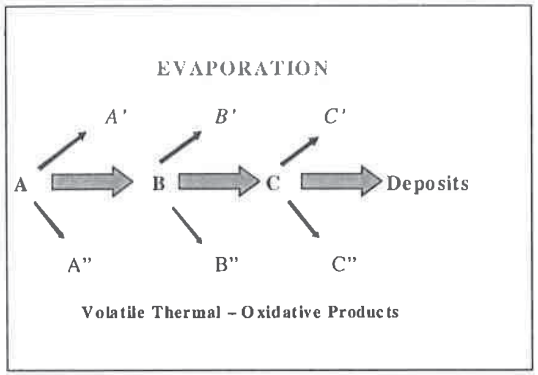

Figure 3. PSMO Deposit Formation Model

Results for the six soybean oils found on Figure 4 indicate that the ESB was the most stable, with an induction period of over 60 minutes before a significant increase in deposits. The TMP and HOSB with the high oleic content showed similar results and both exhibited a slower rate of deposit formation than the NSB. Increasing the oxidation temperature for the ESB by 25°C did increase the oxidation rate by a factor of four, or better, eliminating the induction time. The results suggest that the primary effect of structure on the formation of deposits is the presence of linolenic acid moieties in the triglyceride esters. The general trend is an increase in

oxidative stability as the number of double bonds is decreased. However, the rate of oxidation does increase with the linolenic content. The total no. of double bonds and the number of linolenic acid moieties present per 100 molecules are indicated on Figure 4. The ESB, fluid C, shows good stability for 60 minutes at 175 °C. Fluid E the partially hydrogenated soybean oil with 66 percent oleic and only 1% linolenic acid content exhibits a reduced oxidation rate when compared to the other fluids with increased oleic acid content. All of the fluids show an improvement over the NSB oil.

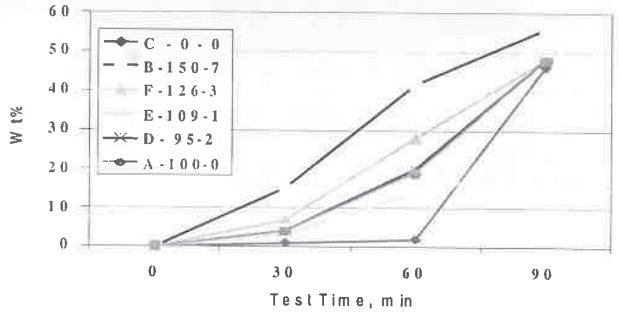

Figure 4. Deposit forming tendencies of several soybean oils.

Conclusions

- The epoxidized soybean oil used in this study exhibited excellent stability. Both the volatility and deposit forming tendencies were significantly improved over the normal, genetically modified and partially hydrogenated oils used in this study.
- The volatility of the vegetable oils studied is significantly increased by oxidation. The oxidative volatiles, that is, the volatiles resulting from fragmentation of the fluid as the result of oxidation, exceed the loss due to evaporation by a factor of over two.
- The rate of oxidation and the deposit forming tendency of the soybean oils studied depends on the total number of double bonds present in the molecule.
- The presence of double allylic hydrogens in linolenic acid appears to be a significant factor.

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Applications of soybean lipids as energy fuels for residential heating and jet aviation

S. Bist and B. Y. Tao[1]

The chemical similarities soybean oil and petroleum has led to many alternative products. Due to changing economic and social conditions we have been exploring the use of soybean lipids as alternative liquid energy fuels. This presentation reports our current work in the development of home heating fuels and jet aviation fuels, which meet current regulatory specifications and have economic, environmental, and domestic benefits vs. petroleum-derived products.

Approximately 6 billion gallons of liquid fuel oils are used for residential heating applications annually in the U.S., primarily composed of #2 fuel oil, which sells for approximately the same price as gasoline and contains approx. 2000-5000 PPM of sulfur. Use of degummed soybean oil blends provides both an economic benefit as well as reduction of sulfur dioxide emissions, in addition to a new market for soybean producers. Our laboratory has examined the physical and chemical combustion parameters involved with use of blended fuels in residential furnaces, quantifying the effects of viscosity, pump pressure, atomization, energy efficiency, and emissions, in addition to storage and commercial testing. Our results indicate that blends of up to 20% soybean oil with #2 heating fuel can meet all current heating fuel specifications, and result in significant reductions of SOx and potentially lower fuel costs, using existing equipment. Higher levels of soybean oil can also be used in current equipment, with commensurate reduction in SOx and fuel costs, however, modifications in heat exchanger design and atomization are needed for optimal energy efficiency.
Methyl esters of soybean oil have also been developed for use as jet aviation fuels. Current economic, environmental, and social conditions in the U.S. favor the development of alternative domestic renewable jet aviation fuels. Jet fuels have highly regulated strict performance and physical specifications, including viscosity, cold temperature performance, and energy density, which other renewable liquid fuels, such as ethanol cannot meet. The use of soybean methyl ester blends can meet many of these specifications, however cold temperature behavior has always been a limitation, since jet fuel specifications require crystallization limits down to -47 degrees C. In this paper, we report the development of an aviation jet fuel composed of

1 Dept. of Agricultural and Biological Engineering, Purdue University, West Lafayette, Indiana, USA

soybean methyl esters that meets FAA specifications including cold temperature behavior. This fuel is developed from a urea-based fractionation process for soy methyl esters that has yields of up to approx. 80% allowing economical fuels. Jet fuel blends incorporating up to 30% methyl esters have been produced that meet FAA jet fuel specifications, including cold temperature performance. These fuels are currently being tested in commercial jet aviation engines for functional performance. This presentation will describe the fractionation process and fuel properties, as well as report on the jet engine performance testing.

Bioprocessing of oils and fats to higher value-added products

T. A. Foglia; R. Ashby; D. Solaiman; M. J. Haas;
G. Piazza and W. N. Marmer[1]

Abstract

Fats and oils were once the primary sources of aliphatic carbon compounds used by industry. Despite their ready availability and competitive price the non-food use of fats and oils continues to decline in almost all applications. To reverse these trends, our laboratory is evaluating the application of biocatalysis and biomimicry (chemical reactions that mimic enzyme reactions) to fats and oils. The goal of the research is to expand current uses and identifying new uses of fats and oils in higher-value added applications. Particular areas of research in which we are exploring and developing include: the lipase-catalyzed synthesis of structured and neutraceutical triacylglycerols; the biocatalytic oxygenation of fatty acids; and the microbial production of biodegradable polymers and surfactants from fat and oil substrates.

Lipase-catalyzed synthesis of structured low-calorie triacylglycerols

Medium-chain triacylglycerols (TAG) [tributyrin (1,2,3-tributyrylglycerol), tricaproin (1,2,3-tricaproylglycerol), and tricaprylin (1,2,3-tricapryloylglycerol)] were subjected to acidolysis with stearic acid or interesterified with hydrogenated soybean oil (HSO) using an immobilized lipase as catalyst for the synthesis of structured lipids(SL), Foglia et al., 2003. Normal phase (silica or cyanopropyl phases; NP_{SIL} or NP_{CN}, respectively) and reverse phase (octadecylsilane, RP_{ODS}) high performance liquid chromatography (HPLC) with evaporative light-scattering detection (ELSD) were used to separate the newly synthesized SL. The NP-HPLC methods fully resolved SL-TAG isomers containing butyryl (C4) and long-chain fatty acyl [stearoyl (C18) and palmitoyl (C16)] residues, but SL-TAG isomers composed of caproyl (C6) or capryloyl residues (C8) and long-chain fatty acyl residues were not fully resolved. The latter SL-TAG molecules were resolved using the RP-HPLC method. The HPLC methods were combined with mass spectrometric detection (LC/MS) to characterize the SL molecular species produced. Whether synthesized in the laboratory

1 United States Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, 600 East Mermaid Lane, Wyndmoor, PA 19038 USA

from vegetable oils rich in linoleic acid or obtained from natural sources, conjugated linoleic acid (CLA) are always obtained as heterogeneous mixtures, containing as many as 16 different positional and stereo- isomers. To define the metabolic effects of each isomer and to prepare dietary supplements enriched in the individual isomers, an effective means of preparative-scale isomer separation is required. One described procedure for this process employs a multi-step scheme requiring urea fractionation and solvent extraction. Since simpler methods are needed for the production of pure CLA isomers, we developed a method that exploits the substrate selectivity of a lipase to also achieve this separation (Haas *et al.*, 1999).

Catalytic oxygenation of unsaturated fatty acids

Our laboratory has an active research program designed to investigate novel methods for introducing oxygen into fats and oils. This research has led to a number of promising avenues for the formation of oxygenated materials, Figure 1. The starting point for the synthesis is the preparation of a fatty acid hydroperoxide from linoleic acid (**1**) using soybean lipoxygenase (LOX) Figure 1.

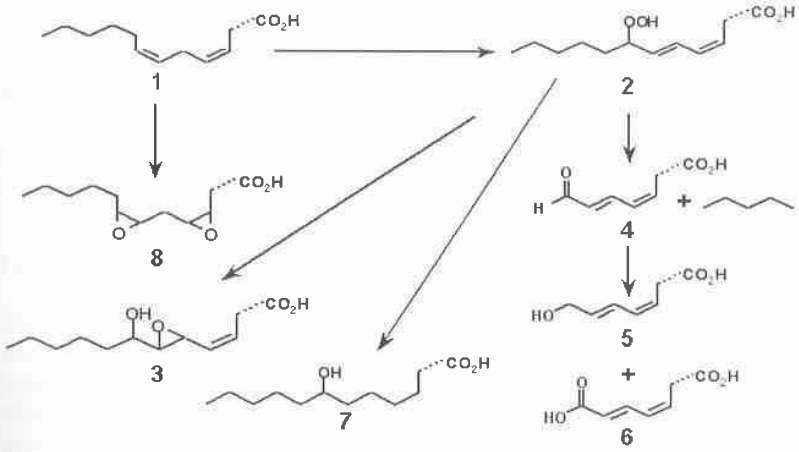

Figure 1. Synthesis of oxygenated fatty acids and their deriviatives.

This enzyme catalyzes the addition of oxygen to linoleic acid to form 13(*S*)-hydroperoxy-9(*Z*),11(*E*)-octadecadienoic acid, **2**, Figure 1 (Piazza, et, al., 1997). Methods were devised to promote the formation of hydroperoxide **2** in organic solvent, and to promote the formation of similar type hydroperoxides in fatty acids such as those found in phospholipids and methyl esters. After obtaining high yields of hydroperoxide **2**, methods of

converting this material to useful chemical intermediates were sought. In the rearrangement of Me-ester of hydroperoxide **2** by titanium (IV) isopropoxide, the predominant methyl ester that was formed was the *threo* isomer, methyl 11(*R*), 12(*R*)-epoxy-13(*S*)-hydroxy-9(*Z*)-octadecenoate (24) epoxide **3** Figure 2, whereas $Nb(OC_2H_5)_5$ gave its *erythro* analogue, demonstrating that both catalysts selectively promoted the formation of an É-epoxide (Piazza at al., 1997).

Hydroperoxide **2** also can be cleaved by the enzyme hydroperoxide lyase (HPLS) to a É-oxo-fatty acid (component **4**, Figure 1) and a volatile short-chain fragment. For example, HPLS form *Chlorella*, cleaves **2** into the C13 É-oxo-fatty acid and a C5 fragment (pentane) whereas HPLS from higher plants cleaves HPODE into a C_{12} É-oxo-fatty acid and a C_6 fragment (hexanal). The oxo-fatty acids were converted to É-hydroxy and É-dicarboxylic acid derivatives (**5** and **6** respectively, Fig. 1) by chemical or enzymatic methods.

Biodegradable polymers and surfactants

Agricultural fats, oils and coproducts (FOCs) are attractive fermentation feedstocks because of their structural diversity and renewable characteristic. This particular research project aims to utilize FOCs as substrates for the fermentative production of biobased products such as poly(hydroxyalkanoates) (PHAs) and sopohorolipids (SLs). PHAs are biodegradable polyesters produced by many microorganisms. The two major types of these biopolymers, Figure 2, are short-chain-length (scl-) and medium-chain-length (mcl-) PHAs.

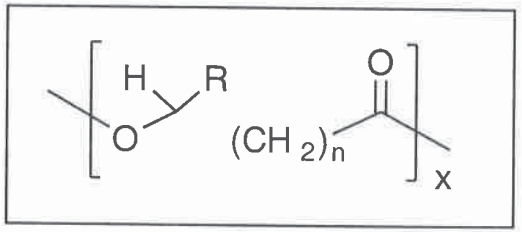

Figure 2. β-Hydroxy acid repeat unit structure for PHA, where R = C4-14.

PHAs could substitute for the petroleum-derived nondegradable plastics, adhesives and elastomers in many applications. Others and we have identified strains of *Pseudomonas* species capable of producing either or both the scl- and mcl-PHAs. Using various pseudomonads, our lab showed that various FOCs could support cell growth and PHA production (Ashby et

al.,1998). [Glycerol also was tested as a model substrate for the crude glycerol coproduct stream of biodiesel manufacture] We further showed that depending on the feedstock and bacterial strain used, PHAs with different repeat-unit compositions are produced. Most notable is the high content of unsaturated monomer repeat units found in soy oil-derived PHA (Soy-PHA) in comparison to the biopolymers obtained with the other FOCs. Because of its high degree of unsaturation, Soy-PHA is amorphous. This was reflected in its thermal properties in which the melting-transition temperature (T_m) is not measurable. The unsaturated bonds of PHAs could also provide active sites for further derivatization such as epoxidation and radiation crosslinking (Foglia, et al., 2003)].

We also investigated the use of FOCs as substrates for producing sophorolipids, a class of biosurfactants. Sophorolipids are glycolipids produced as a mixture of the structural variants of the molecules by several *Candida* species when fed both a sugar and lipid substrates (SLs; Figure 3)

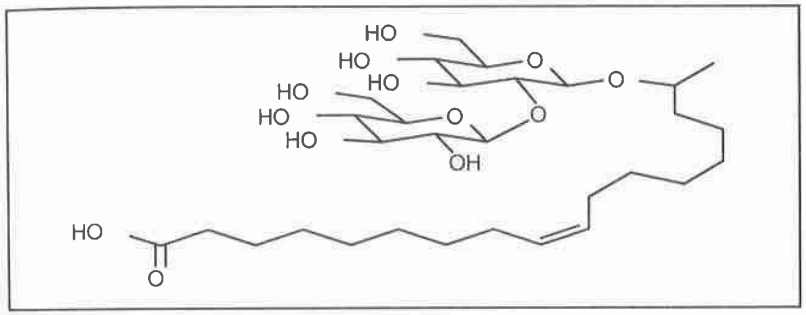

Figure 3. Acid form of sophorolipid produced by *C. bombicola*.

These bioproducts are useful as surfactants and emulsifiers in cosmetics and detergent applications. We showed that various FOCs are good substrates for *C. bombicola* ATCC 22214 to produce SL (Solaiman et al., 2003). Our laboratory developed a liquid chromatography/mass spectrometric method to facilitate the analysis of the SL products. Using this method, we showed that the distribution of SL variants in the isolated products vary according to the type of FOC used in the fermentation (Table 5). This study demonstrates that FOCs are viable feedstocks for the production and manipulation of SL products.

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Production of novel oxygenated fatty acids from soybean oil and pufas by biocatalysis

C. T. Hou[1]

Summary

We are working on new uses for soybean oil by developing new value-added products through bioprocesses. Our microbial culture, *Clavibacter* sp. ALA2 produces many new oxygenated products from linoleic acid such as: 12,13-dihydroxy-9Z-octadecenoic acid (12,13-DHOA), 12,13,17-trihydroxy-9Z-octadecenoic acid (12,13,17-THOA), 12:17; 13:17-diepoxy-9Z-octadecenoic acid (DEOA), 7-hydroxy-DEOA, and two tetrahydrofuranyl unsaturated fatty acids (THFAs). We also found that strain ALA2 converts other n-3 and n-6 unsaturated fatty acids (PUFAs) into novel products. Products obtained from n-3 fatty acid, such as a-linolenic acid were converted to tetrahydrofuranyl fatty acid products. Similarly, eicosapentaenoic acid and docosahexaenoic acid were converted to 15, 18-dihydroxy-14,17-epoxy-5(Z),8(Z),11(Z)- eicosatrienoic acid and 17,20-dihydroxy-16,19-epoxy-4(Z),7(Z),10(Z),13(Z)-docosatetraenoic acid, respectively. In contrast, n-6 fatty acids, such as linoleic acid, ±-linolenic acid and arachidonic acid, were converted to diepoxy bicyclic fatty acids, tetrahydrofuranyl monohydroxy fatty acids, and trihydroxy fatty acids. Therefore, the structures of bioconversion products were different between n-3 and n-6 PUFAs. Furthermore, strain ALA2 places hydroxy group and cyclic structure at the same position from the É-terminal despite the number of carbon chains and double bonds of PUFAs. All of these new products have high potential of being used in biomedical applications.

Introduction

The United States produces more than 18 billion pounds of soybean oil (SBO) annually with a yearly carryover of more than 300 million pounds. How to utilize this surplus oil effectively becomes a big issue in our agricultural community. SBO is relatively cheap at 22 cents per pound and is an attractive raw material for bioindustries. The content of unsaturated fatty acids, such as oleic and linoleic acids, are 22% and 55%, respectively.

Currently, the major use of SBO is for food products such as shortenings, salad and cooking oils, and margarines, with small quantities serving

1 Microbial Genomics and Bioprocessing Research Unit, National Center for Agricultural Utilization Research, ARS, USDA. 1815 North University Street, Peoria, Illinois 61604, USA

industrial applications. Total non-food uses of SBO have grown little during the past 30 years. For applications such as cosmetics, lubricants, and chemical additives, soybean oil is too viscous and too reactive toward atmospheric oxygen to establish significant markets. For other uses, including coatings, detergents, polymers, flavors, agricultural chemicals, and pharmaceuticals, reactivity of SBO needs to be enhanced by introducing additional functionalities or cleaving the fatty acid molecules.

The NCAUR biocatalysis research project aims at producing new value-added products such as hydroxy fatty acids from SBO through bioprocesses. Because of the anticipated commercialization of biodiesel, and the anticipated huge amount of by-product glycerol, we are also working on the utilization of glycerol to produce value-added products.

Hydroxy fatty acids are common in nature. In mammals, fatty acids are oxidized in mitochondria by a sequence of reactions in which the fatty acyl chain is shortened two carbon atoms at a time (b-oxidation). During the b-oxidation, it produces b-hydroxy fatty acids. Although b-oxidation is the major fate of fatty acid metabolism, two other types of oxidation also occur, a- and w-oxidation.

Plant systems are also known to produce hydroxy fatty acids. In these cases, the hydroxy groups are usually located in the middle of the fatty acyl chain. These types of hydroxy fatty acids are important industrial materials. Castor and lesquerella oils are the two major sources. Ricinoleic and sebacic acids, two of the castor oil derivatives, are classified by the Department of Defense as strategic and critical materials.

Microorganisms oxidize fatty acids either at the terminal carbon or inside of the acyl chain. Microbial conversion of oleic acid to 10-hydroxy and 10-keto fatty acids are known. Hydroxy- and keto-fatty acids are useful industrial chemicals. Recently, several microorganisms and biocatalytic processes have been identified that introduce new functional groups to the inner carbon atoms of fatty acids. These hydroxy fatty acids are classified into three types, namely: monohydroxy, dihydroxy and trihydoxy fatty acids [1]. In this paper we reported the conversion of additional n-3 and n-6 PUFAs into novel products by *Clavibacter* sp. ALA2.

Materials and methods

Microorganism and bioconversion. *Clavibacter* sp. ALA2 (NRRL B-21660), isolated from a dry soil sample collected from McCalla, Alabama, was cultivated aerobically at 30°C with shaking in 50 ml of culture medium [2].

Bioconversions were carried out by adding 125 ¼l PUFA into a one day-old culture and shaking the flasks at 200 rpm at 30°C for an additional 7 days. After incubation, the culture was acidified to pH 2 with 6 N hydrochloric acid. A crude lipid fraction was extracted with 100 ml of ethyl acetate and then with 100 ml of diethyl ether using separation funnel. The solvent was evaporated to obtain the crude lipid extract. The crude lipid extracts were methylated with diazomethane for gas chromatography (GC) analyses.

Isolation and identification of products. The crude lipid extracts were fractionated by column chromatography (2.5 cm i.d. x 35 cm length) packed with silica gel 60. Elutions were carried out using 500 ml of methylene chloride, 500-1000 ml methylene chloride:methanol (97:3, v/v), and 500-1000 ml methylene chloride:methanol (95:5, v/v). Product 1-4, product 6, and product 7 were obtained from methylene chloride:methanol (97:3, v/v) fraction. Product 5 and product 8 were eluted by methylene chloride: methanol (95:5, v/v). Fractionated products were methylated with diazomethane and further purified by preparative Silica Gel 60 F_{254} TLC plate. Product 1 (converted from EPA): (developing solvent) *n*-hexane: ethyl ether (1:9, v/v), $R_f = 0.44$, purity (by GC analysis) 96%; Product 2 (converted from DHA): *n*-hexane:ethyl ether (1:9, v/v), $R_f = 0.43$, purity 92%; Product 3 (converted from ±-linolenic acid): methylene chloride, $R_f = 0.22$, purity 95%; Product 4 (converted from É-linolenic acid): *n*-hexane:acetone (7:3, v/v), $R_f = 0.52$ purity 87%; Product 5 (converted from ±-linolenic acid): ethyl ether:acetone (4:1, v/v), $R_f = 0.52$, purity 97%; Product 6 (converted from arachidonic acid): methylene chloride:methanol (99:1, v/v), $R_f = 0.70$, purity 99%; Product 7 (converted from arachidonic acid): *n*-hexane:ethyl acetate (7:3, v/v), $R_f = 0.53$, purity 96%; Product 8 (converted from arachidonic acid): methylene chloride:methanol (9:1, v/v), $R_f = 0.43$, purity by GC-MS 95%.

The structures of bioconversion products were analyzed with GC-mass spectrometry (MS) and proton and ^{13}C nuclear magnetic resonance (NMR).

Results and discussion

Bioconversion of n-3 polyunsaturated fatty acids by *Clavibacter* sp. ALA2. Strain ALA2 converted EPA to a major product (product 1) with GC retention time of 24 min. The electron impact mass spectrum (EI-MS) of the methyl ester/trimethylsilyloxy ethers (OTMSi) of product 1 gave a molecular ion at (relative intensity) *m/z* 510 $[M]^+$ (1). Typical fragment ions were interpreted as follows: 379 $[M\text{-}CH_3CH_2CHOTMSi]^+$ (0.7), 289 $[M\text{-}(CH_2CH{=}CH)_3CH_2)_3COOCH_3]^+$ (4), 271 (2), 239 (2), 199 [289-TMSiOH]$^+$ (29), 181 (6), 157 (20), 145 (21), 131 $[CH_3CH_2CHOTMSi]^+$ (59), 73 $[TMSi]^+$ (100). The

structure of product 1 (methyl ester derivative) was further confirmed by ^{13}C and ^{1}H NMR analyses. The ^{13}C NMR signals at 71.2 ppm and 74.2 ppm corresponded to C-15 and C-18 positions hydroxy group in the molecule, respectively. ^{13}C NMR signals of 129.8 ppm (C-5), 128.9 ppm (C-6), 128.2 ppm (C-8), 128.1ppm (C-9), 128.8 ppm (C-11), and 125.9 ppm (C-12) indicated the presence of three double bonds. The carbon peaks for C-6 (25.7 ppm), C-10 (25.6 ppm) and C-13 (27.1 ppm) indicated the three double bonds remained in cis configuration. Epoxy structure was found at C-14 (83.6 ppm) and C-17 (79.1 ppm). The positions of hydroxy group and double bonds were also supported by ^{1}H NMR signals. From these data, the structure of product 1 converted from EPA was identified to be 15, 18-dihydroxy-14,17-epoxy-5,8,11-eicosatrienoic acid (Figure 1). Strain ALA2 hydroxylated the two double bonds between C-14 and C-15, C-17 and C-18, but did not alter the double bonds at C-5, C-8 and C-11 position.

Strain ALA2 also converted DHA to several products: a major product (product 2) with GC retention time at 37 min. Using similar GC/MS and proton and ^{13}C NMR analytical procedures, we identified Product 2 as: 17, 19-dihydroxy-16,18-epoxy-4,7,10,13-docosatetoraenoic acid. (Figure 1).

Bioconversion of n-6 polyunsaturated fatty acids by *Clavibacter* sp. ALA2. Strain ALA2 converted ±-linolenic acid to two major products with GC retention time at 11 min (product 3) and 22 min (product 5), and a minor product at 13 min (Product 4). These products were identified as: 12,17;13,17-diepoxy-6,9-octadecadienoic acid, 12,13,17-trihydroxy-6,9-octadienoic acid, and 12-hydroxy-13,16-epoxy-6,9-octadecadienoic acid, respectively. (Figure 1).

Strain ALA2 also converted another n-6 PUFA, arachidonic acid to cyclic fatty acids and trihydroxyl fatty acid. Their structures are: 14,19;15,19-diepoxy-5,8,11-eicosatrienoic acid (product 6, GC retention time of 15 min), 14-hidroxy-15,18-epoxy-5,8,11-eicosatrienoic acid (product 7, GC retention time of 18 min), and 14,15,19-trihydroxy- 5,8,11-eicosatrienoic acid (product 8, GC-MS retention time of 18 min),(Figure 2). Therefore, the products obtained from ±-linolenic acid and arachidonic acid by strain ALA2 consists of multiple products with diepoxy bicyclic structure, tetrahydrofuranyl ring or trihydroxy group in their molecule. These products had very similar structure to those products obtained from another n-6 fatty acid, linoleic acid by strain ALA2 (3).

It is interesting to note that the structures of products are remarkably different between those obtained from n-3 and from n-6 PUFAs. It seems that substrate PUFAs with a double bond at n-3 position affects the outcome

of the bioconversion products by ALA2 enzymes. In addition, both the hydroxyl group and cyclic structure are placed at the same position from É-carbon terminal, despite the differences in the number of carbons and double bonds of the substrate PUFAs. All of these products are new and might have high potential of being used in biomedical applications

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Biosynthesis of soybean oil in isolated microsomes

T. McKeon; X. He; C. Turner; M. Ikeda; G. Chen and J.-T. Lin[1]

Introduction

Although soybean oil accounts for over half the vegetable oil produced in the US, the soybean itself is not especially rich in oil, producing approximately 20 % oil. In contrast, the castor seed produces up to 60% oil. Although there is usually a surplus of US produced soybean oil with an annual carryover of 1 to 2 billion pounds, in the shift to a biobased economy, biofuels such as biodiesel will become more important in the economy. The current usage of diesel fuel in the US is approximately 300 billion pounds, while the optimal use of biodiesel is as a 20% blend with petroleum diesel. This mixture provides suitable lubricity, eliminating the need for added sulfur compounds that are usually used. Thus biodiesel helps to reduce the sulfur emissions from standard petroleum diesel. To meet the need for 20% biodiesel would require nearly three times the annual production of soybean oil. While it is therefore unlikely that all diesel sold will contain such a high content of the vegetable oil alkyl esters, it is feasible to approach 10% or more if yields can be boosted to a suitable level. To this end, we have been carrying out a comparison of castor oil biosynthesis with soybean oil biosynthesis, using microsomes derived from the oilseed, a technique that we have shown to be an excellent simulation of the oil biosynthetic process in castor (Lin et al. 1998).

Methods

Microsomal preparation. Microsomes were prepared from Daizu variety soybean in mid-maturity. Immature seeds were harvested, frozen in liquid nitrogen and stored at -80C until needed. Microsomes were prepared by the same procedure used for castor microsomes (Lin et al. 1998). Due to poor oil biosynthetic activity, more protein was used in incubations.

HPLC analysis. All analyses were carried out as previously reported (Lin et al. 1998, 2002) and lipid peaks tentatively identified on the basis of relative retention time in the separation system.

Results

Soy is much less efficient at incorporating 14C-labelled fatty acids than castor. Table 1 shows the limited amount of label incorporated in each lipid fraction by soy vs. castor.

[1] USDA/ARS Western Regional Research Center, Albany, CA 94710 USA

Table 1. A Comparison of Incorporation of ^{14}C-Oleate and ^{14}C-Ricinoleate by Castor and Soy

	OXPC	RXPC	^{14}C-OXX' TAG	^{14}C-OX' DAG	^{14}C-RXX' TAG
SOY	3.1	2.4	0.2	0.15	0.3
CASTOR	64.0	19.4	15	4	81
RATIO, CASTOR / SOY	20	7	75	27	270

In each case the amount of label incorporated by soy microsomes was far exceeded by castor microsomes. Since we have a research program examining the individual enzymes involved in castor oil biosynthesis, we plan to use these enzymes to supplement the soy system in order to elucidate the limiting factors in soy oil biosynthesis.

Surprisingly, the soy microsomes, incorporated ricinoleate slightly more effectively than oleate. However, both are very low compared to castor. The indication that soy DAG is labelled nearly at the same level TAG is suggests that the final acylation step may be limiting in soy oil biosynthesis. With our recent cloning of the castor DGAT (unpublished) we hope to test this idea directly in soy microsomes.

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IV INTERNATIONAL SOYBEAN PROCESSING AND UTILIZATION CONFERENCE

MARCH 2

Soybean allergenic proteins and hypoallergenic soybean products

T. Ogawa[1]; T. Moriyama[1]; M. Samoto[2] and K. Takahashi[3]

Introduction

Of many foods eliciting adverse reactions, soybean and soybean products as an important protein source are known as one of the major allergenic foodstuffs in common with other protein-rich foods such as milk and eggs (1). In recent years, soybean protein isolate (SPI) prepared from defatted soybean meal has been increasingly applied in a field of food processing as ingredients of minced meat, stuffing, pasta flour, yogurts, sauces, and flavor enhancer, because of their high nutritional quality, processing functionality and economical basis. Accordingly, it has been difficult for the patients allergic to soybean to get the allergen-free products from commercially available processed foods and also the patients have to have wide knowledge about the ingredients in the processed foods in which allergenic food residues are incorporated as processing aids. Recently, we demonstrated the occurrence of about 15 protein components binding with IgE antibodies in sera of the soybean-sensitive patients with atopic dermatitis, three of which named as Gly m Bd 30K, Gly m Bd 28K, and Gly m Bd 60K were shown to be major allergenic proteins (1). Based on these information about allergenic proteins in soybean as the target to be removed, many approaches to reduce the allergenicities of soybean and soybean products have been proposed; (a) physico-chemical procedures such as heat denaturation and precipitation, (b) destruction and modification of allergenic structures such as an introduction of polysaccharide moieties and enzymatic digestion, (c) breeding (selection of allergen-deficient variety or induction of mutant), (d) genetic engineering, and (e) fabrication of non-allergenic constituents. In this Symposium, a recent knowledge on soybean allergens and development of hypoallergenic soybean products are reviewed briefly.

[1] Graduate School of Agriculture, Kyoto University, Gokasyo, Uji, Kyoto 611-0011, Japan.
[2] Tsukuba R & D Center, Fuji Oil Co., Ltd., 4-6 Kinunodai, Yawara, Tsukuba-gun, Ibaraki 300-2497, Japan.
[3] National Agriculture Research Center, Kannon-dai, Tsukuba, Ibaraki 305-0856, Japan: ogawa@food2.food.kyoto-u.ac.jp

Major soybean allergens

Gly m Bd 30K

The soybean allergenic protein, Gly m Bd 30K (1) which is most strongly and frequently recognized by the IgE antibodies in sera of the soybean-sensitive patients with atopic dermatitis, has been characterized to be a soybean seed 34kDa oil-body-associated protein (2). This protein had been identified by Karinski et al (3) from the fractionated soybean oil body membrane, whereas the cDNA was isolated and cloned as a vacuolar storage protein P34 with close homology to thiol proteases classified into a group of papain super family. The primary structure of Gly m Bd 30K was shown to have about 30% homology or 54% similarity with Der p 1, a house dust mite allergen that is thiol protease found in feces of *Dermatophagoides preronyssius* (4). In recent years, it was shown that IgE binding sites (B-cell epitopes) located on Gly m Bd 30K was investigated by using synthetic peptides and identified (5). Interestingly, all the epitopes recognized by human IgE antibodies were shown to be quite different from those on house dust mite allergen, Der p 1.

Gly m Bd 28K

One of major allergens named Gly m Bd 28K (25% incidence) was isolated and purified from 7S-globulin fraction of SPI prepared from defatted soybean flakes (6). The purified allergen was shown to be a glycoprotein with the molecular mass and isoelectric point of 26 kDa and 6.1, respectively. In 2001, a cDNA clone encoding Gly m Bd 28K was isolated by Tsuji et al (7) and it was shown that the polypeptide for the cDMA exhibits high homology with the MP27/MP32 proteins in pumpkin seeds and the carrot globulin-like protein, and a vicilin-like protein similar to that reported from peanuts (7).

Gly m Bd 60K (}αsubunit of β-conglycinin)

The other major allergenic protein, which was recognized by about 25% of sera from soybean-sensitive patients with atopic dermatitis, was identified as α subunit of β-conglycinin with the molecular weight of 57,000, and with pI of 4.90 (8).

Other allergenic proteins

Soybean low molecular weight proteins were identified as allergens eliciting Barcelona asthma by Rodrigo et al. (9) were identified as Gly m 1.0101 (Gly m 1A) and Gly m 1.0102 (Gly m 1B) which are isoforms with different molecular weights of 7,500 and 7,000, respectively (10). Gly m 2 is also demonstrated as a hull-derived allergen related to Barcelona asthma with

molecular weight of 8,000 and pI of 6.0, which is homologous to a storage protein from cotyledones of *Vigna radiata* (cow pea) and with a disease response protein from *Pisum sativum* (green pea) (11). Soybean profilin was identified as another allergen (Gly m 3) with a molecular weight of 14,000 and pI of 4.4, which is homologous to Bet v 2, a birch pollen allergen, with a sequence identity of 73% and other 11 plant profilins with 69 to 88% identity (12). These three allergens are recognized as inhalant allergens eliciting asthma in Barcelona. Several reports on glycinin as allergens could be found, and acidic subunits A1a, A1b, A2, A3 and A4 were identified to be allergenic (13).

Development of hypoallergenic soybean products

Determination of allergenicity

It is eagaly requested to establish a convenient method for determination or evaluation of an allergenicity of soybean and soybean products. The immunoblotting method (Western blot) is one of very useful techniques to detect small amounts of allergens, but it is not a quantitative way to evaluate and compare the allergenicity (amounts of allergen) of various soybean products. Most suitable method for selective and quantitative analysis of allergens is considered to be the enzyme-linked immunosorbent assay (ELISA) using allergen-specific antibodies or patient's sera.

Molecular breeding

The target molecular species to be removed are three major allergens, such as Gly m Bd 60K, 30K, and 28K. Among them, a new soybean line (*Glycine max*, Tohoku 124) lacking both að and að' subunits of bð-conglycinin (Gly m Bd 60K) was induced by irradiation with 20kR (1.0 kR/hr) gð-ray to Karikei 434 with the marked decrease of the level of the að, að' and bðbNäNOJ'c_ !äarf_hoku 124 also lacks another major allergen, Gly m Bd 28K, indicating that an application of Tohoku 124 is beneficial for developing hypoallergenic soybean products. In 2000, Tohoku 124 has been registered as a new variety named Yumeminori.

Reduction of Gly m Bd 30K by genetic modification

Herman et al. has been achieved the removal of a most immunodominant soybean allergen Gly m Bd 30K by using a genetic modification technique. They developed a genetically modified soybean lines by transgene-induced gene silencing of the Gly m Bd 30K gene (15). The introduction of this transgenic technique into a hypoallergenic soybean variety, Yumeminori, may provide a powerful hypoallergenic material for patients to be.

Physicochemical reduction

Heat treatment is a general way for a food processing and induces the destruction of protein structures. Epitope, that is IgE binding sites of allergens, are, however, assumed to be sequential structure on a peptide chain (about 10 amino acid residues), so that the reduction of allergenicity due to heat denaturation of proteins (unfolding of polypeptide chains) would not be expected. In the case of defatted soymilk, about 97% of Gly m Bd 30K could be removed as the precipitate in the presence of reducing agent (10mM sodium bisulfite) under the specified condition (1M Na2SO4 in acidic pH of 4.5). The major storage soybean proteins, both glycinin and bð-conglycinin, remained in the supernatant after centrifugation (16). Since these procedures for reduction of allergenicity do not include the methods modifying protein structures and, especially, the enzymatic digestion, the processing functionality of soybean storage proteins could be retained to be applicable for making the traditional soybean products, for example, Tofu (soybean cake) and other products.

Enzymatic digestion

An enzymatic treatment of whole soybean seeds effectively reduces the allergenicity. Autoclaved soybean was treated by certain proteases from *Bacillus* sp. at 37ýÿ for 20 hr, which is the same condition as that of the fermentation procedure for Natto with *Bacillus natto*, the Japanese traditional fermented soybean product (17). When the residual allergens were examined by the immunoblot and ELISA (inhibition ELISA), the product showed no binding activity against F5 mAb and patients sera. Obata et al (18) reported the reduction of allergenicity of Tofu (soybean curd) by an enzymatic digestion with enzyme from *Aspergillus soyae.*

Recently, a novel hydrolytic processing of soybean proteins was reported by Tsumura et al (19). Under the limited hydrolytic conditions (pH and temperature), the selective digestion of b*Bacillus*b.

Chemical modification

An attempt to mask the allergenic sites (epitopes) of soybean proteins using the Maillard-type polysaccharide conjugation at 60ýÿ under 79% relative humidity was examined. The allergenic potential of the acid-precipitated soybean proteins was shown to be reduced by the conjugation of galactomannan molecules to the lysine residues of APP (20), which was confirmed by dot blotting of treated APP with patients sera and inhibition ELISA.

Extrusion cooking

Ohishi et al. (21*)* reported that antigenicity of soybean meal against calves sera was reduced to 0.1% of the original activity by an extrusion cooking with screws containing kneading-disc elements and die-end temperatures exceeding 66ýÿ.
Frank et al (22) reported that texturized soy proteins, processes of whose production include heating, mechanical pressure and acid treatment (pH 4.5) leads to the disappearance of the major allergen Gly m Bd 30K.

Evaluation of hypoallergenic soybean products and perspectives

The hypoallergenic soybean products have been developed and subjected to the evaluation of usefulness under the observation of physicians and dieticians. In the case of in vivo examination of allergenicity, a single blind food challenge test or open challenge test are practical in evaluating the processed foods. By the preliminary challenge trial it was confirmed that at least 80% of the soybean allergic patients could be used the above mentioned hypoallergenic products without any adverse reactions. Some of the products, evaluation of which have been completed, await a chance of the distribution to the soybean-sensitive patients through the physicians. Detailed information is also available in ref. 23.

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Physiologically active peptides in soybean and soy products

E. G. de Mejia[1]; W. Wang[1]; M. Vasconez-Costa[1];
R. Nelson[2] and B. O. de Lumen[3]

Abstract

The goal of this research is to identify and quantify biologically active peptides with functional properties, for the prevention of age-related chronic diseases, in soy proteins. One of the main outcomes of this investigation has been the identification and mapping of bioactive peptides with health benefits in commercial protein products. Furthermore, we have established the presence of and quantified lunasin™ a peptide with anti-cancer properties, in various soybean genotypes, soy products and industrial down-streams. The results obtained in these studies will provide food processors with valuable information concerning product benefits, information on how to optimize the content of bioactive peptides and points of functional differentiation. At the same time, the consumer will have better availability and alternative options for consuming soy products that will benefit their health.

Introduction

The number of Americans aged 65 and older climbed to 35 million in 2000, compared with 3.1 million in 1900. Between 1990 and 2020, the population aged 65 to 74 is projected to grow 74%. This population will be the beneficiaries and customers of the newly identified compounds.[1] Bioactive peptides, such as lunasin™ occur naturally, or can be produced by hydrolysis of proteins during industrial processing. These peptides, are resistant to digestion and can act as physiological modulators of body functions related to aging and the development of chronic disease conditions typical of the elderly such as cancer, hypertension, obesity, hypercholesterolemia and decrease immune function, among others. In addition, the bioactive peptides from soybean may delay the process of aging due to free radical scavenging and the inhibition of enzymatic and non-enzymatic lipid peroxidation. Antihypertensive peptides are the most common occurring bioactive peptides. Soybean paste has shown antihypertensive activity by inhibiting angiotensin-converting enzyme. Other biological activities include anti-cancer properties, enhancement of immune function against viral infections, opioid action against pain, antioxidant protection, anticholesterolemic effects and

[1] Department of Food Science and Human Nutrition, University of Illinois.
[2] Department of Crop Sciences, and USDA-ARS.
[3] Nutritional Sciences and Toxicology, University of California, Berkeley

anorectic properties. Food processing can also impact the profile of peptides formed during subsequent digestion and these are promising prospects for exploitation as a functional food. Thus, the generation of bioactive peptides from soy proteins could also provide new food processes that the industry can utilize in optimizing the content of bioactive compounds in their soy products or that they can use to add to other food products or dietary supplements. These new peptides will enhance the value of soy and will increase the business opportunities for food processors in the rapidly growing functional food industry.

Materials and methods

One hundred and forty four soybean lines from the USDA Soybean Germplasm Collection at the University of Illinois (USDA, Agricultural Research Service, Urbana IL) were classified into categories according to country (9 countries), province (China alone, 15) and maturity group (13, from 000 to X). Lines were also categorized as either U.S. ancestors, U.S. modern or exotic (selected to represent the variability of the larger collection). Synthetic lunasin™ and monoclonal antibody for lunasin™ developed in mice, were provided by FilGen, BioSciences, Inc.[2, 3] The ground soy meal was defatted and extracted with 0.05 M Tris-HCl buffer, pH 8.2. The commercial samples were extracted using buffer 0.1 M PBS pH 7.4. An ELISA method was developed for the analysis of lunasin. A standard curve of pure synthetic lunasin™was used (100 ml/well) with concentrations in the range of 24-72 ng/ml ($y = 0.0117x - 0.0485$, $R^2 = 0.9626$). The total content of extracted protein was measured at 280 nm using a BSA standard curve ($e = 0.66$ ml/cm g). Amino acid sequences of main soybean protein components [2]-conglycinin, glycinin precursors, Kunitz trypsin inhibitor, Bowman-Birk inhibitor precursors were obtained from Swiss-Prot database. The BIOPEP database[4] was used for searching the potential bioactive peptides.

Results

Figure 1 shows the standard curve for the recently developed ELISA method for lunasin. This method has a good reproducibility and better sensitivity than electrophoresis. The detection limit was 8 ng/ml of lunasin, with a recovery of 90%. Lunasin content in defatted soybean flour from the different genotypes studied ranged from 0.1 to 1.33 g/100g flour. Diversity for lunasin concentration does exist within commercial soybean cultivars (0.33-0.95 g/100g flour). A wider range of lunasin concentration exists within the exotic *Glycine max* species. Table 1 presents the minimum and maximum values of lunasin in each maturity group for all the soybean accessions. Interestingly, the Bowman-Birk inhibitor null accession PI 321393 *Glycine latifolia* did not contain lunasin. Soy protein concentrate

(60%-76% protein), isolated soy protein (84.6%-88.2% protein) and hydrolyzed soy protein (82% protein) contained 2.19 ± 0.99, 3.74 ± 0.19 and 4.43 ± 0.59 g lunasin/100g flour, respectively. These values represent 6.11 ± 2.47%, 8.09 ± 0.57% and 7.70 ± 0.64% of the extracted protein, respectively. Soy flour (23% protein) and soy flakes (40%-50% protein) contained a combined average value of 1.24 ± 0.17 g lunasin/100g flour equivalent to 1.77 ± 0.53 g lunasin/100g extracted protein.

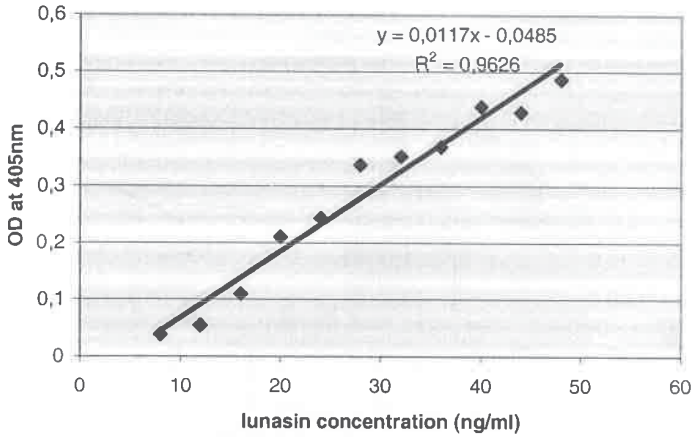
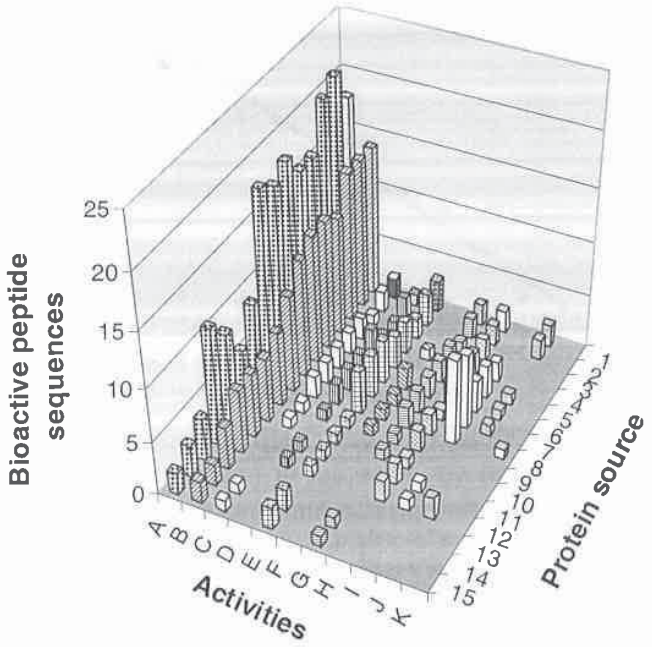


Figure 1. Lunasin Standard Curve

Table 1. Minimum and maximum values for lunasin content (mg/g extracted protein, and mg/g defatted flour) in each maturity group.

Maturity Group	N	Min mg/g extracted protein	Max mg/g extracted protein	Min mg/g flour	Max mg/g flour	Mean ± SD
0	9	2.03	10.97	1.84	9.54	5.13 ± 2.68
00	6	1.14	10.19	1.00	7.75	4.73 ± 2.31
000	2	6.33	7.17	6.24	5.77	6.00 ± 0.33
I	6	2.44	7.34	2.19	7.09	4.47 ± 2.12
II	20	2.03	9.84	2.11	8.91	5.40 ± 2.01
III	19	2.19	9.83	1.91	9.69	4.74 ± 2.27
IV	21	1.71	8.83	1.50	7.58	4.52 ± 1.94
V	13	2.19	12.64	1.69	10.44	5.70 ± 3.11
VI	16	1.83	13.21	1.48	11.77	6.37 ± 3.28
VII	14	2.70	11.30	2.26	10.37	6.46 ± 2.35
VIII	9	2.50	11.24	2.22	11.43	7.70 ± 2.51
IX	5	5.17	13.97	6.26	13.27	8.89 ± 2.79
X	4	6.91	9.85	6.43	10.14	8.08 ± 1.55

The results of the profile of peptides in soy protein with potential biological activities are shown in Fig 2. Amino acid sequences with antiamnestic, antithrombotic, opioid, dipeptidyl peptidase IV (DPPIV) inhibitory, antioxidative, immunostimulating, and antihypertensive activities were found in soybean proteins. Antihypertensive activity and DPPIV inhibition were the most potent. Soy protein components 7S and 11S were good potential sources of bioactive peptides.



Soybean sources:
1. Glycinin G1precursor; **2.** Glycinin G2 precursor; **3.** Glycinin G3 precursor; **4.** Glycinin G4 precursor; **5.** Glycinin G5 precursor; **6.** b-Conglycinin a chain; **7.** b-Conglycinin a' chain; **8.** b-Conglycinin b chain; **9.** Trypsin inhibitor A/C precursor; **10.** Trypsin inhibitor B ; **11.** Trypsin inhibitor KTI1 precursor;
12. Trypsin inhibitor KTI2 precursor; **13.** Bowman-Birk inhibitor precursor; **14.** Bowman-Birk inhibitor C-II precursor; **15.** Bowman-Birk inhibitor D-II precursor
Activities:
A: Antihypertensive; **B:**Dipeptidyl peptidase IV inhibitor ; **C:** Antithrombotic; **D:** Opioid; **E:** Immunostimulating; **F:** Regulating; **G:** Ligand; **H:** Antiamnestic; **I:** Activatiing ubiquitin-mediated proteolysis; **J:** Antioxidative; **K:** Opioid agonist

Conclusions

In summary, the availability of bioactive peptides will provide new choices to the elderly population for improving their health. The communication of the benefits of soy not only will help to educate the consumer but will also enhance the value of the soy industry in general, favoring both, food industry and soy farmers. It is also likely that the levels of lunasin in soybeans can be genetically manipulated. Further studies are needed to explore the mechanism and the potential of these peptides as chemopreventive and therapeutic agents.

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The role of INTSOY in extending soybean utilization

K. Weingartner[1]

Introduction

The International Soybean Program (INTSOY) at the University of Illinois in Urbana-Champaign is part of the National Soybean Research Laboratory at the university. INTSOY's mission is to improve human nutrition and promote soybean utilization. With an active network of collaborators in more than 132 countries, it is recognized worldwide as a center of excellence for soybean processing and utilization technology. Each quarter, INTSOY responds to more than 100 technical inquiries from both U.S. and international clients. These inquiries come from public, private, educational, and non-governmental institutions. In addition, INTSOY has been able to extend its expertise to an even wider audience through its university Web site on soybean utilization. It receives electronic enquiries, sometimes referred to as "hits," on its site at a rate that exceeds 22,000 per month. In sum, INTSOY's activities represent a comprehensive program that has enhanced the goals of a land-grant university in the areas of interdisciplinary research, teaching, and outreach. This paper outlines the integral role that INTSOY has played, and continues to play, in the field of soybean processing and utilization, and the significant and positive impact that its activities has had on soybean processing and utilization.

When INTSOY came into being in 1972, over 30 years ago, processing soybeans was a large-scale, high-capacity, capital-intensive industry, limited to only a few very large companies. Three faculty members from the Department of Food Science and Human Nutrition †Professors A.I. Nelson, M. Steinberg, and L.S. Wei †envisioned the potential of an entity such as INTSOY to extend the benefits of soy to more beneficiaries. During the 1970s and 1980s, they and other faculty at the University of Illinois developed systems for medium- and small-scale processing and utilization of soy. Embedded with the applied research program was a strong educational and outreach component. Today, INTSOY's 6,000 ft^2 pilot plant and test kitchen, part of the Department of Food Science and Human Nutrition pilot plants, are used constantly by students and faculty for applied soybean research and product development. Current activities at INTSOY include fostering interaction between international food processors and American agribusiness. Dissemination of information by electronic means

[1] National Soybean Research Laboratory, University of Illinois, USA

is also an important current activity. These activities, as well as the current status of soy food processing and utilization at University of Illinois, are discussed in the following paragraphs of this paper.

Applied research

Since its inception, INTSOY has conducted a program of applied research, carried out by its core staff in collaboration with faculty members and graduate students in the Department of Food Science and Human Nutrition. It has undertaken research on a diverse array of topics: chapattis, soy cheese, co-extrusion, dairy analogs, developing new markets, extrusion, extrusion-expelling, functional properties, home-level processing, new product development, nutritional properties, okara utilization, protein quality, silken tofu, soymilk, textured soy protein, soy pizza, tofu, vegetable soybeans, and village-level processing. The applied research component has significantly influenced the development and application of soybean technology globally. The following paragraphs describe research projects that have had a significant impact on soybean processing.

• Soy beverage, also called soymilk, has been produced and consumed in Southeast Asia for centuries. However, the soymilk from this traditional process has a flavor profile that is unacceptable to consumers in many non-Asian countries. Over the course of 20 years, research at the university developed an "Illinois," and later, "INTSOY/Food Science" method for soymilk. The process eliminated the traditional step of overnight soaking. In its place was substituted the direct blanching, hot grinding, and filtration method. This process inactivated the enzymes producing the off-flavor, eliminated microbiological problems arising from soaking under ambient conditions, and improved the mouth feel and texture of the soymilk. The critical innovations of the Illinois process have been widely adopted in commercial soymilk processing systems. More process refinements have since been made to produce soymilk with protein and solids content suitable for soy yogurt and ice-cream manufacture.

• In many countries, soybeans are not grown in sufficient quantity to justify the use of large-scale solvent extraction equipment. INTSOY and the Department of Food Science and Human Nutrition developed a medium-scale process for transforming whole soybeans into edible oil and soybean meal. The process involves conditioning soybeans by extrusion cooking and pressing with a mechanical screw press. This method is affordable, does not use solvents, and results in food-grade soy flour and oil. The process was commercialized within five years; it is utilized today by more than 160 commercial operations globally. In a separate but related project,

INTSOY collaborated with a Midwest agribusiness to develop a non-solvent process for making textured soy protein. This process has also been commercialized in the U.S. and globally.

• Thirty years ago, soy products were not a traditional part of most people's diets. With the collaboration of many international home economists and food technologists, INTSOY has developed recipes that are acceptable to many regional preferences and incorporate regional food preparation techniques. Soy was added to such regional staples as tortillas, chapattis, and nshima, as well as used as an ingredient in main courses, side dishes, weaning foods, and desserts.

Teaching and training

Training is an integral part of INTSOY's total program. A month-long course on soybean processing and utilization was held annually for 17 consecutive years, on the campus of the University of Illinois. The course involved classroom instruction, delivered by the faculty and personnel from U.S. businesses, hands-on laboratories in the soybean pilot plant and kitchen, and visits to regional soybean industries. The course was timed so participants could attend the annual meeting of the Institute of Food Technologists. More than 200 people have benefited from attending this course over the 17 years that it has been offered.

In the first 20 years of its existence, INTSOY's core recipients were primarily from the public sector. Since the 1990s, there has been a consistent **(steady??)** increase in the number and percentage of contacts from the private sector. In 1999, INTSOY redesigned the course to reflect current needs. Many attendees, both American and international, were entrepreneurs from the private sector. INTSOY now offers more-focused, three- to ten-day courses that address one particular aspect of soybean technology. In 1999, INTSOY offered a ten-day course in conjunction with the Global Soy Forum 99 held in Chicago. The course attracted 59 participants from the U.S. and 20 different countries.

Over the years, INTSOY staff have participated in a wide range of teaching activities and have served on the graduate committees of Food Science students, especially those whose research involves soybean. Between 1990 and 1995, 225 person-months of short-term training were provided to 128 candidates from 30 countries. Six master's degree programs, one doctoral program, and five postdoctoral programs were completed during that period. Staff have also supervised programs of study in soybean utilization for visiting scholars from several different countries. Some participants from

INTSOY's short-term training courses have even returned to the university and entered graduate degree programs in Food Science and Human Nutrition.

In addition to its regular courses, INTSOY provides short-term training in postharvest processing and utilization of soybeans. Since 1997, INTSOY has offered a visiting scholars' program for students from Latin America. The program offers scholarships and advanced training to students from Zamorano, in Honduras, who are interested in working with soybeans. Zamorano is the most highly respected agricultural school in Latin America, where the students "learn by doing." It trains about 800 undergraduates from 17 countries. So far, INTSOY has hosted four students, who train for a semester at the University of Illinois. Another student has been selected for 2004.

INTSOY also offers short-term training programs at various international locations in collaboration with partner institutions. Its long-term perspective has resulted in the development of physical facilities and expertise in several countries where short-term regional training can be held. For example, in-country training has been offered in Botswana, Egypt, Sri Lanka, Zambia, and Zimbabwe. Three hundred people from 22 countries have taken advantage of these overseas workshops. In addition, in partnership with soybean industry groups and international collaborators, INTSOY has played a key role in organizing several international workshops and conferences. For example, in 1995, INTSOY co-hosted a regional workshop in the West Indies on soybean utilization. More than 75 participants from 20 different countries attended. Similar workshops were held in Peru (1975), China (1990), and at the Global Soy Forum in Chicago (1999).

Outreach/extension

INTSOY provides extension and outreach, both long- and short-term, to national-level programs, non-governmental organizations, and private agribusinesses.

Long-Term Technical Assistance

1970s: Sri Lanka. INTSOY was the prime contractor for a seven-year postharvest project sponsored by UNDP/FAO. The project established a soy foods research center, provided degree training, developed in-country training programs for extension staff, and provided outreach to private agribusiness.

1980s: Nigeria. INTSOY assisted the International Institute of Tropical Agriculture (IITA) in developing the postharvest processing and utilization component of the institute's soybean program. With continued support from the Canadian International Development Research Centre (IDRC) and

the Japan International Cooperation Agency (JICA), IITA maintains its emphasis on soybean utilization for sub-Saharan Africa.

1990s: Egypt. INTSOY implemented a four-year contract with USAID and the Egyptian National Agricultural Research Project to strengthen soybean processing for the Middle East region. A fully equipped, pilot soybean processing center was established at the Food Technology Research Institute (FTRI) in Giza. Human resource development was carried out through degree, non-degree, and postdoctoral training. Egyptian agribusinesses and entrepreneurs were selected to tour leading U.S. soybean companies.

1990s: Ukraine. INTSOY designed and installed a factory to process soybeans and corn grown by a U.S./Ukrainian joint venture. Selected privatized farms collaborated with U.S. agribusinesses to develop a system for grain production, postharvest handling, and storage; add value to on-farm processing, and marketing. INTSOY assisted with project management, establishment of a commercial soy processing facility, transfer of technology, and training of personnel.

Short-Term Technical Assistance

The following projects highlight the extensive locations and scope of INTSOY's short-term technical assistance. Over the years, there has been a change in the recipients of soybean technology. In the 1980s, many recipients were public institutions, whereas currently, in the new millennium, the majority of requests come from entrepreneurs and companies. The following are examples of technology transfer conducted by INTSOY in 1992, a year of transition. This list includes countries, companies and organizations, and description of technology transferred, highlighting only one company from each country: Bangladesh, Mennonite Central Committee, commercial application of extrusion; Botswana, SACCAR, blended weaning food development; Bulgaria, Bulgaria Interuniversity, introducing soymilk technology; Cambodia, Ministry of Agriculture (MOA), technical assistance for commercial soymilk factory; China, Me Le Fu factory, extrusion; Egypt, FTRI, turnkey project for establishing a research and technology transfer center for wet and dry processing; El Salvador, Planta Soyavyn, technical assistance soymilk processing; Ethiopia, FAFA Food Company, enterprise development; India, CIAE Bhopal, human resource development; Indonesia, Bandung University, technology transfer; Italy, FAO, soybean utilization network; Ivory Coast, MOA, private sector development; Kenya, Soy Afric, technology transfer; Laos, MOA, soy processing; Philippines, PCARRD, soybean utilization; Sri Lanka, Plenty Canada, soymilk and dairy analogs; Tanzania, Sokoine University, soybean utilization; Thailand, C.P. Company,

soy food utilization; Turkey, Ege University, soy food utilization, and Vietnam, Bich Chi Powder Factory, extrusion expelling.

Current and future activities

In the past five years, Americans in larger numbers have been consuming soy foods in the context of recent research linking the beneficial effects of soy on chronic disease. Sales of some soy foods in the United States have increased at a rate of more than 10 percent annually. In 2001, the Illinois Center for Soy Foods was established. It is funded by the State of Illinois through the Illinois Council on Food and Agricultural Research (C-FAR). Its goal is to promote the consumption of soy foods, thereby providing financial benefits to growers and processors in Illinois, technological innovations to large- and small-scale processors, as well as health benefits to consumers. The center comprises "action teams," drawn from faculty and staff with expertise in food technology, food product development and testing, nutrition and safety, and marketing and education, to meet industry needs.

In 2003, INTSOY held two 10-day courses (Soymilk and Dairy Analogs, Marketing and Product Development of Soy Foods Sensory and Chemical Aspects of Soy Flavor) and four short-term training courses on campus (for participants from Ivory Coast, South Africa, Turkmenistan, and American private voluntary organizations). In 2004, INTSOY will be extending its technical knowledge to the following countries: Angola, Botswana, Guatemala, Haiti, Honduras, Kenya, Malawi, Senegal, Uganda, and Zimbabwe.

Introduction and the use of soybean in Kenyan food chain and the experiences of soy Afric in un feeding programmes to refugee camps, draught victims, slum dwellers and HIV/AID victims

C. Muthuri[1]

Soy Afric

Who is Soy Afric?

- Soy Afric is the leading Soybean processing factory in East Africa.
- Has a daily capacity of 40 MT Full Fat Soya Cake, and 30 MT production of Unimix or Cereal based/soya Extruded powdered products.
- 15 – 20 MT Full Fat, Full Enzyme Soya Flour.

Processing:

Extruded products for relief agencies:

Millet	)
Cleaned whole Maize	)
Water	)
Cleaned Soya	)
Sorghum	) Extrusion process
Super Unimix	)
Mixplus	)
Unimix	)
Sudan Mix	)

For retail and local markets:

Other Cereal	)
Cleaned Maize	)
Sorghum	)
Clean Soya	)
Millet	) Extrusion process
Full Fat Soy Meal Cake	)
Ujimix	)
BoraBora Nutrimix	)

[1] C.E.O SOY AFRIC LTD

Factory

The factory products

- Unimix, Super Unimix, Sudan Mix mainly for UN Agencies, NGOs and Church Organizations.
- BoraBora Nutrimix for institutions.
- BoraBora Recovery Porridge.
- Ujimix – for Retail Outlets.
- Full Fat Full Enzyme Soya Flour.
- Extruded Full Fat Soya Flour.
- Full Fat Soybean Cake.

Product is custom packed according to customer needs.

Location

The factory is located in the Industrial Area of Kenya's Capital City, Nairobi.

There the products can leave by road or by Air through Jomo Kenyatta International Air Port to remote destination and Areas un accessible by road. (Especially rainy seasons).

Entry of soy bean in Kenyan market

Prior to the start of Somalia War of 1992, nothing was known about Soybean in Kenya Market. Soy Afric got involved in early Extrusion of Soybean making Unimix for UN Agencies to feed the influx of refugees from neighboring Somalia and latter from Ruanda and Sudan. The need for Unimix was as high as 2,000 MT per month, but Soy Afric was able to extrude and supply only 600 MT per month maximum.

However, this gave the birth of Extrusion technology in the country.

After the need for Refugees went down, there was out-break of severe drought in the Horn of Africa affecting even Northern Kenya. Again we swung into action supplying Unimix and saving lives from Hunger. This gave Soy Afric immense experience to respond to Emergency needs and to deepen our knowledge in Cereal Extrusion.

Since then Soy Afric has diversified to lead in introduction of Cereal based Soybean and fortified foods in leading Supermarkets.

The real formation of Soy Afric began with an offer of INTSOY offering to send the current C.E.O to a Soybean processing course to university of Illinois Champaign – Urban, U.S.A. summer of 1993. Thereafter, I got into contact with InstaPro who provided the first extruder in 1994.

Soy Afric has been the pioneer to many programs ever since, such as:-

1. GTZ Programme in promoting of growing Soybean in Kenya.

2. Government of Kenya Health Education Extensions, promoting utilization and use of soya in Rural Kenya.

3. Soy Afric involvement in promotion of use of Soya Flour in the Bakeries working together with WISSH.

Challeges, planting

There are various challenges we have faced in all these:

1. Planting: Soybean yield less than maize, or kidney beans in rural Kenya. Therefore, farmers lost the incentive to plant.

2. Soybean is hard to cook and cannot be readily utilized in homestead.

3. Production costs per hector are high in Kenya (intensive labour) and soybean is a World Commodity.

4. Rainfall is not so good in draught and marginal areas.

Utilization

1. Soybean is not appealing to African taste.

2. Methods of preparation to match the local dishes are poor.

3. Need more Education about Soybean.

Achievements

1. Successfully sensitized Bakery users to put Soya Flour in baking Flour.

2. Sensitized Flour millers to start using Soya Flour in Porridges.

3. Created awareness in rise of Full Fat Soya Cake (limited extent).

4. Sensitized the Government to promote use of Soybean through Health Education extension.

5. Together with Catholic Church in Kenya, we have introduced the use of Soy based Extruded Cereal to be used in Slums and Children Homes.

Future

1. To continue with current campaign of use of Soya flour in Bakeries.

2. To start joint campaign with NGOs, Government and Church Organizations to intensify the use of soybean and fortified cereal in HIV/AID victims and families.

3. To encourage the growing of Soybean in the Country because all what is processed is imported.

Current status and future perspectives of soybean processing in Russia

M.L. Domoroshchenkova[1]

Abstract

The current processing of soybeans in Russia doesn't meet the demand of domestic feed and food industries neither in volumes of produced products neither in their assortment. The largest volumes of soybeans in Russia are processed by crushing enterprises of oil & fat industry. Domestic production of edible soy proteins is limited to different types of soy flour and textured soy proteins. To cover the growing demand in high protein feeds, vegetable oils and edible proteins Russia is importing large volumes of soybean meal, oil and edible soy proteins. Main volumes of soy proteins are currently used by meat processing industry. In the last years a domestic production of traditional soyfoods (soy milk, tofu, etc.) has received a certain level of development. But these products occupy only a niche market in modern Russia. Serious marketing activities are required for further growth of this market. With the improvement of the economic situation in Russia the demand in soy protein products will grow. In the near future Russia has to switch from an extensive way of supplying local customers by imported soy products to the intensive way of development of domestic soybean processing industry accompanied by a construction of new processing facilities. Such transition could become successful on the basis of the special state support programs and/or through a foundation of integrated business structures with consolidated capitals.

Meeting the demand of the population in high quality protein products and production of high-protein feeds are among the most serious objectives of the modern agro-industrial complex of Russia. Production and consumption of principle protein products of animal origin (meat, fish, eggs, milk) in Russia has seriously decreased in the last years (1, 2). The average per capita consumption of proteins in 2000 was evaluated as 64 g per day. The shortage of proteins in diets of Russian citizens based on comparison of recommended and actual consumption levels could be evaluated as more than 25% or more than 1 million MT per year.

[1] All-Russia Scientific & Research Institute of Fats, St.Petersburg, Russia, wniig@peterlink.ru

The observed deficit of proteins in diets of Russians can be covered by a growth of production and consumption of soy protein products. The attractiveness of development of domestic soy protein industry in Russia is enhanced by the current market situation and constantly growing demand for all main varieties of soy products such as soybean meal, soybean oil and all types of edible soy proteins.

Production of soybean seeds

The main type of oilseeds grown in Russia is sunflower seed. It occupies more than 80% of the total oilseed sowing area and provides more than 85% of the total oilseed production. Soybeans are on the 2nd position but volumes of their production are far behind sunflower seeds and can't satisfy the demand of the growing market of soy products. The production of soybean seeds versus total oilseed and sunflower seed volumes in the recent years is demonstrated in Table 1.

In 1970-2001 the decrease of soybean growing areas was observed in Russia. They've decreased from 850 thou. hectares in 1970 to the lowest area of 404 thou. ha in 1997. Most seriously soybean areas were decreasing in the 1990s due to the total crises experienced by the agriculture in Russia in those years. In 2002 the total soybean sowing area reached 476 thou. hectares. About 80% of soybean sowing areas were located in the Far East of Russia and more than 15% - in the North Caucases. The total production of soybeans in 2002 reached a maximum level for the last 9 years.

Table 1. Total Production and Yield of Oilseeds, Sunflower and Soybean Seeds in 1998-2002 (for all types of farms)

Years	Total Production, thou. MT			Yield, c/ha		
	Total	Included		Total	Included	
		Sunflower	Soya		Sunflower	Soya
1998	3,435	2,998	297	6.9	7.2	6.5
1999	4,674	4,151	334	7.3	7.4	7.6
2000	4,500	3,913	342	8.2	8.5	8.1
2001	3,185	2,685	350	7.9	7.8	9.4
2002	4,271	3,684	423	9.8	9.7	11.7

Processing of soybean seeds

Processing of soybean seeds is accomplished in Russia in the following directions:

- production of soybean meal and oil by solvent extraction and pressing operations;

- production of edible soy protein products;
- production of traditional soyfoods.

The largest volumes of soybeans in Russia are traditionally processed by oil extraction plants. Russia has a well-developed oil & fat industry. According to the data of the department of oil & fat industry of the Ministry of Agriculture of Russia the total annual crushing capacity of all types of enterprises was estimated as 4,560 thousand MT on 01.01.2003 (3). In 2002 the Russian oil & fat industry produced 1,186 thousand MT of vegetable oil, 1,270 thousand MT of meals and cakes, 515.5 thousand MT of margarine products and 317.5 thousand MT of mayonnaise (4). Processing of oilseeds was accomplished by 57 large oil mills (90% of the total crushing capacity) with a maximum capacity 1,000 MT/day and by approximately 1,200 small shops and operations. Capacity utilization ratio of these enterprises was about 55% in 2000-2002. The main reasons for inadequate usage of crushing capacities were a shortage of raw materials and lack of financial capabilities of enterprises to purchase raw materials in due quantities; poor technical performance of old items of equipment; and inadequate supply of necessary utilities and shortage of storage capacities.

2,076 thou. MT of oilseeds (1,755 thou. MT of sunflower seeds and 265 thou. MT of soybeans included) were processed by the Russian oil mills in 2002 (table 2). They produced 208 thousand MT of soybean meal and 46 thousand MT of soybean oil. It's far behind the potential of the Russian oil & fat industry and the demand in high protein feeds and soybean oil.

Table 2. Processing of Soybean Seeds Versus Other Oilseeds in Russia in 2000-2002, thou. MT

	Years		
	2000	2001	2002
Total oilseeds (100%)	2,829.36	2,284.69	2,075.86
Soybean seeds	177.37 (6.3%)	176.87 (7.7%)	264.54 (12.7%)

The current volume of soybean processing is much lower than in the Soviet years when oil mills crushed more than 900 thou. MT in 1980 and it is substantially lower than the volume of soybean processing in 1992 when 19 oil mills crushed about 700 thou. MT of soybeans.

A growth of volumes of processing of soybeans at the vacant capacities of oil mills could be very beneficial for the national economy and individual enterprises. In absence of a developed credit system in modern Russia and shortage of financial recourses of individual enterprises to purchase ship quantities of soybeans an allocation of special credit programs for acquisition

of soybeans could be helpful for a growth of soybean utilization and processing by local oil mills.

Industrial production of edible soy proteins in Russia is limited to different types of soy flour and textured soy proteins. The absence of white flake industrial operations negatively influences the development of edible soy protein industry. The local producers of soy proteins have to use toasted soy flour or to rely on import of raw materials for further processing.
Russia has no industrial production of soy protein isolates and functional concentrates. Some Russian companies are trying to arrange a local production of soy concentrates but their quality parameters don't meet the requirements of the modern market. There is also a tendency of production of functional protein blends from soy proteins. Usually companies- traders of protein products are involved in this business.

Total annual volume of industrial production of different types of soy flour and textured soy proteins doesn't exceed 50 thousand MT and doesn't meet the requirements of the Russian market in volumes, varieties and functional characteristics of available soy proteins.

In absence of domestic production of soy isolates and concentrates and shortage of edible proteins a number of companies investigate an opportunity to construct a soy protein plant. High social necessity, large investments and financial risks associated with a foundation of soy protein industry in Russia requires state support through allocation of state credit lines, facilitation of tax policy and/or through a foundation of integrated business structures with consolidated capitals.

The program "Soya of Russia" approved in 2003 is one of the most recent and detailed programs devoted to the development of soy protein industry. It deals with a construction of several soy protein plants to 2010.

Soyfoods occupy only a niche market in modern Russia. The range of traditional soyfoods produced in Russia varies from plain soybean milk and regular firm tofu to different flavored soya drinks, yougurts, mayonnaise and dressings, frozen desserts, powdered and condensed soy milk, soy nuts, different types of tofu, okara and tofu based soya pastes and mashed meat replacers. In the past 5-8 years several high capacity modern soya milk installations (500-3,000 l/hour) and dozens of middle scale installations with capacity 200-300 l/hour appeared in Moscow, St.Petersburg, Komsomolsk-on-Amour, Volgograd, Astrakhan and other cities of Russia. Hundreds of small "soy cows" with the capacity 30-40 l/hour were installed in 1998-2000 in different social institutions, churches, orphanages,

children's and women's shelters and prisons as the result of the international charitable program "Feed the Children". Bringing of US soybeans for feeding "soy cows" supported the development of this program in Russia.

Production of traditional soyfoods experienced some decrease during the last couple years. A number of soymilk plants have lowered or closed their operations due to the unstable demand of the local markets, problems of purchasing of good quality soybeans in necessary volumes and inadequate marketing policy. Currently the total processing of soybeans for soymilk, soy drinks and tofu doesn't exceed 5 thousand MT a year.

Better development of soyfood market could become possible on the basis of reliable supplies of soybeans (probably through establishment of specialized soybean storage and distribution centers), better adjustment of the appearance and flavor of final products to the tastes and preferences of local customers, introduction of soyfoods to the formulation of regular food products, and a good marketing and promotion campaign with a special emphasis on the health benefits of soya.

Consumption of soy protein products and soybean oil

According to the estimates of agricultural specialists the deficit of soybean meal for poultry and swine feeds exceeds 800 thousand tons. The estimated potential demand of the rapidly growing Russian poultry industry can reach 1.2 million MT of soybean meal in the near years. With further projected growth and development of domestic poultry and swine industries the demand in soybean meal will correspondently grow. Given the shortage of local soybean processing Russia will need to import more soybean meal or up-grade and better use the capacities of the local crushing industry.

In modern development of food industry soy proteins can be regarded not only as nutritional supplements but also as functional additives in production of a number of regular foods.

Main volumes of soy proteins are used by meat processing industry. Usage of soy proteins in meat recipes is constantly growing. Different types of soy isolates, concentrates, TVP or defatted soy flour can be found in more than 90% of recipes of boiled and semi-smoked sausages, minced meat products, semi-prepared meats, pastes and other types of meat products. Usage of soy proteins in mayonnaise formulations received a good level of development in recent years. Current usage of soy proteins in dairy, bakery and confectionery industry is far behind it's potential. Soy proteins can be also used in production of infant and baby foods, in production of food

concentrates, in specialized nutritional products and for other applications. But all these applications of soy proteins are less developed in Russia yet. According to some market estimates the total potential demand of Russia in edible soy proteins can be evaluated as 400-450 thousand MT.

The consumption of soybean oil experienced a substantial growth in recent years. It has increased from 261 thou. MT in 1999/00 MY (11% of the total oil & fat consumption) to 536 thou. MT in 2001/02 MY (20.7% of the total consumption) (5). The growth of consumption of soybean oil increases the attractiveness of processing of soybean seeds by the domestic oil mills.

Special marketing efforts are required for introduction of GM soy products to general public. Three types of GM soybeans are certified for food applications in Russia. Planting of GM soybeans is not permitted in Russia. The food labeling starts from 5% and higher level of GM soy protein ingredient in a food formulation. In spite of numerous publications and presentations of leading Russian scientists from the State Institute of Nutrition devoted to the safety issues of certified GM soy products many people are concerned on consumption of transgenic foods.

Import of soybeans and soy products to Russia

Domestic production of soybeans at the end of the XXth century never corresponded to the demand of Russia in high protein feed additives. Many years USSR imported large quantities of soybeans and soybean meal. Thus in 1988 more than 2 million MT of soybeans were imported to Russia. About 200 thou. MT of soybeans and 300 thou. MT of soybean meal were delivered to Russia from USA under PL-480 credit program in 1999. But volumes of commercial import of soybeans in the following years were very low and varied from 40.9 thou. MT in 2000 to 12.5 thou. MT in 2001 and 64.4 thou. MT in 2002.

Though Russian importers preferred to import soybean meal versus soybeans after the "perestroika" period current volumes of import of soybean meal don't cover the potential demand of local poultry and swine industry. According to the data of State Customs' Committee less than 200-300 thou. MT of soybean meal was imported annually to Russia in 2000-2002 (table 3).

Table 3. Import of Soy Protein Products to Russia in 1999-2002, thousand MT

	Years			
	1999	2000	2001	2002
Import of soybean meals and cakes, (6)	389.9	178.1	232.1	287.5
Estimated total volume of imported soy proteins	45-50	50-55	65-70	80 +

To cover the demand of food industry large volumes of edible soy proteins are imported to Russia from other countries. Analysis of the customs' statistics showed that the volumes of import of soy proteins were constantly growing in the last years (table 3) and they've exceeded 80 thousand MT in 2002. Many soy protein products were imported to Russia through middle countries (Estonia, Poland, Latvia, etc.) and middle trading companies.

Soybean oil occupied the leading position in the structure of import of vegetable oils to Russia in the last years. According to the data of the State Customs Committee of the RF volumes of import of main types of vegetable oils to Russia in 2002 were 1,054 thousand MT. The volume of import of soybean oil in 2002 was the highest for the observed period – 473 thousand MT (6). It's share in the total volume of imported oils grew up to 45%.

One of the main reasons of the growth of import of vegetable oils and soybean oil to Russia is the growth of consumption by enterprises of the Russian food industry. The difference between the demand and domestic supply was covered by imported oils with the prices lower than the local sunflower oil prices. Soybean oil competed mainly with sunflower oil of domestic origin and imported cheap palm oil. This competition will continue to grow next seasons and import of soybean oil will depend on domestic sunflower crop and world vegetable oil market situation.

Conclusion

The current processing of soybeans in Russia doesn't meet the demand of local feed and food industries neither in volumes of produced products nor in their quality and assortment. Supplies of soy products to the Russian food, poultry and husbandry industries are characterized by an extensive approach when finished products of soybean processing are imported to Russia in growing quantities and Russian customers pay an extra price to cover the expenses of foreign farmers, processors, traders and middle companies.

With improvement of the economic situation in Russia the demand in soy protein products will grow. In the near future Russia should switch to the intensive way of development of domestic soybean processing industry accompanied by a construction of new processing facilities. The necessity of construction of facilities for processing of soybeans and development of a soy protein industry in Russia is based the growing demand in soybean meal and edible soy proteins and on the developed domestic soy oil market. These facilities can use both local and imported soybeans as raw materials.

Establishment of a soy protein industry in Russia should be considered as a primary priority for the further development of the national economy. It will contribute to the solution of a number of social and economical objectives such as decrease and elimination of existing deficit of proteins and vegetable oils, creation of extra working places, import replacement, improvement of structure of nutrition of local population, and growth of production and enhancement of biological value of feeds.

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Introducing soybeans in Argentina within crisis and poverty

A. Kossoy[1] and M. V. F. de Picardi[2]

2002, was the worst economic and social crises ever known in Argentina: increasing unemployment and poverty enabled thousands of citizens the access to food. Within this context, a farmers' association for non-tillage practices create SOYBEAN SOLIDARITY PLAN consisting of soybean donations for the impoverished population afflicted by hunger. 1200 tons of soybeans were distributed to community entities all around the country .The "soybean phenomenon" brought about a large number of controversies around the crisis: Society debated over productive politics and a country model, over responsible of hunger, over food safety nutrition and eating habits, over ecology and science, over the term "soy milk".

Social Actors from the private sector (soybean producers, seed producers and carriers), the third sector (specialists in soy related issues or community activities, and more than 2000 NGOs) and from the public sector worked together on the cooks' training and soy distribution across community canteens.

Beef accounts for one of the major supplement in people's daily diet, and Soybean food was little known, only by a small wealthy elite interested in natural eating habits, so training was challenged to overcome this cultural barrier and introduce a new cheaper food for the population. Other experiences were developed in the 70's, and recently in the northwest of the country by a nutritionist who trained community members with the donations given to a hospital by a farmer. Both projects encouraged other farmers to implement the Plan across the country.

Workshops for cooks' canteens and homemakers were organized, including food safety and nutrition notions, information about soybean health benefits and how to process and elaborate beverages and dishes from okara or grinded bean. The meals prepared during the workshops were tasted, promoting trainee's interest in experimenting the new learned recipes. In big cities, especially in the Buenos Aires area, which concentrate 40% of poor people, soybean is distributed by 30 distribution centers located in different neighborhoods that operate within civil organizations, religious institutions and public entities.

[1] Sociologist, akossoy@fibertel.com.ar.
[2] Agronom. Engineer, vfrancoman@interserver.com.ar

The first results are widely encouraging in such a short time, including geographical coverage, nutritional education and solidarity initiatives. The Plan has contributed with more than 600,000 people in need, facilitating the access to a new nutritional and cheap food rich in proteins. Also, the nutritional training impact on unemployed people that joined the workshops., increasing nutrition culture the use of vegetables foods rich in vitamins and nutrients and empowering self-esteem, and work culture of In the health area volunteer nutritionists encourage food safety among community institutions, and developed some projects with ill-nourished children in the last two years.

Strategic issues to expand soybean utilization in India

R.T. Patil[1]; S. D. Kulkarni[2] and N. Ali[3]

Introduction

Though ancient record of soybean cultivation dates back to 100 AD, its cultivation was limited to hills of Assam, Bengal, Manipur, Nagaland, Sikkim, Kumaon and Garwal regions. The production till 1970-71 was only 0.01 Mt, which increased to 5 Mt in year 2002-2003 (Fig 1). Traditionally soybean in Himalayan states was known as 'Bhat' and it was consumed after roasting or boiling.

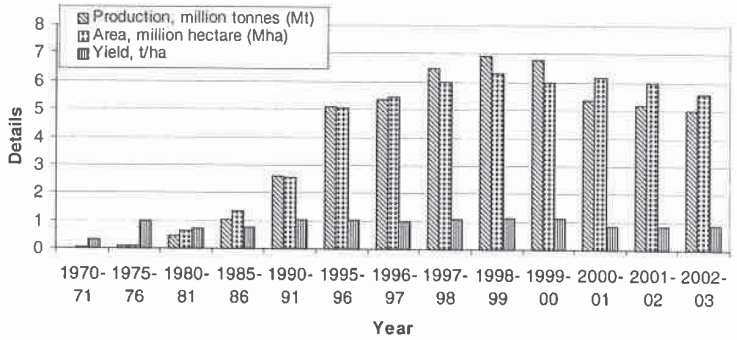

Figure 1. Trend in soy cultivation in India in last 3 decades

Even Mahatma Gandhi appreciated importance of soya for human health and its good quality protein, but due to lack of proper processing technology and general awareness about the method of its consumption, it could not be made popular. However, 1970 onwards cultivation of soybean got momentum when it was considered as an oilseed crop. Though, it contained only 20% oil as compared to larger portion of (40%) protein, it became a boon for solvent extraction plants, as it could be processed without pre pressing. Soybean was a best raw material with three times more oil compared to pre pressed cake of soft oilseeds and hence it proved more economical for extraction. This provided an assured market for soybean

[1] Principal Scientist.
[2] Project Director, Soybean Processing & Utilization Centre
[3] Director, Central Institute of Agricultural Engineering, Bhopal

grown by local farmers. The oil extraction from soybean was also encouraged by the Government to meet the shortage of edible oil in the country. However, due to lucrative oil business not much attention was paid to develop technologies for its utilization as food.

However, when the quality of DOC deteriorated due to drought and untimely rain, its export got affected. Even some consignments from export destination were rejected. The results were disastrous, farmers did not get much return, the productivity of soybean gone down and ultimately the area under soy cultivation was also reduced. (as seen from the trend in last five years). This posed a grave problem for already over capacity soy processing industry. Hence, it became important that alternate ways for soy utilization as food either from whole soybean or de oiled cake must be developed.

Another reason why soybean utilization as food is important for India is protein-calories malnutrition prevailing in the Indian population. The rising prices of conventional source of protein i.e. pulses also aggravated this problem. About 55% of our children are malnourished, 65-70% of women are anemic and about 30% of population does not get two square meals a day. This has also reflected in high incidence of diabetes, heart patients due to consumption of unbalanced diet. As rule of thumb average protein requirement of body is 1g/kg of body weight. By this rate individual weighing 50 kg and having only carbohydrate based diet should take to about 60-80 g dhal per day. That means in a family of four/five with average body weight of 50 kg should consume 300 g dal/day or 9 kg/month. But average dal consumed in household is found to be 3-4 kg at the maximum, thus having deficiency of 60% intake of protein source. Hence it is essential that this gap in protein intake need to be supplemented by high protein product from affordable alterative like soybean.

However the major issues related to soybean, which have inhibiting effect on its popularisation are;

1. Antinutritional factors present in raw soybean requiring precise processing.
2. Beany flavour development if not processed properly.
3. Poor cooking quality for its use as dal
4. Indigestion effect due to possibility of over consumption
5. Lack of awareness about health benefits and processing requirements.
6. Non availability of processed products suiting to Indian taste.

Hence it is essential that package of technology should be developed to take care of these impediments and entrepreneurship at cottage industry level be developed so that soy products can be made available at affordable cost. The adoption of soybean processing by entrepreneurs, therefore, depends on the following strategic issues.

Availability of raw materials

The need for soy products is growing throughout the country, but soybean is not available in local market like other pulses and food grains. This is mainly due to the fact that it is a cash crop and sold immediately after its harvest. This is the case also in the states where it is grown. Hence, government or Food Corporation of India should make available soy from some central point for the food processors. The price of the raw material is another factor. When the soybean is being used for oil expression the price is very low i.e. about Rs. 8-10 per kilogram but as soon as the small scale or cottage industry starts using it as raw material for food, the price goes up to Rs. 25 per kg. It is this necessary that soybean is made available in sufficient quantity and through out the year through government regulated shops or other arrangements.

Availability of technology

Another important factor that was coming in the way of soybean utilization as food was that of non-availability of indigenous technology. The technology developed in Japan for the products like milk and tofu though was apparently considered suitable for adoption in India, however, the capacity and cost was not economical to set up a business based on it. Few companies comparing the scope of soymilk with other cold drinks and beverages imported such plants. However, the consumption did not increase at the expected rate and hence they could not sustain. The sale of such product in comparison to conventional milk also did not succeed because of price appreciably higher than dairy milk due to high packaging cost in tetra packs. In ploy bags cost was equal or little higher but the product shelf life has less due high perishability of the product. On the other hand high perishability and un controlled vending of the spoiled material, made the soymilk unpopular. It is therefore essential to develop indigenous and easy to adopt technology. Its capacity should also be such that full load can be absorbed in the market in the reasonable time.

Availability of skills

The general food catering establishments in India are of small size and generally fresh material is produced every day. This avoids the need for costly preservation and packaging. The high density of population makes possible such enterprises to survive. Thus there is a greats scope for many small capacity ventures compared to one large capacity plant as would be feasible in developed countries. Such small capacity plants can also utilize family labour and could be very cost competitive. The production of soy foods requires certain degree of skill. If the skill is not available in making the acceptable quality soy products, then it creates a wrong impression

and can even make soy products unpopular. This has been the experience of many when they tried to produce soymilk and tofu just by following the published literature. The flavour development was so high that the producer himself could not accept the product for second time even though it was better than the first time. Hence, it is very important that the skilled manpower be developed in larger number to make available the acceptable quality soy products in the market on sustainable basis.

Government priority

Another important criteria that can be helpful for soy utilization is the government priority. Due to concern about growing malnutrition among the urban as well as rural population, the government has started many supplementary feeding programmes. The recipe to be high protein and nutritious one at affordable cost, the soy is the ultimate choice. Many state and central government schemes are providing soy cereal based either extruded or roasted products with wheat as cereal component. The publicity of soy through electronic and print media and also the health benefits of soy have generated interest among the population towards the soy foods. To sustain this interest it is essential that proper soy products are made available at affordable price. However, more efforts on wider publicity on health and clinical benefits of soy through advertisement in electronic and print media are needed.

Ease of implementation

Since the targetted soy processing will be a cottage industry, the technology and equipment developed should be easy to install and operate in that scale. The quality control techniques be easy to understand and implement.

To expand the soybean utilization as food needs major components as follows:

1. Development of suitable products and equipment to establish cottage level industry.
2. To popularise preparation of soy products at domestic level in soybean growing areas.
3. Conduct technology based entrepreneurship development programme for up coming entrepreneurs.
4. To popularise nutritional and health benefits of soybean through extension leaflets, electronic and print media.

Technology development:

Since no technology available in developed countries would meet the criteria or strategies mentioned above, the onus of developing the suitable

technology and equipment fell on ICAR and hence the SPU centre was established to conduct research and develop suitable technologies and equipment so that the soy products suiting to Indian taste and also at affordable price could be produced.

The recent scientific evidence on health benefits of soybean for controlling diabetes, preventing cancer, lowering cholesterol, thereby reducing the risk CHD/CVD, suitable for lactose intolerants, preventing menopausal syndrome in ladies and preventing osteoporosis, has made USFDA recommend 25 gm soy consumption per day for good health. Due to this reason the processing of soybean for foods has great potential. However, the products to be developed should be suiting to the taste and pallet of our population.

This would indirectly provide the income generating opportunities to the upcoming entrepreneurs along with alternate market for soybean as food crop. Some of the important products, technologies and equipment developed at SPU centres are given in Table 1.

Table 1. Some of the soy products, processing equipment and technology developed in India for food uses of soybean

Soy products and Technology (20)	
• Soydal	• Soymilk
• Soyflakes	• Soypaneer
• Soyflour	• Soy-yogurt
• Soyfortified biscuits (biscuits)	• Soy-ice cream
• Soyfortified biscuits (salty)	• Tempeh
• Soyfortified bread	• Soy-sattu
• Soyfortified muffins	• Okara based burfi & Gulabjamun
• Soyfortified bun	• Soy-sauce
• Soy-nuts (roasted/fried)	• Soy-srikhand
• Soy-amrakhand	
Soybean Processing Equipment (20)	
• Grader	• Plate type wet grinder
• Manual dehuller	• Modified oil expeller
• Power operated dehuller	• Low-cost steam generator
• Blancher	• Soybean cake grinder
• Natural convection tray dryer	• Dough mixture
• Forced convection tray dryer	• Loaf volumeter
• Multipurpose LSU type dryer	• Lever-type paneer pressing device
• Three-roller flaking machine	• Screw-type paneer pressing device
• Two-stage roller mill for soyflakes	• Cottage level soypaneer plant
• Low-cost single screw forming extruder	• Biscuit-cutter/moulder
Limited Scale Pilot Production Facilities for (6)	
• Fullfat soyflour	• Soypaneer
• Partially defatted soyflour	• Soyfortified bakery products
• Soymilk	• Soy ice-cream

Entrepreneurship development based on soybean

Soy foods, especially due to their characteristics flavour are not instantly liked. It will take some time for the people go get adjusted to it. Another draw back in popularizing soybean is lack of easy availability of soyfoods. It is therefore essential that the upcoming entrepreneurs should be trained in production of good quality acceptable raw products. Hence technology based entrepreneurship course based on the products suiting to Indian taste was a need. Our programme has generated confidence among the entrepreneurs in soy products. The course has been designed for one week duration covering the major topics like technologies for soymilk and paneer production, and other soyfoods, demonstration of plants for production of soymilk and paneer followed by practicals or hands on experience on production of paneer tofu by domestic as well as cottage level plants. The information on enterprise planning, launching and management of the enterprise along with financial details are also provided to the participants by the professionals.

So far we have conducted 45 courses and trained 432 participants. The cottage level plant based on the design perfected by SPU centre has been adopted by 7 manufacturers. These efforts have resulted in establishment of more than 175 cottage level industries earning from Rs. 5000 to Rs. 50000 per month. The state wise distribution of trainees and enterprises are given in Fig. 2.

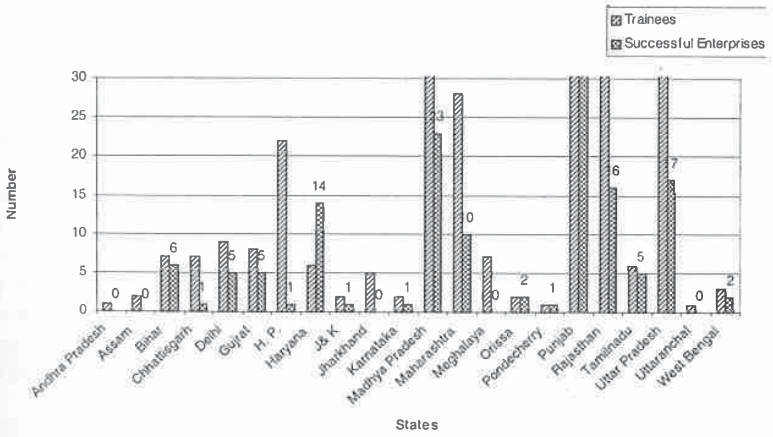

Figure 2. Statewise distribution of trainees and successful enterprises based on soybean

Development of household level entrepreneurship among rural women

The Madhya Pradesh is a soybean state, even then the use soybean as food is negligible and major production goes for oil extraction. This has also resulted in malnutrition in rural areas. The survey conducted in nearby villages indicated that protein deficiency was main cause. Similarly energy deficiency was also prevalent among children. This was mainly due to lack of knowledge about the processing of soybean and the mode of its consumption. Hence one-day awareness camps were conducted in nearby villages where preparation of products like soymilk, soypaneer and recipes were demonstrated using domestic appliances. As a follow up a six-day training programme was organized for women for preparation soy products. So far 317 farmwomen from 18 villages were trained. They were also helped to start the micro level women enterprise by providing the domestic mixer grinder on loan basis. The market as well as service support was provided by engaging them for soymilk distribution programme for the village children. As effect of it 7 soymilk entrepreneurs and 1 cottage level women entrepreneur are earning their livelihood by processing soybean.

Conclusion

Our experience on processing and utilization of soybean in India had indicated that there was lack of awareness about the processing and health benefits of soybean. Many misunderstanding and misconceptions about soy and its products also existed in the minds of consumers. Even the farmers who produced soybean did not know method of processing and mode for its consumption. Another important factor why soy did not catch up even when consumers were convinced about the benefits of soy products was non-availability of the products in Indian market suiting to Indian eating habits. This was mainly due to non-availability of technology appropriate to Indian socio economic conditions. Hence the main effort to make soybean popular as food were to popularize indigenously developed technology for processing of soybean, conducting awareness camps for farmers, educated youth, doctors and other literate people of the society about the ease of soy processing and its health benefits. This was followed by training programmes for up coming entrepreneurs for commercial production as well as for women for utilization of processed soy products by preparing acceptable dishes. The impact was highly encouraging with 175 cottage level enterprises in last 5 years and 80% utilization of soybean as food in the villages where awareness camps were conducted.

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Recent progress in soybean breeding for processing and utilization in Japan

K. Kitamura and A. Ujiie[1]

Introduction

Since the government decided to encourage soybean production instead of rice in 1978, soybean production in paddy field has dominated. In March 2000, the government launched the New Agriculture Policy to raise food self-sufficiency rate adopting a new grand-aid system to accelerate increase of soybean and wheat production. Soybean production sharply increased again (Fig. 1).

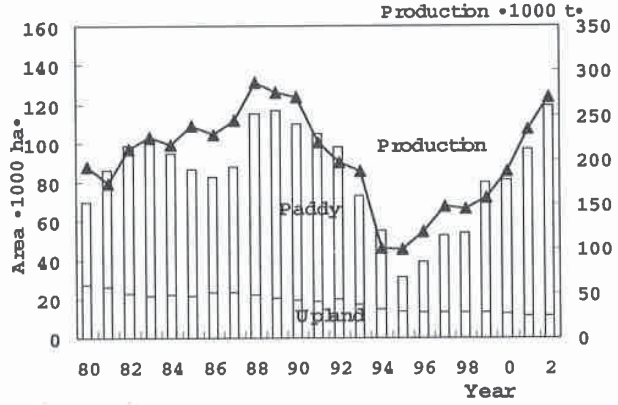

Figure 1. Soybean area and production in Japan

All of the soybeans produced in Japan are used for the traditional soy-foods such as tofu, natto (fermented soy), miso (fermented soy-paste), and nimame (boiled soybeans). The domestic soybeans have good images among the consumers. However, they are expensive in comparison with the imported ones amounting to only 10-20 % of the total consumption of tofu and natto. In order to increase use of the soybeans domestically produced, we have to improve the food processing quality through breeding activity in addition to stabilizing the supply of production and making the soybeans inexpensive.

[1] Hokkaido University, Sapporo, Japan.

In the last 25 years, various null soybean mutants of the seed proteins have been identified in the world soybean germplasm (Table 1). These could be of value to the food-processing industry. All the null characteristics listed in the table have been shown to be controlled by the respective single recessive genes except for the 7S globulin-null one which is under the control of a single dominant gene. Although protein and oil are the two major components of soybeans, limited research works on the oil component have been done in this country. However, some unique studies on the fatty acid and tocopherol compositions have been recently conducting in some laboratories.

Table 1. Null mutants of the seed enzymes and the storage protein subunits in soybean

Nullmutants	Reference
the lectin-null	Pull et al (1978)
SKTI-null	Orfand Hymowitz (1979)
Lox-1-null	Hildebrand & Hymowitz (1982)
Lox-2-null	Kitamura *et al.* (1983)
Lox-3-null	Kitamura *et al.* (1985)
the 7S a ' subunit-null	Kitamura & Kaizuma (1981)
the 7S a subunit-null	Takahashi *et al.* (1994)
the 7S globulin-null	Hajika *et al.* (1999)
the 11S $A_4A_5B_3$-null	Harada *et al.* (1983)
the 11S Group-I-null	Kaizuma *et al.* (1990)
the 11S A_3B_4-null	Kitamura *et al.* (1993)

Breeding for protein component

Genetic elimination of seed lipoxygenases

So far, two soybean cultivars Ichihime and L-star as well as two promising breeding lines lacking Lox-1, Lox-2 and Lox-3 have been released in Japan. They could cover the most cultivation areas of Japan for the practical production of the lipoxygenase-lacking soybeans except for Hokkaido (Fig. 2). The merits of the lipoxygenase-null soybeans over ordinary soybeans are:

1. Low levels of beany flavor and bitter taste suitable for soymilk, soymilk related products and light-plain tofu.
2. Low levels of lipoxygenase-induced oxidative deterioration of soy oil and protein which could enhance storage stability of soybeans.
3. Low levels of hydroperoxides which could maintain vitamin E and sulfhydryl contents in soy homogenate high.

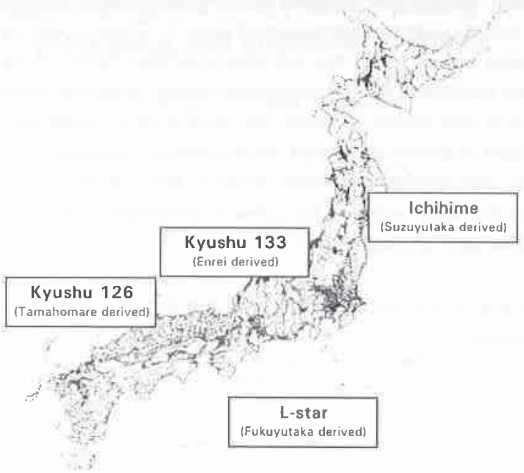

Figure 2. Cultivation region and cultivars/lines of lipoxygenase-lacking soybeans in Japan

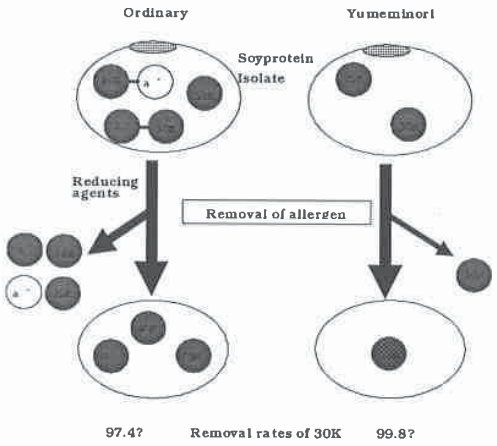

Figure 3. Schematic representation of the effective use of a low allergenic cv. Yumeminori

Genetic modification of storage proteins

A high nutritional and low allergic soybean line Tohoku 124 has been bred using null alleles for α and α´subunits of the 7S globulin. The line showed a high 11S/7S ratio and contained 1.2 times more Cys + Met per unit protein than that of the ordinary cultivars. Further, this could be used as low allergenic soybeans because the α and α´subunits tend to combine with the predominant allergenic protein (*Gly m* Bd 30K) via disulfide bonds and the α subunit itself is one of the major allergenic proteins in soybeans (Fig. 3). From the above mentioned characteristics, Tohoku 124 was registered as a new cultivar Yumeminori in 2001.

Breeding for lipid component

Altering fatty acid composition

In the last two decades, significant progress in modifying oil composition has been made mostly in US. Consequently, some commercial soybean cultivars with improved fatty acid compositions characterized as reduced linolenic and elevated oleic, respectively have been bred and released. Researchers of Saga University, Japan have successfully isolated mutant soybeans with altered fatty acid compositions from about forty thousands M2 plants derived from X-ray irradiated cv. Bay soybeans (Table 2). Among the mutant genotypes, both M23 and M11 with elevated oleic contents are especially valuable to breeding because no such a genotype with the stable heritability of the trait has been identified except the high oleic genotype obtained through genetic engineering.

Table 2. Fatty acid composition of some mutant soybean lines identified in Japan

	Fatty acid (% of total oil)						
Line	Palmitic	Stearic	Oleic	linoleic	Linolenic	Genotype	Character
J3	6.1	3.4	26.5	55.4	8.6	*sop1sop1*	low palmitic
J10	17.2	5	19.8	48.4	9.7	*fap2fap2*	high palmitic
KK7	14.2	5	5	49.5	8	*fapxfapx*	high palmitic
M25	9.5	21.2	21.2	44	9.7	*st2st2*	high stearic
M23	9	4.4	48.6	48.6	8.5	*olol*	high oleic
M11	10.3	4.3	35.8	41.4	8.2	*ol"ol"*	high oleic
KL8	8.9	4.8	24.6	54.8	6.9	*fanxfanx*	low linolenic
M5	9.8	4.5	25.5	55.3	4.9	*fanfan*	low linolenic
Bay	10.6	4.2	25.2	51.7	8.3		normal

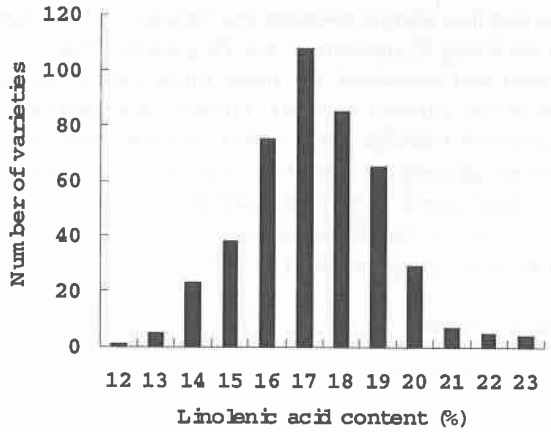

Figure 4. Distribution of linolenic acid content in wild soybean seeds

Recent studies have shown that an adequate intake of the n-3 polyunsaturated fatty acid has many clinical benefits for our health. α-linolenic acid (linolecic acid) is the major n-3 fatty acid. Unpublished data in our laboratory showed that the linolenic acid content of some *Glycine soja* (wild soybean) accessions was higher than that of soybeans. Fatty acid composition was determined from a total of 454 wild soybean accessions by gas chromatograpy. Linolenic acid contents of the wild soybean examined in duplicate ranged from 12 to 23 % of the total fatty acids (Fig. 4), while the average linolenic acid content of 10 soybean varieties was 8.4%. The results confirmed the preliminary data indicating that the wild soybean could be used as a donor parent for breeding to elevate linolenic acid contents of soybeans.

Enhancing α-tocopherol contents

Tocopherols are a class of compounds that function as lipid soluble antioxidants and four different tocopherols, α-, β-, γ -, and δ-tocopherols, are synthesized in nature. Although each of these different forms of tocopherol functions as vitamin E, the relative vitamin E activity associated with each differs significantly. α-tocopherol is the most potent form of vitamin E with β-, γ-, and δ-tocopherols having 40, 10, and 1%, respectively, of the vitamin E activity of α-tocopherol. Among the tocopherols, α-tocopherol is the predominant form in photosynthetic tissues such as leaves and stems. However, in seeds γ- tocopherol frequently predominates. Soybean oil as well as corn, peanut and sesame oils have similarly low ratios of α- to γ- tocopherol, while sunflower and cotton oils have relatively high ratios.

Little is known about varietal differences of tocopherol content in soybeans. The tocopherol composition of soybean varieties was examined from the germplasm collection housed at the National Institute of Agrobiological Sciences in Tsukuba. The tocopherols were separated in a reversed-phased column by using acetonitrile-methanol (75:25) as the mobile phase and detected at 295 nm. So far, the authors have identified cultivars with remarkably high α- tocopherol. The α-tocopherol contents of the cultivars increased two to several times that of an ordinary cultivar based on direct comparison of the respective peak areas of α- tocopherol. This increase has been confirmed by normal phased-HPLC using a fluorescence detector with 290-nm excitation and 325-nm emission in the laboratory of Prof. K.Fujimoto, Faculty of Agriculture, Tohoku University. Further investigations are necessary to evaluate the implication and to clarify the heritability of the high α- tocopherol contents of the cultivars identified in breeding work for processing and utilization.

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Breeding for fatty acid composition of soybean oil

W. R. Fehr and C. F. Curtiss [1]

Abstract

Genetic modification of soybean oil has been achieved by conventional and transgenic methods. Conventional soybean oil has approximately 12% palmitic acid, 4% stearic acid, 27% oleic acid, 50% linoleic acid, and 7% linolenic acid. Genetic variants have been developed that have 3.5% palmitic acid, 40% palmitic acid, 28% stearic acid, 80% oleic acid, and 1% linolenic acid. Cultivars with reduced palmitic acid, elevated oleic acid, or reduced linolenic acid have been grown commercially on a limited number of hectares in the United States. A low-saturate oil with 3.5% palmitic acid content has been sold commercially in the USA since 1997 to reduce saturated fat in the diet. Soybean oil with 80% oleic acid is grown commercially in the USA for use in biolubricants. A 1%-linolenic acid oil currently is under evaluation by the food industry as a possible means of eliminating the need for partial hydrogenation of oil. Soybean genotypes with elevated palmitic acid and stearic acid have not been grown commercially.

Symposium paper

Genetic variation for the five major fatty acids in soybean oil has been developed through hybridization and selection, mutagenesis, and genetic engineering. This paper will address the genetic variation that is available for the five fatty acids, the inheritance of the modifications, the impact of the modifications on agronomic and seed traits, and the commercial adoption of the modifications.

Reduced palmitic acid – 16:0

Chemical mutagenesis has been used to decrease the amount of palmitic acid in soybean oil. The *fap*1 allele in C1726 reduced palmitic acid to about 8.5% (Erickson et al., 1988). The *fap3* allele in A22 reduced palmitic acid to about 7.8% (Schnebly et al., 1994). By combining *fap1* and *fap3*, palmitic acid was reduced to as low as 3.5% (Fehr et al, 1991). A line N79-2077-12 with about 6.0% palmitic acid was crossed with C1726 and produced segregates with about 4% palmitic acid (Wilcox et al., 1994). No transgressive segregation for palmitic acid was observed in the progeny of

[1] Distinguished Professor in Agriculture and Director of the Office of Biotechnology, Iowa State University, 1212 Agronomy Hall, Iowa State University, Ames, IA 50011 wfehr@iastate.edu

the cross of N79-2077-12 with A22 made at Iowa State University, which indicated that N79-2077-12 has a major allele for reduced palmitic acid at the *fap3* locus.

The palmitic acid content of lines with the *fap1 fap3* genotype is influenced by modifying genes (Horejsi et al., 1994). Continuous variation for palmitic acid is obtained from the cross of a parent with reduced palmitic acid to a parent with normal palmitic acid content; therefore, reduced palmitic acid can be considered a quantitative trait in a cultivar development program.

Reduction of palmitic acid to less than 4% has made it possible in the USA to develop cultivars that produce an oil that can be labeled as low in saturated fat. The rules of the Food and Drug Administration (FDA) specify that a food product labeled "low in saturated fat" cannot contain more than 1 g of saturated fat in a 14 g serving, or 7.1% of total saturated fat. When rounding is taken into account, the maximum allowed by the FDA is 1.25 g in a 14 g serving, or 8.9%.

When breeding for a low-saturate oil, breeders generally select for the sum of palmitic acid and stearic acid based on gas chromatography analysis. It is desirable to select individuals that do not have more than 7% of the two fatty acids. This standard takes into account that the other saturated fatty acids not routinely measured in a breeding program; such as 14:0, 20:0, and 22:0; can add 0.5% or more to the total saturated fatty acids. It also is important to consider fluctuations in saturated fat content due to the environment and the increase in saturates caused by mixtures of conventional soybeans with low-saturate grain during commercial production.

The influence of reduced palmitic acid on agronomic and seed traits seems to vary among crosses (Horesji et al., 1994). In some crosses, lines with reduced palmitic acid have yielded as well as normal lines, while in other crosses the best reduced-palmitic acid lines have not yielded as well as the best normal lines.

Low-saturate soybean oil has been sold commercially in the USA since 1997. It also is provided to all schools by the United States Department of Agriculture to reduce the amount of saturated fat in the food served to young people. All of the low-saturate cultivars grown in the USA were developed by Iowa State University or Pioneer Hi-Bred International, Inc..

Elevated palmitic acid – 16:0

Many alleles for elevated palmitic acid have been developed by mutagenesis (Stoltzfus et al., 2000). Mutant lines with one of these alleles have 15 to

17% palmitic acid. Individuals with > 40% palmitic acid have been developed by combining the independent mutations. Soybean oil with elevated palmitic acid has greater oxidative stability than conventional soybean oil (Shen et al., 1997).

There are negative impacts of elevated palmitic acid on seed yield, oil content, and seedling emergence (Hayes et al., 2002). The negative impact on oil content increases as the content of palmitic acid is increased (Stoltzfus et al., 2000). No cultivars with elevated palmitic acid have been grown for commercial production of oil.

Reduced stearic acid – 18:0

No major genes for reduced stearic acid have been reported. Although genetic variation for the trait is small, any increase in the fatty acid is undesirable when breeding low-saturate cultivars. To account for the variation in stearic acid, breeders select for the sum of palmitic acid and stearic acid as evaluated by gas chromatography, instead of selecting only for reduced palmitic acid.

Elevated stearic acid – 18:0

There are multiple alleles at the *Fas* locus that cause an increase in stearic acid (Graef et al., 1985). The fas^a allele elevates stearic acid to about 28%, the greatest increase that has been reported for any of the alleles. Modifying genes also influence stearic acid content.

Elevated stearic acid has a negative impact on seed yield, particularly when it is greater than 15% (Hartmann et al., 1997). Lines with elevated stearic acid also can have reduced germination in some environments. The reduced germination is not consistent among environments, which makes it difficult to determine an appropriate seeding rate.

No cultivars with elevated stearic acid have been grown for commercial production of oil.

Elevated oleic acid – 18:1

Soybean genotypes with mid-oleic acid of 50 to 60% have been developed by conventional breeding. By the use of genetic engineering, oleic acid has been increased to > 80%.

At least three sources of mid-oleic acid are available through conventional breeding. FA22 was developed by chemical mutagenesis at Iowa State University. M23 was developed at Saga University in Japan by X-ray

mutagenesis and has the *ol* allele (Takagi and Rahman, 1996). N98-4445A was developed through hybridization and selection by the USDA-ARS and North Carolina State University (J. W. Burton, personal communication).

Breeding for mid-oleic is difficult because of environmental variation for the trait. There can be differences of greater than 20 percentage units among environments and greater than 10 percentage units among individual seeds and plants of a highly inbred line. No cultivars with mid-oleic acid have been grown for commercial production of oil.

Genetic engineering was used by DuPont to develop a transgene that resulted in 80% oleic acid (Kinney and Knowlton, 1997). Commercial production of 80%-oleic acid oil has been hampered by the lack of approval for the transgenic event by some countries. The selectable marker initially used for transformation was an antibiotic resistance gene. The antibiotic gene is not acceptable to regulatory agencies in some countries. The 80%-oleic acid oil currently is used in biolubricants.

The 80%-oleic transgene has been transformed into soybean without the antibiotic resistance marker. Breeding is underway to develop cultivars with the trait.

Linoleic acid – 18:2

Direct selection for reduced or elevated linoleic acid has not been emphasized as a breeding objective. Selection for elevated oleic acid reduces the amount of linoleic acid because of the strong inverse relationship between the two traits.

Reduced linolenic acid – 18:3

The linolenic acid content of soybean oil has been reduced to about 1% by the combination of the *fan1(A5), fan2, and fan3* alleles (Ross et al., 2000). The three alleles were developed by chemical mutagenesis. Other sources of alleles that reduce linolenic acid also have been reported (Wilcox and Cavins, 1985; Palmer et al., 2003).

Modifying genes have an influence on linolenic acid. There is continuous variation for linolenic acid among the progeny of a cross between a parent with the *fan1(A5) fan2 fan3* genotype and a normal-linolenic acid parent. Reduced linolenic acid can be considered a quantitative trait for cultivar development.

The influence of reduced linolenic acid on agronomic and seed traits seems to vary among crosses (Walker et al., 1998). Lines with reduced linolenic acid have lower yield than normal lines in some crosses, but not in others.

Soybean cultivars developed by Iowa State University and Pioneer Hi-Bred International, Inc., with the *fan1(A5) fan2* genotype were grown commercially in the USA during the mid-1990s. The oil was used in Japan in a blend with canola oil to improve the flavor and odor in frying applications. The demand for the product was not sufficiently large to justify the cost of the 2.5%- linolenic acid oil and commercial production of the oil was discontinued.

In the United States, interest in reduced linolenic acid has become greater with the decision of the Food and Drug Administration to require labeling of food products for their *trans*-fatty acid content beginning Jan. 1, 2006. *Trans*-fatty acids are produced when soybean oil is hydrogenated to improve its stability.

Research on the 1%-linolenic acid soybean oil indicated that its flavor stability is superior to high-oleic acid soybean oil and at least as good as partially hydrogenated soybean oil (White et al., 2003). Extensive testing of the 1%-linolenic acid soybean oil currently is underway by major food companies in the USA.

The first soybean cultivar with 1% linolenic acid was provided by Iowa State University to seed growers for planting in 2003. A second cultivar from Iowa State University will be planted by seed growers in 2004.

Combinations of fatty acids

Development of cultivars for two or more of the fatty acid modifications poses important challenges for the breeder. Two of the most difficult challenges are the number of major and modifying genes that must be combined in an individual and the requirement for yields that are comparable to conventional cultivars. For example, the combination of low saturates and 1% linolenic acid may be desirable nutritionally for lowering saturates and avoiding *trans*-fatty acids by elimination of hydrogenation. When a low saturate/1% linolenic acid parent is crossed to a high-yielding normal parent, there are five major genes and an unknown number of modifying genes segregating in the population. Less than 1% of the progeny will have with the combination of 7% palmitic acid + stearic acid and <1.5 % linolenic acid. To obtain only 50 lines with the desired traits to evaluate for yield and other agronomic traits, at least 5,000 individuals must be evaluated by gas chromatography. It is highly unlikely that one of the 50 individuals would yield as well as the individuals obtained from crosses between high-yielding conventional parents.

Future prospects

The lack of a major market for soybean oil with modified fatty acid content has limited the investment in cultivar development. The modifications that are possible in soybean oil already are available in the oil from other species, particularly canola, sunflower, and maize. These oils put a limit on the amount that can be charged for a modified soybean oil that must be identity preserved. The greatest possibility for modified soybean oils may be when there is increased demand for a fatty acid trait that exceeds the supply available from other species.

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Genetic improvement of soybean saponins

C. Tsukamoto[1]; A. Kikuchi[2]; Y. Takada[3]; Y. Kono[3];
K. Harada[4]; K. Kitamura[5] and K. Okubo[6]

Soybean saponins have been considered as one of major active components contributing to the health benefits of soy products. In addition they affect undesirable soybean flavor. Reducing the saponins possessing undesirable characteristics, and increasing the others with health benefits, would contribute to the improvement of soybean-based foods. The purpose of our research is to manipulate the composition of saponins in soybean seeds by genetic methods. Phenotypes of over 1,000 soybeans were classified into ten saponin types. The variation of saponin composition in soybean seeds is explained by different combinations of 11 alleles of 5 gene loci that control the utilization and the presence of soyasapogenol glycosides as substrates. The function of these genes is variety-specific and organ-specific. The mode of inheritance of saponin types is explained by a combination of co-dominant, dominant and recessive acting genes. Analysis of epistasis of the genes made it possible to predict a part of the biosynthetic pathway for saponins, and to deduce the chemical structures of unidentified saponin components. In this paper, the directed manipulation of soybean saponin composition is discussed.

Chemical structures and distribution of saponins in soybean seeds

The composition of the triterpenoid saponins found in soybean seeds is very complex (Table 1), their direct function is little known (Tsurumi et al., 1992), and their synthesis is poorly understood (Kurosawa et al., 2002). Many soybean saponins have been isolated and characterized (Fenwick et al., 1991; Shiraiwa et al., 1991b and 1991c; Kudou et al., 1992 and 1993; Tsukamoto et al., 1993; Kikuchi et al., 1999). They are divided into two groups, A and DDMP, according to their respective aglycone, soyasapogenol A or DDMP (2,3-dihydro-2,5-dihydroxy-6-methyl- 4*H*-pyran-4-one)- conjugated soyasapogenol B (Fig.1). Group A saponins are only detected in seed hypocotyls.

[1] Department of Agro-Bioscience, Faculty of Agriculture, Iwate University, Morioka, Japan.
[2] National Agricultural Research Center (NARC) for KCS Region, Zentsuji, Kagawa.
[3] NARC for Tohoku Region, Kariwano, Akita
[4] Faculty of Horticulture, Chiba University, Matsudo, Chiba
[5] Graduate School of Agriculture, Hokkaido University, Sapporo.
[6] Graduate School of Agricultural Science, Tohoku University, Sendai. chigen@iwate-u.ac.jp

Table 1. The composition and nomenclature of saponins found in soybean seeds based on the sugar chains attached at the C-3 and C-22 positions of the soyasapogenols, A, B and E[a)]

C-3 position sugars	C-22 position components				
	Soyasapogenol A		B		E[b)]
	AcXyl(1→3)Ara-	AcGlc(1→3)Ara-	DDMP-[c)]	-[d) e)]	-[d)]
Glc(1→2)Gal(1→2)GlcUA-	Aa	Ab	?g	Ba (V)	Bd
Rha(1→2)Gal(1→2)GlcUA-	(Au)	Ac	?g	Bb (I)	Be
Gal(1→2)GlcUA-	Ae	Af	?g	Bb' (III)	Be'
Glc(1→2)Gal(1→2)GlcUA-	Ax	Ad	?a	Bx	Bf
Rha(1→2)Gal(1→2)GlcUA-	(Ay)	(Az)	?a	Bc (II)	Bg
Gal(1→2)GlcUA-	Ag	Ah	?a	Bc' (IV)	Bg'

[a)] Unreported saponins are shown in parenthesis ( ).
[b)] Soyasapogenol E contains a ketone function at the C22 position.
[c)] 2,3-dihydro-2,5-dihydroxy-6-methyl-4*H*-pyran-4-one
[d)] No sugar chain is attached at the C22 position.
[e)] Kudou' s nomenclature systems are shown in parenthesis [ ].

Physiological and taste characteristics of soybean saponins

Group A saponins are responsible for an undesirable bitter and astringent taste (Kitagawa et al., 1988; Taniyama et al., 1988; Okubo et al., 1992). DDMP saponins and their degradation products, or subgroup B and E saponins, have health benefits; e.g. inhibition of the infectivity of the AIDS virus (HIV) (Nakashima et al., 1989), inhibition of the activation of the Epstein-Barr virus early antigen (Konoshima and Kozuka, 1991), antioxidative (Yoshikoshi et al., 1996) and antiviral (Hayashi et al., 1997) activities. Recently a theory of curdling of soymilk has been presented (Ono, 2000; Guo et al., 2002; Ono and Tsukamoto, 2003) and the theory makes possible to reduce undesirable taste of soybean-based foods by processing method (Tsukamoto et al., 2002; Abdullah et al., 2004). However, the reduction by genetic means of saponins possessing undesirable characteristics, and the increase of the others with health benefits, would also contribute greatly to the improvement of soybean-based foods. It has been reported that polymorphisms of saponin composition exist in soybean seeds (Shiraiwa et al., 1990 and 1991a; Tsukamoto et al., 1993), and that some genes controlling the sequence and modification of saponin sugar chains express the polymorphism (Tsukamoto et al., 1992 and 1993; Kikuchi et al., 1999).

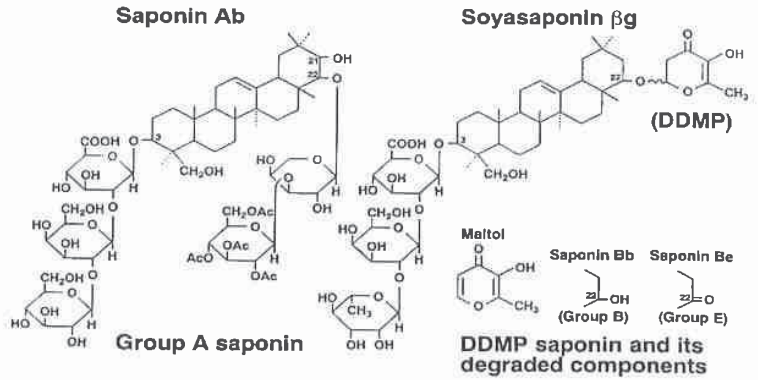

Figure1. Chemical structure of representative saponins, saponin Ab (group A saponin), soyasaponin βg (DDMP saponin) and its degradation products (maltol, saponins Bb, and Be; a part of ring E structure of soyasapogenols), found in soybean seeds.

Mechanism of polymorphism expression in soybean seeds.

The saponin composition of the seed hypocotyl of over 800 cultivated and 300 wild soybeans were examined. Soybean saponins Aa, Ab, Ax, Ad, Ae and Af (group A saponins), and the ag, bg and ba (DDMP saponins) were generally detected. The Ac, Ag, Ah, gg, aa, and ga saponins were minor components, and hardly detected in ordinary soybeans. Soybeans were classified into 10 types according to the saponin composition of the hypocotyl (Table 2). Presence of the DDMP saponins was coincident with it in corresponding subgroup B and E saponins. The AcAf, A0, A0Bc and A0Bc-S types were mutant varieties for the saponin composition (Shiraiwa et al., 1991a; Tsukamoto et al., 1992 and 1993; Kikuchi et al., 1999). Polymorphism of saponin composition in soybean seeds could be explained by combinations of the characteristics of the alleles of the five genes (Table 3, Fig. 2 and Table 4). Recently we have hound two accessions of *G. soja* collection which contain some unidentified saponins. The new saponins seemed to have the same sugar chains as the ordinary saponins at the C-3 position of new soyasapogenol, as the existence and absence of them were controlled by the same genes. The chemical structure of unidentified soyasapogenol is now under investigation.

Table 2. Classification of saponin types of 801 cultivated soybeans (*G. max*) and 330 wild soybean (*G. soja*) accessions according to the saponin composition of the seed hypocotyl[a]

Saponin type	Representative Variety	Group A saponins						DDMP saponin		
		Aa	Ab	Ax	Ad	Ae	Af	αg	βg	βa
Aa	Shirosennari	+	-	-	-	+	-	+	+	-
Ab	Suzuyutaka	-	+	-	-	-	+	+	+	-
AcAf	Mikuriyaao	-	-	-	-	-	+	-	+	-
A0	A-b(F)	-	-	-	-	-	-	+	+	-
AaAb[b]		+	+	-	-	+	+	+	+	-
AaBc	Norin No.3	+	-	+	-	+	-	+	+	+
AbBc	Shin No.4	-	+	-	+	-	+	+	+	+
A0Bc	Gs-125[c]	-	-	-	-	-	-	+	+	+
A0Bc-S	B01082[c]	-	-	-	-	-	-	+	+	+
AaAbBc[b]		+	+	+	+	+	+	+	+	+

[a] +, detected; -, not detected.
[b] The AaAb and AaAbBc types are heterogeneous on the *Sg-1* locus, thus they are supposed to be crossed with the other line.
[c] *G. soja* collections

Table 3. Proposed characteristics of gene products controlling polymorphism of soybean saponins

	Gene	Characteristics of gene products	Strain[a]
		C-22 position of soyasapogenol A	
1.	*Sg-1*[a]	β(1→3)xylosylation of arabinose of C-22	Shirosennari, M & S
	Sg-1[b]	β(1→3)glucosylation of arabinose of C-22	Suzuyutaka, M & S
	sg-1[a]	absent	A-b(F)
2.	*Sg-2*	acetylation of the hydroxyl groups of glucosyl and xylosyl moieties attached to arabinose of C-22	M & S
	sg-2	absent	Gs-125 (*G. soja*)
		C-3 position of soyasapogenols	
3.	*Sg-3*	β(1→2)glucosylation of galactosyl and arabinosyl moieties attached to GlcUA of C-3	M & S
	sg-3	absent	Mikuriyaao
4.	*Sg-4*	α(1→2)arabinosylation of GlcUA of C-3	Norin 4, Shin 4 & S
	sg-4	absent	M & S
		Soyasapogenol A	
5.	*Sg-5*	soyasapogenol A in seed hypocotyl	M & S
	sg-5	soyasapogenol A in seed hypocotyl, absent	B01082 (*G. soja*)

[a] M and S indicate that gene occurs in many *G. max* and *G. soja*, respectively.

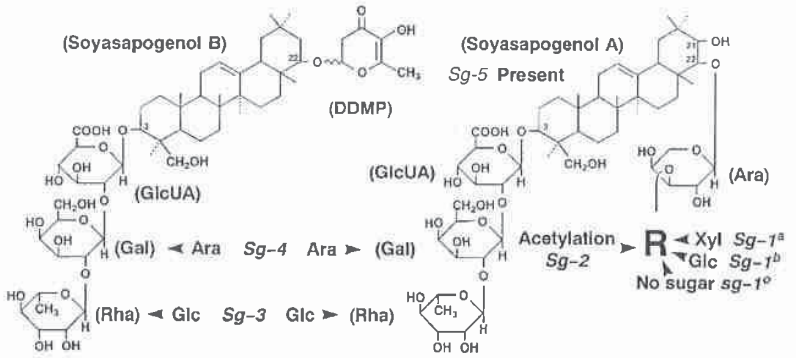

Figure 2. An image of the characteristics of genes controlling elongation and modification of soybean saponin sugar chain attached at the C-3 and C-22 position of the soyasapogenols (*Sg-1, Sg-2, Sg-3*, and *Sg-4*), and the presence of soyasapogenol A (*Sg-5*).

Table 4. Relationship between the phenotype and genotype based on the polymorphism of saponin composition in the hypocotyl of soybean seeds.

Phenotype	Genotype				
Aa	*Sg-1^a /Sg-1^a*	*Sg-2 /Sg-2*	*Sg-3 /Sg-3*	***sg-4 /sg-4***	*Sg-5 /Sg-5*
Ab	*Sg-1^b /Sg-1^b*	*Sg-2 /Sg-2*	*Sg-3 /Sg-3*	***sg-4 /sg-4***	*Sg-5 /Sg-5*
AcAf	*Sg-1^b /Sg-1^b*	*Sg-2 /Sg-2*	***sg-3 /sg-3***	***sg-4 /sg-4***	*Sg-5 /Sg-5*
A0	***sg-1^c /sg-1^c***	*Sg-2 /Sg-2*	*Sg-3 /Sg-3*	***sg-4 /sg-4***	*Sg-5 /Sg-5*
AaAb"	*Sg-1" /Sg-1"*	*Sg-2 / -*	*Sg-3 / -*	***sg-4 /sg-4***	*Sg-5 / -*
AaBc	*Sg-1^a /Sg-1^a*	*Sg-2 /Sg-2*	*Sg-3 /Sg-3*	*Sg-4 / Sg-4*	*Sg-5 /Sg-5*
AbBc	*Sg-1^b /Sg-1^b*	*Sg-2 /Sg-2*	*Sg-3 /Sg-3*	*Sg-4 / Sg-4*	*Sg-5 /Sg-5*
A0Bc	*Sg-1^b /Sg-1^b*	***sg-2 /sg-2***	*Sg-3 /Sg-3*	*Sg-4 / Sg-4*	*Sg-5 /Sg-5*
A0Bc-S	*Sg-1^a /Sg-1^a*	*Sg-2 /Sg-2*	*Sg-3 /Sg-3*	*Sg-4 / Sg-4*	***sg-5 /sg-5***
AaAbBc[a]	*Sg-1^a /Sg-1^b*	*Sg-2 / -*	*Sg-3 / -*	*Sg-4 / -*	*Sg-5 / -*

[a] The AaAb and AaAbBc types are heterogeneous on the *Sg-1* locus.

Epistasis of the genes

If we can obtain information on epistasis of the genes controlling saponin biosynthesis, estimation of phenotype and biosynthetic pathway of soybean saponins become much easier. Mikuriyaao (AcAf type) does not contain saponin Ab and accumulate saponin Af. This mutant carries dominant genes on the *Sg-1^b* and *Sg-2* loci and recessive genes on the *Sg-3* locus. Gs-125 (A0Bc type) does not contain saponin Ab and accumulate deacetylsaponin Af. It carries dominant genes on the *Sg-1^b* and *Sg-3* loci and recessive genes on the *Sg-2* locus. The F_1 seeds of the cross between them can produce saponin Ab by complementation of each characteristics. By a series of these experiments, we deduced that the *Sg-1* genes are epistatic to the

Sg-2, which is epistatic to the *Sg-3* in the Gs-125, A0Bc type mutant. (Tsukamoto et al., 1992). However, there was no epistasis between the *Sg-1* genes and the *Sg-3* or *Sg-4* gene in A-b(F), A0 type mutant (Kikuchi et al., 1999). The *Sg-4* was epistatic to the *Sg-3* in all seeds examined.

The possibility of directed manipulation of soybean saponin composition.

We analyzed more than 800 cultivars and 300 wild soybean accessions, but no mutant was identified for the a(1–›2)Rha and b(1–›2)Gal linkage at the C-3 position of soyasapogenols. Soyasaponin bg, possessing a Rha(1–›2)Gal(1–›2)GlcUA- sugar chain at the C-3 position, was detected widely in many kinds of legumes (Tsukamoto et al., 1994). This saponin was presumed to be a phytochrome killer in *Pisum sativum* (Tsurumi et al., 1992). This specific sugar chain sequence may be conserved in legumes to carry out this physiological activity. The soyasapogenol A-deficient mutant (*G. soja* B01082) showed normal growth during greenhouse and field cultivation, and no negative agronomical or biological observations were noted. Therefore, it is possible that group A saponins do not play a significant physiological role in soybean. The data presented in this report provide an opportunity to improve the saponin composition of soybean. The directed manipulation of soybean saponin composition is a possibility for the future. Studies are under way that will permit us to use these characteristics in breeding programs designed to decrease undesirable tastes and increase health benefits in soybeans.

Materials and methods

Polymorphism analysis of saponin components. After lyophilization of the seeds, the hypocotyls and cotyledons were sampled from each variety and accession. The tissues were extracted for one week (group A, B and E saponins) or 2 hr (DDMP saponins) at room temperature with either 20 volume (hypocotyls) or 5 volume (cotyledons) of aqueous 70% ethanol (EtOH) containing 0.1% acetic acid (AcOH). The extract was used directly for HPLC analysis on an ODS column (YMC Packed Column AM-303 S-5 120A ODS, f4.6x250mm). For analyzing the group A and B saponin composition, the solvent system used was acetonitrile (MeCN)- 2-propanol-H_2O-AcOH (340:60:599:1). The group B and E saponins were separated with methanol-H_2O-AcOH (700:299:1), and the DDMP saponins were separated with MeCN-H_2O-AcOH (420:579:1). The solvent flow rate was 1.0 ml /min. UV absorption was measured at 205 nm for the group A, B and E saponins, and at 292 nm for DDMP saponins.

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Breeding soybeans for the soyfood market: specific traits and strategies for selection

P. Chen[1] and G. R. Buss [2]

Introduction

The expanding soybean-based food market in Japan and the U. S. has generated considerable interest among American soybean producers in recent years. The global demand for food-quality soybeans for direct consumption has been increasing due to proven and publicized health benefits and nutritional values from soy products. Processed soybean foods consist of a variety of products including tofu (bean curd), yuba (soy protein extracts from soy milk), kinako (roasted soy powder), nimane (cooked beans), bean sprouts, and soymilk as non-fermented food products and soy sauce, miso, and natto as fermented food products. Among these soy-based food products, tofu, soy sauce, miso, bean sprouts, and natto are predominant.

Most commercial soybean varieties are not desirable for soyfood production due to their limitations in physical, chemical, or nutritional properties. Very few suitable varieties are commercially available for the soyfood market, and most yield 10 to 20% less than the regular commercial varieties. Thus, farmers are offered premiums as incentives to produce such specialty varieties for the soyfood market. High-yielding potential would make the specialty soybeans more profitable to produce, and improved quality will make the food grade soybeans more marketable. During the last several years, research on specialty soybeans has focused on continuous improvement on yield and seed composition. However, the information on specific quality attributes for soyfood is limited, which hinders breeders in developing quality products that meet the demands of end users. The objectives of this paper are to address the specialty seed quality traits that are important for soyfood production, to discuss methods for testing physical and chemical properties, and to outline selection strategies for specific quality traits.

Selection for agronomic traits such as yield, maturity, plant height, adaptability, standability, and disease and shattering resistance can best be accomplished via conventional methods already in use in the soybean breeding program. All these traits are evaluated through a series of replicated

[1] University of Arkansas, Fayetteville, AR 72701
[2] Virginia Tech, Blacksburg, VA 24061, U.S.A. (P. CHEN, Phone: 479-575-7564; Fax: 479-575-7465;
Email: pchen@uark.edu

field tests that are usually carried out at multiple locations for multiple years before a promising line is released for commercialization. The physical appearance of the seeds is also important for marketing. Tofu and natto makers prefer round seeds with yellow cotyledons, yellow seed coats, and yellow hilum. Seed coat mottling, purple seed stain, splits, insect damage, cracked seed coats, and other physical defects are objectionale. Seeds with strong seedcoat and shining luster are generally desirable. Visual seed quality selections are based largely on freedom from defects such as cracked, mottled, or discolored seedcoats, bleeding hilum, purple stain, shriveled or moldy seed, and any other visible defects associated with breeding lines.

There are several physical and chemical factors that affect the specialty soybean marketing and soyfood quality, such as seed size, seed uniformity, protein content, oil content, sugar content, calcium content, fiber content, ash content, water absorption capacity, and texture. Among these seed quality characteristics, protein, sugar, calcium, water absorption, and hardness are most important for the soyfood products.

The seed size of food-quality soybeans is most important. For example, the Japanese fermented food product, natto, utilizes small-seeded soybeans (usually half the size of the regular soybeans, 7-10g/100, 4.8-5.6 mm in diameter), while tofu production utilizes large-seeded soybeans (usually double the size of regular soybeans, 25-30g/100, 7.0-8.5 mm in diameter). Soybeans for natto production are also suitable for bean sprouting, and tofu beans are also used for miso, soymilk, and soynuts. In addition to average seed size, uniformity of seed size for a given variety is of economic significance to the producer. However, two cultivars with the same average seed size might differ significantly in the proportion of seeds that fall within the desired range. The one with the higher proportion within that range would produce the most marketable product and would be more profitable to grow because less screening is needed and more seeds are acceptable. Thus, selection for seed size uniformity in a breeding program is equally as important as average seed size.

Average seed size and seed size uniformity can be evaluated and selected throughout the breeding process. Desired seed size can be achieved by selecting average seed size in grams per 100 or screening seeds through a specific sieve. Average seed size has fairly high heritability, so selection can be done effectively in the early stages of a breeding process to eliminate the less desired sizes and concentrate on more desirable ones in the segregating population. Then, the derived pure lines can be further evaluated for seed size uniformity, which is measured by the percentage of seed within a specific size range using a series of different size sieves. This

procedure is normally used after lines have been selected for average seed size as well as for important agronomic traits. Although the 100-seed weight is not a direct measure of seed diameter or seed dimensions (length, width, and thickness), seeds between 12/64-inch and 14/64-inch in diameter usually weigh seven to nine grams per 100. Thus, 100-seed weight can be used in making selections for proper seed size.

Yellow hila are preferred for natto and tofu, but light buff is usually acceptable. Hilum color exhibits qualitative inheritance so that lines with unacceptable hila can be discarded in the early stage before any field evaluations are conducted. Breeding for seed size is further complicated by the low yield potential and short statures of breeding lines with extreme seed size, which make production of such cultivars very challenging. The large- and small-seeded cultivars usually yield 10 to 20% less than regular cultivars, and growers are paid $1.50 to $3.00/bu premium for producing such cultivars. Therefore, high seed-yield is critical to maintaining economic production of the specialty soybeans. Every bushel of extra yield would, not only produce extra net income from seed sales, but also gain the additional premium from contracted production of a specific variety.

High protein and low oil are desirable for tofu and natto production. High protein adds nutrition value to the soyfood and also contributes to higher yield of tofu. Nitrogen solubility index (NSI), which measures the ratio of water soluble protein vs. total protein, is also important for tofu makers. It is believed that higher NSI (>70%) increases tofu yield and improves tofu texture. The significant genetic variations in protein content (33.1% to 55.9%) and oil content (13.6% to 23.6%) among soybean germplasm offer breeders opportunities to modify the ratios of protein/oil and protein/sugar contents in soybean seeds. Protein and oil analyses are fairly routine and are readily run by near-infrared reflectance (NIR). The protein and oil contents are highly heritable and selection can be done at any stage of the breeding program. However, maximum efficiency can be achieved by beginning protein and oil evaluations with the first year yield test of the preliminary pure lines. Advanced lines can also be evaluated to confirm the protein and oil contents. The high protein content is generally associated with low yield potential, which makes breeding for high yield and high protein very challenging. However, high yield can be achieved by selection for moderately high protein content (43-45%). The negative correlation between protein and oil contents is advantageous to breeders for selecting high protein and low oil lines.

The carbohydrate content of the soybean seed has been shown to be important in making natto and tofu. Sugars are one of the major components

of the carbohydrates in mature soybean seeds. Sugars, especially sucrose, are the major source of energy for the fermentation and contribute to the sweetness of soymilk and tofu. Increased total sugar and sucrose contents are important to achieve the proper rate of fermentation during the natto-making process and to improve taste and flavor of natto and tofu. Total sugar content has been reported to range from 4.1% to 15.1% of the dry weight in soybean germplasm. Sucrose is the primary component, comprising about 60% of the total sugars. The amount of sucrose in soybean seeds ranges from 1.5% to 10.2% of total dry matter. Recently, germplasm with even higher (13.6%) sucrose content have been identified. The other two sugar components of soybean seeds are stachyose and raffinose, which are not readily digestible and are considered to be a cause of flatulence associated with soybean foods. Stachyose and raffinose contents among soybean germplasm range from 1.4% to 6.7%, and 0.1% to 2.1% of total dry matter, respectively. Thus, there is considerable potential to alter sugar content through the breeding process. Sugar contents can be determined by a High Performance Liquid Chromatography (HPLC) system or enzymatic techniques. Sugar contents have moderately high heritability and selection for high sugar is generally effective. One complication, however, is that there is a positive correlation between sucrose content and oil content, a negative correlation between sucrose content and protein content, and a negative correlation between protein content and yield. Since high protein (above 40%) and low oil (below 20%) are desirable for natto and tofu, care must be exercised in a breeding program to make sure that selection for high sugar does not also select for low protein and high oil. It would appear that some compromise can be reached. The consequences in a breeding program are that more diverse germplasm needs to be used for crossing and that more lines need to be evaluated to find some with the optimal content of all components.

Another characteristic of small-seeded soybeans that could be improved is the texture consistency after processing for natto. Some of the natto soybean varieties receive complaints from the Japanese importers and natto manufacturers because they make firmer and harder natto than desired. In addition, seeds with harder texture tend to produce undesirable ammonia gas and require more soaking and cooking time in the process of making natto, which results in undesirable darker natto products and higher cost of production. The desired natto should have a texture consistency similar to that of the cooked rice with which it is usually served. The seed hardness of soybean is also associated with water absorption and cookability. The Japanese natto makers believe that the hardness of natto is due to the relatively high calcium content of soybean seeds that interferes with the fermentation process. Therefore, it is possible to make significant

improvements in processed seed texture through breeding for lower calcium content. Calcium content in soybean seeds varies widely among varieties (0.19% to 0.52%) but does not vary greatly in different environments. Thus, selection for lower calcium content should be relatively easy if adequate genetic variation is present in a population. The calcium content is readily evaluated in the laboratory using a wet chemistry procedure. High water absorption capacity is also desired by natto manufacturers and is found to be associated with softness of soaked and steamed seeds and with natto yield. The water absorption capacity can be readily measured by the swell ratio which is determined by the seed weight or water volume changes before and after soaking. Seed hardness can be measured by a food texture analyzer or a force testing device. Seed hardness, calcium, and sugar analyses are time-consuming and, therefore, should start when pure lines are obtained.

Ash and fiber content are usually reported as quality factors of specialty soybeans, but the value of these traits is not completely clear. It seems likely that low fiber content might be related to higher tofu yield, but no published evidence for this relationship has been found. It is also believed that the fiber content affects the firmness of tofu, but no hard data have defined optimum range of fiber content. Research is needed to clearly answer the questions associated with ash and fiber contents.

One other trait that would be valuable for the soyfood beans is lipoxygenase-free. Normal soybean seeds contain three lipoxygenase isozymes, L-1, L-2, and L-3, which are controlled by three separate genes, Lx1, Lx2, and Lx3. These isozymes are responsible for the generation of grassy-beany and bitter flavor, which hinder wider utilization of soybean as food ingredients, especially in the U.S. market. The absence of each enzyme is controlled by the null alleles lx1, lx2, and lx3. Genetic elimination of lipoxygenase from soybean seeds has attracted attention as a useful approach to reduce undesirable flavors of the soyfood products. Seven combinations of the lipoxygenase nulls are possible and have been created, including three single-null, three double-null, and one triple-null genotypes. The triple-null genotype leads to no detectable level of the lipoxygenase proteins in mature soybean seeds with a resultant absence of the grassy and beany off-flavor in soyfood products. The absence of the three seed lipoxygenase isozymes does not influence the performance of soybean for agronomic traits and seed composition. The use of lipoxygenase-free soybean has been shown to improve the flavor of tofu and soymilk but has given no marked improvement in the flavor or quality of oil. Lipoxygenase-free small-seeded soybeans are also desirable for bean sprouts. However, the value of low lipoxygenase in natto production is not known. The presence

or absence of three lipoxygenase isozymes can be readily and accurately determined by the sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), by absorbance changes at 234 nm and 280 nm, by polarographic and immunological techniques, or by colorimetric methods.

Recent advances in biotechnology, in particular the development of improved molecular marker technology, have made possible the genetic dissection and characterization of many quantitatively inherited seed quality traits in soybean. Diers et al. (1992) utilized restriction fragment length polymorphisms (RFLP) to identify several molecular markers that were significantly associated with major quantitative trait loci (QTL) for seed protein and oil in an interspecific (*G. max* x *G. soja*) soybean population. More recently, several other research groups have used molecular markers to identify several genomic regions significantly associated with seed protein and oil in several intraspecific (*G. max* x *G. max*) soybean populations (Brummer et al., 1997; Mian et al., 1996). Maughan et al. (1996) and Mian et al. (1996) have identified and characterized several putative QTLs that explained significant levels of phenotypic variation for seed weight. Maughan et al. (2000) also identified seventeen marker loci, mapping to seven different genomic regions, which were significantly associated with sucrose variation in a *G. max* x *G. soja* population. Ongoing research on the inheritance and underlying genetic control of seed hardness, sugar content, and calcium content will bring us closer to a better understanding of the development of the major constituents in soybean seeds and may help breeders accelerate the development of specialty varieties for food quality purposes.

Finally, the natto and tofu quality tests are particularly important in determining if a variety is suitable and has potential for commercialization. Such tests should be conducted in collaboration with marketing groups, distributors, or manufacturers. The sensory test results and other information such as product color, texture, and shelf life are helpful to breeders in correlating natto and tofu quality with seed quality data such as protein, oil, sugar, calcium, water absorption, and hardness.

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The strategy for sweetness increase of vegetable soybeans: maltose, another sugar in boiled seeds

R. Masuda[1]

Abstract

Edamame taste is basically based on sweetness, being sucrose the main component. However, its sweetness may be enhanced during the process of boiling due to the generation of another sugar, maltose. Maltose exhibit 0.4 times the sweetness strength of sucrose, and has more refreshing aftertaste. Soybean starch granule while elevating temperature under the presence of water undergoes an irreversible swelling and disruption of ordered structures (gelatinization), and a followed β-amylase digestion producing maltose. Some early-maturing edamame varieties produced lower maltose than others. DSC analysis revealed that in these seeds high temperature (between 60 and 70°C) of the starch gelatinization causes a reduction in maltose production. The enzyme fraction of homogenized seeds showed no difference in β-amylase activity between low and high production seeds in maltose. The linear glucosyl chain analysis in amylopectin showed that the starches with low gelatinization peak temperature (Tp) contained shorter chain of DP 6-11 than other starches with higher Tp whereas middle chain of DP 13-36 in higher Tp starch increased more. Breeding programs for modified seed starches with gelatinization at lower temperature in the early-maturing groups may introduce sweeter edamame bred lines.

Introduction

The most important taste of boiled vegetable soybean, 'edamame', is sweetness.[1)] The components responsible for sweetness of raw vegetable soybean seeds are sugars, mainly sucrose and free alanine. Results of carbohydrate accumulation in the developing seeds of 54 cultivars revealed that the average of maximum starch is 3.2g/100g fresh weight (FW), with Akagishita (5.0g /100gFW) as the highest among the vegetable soybean type.[2)] During the course of screening high sucrose seeds at the different edamame stages among various domestic cultivars in Japan, we found that some immature soybean seeds with early maturity had produced very low quantity of maltose after boiling, while that of the others contained higher amount.[3)] In this presentation, we examined the differences in gelatinization temperature and amylopectin of starches in the seeds of some early – and late – maturing edamame types of soybean.

[1] National Institute of Crop Science, NARO, Tsukuba, 305-8518 Japan E-mail: masuda@naro.affrc.go.jp

Materials and methods

Soybeans were grown at the fields of Natl. Agric. Research Center (Tsukuba, Japan) using standard cultural practice. Five to ten plants with pods were harvested from early green stages to mature stages of developing seeds. The group with largest number of hulled seeds separated at 0.05 g each was selected for the sample, submerged into liquid nitrogen, and kept at 80°C until analysis. Maltose content was determined by a modified procedure of previous method.[4] Frozen cotyledons were homogenized in a mortar with 10-fold weight of acetate-Na Buffer (pH 5.4). β-amylase activity of the homogenates was measured based on their maltose production. Starch extraction from homogenates and purification were done according to the method of Adkins & Greenwood[5] with some modifications. Differential scanning calorimetry (DSC) of purified starches was measured at the heating rate of 1.0 °C/min. Chain-length analysis of amylopectin was done according to the method of Nagamine & Komae.[6]

Results

Screening of maltose production in immature seeds

Raw seeds of both Hyoukei-kuro 3 (53 days after flowering (DAF)) and Tamasudare (27DAF), at the suitable stages for 'edamame', contained very low maltose, but after pod boiling these edamame seeds showed an increased peak area of maltose. The former seeds produced 0.91% maltose while the latter formed 0.14%. Maltose formation after boiling was also determined in developing soybean seeds of the frozen stock of other cultivars. Developing seeds produced 0.3-1.5% maltose by boiling for 90sec, but in some early-maturing cultivars the seeds produced a fewer amount of maltose, especially Takihime.

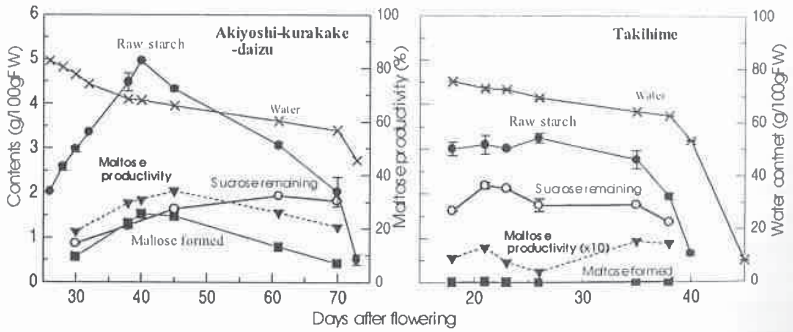

Figure 1. Developmental changes of starch content in raw seeds, and formed maltose and remaining sucrose contents in boiled seeds of Akiyoshi-kurakake e-daizu and Takihime

Maltose production was directly proportional to starch content in the raw seeds. Interestingly, edamame cultivars harvested in summer, containing high sucrose in the seeds, showed low maltose production after boiling, though the starch content in the seeds was enough for a higher maltose production.

Low maltose production of Takihime boiled seeds

We examined low-maltose forming seeds whether the starch was hard to degrade or the β-amylase activity was weak. Homogenates of both Takihime and high-maltose forming Asomusume seeds were prepared, and then their supernatant and precipitate separated by centrifugation. Washed supernatants did not include starch, and washed precipitate fractions showed no β-amylase activity. Maltose productions during heating were determined which was a combination between each of the supernatant and the precipitation. Reconstituted homogenates with Takihime precipitation formed 1/10 maltose of those with Asomusume precipitation. These results mean that maltose formation is mainly influenced by the starch characteristics, regardless of β-amylase activity (Fig. 2).

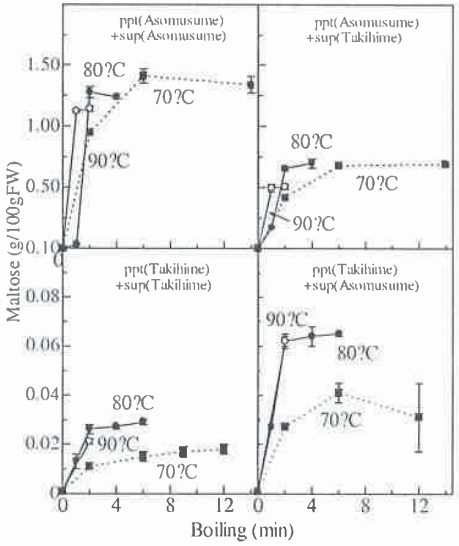

Figure 2. Reconstitution from supernatant an precipitation of homogenate between Asomusume and Takihime

β-Amylase activities in homogenates of Takihime and Asomusume seeds were higher than those in seeds of late-maturing groups. The heat resistance of β-amylase in soybean seeds was extremely higher for few minutes than those in sweet potato tubers. There was no difference in heat resistance of β-amylase activities in crude enzyme extracts between Takihime and Asomusume seeds. Likewise experiments of potato soluble starch added to the seed homogenates showed no difference in starch degradation between the early- and late-maturing soybeans. These results indicated that properties of β-amylase in Takihime seeds were equivalent to those in late-maturing soybean seeds.

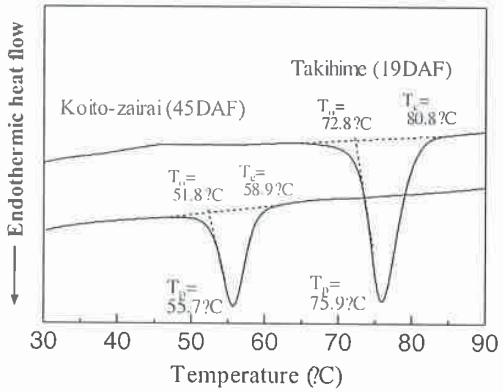

Figure 3. DSC measurement of seed starches

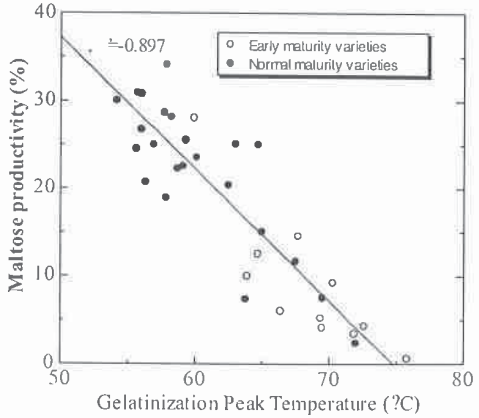

Figure 4. Relation ship between maltose productivity in teh boiled seeds and gelatination temperature of purified starch from the seeds

Properties of extracted soybean starch:

Figure 3 showed heating DSC curves of partially purified starches from seeds with low and high maltose production. The gelatinization peak temperature (Tp) of two starches was different, but in the same cultivar was not changed during the seed development. At the suitable stages for edamame, relationship between maltose productivity (ratio; maltose formation / initial starch content) in boiled seeds and Tp of purified starch from the raw seeds indicated a linear correlation (r=-0.897). Seed starch of various early-maturing cultivars exhibited higher gelatinization temperature (Figure 4).

Starch samples were digested with isoamylase and the resulting linear glucosyl chains were analyzed using high-performance anion exchange chromatography. Result showed that Koito-zairai starch with low Tp contained shorter chain of DP 6-11 than other starches with higher Tp whereas middle chain of DP 13-36 in Takihime starch increased more (Figure 5).

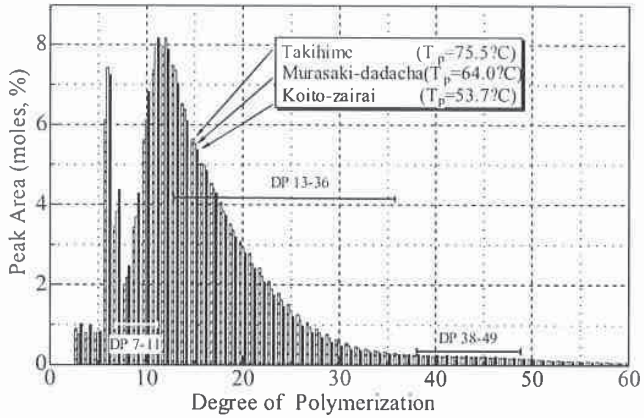

Figure 5. Chain-length profiles of seed starches with different gelatinization temperature

Discussion

Maltose is known to exhibit 0.4 times the sweetness strength of sucrose, and it has more refreshing aftertaste. Boiled immature seeds containing maltose in addition to sucrose are more delicious than seeds with no or small quantity of maltose. Maltose formation during seed boiling could be a new clue for increasing the sweetness of edamame seeds, since maltose production is independent to the storage condition of immature seeds after harvest, while sucrose degradation can not be avoided under practical

storage conditions. To produce large quantity of maltose in edamame seeds in new breeding program, properties of synthesis amylopectin structure with lower gelatinization temperature can be introduced to seeds of early-maturing types.

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Screening and breeding soybeans for isoflavone content and composition

J. Daydé; M. Berger and V. Théodorou[1]

Abstract

In order to certify isoflavone content and composition in dietary supplements or soyfoods, their control in soybean seed and/or germ is needed. Experiments conducted in France and in the USA show that water supply and its interaction with low temperatures enhance isoflavone contents. As far as genotype is responsible of a wide range of variations, isoflavone content and composition in germ or seed could be predicted by crop management, combined with approriate choice of cultivars within a given location. The observed high heritabilities suggest that isoflavone composition is controlled by a few number of major genes, allowing soybean genetic improvement for both isoflavone content and composition.

Introduction

An increasing number of investigations are dealing with soybean isoflavones, mainly concerning human health benefits. Both markets of soyfoods or soy based dietary supplements are consequently increasing. Isoflavone content and composition of these products are directly related to those found in the raw materials used for their process (Table 1). These raw materials can be obtained from whole seed, meal or dehulling by-products containing up to 99% of germs.

Table 1. General variation of isoflavone content and composition in different raw materials

	Content range ($\mu g.g^{-1}$)	Isoflavone type(%)			Isoflavone conjugates(%)			
		Genist :	Daidz :	Glycit	Malonyl :	Acetyl :	Glucosyl :	Aglycone
Seed	1000 - 8000	35-60	35-60	6-12	65-80	0-1	15-25	0.5-1.5
Germ	15000-35000	10-15	35-55	35-55	65-80	0-1	15-25	0.5-1.5
Cotyl.	1000 - 8000	40-70	40-60	0-1	65-80	0-1	15-25	0.5-1.5
Meal	1000 - 8000	35-60	35-60	6-12	5-30	8-30	30-60	1-5

The main factors responsible for the variation of isoflavone content and composition are genotype and environmental parameters during seed maturation. Therefore, isoflavones in soyfoods and even more dietary supplements are subjected to uncontrolled variations. Indeed, compositions

[1] Agrophysiology and Micronutriments Laboratory UMR INRA 1054, Ecole Supérieure d'Agriculture de Purpan, Toulouse, France jean.dayde@esa-purpan.fr

are generally not announced and contents are rarely expressed in aglycone equivalent (Table 2).

Table 2. Comparison of 3 dietary supplements (Daydé et al., 2002)

	% of claimed content	Isoflavone type(%)			Isoflavone conjugates(%)			
		Genist	Daidz	Glycit	Malonyl	Acetyl	Glucosyl	Aglycone
A	50.5	67.0	30.3	2.7	67	0-1	30	3
B	70.0	46.3	53.4	0.4	0	0	1	99
C	135.0	16.5	55.9	27.6	0	10	84	6

In order to certify isoflavone contents and compositions both should firstly need to be controlled in seed and germ separately, within locations and years of production by choosing suitable genotypes and crop managements. Therefore, we focus on screening and breeding soybean for isoflavones by segregating cotyledon *vs* germ variations.

Screening soybeans for isoflavone content and composition by managing genetic and environmental factors of variation

The main factor of variation is obviously the seed fraction (see above) : germ represents 2 to 3 % of the seed, but 8 to 20% of total isoflavone content in the seed. Generally, germ is removed during the dehulling process (crushing for oil extraction or soyfood processing). The dietary supplement compositions reflect the origin of raw material (see Table 2 : supplement A is issued from whole seed, B from meal, C from dehulling by-product).

Environmetal effects and crop management as well as location effects can widely modify the total isoflavone content (Daydé *et al.*, 2002). The complex action of climate and soil can also explain variations, as can temperature and watering at late maturation stage (Hoeck *et al.*, 2000).

Table 3. Effect of irrigation during R5-R6 stage on germ and cotyledon isoflavone content and composition in field and greenhouse experiments in Urbana-Champaign, Illinois, USA (Field: 10 cultivars and 2 replications; Greenhouse: 2 cultivars (Imari and Queen) and 4 replications)

		Germ (means ± SE)		Cotyledon (means ± SE)	
		Content ($\mu g.g^{-1}$)	Glycitein %	Content ($\mu g.g^{-1}$)	Daidzein %
Field	Irrigated	27142 ± 522	37.5 ± 0.3	4147 ± 150	34.1 ± 0.2
	Non-Irrigated	23301 ± 483	36.7 ± 0.3	3265 ± 139	33.9 ± 0.2
	F	29.0**	4.4 NS	18.7**	4.8 *
Green-house	Irrigated	21446 ± 587	41.9 ± 0.4	2948 ± 132	35.8 ± 0.7
	Drought	19758 ± 604	41.4 ± 0.4	2202 ± 136	34.9 ± 0.7
	F	4.1 NS	0.8 NS	15.5**	0.8 NS

NS, *, **: not significant, significant at p = 5% and p = 1%, respectively; SE: standard error of the mean

Irrigation increases significantly isoflavone content (Table 3) and interact with temperature (Figure 1). Indeed low temperatures coupled to late watering (rain or irrigation) increase isoflavone contents.

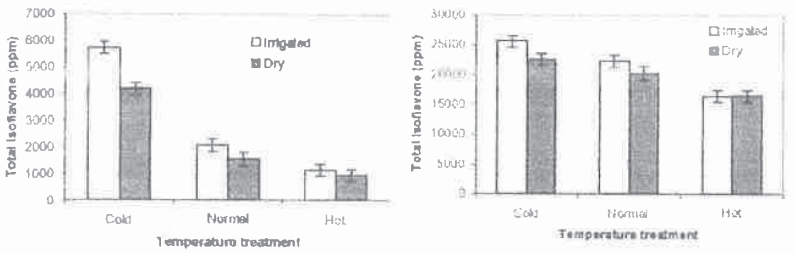

Figure 1. Effect of irrigation and temperature on isoflavone content (a) cotyledon and (b) germ in a controlled experiment in greenhouse (see Table 3) (means ± SE of 2 cultivars : Imari and Queen; 4 replications)

The effects of temperature and water supply on isoflavone content are more pronounced in the cotyledon than in the germ fraction. Nevertheless the isoflavone content in the germ fraction is never below 15000 $\mu g.g^{-1}$. Those factors influence only slightly isoflavone composition.

Genotype is responsible of a wide range of variations: besides influencing isoflavone content, it is the only factor affecting significantly isoflavone composition. Indeed, genetic determination of high or low isoflavone contents are expressed whatever the environmental and crop management factors effects are. Genetic differences concerning isoflavone content are significant but with lower relative amplitude in germs *vs.* cotyledons, as shown in a study comparing 162 cultivars (Table 4). As far as composition is concerned, we can observe in cotyledon that some genotypes overexpress genistein (up to 60%) and others daidzein (up to 60%). In germ, this variability is observed for daidzein *vs*. glycitein with up to 70% glycitein in some cultivars. Nevertheless, there is no correlation between germ and cotyledon profiles (*i.e.* percent daidzein in germ and in cotyledon are not correlated: r = - 0.05; n = 162 cultivars).

Table 4. Statistics on mean values (2 rep. of 162 cultivars) for isoflavone content (DW basis), isoflavone composition (% of total isoflavone in cotyledon or germ), % protein (DW basis), cycle duration (days to maturity) and 1000 seed weight (cultivars were grown in field experiment in Toulouse, France)

	Cot. Content $\mu g.g^{-1}$	Germ content $\mu g.g^{-1}$	Daidzein % (cotyledon)	Glycitein % (germ)	Protein %	Days to maturity	1000 Seed Weight (g)
Mean	4985	28757	39.7	39.0	41.0	162	176
Min	1450	16868	23.6	21.3	35.6	140	64
Quartile 1	3947	25870	36.3	32.7	39.3	155	158
Quartile 3	6011	31734	43.9	44.6	42.3	168	194
Max	9053	40539	58.5	69.5	51.8	187	292

Genotype by Environment (GxE) interaction is always less significant than main factor effects. However, we observe a significant GxE interaction on composition. For example, in various locations, when isoflavone content increase, daidzein percentage increases in cv Queen and decreases in cv Imari (Figure 2a).

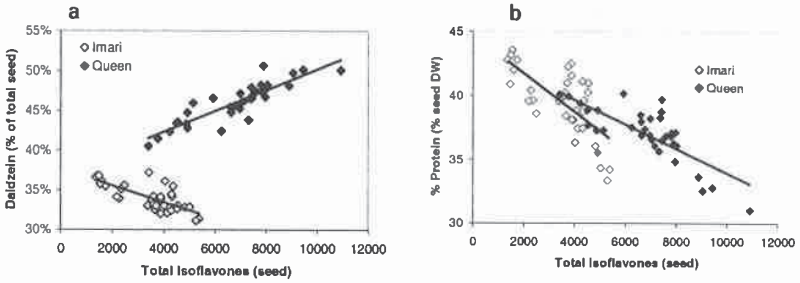

Figure 2. Relationships between isoflavone content and (a) composition in Imari (high genistein profile) and Queen (high daidzein profile), (b) protein content (field experiment : 9 locations in south of France, 4 replications)

As shown in Figure 2b, environmental conditions providing high isoflavone contents are detrimental to protein contents in seeds. In contrast, a significant and positive correlation occurs between 1000 seed weight and isoflavone content (*cv* Imari : r = 0.585 ; *cv* Queen : r = 0.486).

It appears that in European cropping systems, an appropriate crop management combined with a suitable choice of cultivars, within a given location, could allow a predictable isoflavone content and composition in germ or cotyledon.

Breeding for isoflavones: correlations and heritabilities

Table 5 presents the correlations observed between isoflavone contents in cotyledon and germ, percentages of daidzein in cotyledon and glycitein in germ, protein content, thousand seed weight and earliness (days to maturity). The data obtained from two experiments : one comparing 162 cultivars grown in Toulouse, France, and another from 2 locations (Ames, Iowa and Toulouse, France) concerning 38 Recombinant Inbred Lines (RIL) from the cross of Imari x Queen in F6 generation.

Table 5. Correlations between isoflavone content and composition and agronomic traits in two experiments (a) comparison of 162 cultivars in Toulouse, France ; (b) and (c) 38 F6 RILs of Imari x Queen grown in two locations : (b) Toulouse, France; (c) Ames, Iowa

		Cot. Content ($\mu g.g^{-1}$)	Germ. Content ($\mu g.g^{-1}$)	% Daidzein (cot)	% Glycitein (germ)	% Protein	Days to maturity
Germ Content	(a)	0.514**					
	(b)	0.644**					
	(c)	0.505**					
% Daidzein (cot.)	(a)	0.339**	0.043				
	(b)	0.436*	0.132				
	(c)	0.716**	0.188				
% Glycitein (germ)	(a)	-0.009	0.093	-0.050			
	(b)	-0.303	0.171	-0.397*			
	(c)	-0.336*	0.374	-0.483**			
% Protein	(a)	-0.495**	-0.421**	-0.029	0.122		
	(b)	-0.323*	-0.313	-0.202	0.288		
	(c)	-0.435**	-0.341*	-0.109	0.220		
Days to maturity	(a)	-0.045	-0.257	0.254*	-0.160	0.428**	
	(b)	-0.047	-0.137	0.344*	-0.051	-0.182	
	(c)	-0.108	0.041	-0.422**	0.278	0.290	
1000 Seed Weight	(a)	-0.017	-0.021	0.002	-0.278*	-0.190	-0.094
	(b)	-0.091	-0.023	0.020	0.300	0.453**	0.126
	(c)	0.042	-0.105	0.042	0.051	0.404*	0.532**

* , ** : significant at p = 5% and p = 1% respectively

In the 3 datas, we observe significant to very significant correlations between : isoflavone content in cotyledon and germ (probably lower than if both were controlled by the same metabolic regulation), isoflavone content in cotyledon and its composition (% daidzein), isoflavone content in both cotyledon and germ and seed protein content (negative relation).

In apparent opposition with our previous results obtained on near isogenic lines for earliness (days to maturity) (Daydé and Lacombe, 2000), the relationship between earliness and isoflavone content both in cotyledon and germ is never significant. In fact, the comparison between near isogenic lines in the same location was obviously the comparison of the same genotypes with more or less time for seed filling period. In the present datas (larger range of genetic variation), we can hypothesize that the effect of delayed maturity is one out of many factors affecting isoflavone accumulation.

Even if some correlations, obtained from the 2 datas on RILs in France and the USA, are clearly not homogeneous (*i.e.* those involving protein content or 1000 seeds weight), the correlation between US and French datas are always very significant for all traits except protein content. This is also shown by the very high heritabilities obtain from these 2 datas (Table 6). Heritabilities (Table 6) are high for isoflavone content and very high for isoflavone composition, suggesting as previously shown (Meksem et al., 2001), a genetic control by a few number of major genes. This is particularly true for glycitein wich exhibits a bimodal distibution either for RILs or for the comparison of 162 cultivars (Figure 3a & b). The 3 :1 distribution of RILs (c^2 = 0,316 in Toulouse, France ; c^2 = 0,877 in Ames, Iowa, $p<0,001$ in both locations) suggests the contribution of two independent epistatic genes for high or low glycitein relative content in germ. Moreover, this hypothesis is very consistent with the bimodal distribution observed when comparing the162 cultivars (Figure 3b). This last distribution also suggests that favorable allels for glycitein expression are less frequent.

Table 6. Heritabilities (h^2) calculated on 38 F6 RILs of Imari x Queen grown in two locations (Toulouse, France and Ames, Iowa) and in joint data (with $h^2 = s^2_G/s^2_P$ and parameters from mixed model of ANOVA : $MS_G = bs^2_G + s^2_e$; $MS_r = s^2_e$; $?s^2_P = s^2_G + s^2_e$ and b = 2 blocs in each location, or 2 locations in the joint data)

	France	USA	Joint data
Isof. content (Cotyledon)	0.63	0.79	0.60
Isof. content (Germ)	0.77	0.89	0.79
% Daidzein (Cotyledon)	0.84	0.96	0.84
% Genistein (Cotyledon)	0.83	0.97	0.85
% Daidzein (Germ)	0.93	0.99	0.99
% Glycitein (Germ)	0.94	0.99	0.99
1000 Seeds Weight (g)	0.26	0.79	0.32
% Proteins	0.11	0.73	0.03
Days to maturity	0.90	0.96	0.85

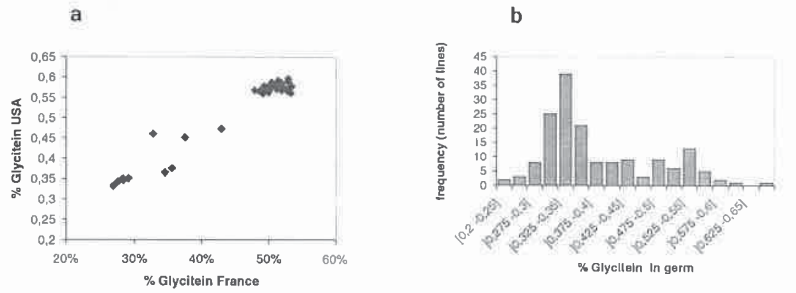

Figure 3. Bimodal distributions for % glycitein in germ (a) correlation between the means of 38 RILs grown in Toulouse, France and in Ames, Iowa (b) histogram of frequencies for 162 cultivars grown in Toulouse, France, with 2 replications

These preliminary results suggest that breeding soybean for isoflavone content could be achieved by screening the segregations in early generation with a relatively low selection pressure due to high heritabilities. Finally, it could be possible to choose the desired isoflavone composition either in seed or in germ, according to the raw material used for dietary supplements or soyfoods.

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Soybean oil milling technology and modified usage of oil and its byproducts

N. Ali[1]

Abstract

Soybean oil is extracted using solvent and expeller technologies. Hexane is the most common solvent used in soybean oil industry. The expander technology has improved the plant capacity and reduced the solvent and energy losses. The technology of supercritical fluid extraction is emerging as a safe and viable extraction. Extrusion aided mechanical extraction of soybean oil has come up as an environment friendly technology for the production of soybean oil and edible grade soy-cake. Membrane technology could be used for separation of oil from miscella. Physical refining offers an increased oil yield and saving in energy consumption. Refined soybean oil is used in various types of cooking and also converted into margarines, shortenings and other products. Soylecithin, has many usages. Soy by-products like soymeal with 50% protein offers a wide range of products for food and livestock feed. Soyhull is a good source for the production of single cell protein for animal feed.

Introduction

The global production of soybean during 2003-04 is expected to be more than 200 million tonnes and that in India would it be around 06 million tonnes (3% of world production). The average structural composition of soybean is 8% hull, 90% cotyledons and 2% germ. On moisture free basis, the chemical composition of soybean is about 40% protein, 35% carbohydrates, 20% oil and 5% ash. However, the actual structural and chemical composition of soybean depends on factors like variety, growing season, geographical location and environmental stresses. Most of the soybean oil is located in cotyledons and germ in the form of oil/lipid bodies. These are encased in minute cells that are deeply embedded in fibrous structures and do not release the oil easily and mechanical action is needed to either forcefully remove it or to make it more accessible to subsequent solvent extraction. Low oil containing seeds such as soybean requires moist heat treatment (Cooking) and flaking to rupture the cells to help release the oil. The changes caused by cooking are complex and are chemical and physico-chemical in nature. One of the changes is denaturation of protein

[1] Director Central Institute of Agricultural Engineering Nabibagh, Berasia Road, Bhopal - 462 038, India

which helps in separation of oil from the solid material. The typical unit operations involved in soybean oil extraction are cleaning, cracking, conditioning/cooking, flaking and then expelling an/or extraction.

Oil milling technology

The purpose of oil milling is to separate oil from the protein-rich residual mass/cake. Oil and cake are then used either for food, feed or fertilizer. Whether the oil is to be separated by pressing, solvent, or a combination of the two, the seed is usually first cooked/flaked to rupture the cell walls, reduce oil viscosity and increase the rate of diffusion (Singh, 1992).

The oil extraction technology has not changed much in the past and both expeller and solvent extraction technology are standard options for the oil industry throughout the world (Anonymous, 1993). The solvent extraction technology is normally preferred for low-oil seeds such as soybean. The better mechanical and thermal designs leading to improvement in processes and equipment for desolventizing and miscella distillation are offering substantial energy savings. A flash-desolventizing technology is available to produce edible grade high protein meal. For miscella distillation, waste heat utilization and 3-stage evaporation with spherical liquid film system is now the state of the art technology to reduce energy consumption as well as solvent loss.

One of the new technological development is in the seed preparation using extruder/ expander. It offers better energy and solvent efficiency and an increased plant capacity. Expander technology for direct extraction plants, processing low-oil bearing seeds like soybean offers steam saving upto 60 kg./tonne of seed/material, lower solvent loss and about 0.3% more oil recovery/yield. This technology is also adaptable for high oil seeds. Membrane separation of oil from solvent offers scope for energy saving. Supercritical fluid extraction is emerging as a safe and viable extraction system. Extrusion aided mechanical extraction of soybean oil has emerged as an environment friendly and decentralized small scale technology suitable for low soybean production catchment. It results in chemical free, high quality edible soybean oil and cake.

Seed preparation

The function of the preparation process of soybean is to prepare the seed for expelling and/or extraction of the oil, either by mechanical methods or using solvent or a combination of the both. It possible, remove the hulls and other materials from the seed kernel or meat. The unit operations involved in the preparation of seed and their purposes are as under:

Major unit operations in seed preparation before extraction

Unit operation	Purpose
Cleaning	It is done **to remove foreign materials** from soybean. It includes the separation of plant tissues, pebbles, dust, etc,. To protect the processing equipment and enable the production of **high quality soyproducts.**
Drying	A moisture content of **about 10% is needed** for an effective removal of soyhull and hence drying of soybean before dehulling
Cracking	Cracking si done to **break soybeans** into **small pieces for dehulling** and **flaking**. It produces 4-6 cotyledon fragments/meat per bean and the hulls are separated from the cotyledon fractions by aspiration. Fines produced in the process are included in the meat for oil extraction to maximize the **extraction yield**.
Conditioning	The cracked soybean grits/meats are conditioned using heat and moisture to obtain the optimum plasticity necessary for soyflakes production prior to oil extraction. Steam heating raises the moisture to about 11%.
Flaking	The conditioned soygrits are flaked to 0.25-0.37 cm thick. It enables better flow of solvent through the bed and improves solvent penetration to oil bodies. It also reduces the diffusion distance to which the solvent or miscella (oil/solvent mixture) moves to extract oil.

An innovative and new technology in pre-extraction is the addition of an expander also known as an extruder or enhancer press to the conventional preparation after flaking. The flakes are extruded to form collets, which enhances extraction an drainage properties of the flakes (Bredeson, 1983). The extruder consists of a worm screw in a barrel. Flakes with about 18% moisture are fed in and high temperature and pressure are generated as the flakes pass through the barrel and when material leaves the barrel, the sudden drop in pressure causes expansion of steam which puffs the final product to produce favourable drainage and extraction properties. Excess moisture is removed and the material is cooled to 60°C before extraction. Increased throughput without loss of oil or hexane, for extraction must be balanced against the increased cost of extrusion. Success has also been found by extruding soybean at 10-14% moisture (Rittner, 1984 and Nelson, et.al., 1987). The subsequent increase in extraction efficiency and oil quality justify the additional cost of an extruder/expander (Kemper, 1995).

Solvent extraction

Oil is removed/extracted from the flakes/collets by an organic solvent (n-hexane) to form an oil/solvent mixture called **miscella**. The oil is recovered from the miscella by removing the solvent. Since hexane is explosive, flammable and expensive, there have been efforts to investigate alternate solvents such as alcohol, isopropanol and carbon dioxide. Pressurized CO_2 named as **supercritical fluid** (Freidrich and Pryde, 1984), extracted oil contains less phospholipids than hexane extracted oil but they are otherwise similar. It is expensive because of equipment cost and need to generate high pressure. The defatted flakes from the extractor contains about 30% hexane by weight. It is removed through a desolventizer-toaster. The process also enhances the nutritional value of soyprotein by inactivating trypsin inhibitors and other naturally occurring toxicants. Steam contacts the flakes and the heat of vapourization released from the condensing steam vapourizes the hexane, which is subsequently condensed and recovered.

Supercritical fluid extraction

The solvent extraction process is favoured over mechanical expellers, because it leaves less oil in the meal. However, the possible escape of solvent from the system is a constant air pollution and explosion hazard. Economic and social factors have revived interest in searching for cheaper and safer solvents, like ethanol and isopropanol. However, it is supercritical fluid technology, which may be a viable alternative to the current extraction methods. Supercritical fluid extraction (SCFE) is a developing technology. It is a way to strip soluble extractives from prepared plant materials without physical damage or chemical change, SCFE is the substitution of a fluid in its supercritical (SC) state for hexane in the conventional solvent extraction process.

Commercial uses of SCFE include decaffeination of coffee, production of spice extracts, and a few costly food components and pharmaceuticals form plant materials. Ammonia, ethylene, toluene and CO_2 all show promise for SCFE. Of these CO_2 offers unique advantages. It is abundant, non-reactive, non-toxic and environmentally harmless. Minor leaks or accidental losses would be of little consequence. All vegetable oils are soluble in SC-CO_2 and optimal performance of SC-CO_2 has been found to be in the easy temperature range 35-70 °C.

Full-fat soyflakes are readily extracted with SC-CO_2 at pressure of 200-700 kg/cm² at 50°C. Soybean oil thus extracted is lighter in colour and contains less iron and about one-tenth the phosphorus of hexane-extracted crude oil from the same beans. Successful use of SC-CO_2 could free over 20 million gallons of costly hexane per year for essential energy uses. It,

therefore, shows that a technology for extraction of oil with SC-CO_2 needs to be adapted/ developed in the country, which may replace the existing technology of oil extraction in coming years.

Mechanical extraction

In mechanical extraction, oil seed is subjected to extreme heat and pressure with oil mechanically forced form oil cell. As the material is subjected to great heat during operation, naturally occurring urease activity is inactivated and protein is denatured, making the product suitable for feed purposes. The quality of mechanical pressed and filtered oil is higher than that obtained from solvent extraction, as less oil-soluble impurities (phosphatides, etc.) are removed and is suitable for direct consumption. Efficiently pressed cake will retain 2-5% residual oil. Solvent extracted meal has less than 1% residual oil. However, the main disadvantage of solvent extraction is high equipment cost and it may not be economically feasible unless its capacity is 50 TPD or more.

Mechanical extraction of soybean oil is often preferred by small enterprises. Screw press is generally used. The advantages are low initial cost and no solvent requirements. However, a disadvantage of this extraction method is the low oil yields. Dry extrusion cooking of whole or dehulled soybean disrupt the cell structure of cotyledons. Consequently, the oil is released from the spherosomes in to the matrix. When such soy-extrudate is passed through a screw-press, about 70% of total soybean oil is recovered in a single pass (Nelson et.al., 1987 and Wijeratne, 1999). This technology could be used for processing soybean into oil and edible cake in low production catchment of soybean. It does not use any chemical and, therefore, it is environment and worker friendly soybean oil expression technology.

Soybean requires careful processing using heat treatment to make it edible. One of such treatments is dry-extrusion. It also aids in expelling of reasonable amount of oil from the extrudate using an expeller. Thus, the extrusion expelling of soybean results in an edible grade soybean cake and oil. The technology is also suitable for small production catchment of soybean (3000-15000 t/year) where solvent extraction plant would not be economical. After successful testing of the technology at laboratory scale, it has been upgraded to a pilot plant of 250 kg/h capacity at CIAE (CIAE, 2000).

Adoption of dry-extrusion as a pretreatment to soybean helps in expelling about 70% of its total oil in a single pass and results in an edible grade soybean cake having about 50% protein and 4-6% oil. The cake can be converted into medium fat soyflour-a good source of protein and calorie for human consumption (Ali, 1994 & 1995).

The cleaned soybean is dehulled and converted into grits and fed to the extruder after conditioning. During the extrusion of soygrits, the cell walls of oil globules are ruptured and oil gets exposed on the surface of feed particles. This helps in easy and quicker expression of oil with relatively lower pressure application on the semi-fluid extrudate fed to the expeller.

The medium fat soyflour could be used for fortification of flour prepared form cereals/pules (10-15% level of soyflour). The blended flour (soy-cereal/ pulse) has been found excellent for making *chapatis, puris, pakoda, idli, dosa,* bread, biscuits, etc. Both, of the soyproducts, oil and flour, would have a distinct market segment which requires better function properties of traditional recipes besides high nutritional value. Oil has ready market among edible oil consumers and can be sold to refining units. The medium fat flour could find ready acceptability in army canteens, hostels, hotels, railway catering services and various other community kitchens as well as among individual households through consumer cooperative retail stores and retail shops.

The extrusion expelling plant has a great potential in India especially in low production catchment of soybean (3000-15000 t/year) or where soybean is likely to be cultivated in near future. In addition to the technical and economical advantages, the system is pollution free and avoids the use of any chemical for extraction of oil. It gives natural oil with better shelf life as it retains most of the lysine amino acid which upon blending (5-15% of soyflour) with cereals/pulses improves the protein efficiency ratio (PER). The blended flour could be used for making various traditional food products including bakery products.

A one tonne/h extrusion expelling soybean plant consisting of building, transport facilities and equipment like grain cleaner, destoner, dehuller, dry extrusion cooker and accessories, oil expeller, oil filter, grinding mill etc. may need about Rs. 6 million capital investment (40 lakhs fixed investment and 20 lakhs working capital) producing 3600 tonnes of medium fat soyflour, 600 tonnes of oil and 600 tonnes of byproducts from 4800 tonnes of soybean by working 16 h/day for 200 days in a year (One Us dollar = Rs. 45 Indian Rupee). The techno-economic feasibility analysis of producing medium fat (4-6%) soyflour and oil using extrusion expelling technology appears viable from financial aspects besides providing high quality and low cost food items to the people.

Refining of crude soyoil

Crude soybean oil contains a lot of non-glyceride impurities. Some of these substances are beneficial and some are not. The most objectionable

impurities are free fatty acids (FFA), gum/phosphatides, volatile/odoroferous compounds, and dark colour pigments. These are removed/reduced as much as possible by refining process to get an edible oil. Refining of crude oil can be done by either chemical processing or physical refining. However, as of now, about 90% of the world soybean crude oil is refined by chemical method and only a small amount is physically refined.

The shelf life of refined soy-oil depends on its fatty acid composition and type of packaging employed. Oils rich in polyunsaturated acids deteriorate faster in the presence of oxygen and light with the passage of time. Therefore, the most appropriate way of packing soy-oil is to use **opaque container** having zero oxygen permeability and fill the oil to the top without any air/head space. Such packaging aids/containers are metal cans, dark glass bottles and opaque plastic containers.

Soybean oil properties and its usage

Physico-chemical properties of soybean oil depends on its fatty acid composition as well as processing conditions. These are measured and monitored in terms of refractive index; iodine valve; smoke, flash and fire points; melting point; crystallization behaviour; polymorphism; and a few others. The oxidative stability of soybean oil has been greatly improved because of the progress made in processing and refining technology. A properly processed soy oil, particularly the hydrogenated soy oil, is now suitable for many food applications.

A wide variety of products based on edible soybean oil is available in the market. Salad and cooking oil, shortenings, margarine, mayonnaise, salad dressing and confectionery coating are some of the available products. With improved flavour stability and stronger emulsion, soyoil has now become the choice of most manufacturers. The soy oil used in mayonnaise is usually partially hydrogenated followed by winterization. Soybean oil is also used in salad dressing. Mono and diglycerides of edible soybean oil and soybean lecithin are among the most commonly used emulsifying agents approved for food uses. Other applications of soybean oil are in canned foods, confectionery coatings, pudding mixes, pancake and waffle mixes, macaroni and cheese mixes, spaghetti sauces, dry breakfast cereals, frozen fried sea foods, meat patties, pizza mixes and others. Partially hydrogenated soybean oil is also used in some whipped toppings.

Soybean oil is also used for industrial applications such as emulsifiers, lubricants, plasticizers, surfactant, plastics, solvent and resins (Erhan, 1999). Soyoil being biodegradable, low-toxic to human and eco-system, renewable

and non-contributor to volatile organic chemicals are superb materials for industrial applications. Soybean oil is successfully used in printing ink, lubricants, pesticide carriers and in paints and coatings. Soybean and its oil represent a large and stable supply of a natural and renewable raw material for various industrial applications.

Byproduct utilization

The major byproducts resulting from soybean oil extraction are soymeal, soyhull and soylecithin. Soymeal has become the standard to which all other plant protein sources are compared and its quality, acceptance and reputation are widely known (Smith and Baldwin, 1986). The meal contains 45-50% protein and 2500-2800 kcal/kg of metabolizable energy depending upon the amount of hull present and the species of animal being fed. Dehulling increases the metabolizable energy value by about 5% for cattle and 12% or more for pigs and poultry (Bell, 1989). Soybean meal is an excellent protein supplement for lactating dairy cattle and for calves following weaning, as it is highly palatable and well digested. In India is used mainly in poultry and aqua feeds. The primary edible products derived from edible grade soymeal are defatted soyflour/grits (50% protein), protein concentrates (70% protein), protein isolates (90% protein). The nutritional quality, availability, price and functionality of these products have resulted in substantial use in a wide variety of food and feed products. Looking in to the present food trend of high plant protein diet, strong growth in food sues of soyprotein is assured (Ali, 1996).

Potential new industrial uses of soy-protein are biodegradable plastics; edible soyprotein films and soyprotein-carbohydrates films to reduce packaging wastes; and a soyflour-recycled paper composite material with the appearance of granite and the handling characteristics of hardwood. Soyprotein-based glues and adhesives and textile fibres are also being developed.

Soyhull is high in cellulose but low in lignin and therefore highly digestible by ruminants. In fact, the digestible energy content of soyhull for ruminants approaches to that of grains. Consequently, soybean hull can be used to economic advantage in high forage diets in lieu of grains with many additional functional advantages. Soybean hull is now finding use in human food as a source of dietary fibre through fibre-enriched pasta and white bread. Soybean Processing and Utilization Centre at the Central Institute of Agricultural Engineering, Bhopal, India has developed single cell protein from soyhull for livestock feed using *Phanerochaete chrysosporium* fungi which yielding significantly higher SCP than the other organism. Supplementation of urea (2%) as the external nitrogen source enhanced the yield of SCP by 30%.

The inoculum size of 3.0 x 1.0^4 spores/100 g dry substrate alongwith urea supplement resulted into maximum SCP yield of 26.4% after 9 days of incubation at 30 °C whereas the soyhull contains only 6.8% true protein. SCP can be sued as animal feed and after further refining it can also be used for human food (CIAE, 2000).

Laboratory scale fermentation of soyhull was taken up for the production of cellulase enzyme./ Out of five fungal strains used, *Phanerochaete chrososporium* was found to be the best. At an optimized pH of 4, 25°C and 2% urea supplementation, the cellulase yield was 74.80 IU/gds. The optimum inoculum size was 1.0 x 104 spores/gds (gds = gram dry substrate).

Lecithin, an edible byproduct of soybean crude oil refining, has a variety of useful functionalities. It is a mixture of phospholipids such as phosphatidyl choline, ethanolamine, serine, inositol and phosphatidic acid and other water-soluble constituents. Soylecithin purification consists of the removal of non-lecithin components such as carbohydrates, proteins and other contaminants. A wide range of functionalities of lecithin has been applied in a variety of industries such as food, pharmaceuticals and cosmetics.

Soy-lecithin is an important natural emulsifier used in foods, feeds, pharmaceutical and technical/industrial products. It has an excellent emulsifying behaviour, colour and taste. Lecithins are essential components of living cell membrane and play an important role in patho-physiology of human disease. Dietary lecithin, as a source of choline is important for the prevention on cancer. Choline deficiency and injury to the liver enhances the development of liver cancer. Soy lecithin is obtained from cruder un-refined soybean oil by means of hydration and centrifugation. The resulting lecithin is dried to a moisture content of about 2%. It is further refined and stored.

Enhancing soybean oil quality

One of the major quality considerations of soybean oil is its Oxidative stability. It refers to resistance of soyoil to oxidative reactions during handling, storage and processing. The outcome of the reaction leads to off-flavour development. A normal soyoil contains about 4% stearic, 8% linolenic, 11% palmitic, 23% oleic and 54% linoleic. Because of a large proportion of polyunsaturated fatty acids (85%), particularly linolenic acid, soyoil often has a problem of lacking flavour stability during processing, storage and application. There are two ways to improve the soyoil stability. One is processing and the other one is through genetic/breeding means (Liu, 1999).

Plant and Environment Safety

Industrial and environment safety is becoming increasingly important to oil processors. Soybean handling and its milling can present a number of hazards. Practical safety precautions must be taken when personnel are involved in cleaning, storage or transportation tanks. Falling and head injuries may occur on slippery surfaces in restricted spaces. Protective clothing, an air supply and breathing apparatus, a worker in attendance in case of emergency and harness and safety line are all sound safety measures. Various emissions and waste products are produced in soy-oil processing that could pose environmental problems if not properly disposed off.

Application of expander technology in solvent extraction, extrusion-aid in mechanical expression and membrane technology in soybean oil refining would rise in future. The extrusion-expelling (EE) provides mechanically expressed soyoil and edible soycake facilitating the sue of both, oil and protein for human consumption. The EE is enviroment and worker friendly technology. Soybean and its constituents provide many health benefits and it, therefore, in future may be treated as a medicinal crop rather than a mere source of oil and protein. Hence, soy future is very bright in the new millennium throughout the world.

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Evaluation of CLA production from soybean oil

R. C. A. Lago[1,2]; P. Villeneuve[2]; G. Piombo[2] and M. Pina[2]

Abstract

Production of conjugated oil from linoleic acid rich oils has been described as early as 1930. Forty years later, conjugated linoleic acids, CLA, started to be regarded as potential anti-cancer and anti obesity agents related to, respectively, the *cis*-9,*trans*-11 and the *trans*-10,*cis*-12 isomers. For economical reasons soybean oil is the preferred starting material for CLA production. Alkali isomerization of linoleic acid is, at present, the most common and less expensive procedure for producing these compounds. The resultant mixture is normally composed of at least four isomers, with equal amounts of the *cis*-9,*trans*-11 and the *trans*-10,*cis*-12 isomers. The need of pure compounds for nutritional studies and/or human or animal consumption makes obligatory purification steps. Apart from commercial scale molecular distillation, enzyme technology appears the most promising tool for effecting CLA isomers enrichment.

Introduction

The methylene interrupted *cis*-9,*cis*-12-octadecadienoic acid, or linoleic acid, is a common component of many vegetable and animal oils.

Conjugated linoleic acids, CLA, represent a group of conjugated positional and geometric isomers of linoleic acid. Therefore, ca. 50 different CLA could exist. The number of these compounds found naturally and/or resulting from available synthetic ways does not overcome a dozen. Nutritionally, the most attractive isomers are the *cis*-9,*trans*-11 and the *trans*-10,*cis*-12-linoleic acids to which it has been attributed, respectively, anticancer and anti obesity activity. The anticancer activity has been described since 1985 (Pariza & Hargraves) and subsequently confirmed (Belury, 1995).

The *cis*-9,*trans*-11 isomer, rumenic acid, occurs naturally mainly in ruminant products such as milk, yoghourt, cheese, butter and meat of cows, goats and sheep (Jahreis and Kraft, 2002).

The very first demand for oils containing conjugated systems are related to their drying properties and dates back to several decades.

[1] Embrapa Labex France
[2] Cirad-Amis, TA 40/16, 73 rue JF Breton, 34398 Montpellier Cedex 5, France

When a technical grade product castor oil continues to have its place, for other purposes linoleic rich oils with prevalence of soybean oil have been utilized as sources for CLA production, under acidic or alkaline catalysis assisted reactions.

The production of pure compounds mainly for carrying on nutritional studies and for products aimed to health food supplement and animal feed additives keeps been a challenge, although purified compounds for research purposes are now available.

This presentation is directed to discuss some of the methods concerning the production of CLA having soybean oil as raw-material and whenever possible to compare them with the CLA production from other sources.

Production of CLA from soybean oil

The production of CLA involves the conjugation of double bonds, through isomerization, or the formation of double bonds through dehydration reaction.

Soybean oil can be employed as such or as one of its derivatives, mainly the free fatty acids, and methyl- or ethyl esters.

The yields of the reaction can be related to the % of conjugation, % loss of starting material and total quantity and/or proportion of different isomers.

The formation of CLA can be catalyzed by bases, acids, metals and more recently by enzymes.

CLA production catalysed by bases

Commercial CLA is produced by alkaline isomerization of linoleate-rich oils, and tends to contain an equimolecular mixture of 9-*cis*,11-*trans*- and 10-*trans*,12-*cis*-octadecadienoic acids, together with variable amounts (up to 30%) of both geometrical and positional isomers (Christie *et al.* 2002).

In a commercial process soybean oil is heated at high temperatures with excess caustic in a high-boiling glycol or water in a pressure vessel. The splitting of fatty acids requires a reesterification and the resultant drying oil possesses no more than 30-50% conjugation (Larock, 2003).

For the process carried out in high alkaline water at ~230°C reaction parameters were carefully investigated but the formation of 11-*cis*,13-*trans* and 8-*trans*,10-*cis* isomers, aim of the investigation, has not been avoided (Saebo, 2003).

When the isomerization is carried out in propylene glycol (preferred to ethylene glycol for safety reasons) and KOH in excess (preferred to NaOH for solubility reasons), at temperatures of 130-180°C, and during 3 to 24h, the use of free fatty acids as raw material is recommended instead of the oil, to avoid contamination of the propylene glycol with glycerol (Sæbø, 2003).

Heating purified methyl linoleate at 180°C in dry ethylene glycol and KOH gave 95% of conjugated isomers, mainly the *cis*-9,*trans*-11 and 10-*trans*,12-*cis* compounds. Starting from 10g of methyl linoleate in 3h reaction 44.4 and 47.2% of these compounds is produced respectively. Starting from 110g of linoleate only after 13hours this same result is obtained (Berdeaux, 1998).

A quantitative isomerization of methyl or ethyl linoleate has been achieved using amounts of catalyst (alkali alcoholate) as low as 2%. In this case more than 92% of the ester remains in its form and the reaction proceeds at temperatures down to below 100°C and levels of undesirable isomers are negligible (Sæbø, 2003). When using mild conditions results <0.5% of the isomers 11-*cis*,13-*trans* and 8-*trans*,10-*cis*. When thermally treated, even in an inert atmosphere CLA isomers give rise to other species through sigmatropic rearrangements. This is the case of 11-*cis*,13-*trans* resulting from the 10-*trans*,12-*cis* isomer.

Berdeaux *et al.* (1997) used a modified procedure to produce an 83% pure 9c,11t CLA concentrate. Methyl ricinoleate (80g), purified from castor oil, was transformed with methane sulfonyl chloride and pyridine, in 4hours reaction, and at low temperature, into the methyl 12-mesyloxyoleate. This compound was treated with 1,8-diazobicyclo[5.4.0]-undec-7-ene, DBU, in toluene, under reflux for 4hours. The resulting CLA mixture (85% yield) contained originally 66% of 9c,11t isomer. Main impurities were 9c,11c and 9c,12t. From conventional dehydration using high temperatures results other isomers, mainly 8*t*,10*c* and *trans*,*trans* isomers. Later on, the expensive dehydrating agent, DBU, has been successfully replaced by KOH, at temperatures not higher than 80°C (Yang *et al.* 2002).

Due to its important presence in natural products, the *trans*-7,*cis*-9 CLA isomer has been synthesized in very small amounts from partially hydrogenated ^{3}-linolenic acid under alkaline catalysis (KOH in ethylene glycol), at 150-160°C, during 90minutes (Delmonte *et al.*, 2003).

CLA production catalyzed by acids

Acid catalysts such as thionyl chloride and toluene sulphonic acid catalyze the isomerization of natural oils and/or methyl linoleate in a very inefficient

way. It is also observed splitting of fatty acids rapidly degrading the oil thus limiting its utility (Larock, 2003).

The dehydration of castor oil is generally carried out under acid catalysis and high temperatures. Catalysts for this reaction include inorganic acids such as sulphuric, hydrochloric, phosphoric, and p-toluenesulphonic; sodium bisulphate-sodium bisulfite, acid-activated clays, etc. The reaction acid catalyzed occurs via ion hydroxonium (H3O+) and the resultant dienes can be either in the conjugated or in the non conjugated form (Naughton, 1974). Commercial sample analyzed in the authors' lab exhibited 40% conjugation (complex mixture of CLA isomers) and up to 45% of linoleic acid (mixture of 9-*cis*,12-*cis* and 9-*trans*,12-*cis*). The original linoleic acid content was <6%.

CLA production catalyzed by metals

This type of catalysis is used for methyl or ethyl esters and natural oils including others than linoleic rich oils, such as linseed and fish oils. The main purpose is drying properties of the products, and their use as ingredients for bioplastics made from renewable sources. A number of transition metals catalysts, through different complexes, will efficiently conjugate oils, but many of the current processes require high temperatures (165-195°C) and pressures (70-200psi), produce side-products, degrade the oil, and/or are prohibitively expensive to use (Larock, 2003).

Attempts to use low levels of rhodium, ruthenium and platinum catalysts under mild conditions have been undertaken (Larock et al. 2001).

CLA production catalyzed by enzymes

Starting with linoleic acid as substrate washed cells of *Lactobacillus plantarum* AKU 1009a produced 40mg CLA/ml reaction mixture (33% molar ratio) in 108h. A mixture 1:1.5 of two CLA isomers 9*c*,11*t*-(or 9*t*,11*c*-) and 9*t*,11*t*-, accounted for 50% of the total fatty acids obtained (Kishinos. *et al.* 2002). Following the assumption that 10-hydroxy-12-octadecenoic acid was a possible intermediate of the reaction, the research group using washed cells of *L. plantarum* JCM 1551 as catalyst was able to obtain 2.4mg/ml CLA from 3.4mg/ml ricinoleic acid in 90h, with a molar yield with respect to the acid of 71%. The two isomers 9*c*,11*t*- and 9*t*,11*t*-, (1:4 ratio) accounted for 72% of the total fatty acids produced (Ando *et al.*, 2003).

Identification/analysis of CLA isomers

The basic tool for analyzing CLA isomers is HRGC, a 60m Supelcowax 10 column offering a good peak resolution (Kramer *et al.* 2002). Methyl esters should not be prepared by acid catalysis since this causes isomerization of CLA.

Silver-ion impregnated high-performance liquid chromatography (Ag+-HPLC) seems to offer a clearer separation of different CLA isomers (Sehat *et al.*, 1998).

Infrared spectroscopy can be used to determine the geometry of the double bonds. The *trans,trans* isomers have distinct infrared absorption at 988cm^{-1}, whereas *cis,trans* isomers absorb at 948 and 982cm^{-1}(Reaney *et al.* 2002).

Mass spectrometry (MS) can be used to locate double bonds, in addition to the simple overall chain lenght and number of double bonds determination, through nitrogen derivatives either on the carboxylic acid group (DMOX, 4,4'-dimethyloxazoline) or at the double bonds (MTAD, 4-methyl-1,2,4-triazoline-3,5-dione), with electron impact techniques (Delmonte et al. 2002; Christie *et al.* 2001).

Jung & Jung (2002) combined some of these techniques to identify CLA in hydrogenated soybean oil.

But the best method of analysis is ^{13}C NMR, which allows for the identification and quantification of all possible positional (7,9 - to 11,13) and geometrical (*c,t-*, *t,c-*, *c,c-* and *t,t-*) CLA isomers (Lie Ken Jie *et al.*,1997; Lie Ken Jie *et al.*, 1999).

Purification/enrichment of CLA isomers

Normally, in a commercial scale the isomerized product is purified, or enriched, by deodorization and molecular distillation. At lab scale, a combination of techniques can be utilized.

High yields and high purity can be obtained by repeated crystallization of the methyl ester forms in acetone at temperatures as low as –60°C (Berdeaux *et al.*, 1998).

Berdeaux *et al.* (1997) using urea inclusion technique were able to obtain a concentrate with 83% 9-*cis*,11-*trans* isomer, in a 50% yield. The starting material contained 66% of the isomer.

On a kilogram scale McNeill *et al.* (1999) obtained an ester fraction containing 91% of 9c,11t and a free fatty acid fraction consisting of 82%

10t,12c based on total CLA content using selective esterification property of *Geotrichum candidum* lipase. A 98% concentrate of 9c,11*t* was also reported by using this lipase (Haas *et al.* 1999). Using *Candida rugosa* lipase in a two-step procedure Nagao *et al.* (2002) prepared >95% pure concentrates of 9c,11t or 10t,12c, and in better yields than the ones reported previously for *G. candidum*. Purified isomerase preparations from *Propionibacterium acnes* and *Clostridium sporogenes* were able to quantitatively isomerize linoleic acid into the 10-*trans*,12-*cis* isomer (Rosson *et al.* 2001).

Conclusions

Several different linoleic rich oils such as sunflower, corn, safflower etc. can be used as raw materials for CLA production. For some countries, the low cost and ready availability of soybean oil makes it the raw-material of choice.

Due to the generally high temperatures involved in thermal procedures degradation and polymerization of starting oil and intermediary products are common features. Sigmatropic rearrangements do occur causing the formation of a complex mixture from where the nutritional important CLA isomers are difficult to separate.

Alkaline catalysis, although simple, is performed at high temperature, saponification requires reesterification, is unsuitable for oils directly and has modest efficiency.

Procedures using metals as catalysts are still in progress. Some of them are expensive and water sensible. Others are toxic. In some cases the separation of the catalyst at the end of the reaction is not feasible.

Methods using enzymes as the catalyst are being developed. Enzymes also appear as a very promising tool for CLA isomers enrichment.

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Physical refining and performance of specialty soybean oil

W.B. Wijeratne[1]

Abstract

Soybean processing into oil and meal by the use of extrusion cooking and screw pressing has been recently developed and commercialized. The meal produced by this technology has been proven to be nutritionally superior to the solvent extracted counterpart. However, the viability of the technology depends also on the quality and value of the oil.

The oil was shown to contain relatively low levels (0.2%-1.0%) of phosphatides and unsaponifiable matter compared to solvent extracted crude soybean oil. The color, clarity, and flavor of the oil were rated superior to that of solvent extracted oil. Because the oil was extracted by a chemical-free method, it was interesting to investigate refining of the oil also by physical means. This would enable both the oil and meal to be truly natural products. Field level research has lead to successful refining of this oil by de-gumming, bleaching, and deodorizing with fatty acid recovery. A 30 tons/day physical refinery has been built alongside the extrusion and screw pressing facility of a farmer's cooperative. The whole processing facility is dedicated to processing identity preserved soybeans produced by the farmers. The physically refined soybean oil without hydrogenation, is being marketed as specialty oil and is being used as cooking and even as frying oil. The value traits are chemical free processing and the lack of trans fatty acids. The users of the oil in restaurants have reported that the oil has a longer fry life compared to partially hydrogenated liquid frying oil. Customers consuming the products fried in the new oil have reported that the food is less greasy and have better appearance and flavor.

In order to validate the anecdotal evidence, a comparative fry life study was conducted with the physically refined soybean oil and partially hydrogenated liquid frying oil. Progressive deterioration of the oil with consecutive frying cycles was monitored by determining free fatty acids, peroxide value, p-anisidine value, absorbance at 520 nm, color, polymerized triglycerides, and oil stability index. It was found that except in the case of the p-anisidine value, the physically refined oil performed better than the partially hydrogenated liquid frying oil. Further studies are needed to elucidate why the un-hydrogenated physically refined oil performed better than the solvent extracted and partially hydrogenated oil.

[1] Insta-Pro International, Des Moines, Iowa, U.S.A. Wilmot@insta-pro.com

Demand and quality aspects of the use of soya in animal feed

C. Bellaver and G. J. M. M. Lima[1]

Sector demand

The swine and poultry productions within the Brazilian agribusiness are very well organized activities in the South and Southeast regions of the country. It is expanding quickly to the Central West region, where there are new areas of grain production, of lower prices in relation to the remaining of the country (Table 1). The Brazilian swine production is increasing along the lasts years, recording an annual growth of 6%, representing almost twice the world rate, result mostly of the exports performance. The country occupies the 4th. place in the world production and on exports (476 thousand tons in 2002); it stays behind of the European Union, Canada and United States (Embrapa, 2003). The gross revenue of the sector is near to US$ 5 billion/yr., employing 2.7 million people, which depend on the swine sector, according to ABCS (2003). Commercial poultry production is formed by distinct sub-sectors, including broilers grand parent supply, commercial eggs for chick production and the production of broilers, itself. Broiler breeder parent hosing reached 31.6 million heads in June 2003, with a 2% growth in the previous twelve months. Southern states of Brazil are specialized in broiler production, detaining 56% of the national production. By June 2003, the production was 3.82 billion of chicks and 7.45 million tons of meat production. Compared to 2002, there was 1.6 million tons of broiler meat exports. In the same year, egg hens population for table eggs production was 50.2 million of heads, resulting in a production of 1.36 billion of dozen of eggs. The poultry sector has participation in the national GDP, in employing direct and indirectly more than 4 million people and contributes to strength the family agriculture. It is possible to preview the continuous growth of swine and poultry production in Brazil, as well as, the greater responsibility in producing animals with sustainable perception. This means: focusing social aspects, environmental preservation, animal well-being, feed and food safety and a general and accepted warranty for product quality at the consumer table.

[1] Ph.D., Researchers at Embrapa Swine and Poultry National Center. 89700-000 Concordia SC – Brazil; mail to: bellaver@cnpsa.embrapa.br

Table 1. Total production of grains 2002/2003 – Thousands tons

Region	Corn and Sorghum	Soya
North	979	369
Northeast	3,646	2,653
Central West	10,128	23,987
Southeast	10,343	3,994
South	22,068	21,019
Brazil	47,164	52,022

Source CONAB 2003

Therefore, swine and poultry are the biggest consumers of soya (and its by-products) and corn, which are complementary ingredients allowing formulation of diets of excellent quality. The production of these grains contributes to poultry and swine chain expansion. However, should be considered also the possibility to use summer cereals such as sorghum and milo, as well as, winter cereal production like wheat, barley, triticale and oat; all of them with good nutritional characteristics, but they need to have competitive prices compared to corn to be used. Among other factors, domestic availability of soya and corn should strengthen the competitive advantage of Brazilian poultry and swine productions. The world feed production is estimated to be 602 million tons and Brazil demands 44 million tons of feed (Table 2). Thus, total production of feed will require about 120 millions tons of soybean meal, where 9 millions tons of soybean meal and around 1 million tons of degumed vegetable oil or animal fat will be consumed in Brazil. Poultry and swine production growth in Brazil has been consistent and it is related positively with the growth of meat exports (Table 3). On the other hand, the agricultural sector needs to assume that poultry and swine are the main grain consumers/clients. Grains need to be genetically improved and in addition, it is important to maintain the quality before and during processing phases of the grain for feed utilization. With that in mind it is possible to ensure gains in quality and the improvement of all the meat chain.

Table 2. Feed production in Americas – Million tons

Countries	2002 [1]	2003 [2]
Brazil	38.8	43.9 [3]
Canada	21.5	21.9
Mexico	21.4	21.8
Argentina	5.5	5.6
Chile	3.5	3.6
Venezuela	2.8	2.9
Others	10.3	10.5
Total Latin America	103.8	110.2
USA	143.4	146.3
Total Americas	247	256
Total World	590 [4]	602

[1] Gill and Migone (2003); [2] Estimates are based in 2% yearly growth; [3] According Sindirações (2003); [4] Production in 1995.

Table 3. Poultry and swine production by region in Brazil

Region	Poultry 2002		Swine 2003	
	Hen herd Million heads (%)	Meat prod. Thousands ton *	Million heads (%)	Meat Thousands tons
North	55 (1)	75	0.72 *(2)*	47
Northeast	301 (8)	596	2.90 *(8)*	204
Central West	342 (9)	670	5.45 *(15)*	429
Southeast	988 (26)	1,937	6.37 *(18)*	500
South	2,131 (56)	4,171	20.14 *(57)*	1,607
Brazil	3,817 (100)	7,449	35.57 *(100)*	2,787

Sources: APINCO in Avisite; * estimated by region based on carcass weight 1.95 kg; Abipecs, ABCS, Detailed: Instituto CEPA. In: ABCS 2003 - J.Braum

Grain quality for animal feeding

In a general manner, the grain market (*e.g.* corn) in Brazil does not rate the quality because the premium payment is of little or no significance. Therefore, despite some few criteria, quantity is more important then quality. Increase costs based on added value of a nutritional characteristic may, sometimes, implicate that a common grain will be preferred after a cost analysis using a least cost feed formulation program. This fact implies in loss of competitiveness of a better grain based in nutritional quality. Thus, it is necessary that grain producers do not underestimate this aspect and consider that: *1)* it is desirable the market appeal for higher nutritional quality but, at the same time, it should come associated with lower prices to ensure competitiveness with common grains and, *2)* it is undesirable to

lose grain quality due to the inappropriate or imperfect grains pre-processing to use in feed production (harvest, sifting, drying, storage and transport). A price fine has to be established for common grains not reaching the standards. According to Biaggi et al. (1996), the international trade of grains establishes the quality standards by variables like humidity, broken grains and foreign material. In fact the quality is also associated to other indicators such as: density, thermal damages, imperfect grains, hardness, protein, oil, insects and/or presence of grain molds and origin (trace ability) of the grains.

It is possible to forecast that soya will be one of the main ingredients for nourishing the future world population, due to the nutritional quality, the adaptation to large areas in the globe, the simple cultivation systems which guarantee high production crops. There is high protein quality and moderate to high quantity of metabolizable energy in soybean grains. However, it introduces some anti-nutritional factors (protease inhibitors, lectins, glycinin, ß-conglycinin, lipase, lipoxigenase and non-starch polysaccharides) that interfere on the possibility to use *in nature* soya for feed formulation. However, heat processing allows the degeneration and/or digestion of the inhibitor molecules allowing, thus, the use in feeding. The most important processing treatments to ensure nutritional quality of soya by-products are: soya oil extraction with solvent, toasting, micronization and extrusion. In the soya processing there are several by-products that can be used as ingredients in animal nutrition: soybean meal, oil, lecithin, stains, soya hulls and protein concentrate. The ingredient quality is measured in the industry by analytical composition, urease activity and protein solubility, reviewed by Bellaver and Snizek (1999) and Swick (1998). Among ingredients, extruded soya is the one with the best nutritional characteristics followed by high protein soybean meal (Hipro); however, the decision to use ingredients with better processing depends on market prices of grain and meat.

In vitro solubility of proteins

The *in vitro* protein solubility has been tested to estimate *in vivo* digestibility (Bellaver et al., 1991; Han, 1990). However, factors like protein source, enzymatic concentration (Johnston and Coon, 1979; Parsons et al. 1997), used enzyme and pH (Cone, 1993) and processing (Zombade and Sathe, 1977) are correlated with *in vivo* values. The technique of *in vitro* digestibility of products of animal origin was developed between 1950 and 1975 with method described in its final form by AOAC (1995), that recommend 0.2% of pepsin in a solubility test. With aim to adjust methodology, few steps were introduced and the final result indicated that best concentration of pepsin to obtain solubility of crude protein in animal protein is 0.0002 %.

The *in vitro* protein solubility paradigm for vegetable origin ingredients is the nitrogen solubility in KOH 0.2%, as recommended by Pomeranz and Lindner (1960) and AOAC (1995). Based on the information obtained by Parsons et al. (1997) and Bellaver et al. (2000), it is known that the digestor substance concentration must be smaller than the originally recommended by the AOAC (1995) for animal origin meals and also that the enzymatic method could be used to vegetable ingredients. Soybean meal has been analyzed more frequently than the rest of vegetable ingredients for the digestion in KOH. However, other methodology proposed by Prosky et al. (1992) is more useful in the establishment of relationships among *in vivo* and *in vitro* variables. This method uses three enzymes and the total dietary fiber (TDF), generated in the procedure, introduces high repeatability and vegetable species show significant differences regarding TDF's contents. Recently, it was observed that the better protein solubility among 12 sources of vegetable ingredients, including two SBM, and one soybean hulls, was obtained with NaOH than with KOH. It was also shown for both alkalis that the concentration of 0.02% allows larger discrimination in the protein solubility (amplitude of 68,61%) in comparison to 0.2% (37,97 %). Therefore, the best discrimination was obtained with NaOH at 0.02%, considering the existing interaction between alkali and concentration.

Genetically modified soya

Regardless the scientific and political discussion established among actors in favor and against transgenic soya varieties, herbicide resistant soya varieties were developed in order to reduce production cost, which is an agronomical appeal. This happens as consequence of smaller need for culture management with herbicides, which brings the advantage of decreasing the quantity of agrochemical substances in the environment. The use of this soya made producers realize the economical advantage, with no nutrition restriction in relation the conventional soya. That can be confirmed by Hammond et al. (1996), who carried out studies with mice, broilers, fishes and bovine comparing the nutritional value of the genetically modified soya variety. In all studies no significant differences were obtained between herbicide tolerant varieties and conventional varieties. Also, Cromwell et al. (2002) determined that conventional and RR soya varieties presented essentially the same chemical composition and produced similar results of performance and carcass traits in fattening swine. Recently, Jennings et al. (2003) tested if the DNA from RR soya plants, used in animal feeding, could be detectable in the swine muscle. They found that there is no transference of fragments of DNA or imunereactive fragments of the plant protein to the pork.

There are other important negative traits in conventional soybeans that could be solved by biotechnology. For instance, trypsin inhibitors and others anti nutritional factors play an important negative role in the use of soybean for non-ruminant animals. Removing it from the plant and maintaining other positive traits will enable a tremendous advantage in the meat chain. Another area for improvement, directed mainly for broilers, is to increase metabolizable energy of soybean products by reducing and (or) modifying the relation of soluble: non-soluble polysaccharides. This improvement could increase metabolizable energy of soybeans by 25%, bringing a reduction in the use of soybean oil, and, off coarse the cost of the diet. Any improvement in nutritional quality of soya should add extra value to the grain and, therefore, lead to new markets.

Simulation of diets for broilers using different alternative of soya ingredients

Feed formulation was carried out to demonstrate economical benefits of nutritionally enhanced soybeans. Prices for soya ingredients were considered equal to demonstrate their specific nutritional advantages, if existing. Poultry diets were formulated for growing phase (21 to 35 days of age), according with NRC recommendations (1994) and concepts of ideal protein. Formulation constraints were established to supply at least 19% of protein, 3200 kcal metabolizable energy/kg, 0,85% calcium, 0,46% available P, and balancing digestible amino acids in the different diets. It was assumed that diets were essentially the same, regarding nutritional characteristics. Therefore, differences that were found were in diet costs, only, since it was assumed similar animal performance among calculated diets. The results showed that diet costs increase in the following order: 48% crude protein (Hipro) soybean meal with added energy value (less expensive), full fat soya, 47% crude protein soybean meal and 44% crude protein soybean meal (more expensive). These simulations originate an important point: the economical advantage of the soya in feed occurs when metabolizable energy concentration is increased. This happened in Hipro soybean meal with extra energy value (this is another trait in soya that could be improved by conventional or transgenic genetics). Full fat soya is another competitive ingredient in which processing (*e.g.* extrusion) allows to eliminate anti nutritional factors and retaining the oil in the grain, and, therefore, increasing the metabolizable energy of the ingredient. This process adds nutritional value to the grain, and, however, it also increases the ingredient cost in about 30%, reducing its competitiveness.

Conclusions

Poultry and swine productions are growing consistently in Brazil and it is positively related with meat exports. Grain producers have to face that the

main grain consumers/clients are these species, worldwide. Therefore, they must direct genetic improvement of grains to the betterment of meat and egg chains.

One of the quality parameters to evaluate soya products is its protein solubility in alkali. A better discrimination of solubility (range) was obtained with NaOH at 0.02%.

Two important appeals for soya ingredient in feeding are: *a)* to decrease trypsin inhibitors and other anti nutritional factors that play an important and negative role on the use of raw soybean for non-ruminant feeding and, *b)* to increase the metabolizable energy values for broilers, that can be achieved by modifying genetically (conventional or transgenic) the soluble: non-soluble polysaccharides fraction, for instance.

Diet costs are sensitive to ingredient cost and quality. Diets formulated with simulated soybeans with enhanced nutritional value reduced diet costs in the following order: Hipro soybean meal with added energy value (less expensive), full fat soya, 47% crude protein soybean meal and 44% crude protein soybean meal (more expensive). The most economical advantage of the soya in the feeds occurs when metabolizable energy concentration is increased in the grain.

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Protein dispersibility index analysis: a tool to analyze the nutritional value of soybean meal

I. Debruyne[1]

Protein Dispersibility Index (PDI) has been developed as a tool for soy protein quality analysis of soybean meal for more than 30 years ago. The method, which is based on the use of an upgraded cocktail mixer, gives the amount of protein soluble after sufficiently long intensive mixing of a sample of soybean meal in distilled water. In many countries, the special mixer required isn't available. Consequently, crushers and feed producers had to import such a machine from the USA, or look for an alternative method. Recently though, the original mixer adapted to the official AOCS Method Ba 10-65 has been made available in other markets, such as Europe, North Africa and the Middle East.

New nutrition studies have shown that PDI is a superior method to Nitrogen Solubility Index (NSI) and other quality analysis methods, especially with respect to predicting the value for use in poultry feed. In reality, it is the Trypsin Inhibitor activity (TIU; Ba 12-57) in combination with the degree of protein denaturation that will determine the real nutritional value of a soybean meal. The TIU analysis method is not suitable for routine analysis, since too complex and open for improvement. To avoid such lengthy TIU analysis, crushers control the toasting process using an independent analysis of remaining urease activity (Ba 9-58) as an indicator of residual trypsin inhibitor activity. If urease activity is significantly down, TIU will be reduced to the required level of 2-5 mg/100 g.

While such low TIU normally corresponds with properly cooked soybean meal, it often is found also for overcooked or insufficiently cooked product. For determining the final value of the soybean meal in feed rations for poultry and other sensitive animal groups, residual protein solubility is used as a second quality specification. Often NSI analysis is used for this.

Two different NSI methods are used: stirring a soybean meal suspension in distilled water, or in a lightly alkaline solution (Ba 11-65). The analysis of the soluble nitrogen gives an idea of protein solubility. The alkaline method tends to give a rather high figure, since soy protein is more soluble at alkaline pH. In practice, alkaline NSI gives values in a very narrow critical range of 78 to 82%.

[1] Ignace Debruyne & Associates, Izegem, Belgium – ignace.debruyne@advalvas.be

NSI values, measured after stirring in water, tend to be quite low and difficult to repeat. The PDI method uses ten minutes of high-speed mixing (8,500 rpm) in distilled water, and gives values between 24 and 32 % in the critical range. Lower values indicate overcooking of the soybean meal, and consequently poor conversion when used in feed. Higher values are a sign of insufficient cooking, which will result in digestibility problems.

In the presentation, the various methods of analysis will be compared in more detail, with special attention to protein quality (overcooking vs. undercooking), and the link to urease and trypsin inhibitor activity. An improved method for Trypsin inhibitor analysis will be presented as well, based on the measurement of linear reaction kinetics, which are used to calculate the TIU.

The use of soybean protein sources in swine and poultry feeding

F. Goldflus[1]

Abstract

The animal feed industry has become increasingly dependent on plant protein sources, a situation also associated to the decrease in fish (meal) supplies and to the strong international / European Union pressure to ban the use of offal and meat & bone meals as raw materials. The main advantages of soybeans *(Glycine max)* in animal nutrition are its high protein content, proper amino acid profile, and excellent energy availability. Among the major problems of soybeans in terms of nutrition are their flatus-producing potential, deficiency of sulfur-containing amino acids, low digestible energy relative to gross energy, presence of anti-nutritional factors and negative correlation of crop yield with oil and protein content.

Heat-stable and heat-labile anti-nutritional factors of soybeans can significantly influence the performance of some animal species and categories. The presence of these anti-nutritional components in raw soybeans require different processing technologies in order to produce higher added value alternative soybean products. Therefore, it is essential to thoroughly understand each anti-nutritional factor present in soybeans, as well as their properties, sensitivity, and the physical-chemical effects of the steps of the process used on the nutritional value of this ingredient when selected as dietary protein source.

Anti-nutritional factors present in soybean grains

The chemical composition and average energy values of soybean grains (Table 1) (Lima, 1999), shows that, despite presenting low levels of sulfur amino acids, soybeans is the main lysine source in swine diets, complementing corn in the formulation. In addition, its significant oil content makes it an excellent energy source, although it does not present a high degree of metabolization as compared to its crude energy content.

[1] ADM BioProducts, Rua Padre Anchieta, 2454 – Cj. 1706/1707 – 80730-000 – Curitiba/PR – Brazil fabio_goldflus@admworld.com

Table 1. Nutritional value of soybean grains (Lima, 1999).

Nutritional Parameter	N	Average	Nutritional Parameter	N	Average
Energy, kcal/kg (swine)			*Protein/Amino acids, %*		
Crude Energy	14	5155	Crude Protein	40	36.63
Digestible Energy	4	4025	Lysine	6	2.34
Metabolizable Energy	4	3824	Methionine	5	0.47
Others, %			Methionine + Cystine	5	1.09
Ether Extract	28	15.62	Threonine	6	1.32
Crude Fiber	15	8.54	Tryptophan	6	0.45

N: number of samples.

Among the factors that impair soybean protein source composition and quality, the quality of the grain used is emphasized. The grain must contain an optimal nutritional profile in order to ensure a good quality raw material. Grieshop and Fahey (2001) verified nutritional differences among soybean samples derived from the USA, Brazil, and China (Table 2). Although the sample from China presented higher crude protein content (42.14%) as compared to the Brazilian sample (40.86%), its lipid content was lower (17.25%) than that found in the sample from Brazil (18.66%) or from the USA (18.70%). North-American samples also contained higher concentrations of essential and non-essential amino acids. In addition, the quality of the protein of the North-American soybeans was higher as compared to the others, as indicated by KOH solubility analyses.

Table 2. Mean nutritional composition of soybean grains from Brazil, China, and USA (Grieshop and Fahey, 2001).

	Country of Origin			
Item (%)	Brazil	China	USA	SEM[2]
Dry Matter	86.98 A	92.33 B	93.86 C	0.29
Organic Matter	94.89 A	94.58 B	94.56 B	0.05
Mineral Matter	5.10 A	5.42 B	5.44 B	0.05
Crude Protein	40.86 A	42.14 B	41.58 AB	0.31
Ether Extract	18.66 A	17.25 B	18.70 A	0.19
Protein Solubility[1]	27.68 A	29.18 B	30.80 C	0.47
Essential Amino Acids	16.64 A	17.39 B	18.51 C	0.23
Non-Essential AAs	21.52 A	22.12 A	23.29 B	0.29
Total Amino Acids	38.17 A	39.15 A	41.80 B	0.52

[1] Protein Solubility in KOH; [2] SEM = Standard Error of the Mean. Means followed by different letters are significantly different ($P<0.05$).

Raw soybeans present a series of anti-nutritional factors in their composition, particularly protease inhibitors, goitrogenic substances, lectins, estrogens, antigenic or allergenic proteins, flatulence factors/ oligosaccharides, saponins, tannins, and phytate. The basic characteristics of the anti-nutritional factors that have the largest impact on swine and poultry performance are described below.

Heat-sensitive anti-nutritional factors:

Protease Inhibitors: These protein can specifically react with some proteolytic enzymes derived from the digestive/endogenous secretions of animals, impairing the digestive efficiency and/or enzyme activity (Liener, 2000 and Silz, 2000). In poultry and weaning piglets, pancreatic trypsin inhibitors can significantly reduce the digestibility of dietary amino acids.

Lectins/Hemagglutinins: These glycoproteins are able to agglutinate erythrocytes of several animal species and to bind to sugar molecules on the grain, with no changes in its covalent structure (Pusztai *et al.*, 1990 quoted by Clarke and Wiseman, 1999). As they are highly resistant to the physical and chemical action of the digestive system, Liener (2000) observed that this class of anti-nutritional factors can affects the cells of the mucosa, by increasing the wear of the brush border, and causing atrophy of the microvilli, with consequent reduction of the surface area of absorption of the small intestine. They can also impair the immune system of the animal (Russett, 1999). This author mentioned that swine under the action of lectins develop coliform bacteria in the small intestine (Wilson *et al.*, 1980; Banwell *et al.*, 1985).

Under heat treatment, urease present in the grain looses its activity at a rate similar to the destruction of heat-sensitive anti-nutritional factors. Therefore, the industry uses the "urease value" to evaluate its soybean products. Values near zero reflect good processing conditions, but may also indicate excessive heat and possible changes in the specific original protein structure, and lower amino acid availability due to structural changes and formation of products deriving from the reaction of Maillard. Thus, additional analyses are recommended, particularly protein solubility and lysine percentage in the protein. The Brazilian Food Compendium (1998) suggested for soybean meal values of urease activity and protein solubility (KOH 0.2%) of 0.15 D pH (max.), and 80% (min.), respectively. Interestingly, the mean values of urease and solubility for soybean meal required by a large number of Brazilian companies are 0.03 to 0.16 D pH and 79.23%, respectively.

Heat-stable anti-nutritional factors:

Phytic acid: A large proportion of phosphorus in plants is present as phytic acid (*myo*-inositol-hexa-cys-di-hydrogen phosphate), or as its salt, phytate. Non-ruminant species are not able to digest the phosphorus included in the phytate structural complex, as they do not produce endogenous enzymes capable of breaking down the molecule. The use of the exogenous enzyme phytase renders the inorganic phosphorus available to monogastric digestion by hydrolyzing the phytic acid present in the grains.

Antigenic factors: These are globular proteins present in the germ of legumes, which are able to cross the epithelial barrier of the intestinal mucosa and can have adverse effects on the animal's immune function, such as "Immune Hypersensitivity Syndrome" (Liener, 2000 and Silz, 2000). This allergic reaction to soybean protein is possibly responsible for the lower performance of early weaned piglets, as the piglet undergoes several digestive, metabolic, and immune changes from birth up to eight weeks of age. These proteins, according to Stokes *et al.* (1987) quoted by Silz (2000), cause changes in the intestinal morphology, such as decrease in villi height and increase or not of crypt depth. The resulting effects include abnormalities in the movement of the digesta, impairment of nutrient absorption, and predisposition to enterotoxins and diarrhea (Li *et al.*, 1991 quoted by Thomaz, 1996). The main antigenic factors of soybeans were identified as glycinin and b-conglycinin.

Oligosaccharides: Although the poor digestibility of soybean carbohydrates can be attributed to polysaccharides, including some structural components of the cell wall that are insoluble, the soluble fraction of the oligosaccharides, raffinose and stachiose, may pose special problems. The largest of them is the lack of a-galactosidase secretion in non-ruminant species and humans (Clarke and Wiseman, 1999). These sugars, particularly raffinose, have been considered responsible for the poor utilization of soybean energy by poultry (Liener, 2000). The microbial population of the large intestine of swine is capable of breaking down non-starch polysaccharides and generating energy. However, Veldman *et al.* (1993), quoted by Clarke and Wiseman (1999), reported that the presence of a-galactosides causes fluid retention and microbial activity increase, leading to systemic effects, such as stimulation of intestinal motility, damage to the intestinal wall, and reduction of the hydrolysis of dietary components, affecting feed digestion as a whole. Table 3 shows the mean content of the main anti-nutritional factors present in soybean products used in animal nutrition.

Table 3. Anti-nutritional components in soybean, soybean meal, soy flour and soy protein concentrate (Russett, 1999 and Peisker, 2001).

Anti-nutritional/Product	Soybean	Soybean Meal	Soy Flour	Soy Concentrate
Trypsin inhibitor (mg/g)	45-50	1-8	5-8	<4
Glycinin antigen (ppm)	180000	66000	66000	<30
Lectins (ppm)	3500	10-200	10-200	<0.1
Oligosaccharides%	14	15	15	3
Saponin	0.5	0.6	0.6	0

The presence of anti-nutritional components in raw soybeans requires the use of different technologies for the production of added-value products.

These technologies include toasting, (dry or wet) extrusion, micronization, "jet-sploder", alcohol extraction, protein precipitation, and others. The characteristics of the process, and the quality and type of original grain employed, as well as the temperature and the pressure of the treatment used, must be taken into consideration when evaluating the nutritional value of the obtained product. The use of exogenous enzymes, such as phytases, amylases, and proteases, must also be considered to improve the final nutritional quality of soybean products.

Soybean meal (SBM) and de-activated full-fat soybeans

The main form of soybeans used in animal feeds is the meal, which results from the extraction of oil by solvent and heat treatment/solvent removal. The use of de-activated full-fat soybeans (toasted, extruded, and micronized) has increased as protein and energy alternatives in feeds. The different processes can have positive as well as negative effects on the quality of the processed protein source. The de-activation of heat-sensitive anti-nutritional factors by heat usually favors the digestibility of nutrients (amino acids and lipids) present in the grain (Penz and Brugalli, 2001).

Soybean meals with high protein contents, due to the lower inclusion of hulls during production, present higher amino acid digestibility for swine as compared to those with lower crude protein content, as seen in the results presented in Table 4 (Bellaver *et al.*, 1998).

Table 4. True digestibility coefficients of essential amino acids (mean %) obtained for soybean meals with different protein levels (Bellaver *et al.*, 1998).

Ingredient	N	Lysine	Methionine	Threonine	Tryptophan
SBM 44% CP	13	97.01	95.43	95.18	93.94
SBM 46% CP	10	98.26	97.41	97.52	97.66
SBM 48% CP	24	98.35	97.28	97.29	97.65

[1] grower pigs.

The energy level in soybean meal depends on the residual oil of the process, as well as on the fiber and mineral contents. Lower fiber and mineral levels mean not only higher protein and amino acid levels, but also higher available energy content for poultry (Dale, 2000).

The physical aspect of soybean meal also has a significant influence on its nutritional value. As mean particle size increased in swine feeds, from 150 to 900 microns, Fastinger *et al.* (2001) quoted by Mahan (personal communication) found linear losses in true digestibility coefficients of lysine, methionine, threonine, and total essential amino acids, as shown in Table 5.

Table 5. Effect of soybean meal particle size on amino acid digestibility[1] (Fastinger *et al.*, 2001 quoted by MAHAN, personal communication).

AA (%)	Mean Particle Size (microns)				SEM	P
	900	600	300	150		
Lysine	90.5	91.4	91.6	91.8	0.60	0.16
Methionine	92.8	94.4	94.9	94.8	0.50	0.10
Threonine	88.7	89.6	90.6	89.5	1.10	0.15
EAA (10)	91.2	92.0	92.5	92.5	-	-

[1] Swine; [2] SEM = Standard Error of the Mean

The Brazilian Ministry of Agriculture has only 3 classes of commercial soybean meal as a function of crude protein level: 44, 46, and 48%. However, some crushing units also offer SBM with 47 and 47.5% crude protein. Occasionally, it is possible to find products with 42-43% protein, which are mainly employed in ruminant feeds. Minimal ether extract is not guaranteed by the specifications, but maximum levels of crude fiber must be ensured.

Among the several types of heat de-activation of soybeans, extrusion has produced the best results for weaning piglets (Faber and Zimmerman, 1973), as this process used short periods of time (10-30 sec), high temperature (130 to 140°C) and pressure (30 a 60 atm), and, in some cases, humidity levels of 19 to 25% (Miyada *et al.*, 1993 quoted by Thomaz, 1996). However, the results obtained by SAKOMURA *et al.* (1998), during 49 experimental days with broilers, did not show significant differences ($P > 0.05$) among the evaluated full-fat soybeans, either extruded or toasted, as presented in Table 6.

Table 6. Effect of soybean meal (SBM) with oil, extruded full-fat soybeans, and toasted full-fat soybeans on the performance of 1-49-day-old broilers (Sakomura *et al.*, 1998).

Parameter/Product	SBM + Oil	Extruded	Toasted
Feed Intake, g	4753	4787	4692
Weight Gain, g	2316 A	2405 B	2344 AB
Feed Conversion	2.057 A	2.002 B	2.004 B
Carcass Yield, %	79.13	79.70	78.62
Abdominal Fat, %	3.64	3.90	3.89

Means in the same line followed by different letters are significantly different ($P < 0.05$).

Faria *et al.* (1995) studied the effect of the use of extruded or toasted soybean grain on layer performance. Urease activity (DpH) and protein solubility (%) were 0.2 and 87.71 for soybean meal, 1.24 and 91.87 for

toasted soybeans, and 0.01 and 83.98 for extruded soybeans, respectively. The authors concluded that the extruded soybeans could totally or partially replace soybean meal in the diet (Table 7). However, when 100% soybean meal was replaced by toasted soybeans, layer performance was reduced (Table7). The worst result was observed with the use of toasted full-fat soybeans, which was probably caused by under-processing, as indicated by urease and protein solubility results.

Table 7. Effect of the substitution of soybean meal (SBM) by toasted or extruded full-fat soybeans on the performance of 33-50-week-old layers (Faria *et al.*, 1995).

Diet	Substitution, %	Egg Production, %	Feed Intake, g	Egg Weight, g	Feed Conversion
SBM	0	63.67	81.57	52.66	2.59
Toasted S.	50	60.60	78.78	53.14	2.58
Toasted S.	100	59.56	78.47	52.32	2.67
Extruded S.	50	63.88	80.57	51.67	2.56
Extruded S.	100	62.27	78.69	51.34	2.57

Soybean protein concentrate (SPC) and soybean protein isolate (SPI)

The positive aspects related to the processes of soybean protein concentrate and isolate, and hence the absence of heat-stable or heat-sensitive anti-nutritional factors in these products, makes them excellent protein sources, which can be used in high added-value diets.

Soybean protein concentrate is obtained by the almost complete removal of oil and of the water-soluble non-protein fraction by washing with alcohol/ethanol in water medium (El-Dash, *et al.*, 1994). Due to the extraction of oligosaccharides (raffinose and stachiose), which are present in soybeans flakes and have anti-nutritional properties for some animal classes, the product offers a relatively stable protein level of 64%, with high nutritional quality.

The digestibilities of the protein content of the soybean protein concentrate and isolate for swine are similar that of high quality skimmed powdered milk (Visser and Bremmers, 1999), and higher that of soybean meal (Sohn *et al.*, 1994). Experimental studies with broilers, carried out by Batal and Parsons (2003), indicated that the apparent digestibilities of energy and amino acids those products were higher than that of soybean meal, probably due to the extraction of soluble carbohydrates. These ingredients reduce the transitory hypersensitivity reaction in piglets caused by other heat-processed soybean protein sources, which is manifested as increase in intestinal villi height (Hancock *et al.*, 1990).

The soybean protein isolate is the main protein fraction of the soybean grain, as defined by Cromwell (2000). Further processing to soybean protein isolate involves an alkaline extraction and centrifugation to remove cotyledons, followed by an acid precipitation, after which soy whey is removed (Johnson and Kikuchi, 1989 quoted by Batal and Parsons, 2003).

When working with increasing levels (0, 25, 50, 75, and 100%) of skimmed powdered milk substitution by soybean protein isolate in diets for 21-35-day-old piglets (5.78 ± 0.15kg), which the same protein (20.50% CP), same energy (3420 kcal DE/kg), and same lactose (8.16%) levels, SILZ (2000) verified that, up to 32.01%, the substitution improved weight gain, and that up to 42.15%, it improved feed conversion ratio. Higher substitution levels impaired performance. However, intestinal morphometry, and the relation of pancreas weight to carcass weight in 35-day-old piglets remained unchanged as milk protein was replaced by soybean protein.

In a comparison of alternative protein sources (sugarcane yeast - SCY; soybean protein isolate - SPI ; powdered egg - PE; blood plasma - BP and corn gluten - CG) used along with soybean meal in the composition of pre-starter (1-7 days of age) diets for broilers, Longo *et al.* (2003) found that the addition of yeast increased feed intake, whereas the treatment with soybean isolate reduced feed intake. These changes in intake, associated to similar weight gain among treatments, resulted in differenced in feed conversion ratio: birds fed soybean isolate and blood plasma improved feed conversion, whereas those fed yeast had worse feed conversion (Table 8). Batal and Parsons (2003) suggested that the use of soybean protein concentrate and isolate may have a good potential in starter feeds for 1-3-week-old broilers as compared to soybean meal.

Table 8. Performance of 1-7-day-old broilers fed different protein sources (Longo *et al.*, 2003).

Treat.	Weight Gain (g/bird)	Feed Intake (g/bird)	Feed Conversion (g/g)
SCY	119.73	144.2 A	1.21 C
SPI	123.77	124.2 C	1.01 A
PE	112.24	125.4 BC	1.12 B
BP	122.47	128.0 BC	1.05 A
CG	115.71	130.2 BC	1.13 B
Pr>M	0.1236	0.0003	0.0001

Means in the same column followed by different letters are significantly different ($P<0.05$).

Final considerations

The differences among the several processes employed to eliminate the anti-nutritional factors present in the soybean grain have significant influence on quality of the finished products. Therefore, it is essential to thoroughly understand each anti-nutritional factor present in soybeans, as well as their properties, sensitivity, and the physical-chemical effects of the steps of the process used on the nutritional value of this ingredient when selecting the appropriate product for feeding each animal species and class.

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Novelties in formulation of soybean products with functional compounds

S. S. Koseoglu[1]

Soy foods have been consumed in Asian countries for centuries. The US currently produces more soybeans than any other country. Up until late 1980's, the soybean production in the U.S. was mainly geared towards export business selling the beans, soybean meal and crude oil. Limited niche markets such as protein isolates / concentrates and lecithin have taken advantage of abundant soybean by-products to produce variety of ingredients for the food manufacturers and formulators. However its utilization in main food systems did not reach to a significant level until 1990's when researchers began to discover a myriad of disease-preventing properties of this functional food. There is conclusive evidence that soy protein can significantly reduce the risk of coronary heart disease, and decrease the oxidation of LDL cholesterol and improve vascular reactivity. A number of publications dealing with soy also show a significant amount of evidence on the role of soy in improving bone health and alleviating symptoms associated with the menopausal transition.

"Functional foods" has become a familiar term with consumers today. Soy, a functional food, is a success story as it is being utilized in many different forms and products. Many of the methods used in production, characterization of individual bioactive, and functionality, can easily be adapted to other specialty food protein products. Soy is viewed as a functional food because of the numerous health benefits attributed to its consumption. This presentation will provide a review of functional food ingredients from soyfoods and their utilization in commercial food products in the USA.

[1] President, Filtration and Membrane World LLC 209 University Drive East, Suite B-2 College Station, Texas 77840 Tel: 979-846-1951 Fax: 979-690-7309
E-mail: membrane@membraneworld.com URL: www.membraneworld.com

Soybean as a multiple source of biomolecules in food and health products

F. Shahidi[1]

Soybean has been a main food item for many centuries. Recent advances in the areas of agriculture and nutritional biochemistry have resulted in the production of increasing amounts of soybean for its oil, protein and other biomolecules with potential health benefits. Isoflavones in soybean have been well-recognized for their myriad of health-related advantages related to estrogenic/anti-estrogenic activities and anticarcinogenicity. However, other biomolecules such as lecithin, saponins, phytic acid and tryprin inhibitors also occur in soybean. The role of these phytochemicals in health promotion and disease prevention is of much interest. The use of soybean and its components in production of food and nutraceutical applications is also discussed.

Introduction

The Materia Medica of the Chinese Emperor Shen Nung first recorded the use of soybean in 2838 BC (Adolph and Kiang, 1920). Tofu, a protein-rich curd made from hot-water extract of soybean, was developed in China and then introduced into Korea and Japan between the 2nd and 7th centuries. The use of soybean in food in the United States occurred during the second half of the 19th century by Chinese immigrants and its cultivation began in 1890.

The current world production of soybean is over 180 million metric tons, with US, Brazil and Argentina contributing 43, 24 and 16% to the total amount, respectively. The South American production of soy in terms of growth has been more than that of North America over the last 7 years. While the overall growth is 5.6/year, US growth has been at 3.4% while that of Brazil and Argentina averages 10.5%. Nearly 84% of soy is crushed for oil and meal production, and 8.6% of it is used directly for food applications, mainly in the Asian countries. The defatted meal is also used in the preparation of textured soy flour, protein concentrate and protein isolate. The direct food uses of soybean include tofu, soymilk, miso, soy sauce, natto and tempeh, among others. In addition, hydrolyzed soy proteins are used in baby formulas as a protein source.

[1] Department of Biochemistry, Memorial University of Newfoundland, St. John's, NL, Canada, A1B 3X9

Because of soy's effectiveness in lowering total cholesterol (9.3%), LDL-cholesterol (12.9%) and triacylglycerols (10.5%) with a small increase (2.4%) in HDL-cholesterol (Anderson *et al.* 1995), the US Food and Drug Administration (FDA) approved a health claim for relationship between the consumption of soy protein and reduced risk of coronary heart disease (Department of Health and Human Services, 1999). The following claim may now be used on qualified soy products: "Diets low in saturated fat and cholesterol that include 25g of soy protein a day may reduce the risk of heart disease. One serving of [name of food] provides [amount] g of soy protein." While the FDA-approved health claim is based on soy protein content, a number of other physiologically active components may contribute to its cholesterol-lowering effect. These include amino acids, isoflavones, saponins, phytic acid, trypsin inhibitors, fiber and globulins (storage proteins in soy).

Soybean, following oil extraction, contains 44-48% protein. Meanwhile, defatted soy flour and grits as well as textured soy flour contain 52-54% protein and these are used in foods and baking as well as extenders and meat alternatives, respectively. Soy protein concentrates and textured soy protein concentrates have a protein content of 62-70% and these are used as milk replacer, food ingredient and meat alternative/extender, respectively. In addition, soy protein isolates have a protein content of 86-88% and these are used as food ingredients, nutritional products, beverages, meat alternatives, soyfoods and energy bars.

While 95% of all soybean used for food is in Asia, Africa uses 3.7%. In comparison, North America, Europe, South America and Central America use 0.6, 0.5, 0.2 and 0.1%, respectively. In this regard, soybean has played a major role in providing the necessary protein for feeding the world over the past 40 years or so.

While soybean-based products are of much interest in many functional foods and as ingredients, challenges related to undesirable flavor of soy-based products must be considered. The co-products of soybean such as lecithin, isoflavones, saponins, phytic acid and trypsin inhibitors are discussed in the following sections.

Lecithin

Lecithin is a main by-product of soybean oil refining process and constitutes 0.5-1.5% of soybean seed, as such. Crude lecithin is a mixture of phospholipids, glycolipids, carbohydrates and triacylglycerols. However, lecithin is generally referred to a group of phospholipids that act as natural surfactants and are diacylglycerols with a phosphate ester attached. The major variables are the fatty acids attached and the base (choline, ethanolamine, serine or inositol) attached to phosphate.

In soybean oil, lecithin is often referred to phosphatidylcholine (13% choline) which represents 23% of the total phospholipids present (Canty and Zeisel, 1994). Lecithins is an important source of choline which is essential for the structure integrity and signaling functions of the cells and also provides a source of methyl groups necessary for normal metabolism (Zeisel and Blusztain 1994).

Dietary sources generally provide up to 6g of lecithin, equivalent to 0.6-1.0 g of choline per day; the richest source being beef liver. The therapeutic benefits of lecithin include lowering of cardiovascular disease risk, preventing abnormal fetal development, reducing some form of male infertility, promoting healthy liver, improving memory and cognition and preventing or lowering adverse reaction to various drugs. Some benefits of lecithin/choline are described below. On the basis of what is given below and the safety record as well as its reasonable cost, lecithin may be recommended for reducing the risk of coronary heart disease, fatty liver and mild memory impairment.

Plasma homocysteine reduction by lecithin

Increased coronary heart disease (CHD) and risk of stroke have been associated with high plasma homocysteine levels (Wold *et al.*, 1998). Homocysteine is formed via demethylation of methionine. Plasma levels of homocysteine may increase due to deficiencies in vitamins B6, B12 and folate. It appears that choline deficiency may serve as a risk factor for hyperhomocysteinemia.

Hepatoprotectant effect

The National Academy of Sciences in 1998 recommended choline intake of 425-550 mg/day. Choline deficiency may result in fatty infiltration of the liver (Ghoshal and Farber, 1993). In humans, the primary ethiology of fatty liver is excessive ethanol consumption (Eaton *et al.*, 1992). Thus, men that consumed 1350 mg phosphatidylcholine per day had a significantly improved liver function as compared to the placebo group (Kneuchel, 1979). Fatty liver is not only caused by ethanol consumption as patients receiving total parenteral nutrition (TPN) also suffer from choline deficiency. Thus, consumption of 20g of lecithin every 12 h for 6 weeks showed a significant decrease in hepatic steatosis occurring as shown by a CT scan (Buchman, 1992).

Memory Enhancement by Lecithin

Lecithin is known to provide choline to neurons in the central nervous system. Acetylcholine has long been recognized as a neurotransmitter in the mammalian brain. The effects include controlling of the movement,

sleep and memory (Whittaker 1998). While lecithin has little therapeutic value for Alzheimer's dementia, it might be able to improve memory in non-demented individuals (e.g. Ladd, 1993).

Isoflavones

Isoflavones occur naturally in soybean and are strikingly similar in their chemical structures to mammalian estrogens. In soybean, they are present at 0.1-0.5% and have gained considerable attention for their potential health benefits in the prevention of cardiovascular disease, cancer, osteoporosis, premenstrual and postmenopausal symptoms and hypocholesterolemia, among others (Zubik and Meydani, 2003). Isoflavones in soybeans are found in the aglycone, glucoside, acetylglucoside and malonglucosides, but the glucoside forms predominate (US Department of Agriculture, 1999). The major isoflavones in soybeans are daidzin (7, 4'-dihydroxyisoflavone-7-glucoside) and genistin (5, 7, 4-trihydroxy isoflavon 7-glucoside) and their corresponding aglycones daidzein and genistein. The total content of isoflavones in different soy products is varied. Thus, soy milk contains 80-165, tofu 202-347, soy sauce 1-14, miso 228-235, and tempeh 531-538 mg of isoflavones per kg of sample (Murphy *et al.*, 1999). These values reflect a clear loss of isoflavones during processing. Comprehensive analyses of the isoflavone content of numerous soy foods generally indicate that most products contain 0.1-0.3 mg/g of total isoflavone (Coward *et al.*, 1993). The health benefits associated with soy isoflavones are described briefly below.

Cardiovascular disease

The first report on plasma cholesterol lowing effect of soy protein in hypercholesterolemic rabbits was communicated by Hamilton and Carroll (1976). However, the meta-analysis of Anderson *et al.* (1995) paved the way for general acceptance of cholesterol lowering effects of soy protein. These effects were found to be due to the presence of isoflavons in soy (Siddiqui and Siddiqui, 1976).

The oxidation of LDL cholesterol is a key event in the pathogenesis of atherosclerosis. It was found that 56 mg isoflavone (15 g soy protein/day; 17 days) reduced LDL oxidation by extending the lag time by 8% (Wiseman *et al.*, 2000). The effects were not observed when low isoflavone concentrations (1.9 mg) were consumed, suggesting that isoflavones were responsible for the antioxidant activity of soybean. Similar results were obtained by Jenkins *et al.* (2000) reflecting reductions in LDL cholesterol oxidation by 8.5 ∀ 3.3% and increasing the concentration of HDL cholesterol by 6.4 ∀ 2.4%. Isoflavones, being estrogen-like, may also enhance branchial

artery flow-mediated dilation and hence reduce stiffness of the arteries that could contribute to cardiovascular disease risk.

Cancer

It appears that the incidence of breast cancer is lower in Asian women primarily due to their higher consumption of isoflavones from soy foods and also having a longer menstrual cycle. In countries with high consumption of soybean products the risk of hormone-related prostate and breast cancer is also much lower than in populations with a low consumption of soybeans (Morton *et al.*, 1997). In addition, a high consumption of isoflavone diet has been shown to inhibit tumorigenesis in several animal models for prostate cancer (Messina *et al.*, 1994). Isoflavones are also known to protect against bowel, colon and other non-hormonal cancers as evidenced by both animal and clinical studies (Bennink, 2001).

Isoflavones and Menopausal Symptoms

Menopause is associated with the development of several conditions, ranging from the incidence of hot flashes to osteoporosis and bone health, associated with acute ovarian estrogen loss. The incidence of hot flashes was found to be much lower in women from Asian countries, where consumption of soy products is higher (Blair *et al.*, 1996). Blair *et al.* (1996) have shown that genistein administered in the diet can significantly increase the mass of weight bearing bones compared to control in rats made surgically postmenopausal. The effects observed in animals are similarly observed in humans (Potter *et al.*, 1998).

Soy saponins

Saponins are composed of sugars bound to alkaloid, steroid or triterpene compounds and have detergent surfactant properties. The aglycone portions of saponins are known as genin or sapogenin. Soy proteins contain 0.1-0.3% saponins, at least five of which have been isolated (Arditi *et al.*, 2000). Many studies have shown that saponins have blood cholesterol-lowering properties rendered by their binding of cholesterol. The bound cholesterol is then passed to colon and excreted. Saponins have also been known to reduce the risk of cancer and heart disease. The binding of bile acids by saponins removes cholesterol metabolites from the colon area and hence reduces the risk of colon cancer. In addition, saponins inhibit cancer cell proliferation by binding to them (Lipkin, 1995).

Phytic acid

Phytic acid, a simple ringed carbohydrate with six phosphate groups attached to each carbon is present in soy protein at 1-2%. Phytic acid shows a

remarkable antioxidant function by chelating pro-oxidant divalent metal ions such as those of iron and copper. Both *in-vivo* and *in-vitro* studies have demonstrated the striking anticancer effect of phytic acid (Shamsuddin, 2002).

Trypsin inhibitors

Natural proteinase inhibitors have the ability to specifically inhibit the catalytic activity of proteolytic enzymes in biological systems. Soybean Bowman-Birk inhibitor (BBI) has been found to possess chemopreventive properties (Meyskens, 2001). Inflammation associated with tumor promotion is also inhibited by BBI (Kennedy, 1998). Soybean BBI may exert its effects via different mechanisms, including antiangiogenic activity.

Conclusions

Soybean and its products provide important biomolecules which contribute to health promotion and disease prevention. While nutraceutical use of these biomolecules, in the isolated form, may be practiced, soybean protein-based products may serve as functional food/functional food ingredients for which health claims could be made. However, challenges related to potential undesirable flavors in soy-based products needs to be addressed when formulating such products.

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Physiologically functional effects of soy protein and extracts on hypertension and bone metabolism in the model mouse

Y. Kawamura[1]

Abstract

Effects of orally administrated soy proteins extracts on blood pressure of the spontaneous hypertension rat(SHR) and of soy extracts on bone metabolism of ovariectomic mouse, were investigated to know the physiological function of soybean based foods. The possible components and mechanisms responsible for these function were discussed. Of 4 kinds of proteins and a disaccharide administrated orally to SHR, only soy protein isolates exhibited the significant anti-hypertensive action. The angiotensin I-converting enzyme (ACE) inhibitory activity of peptic hydrolysates of soy protein was postulated as an possible mechanism for that effect. The four angiotenshin I-converting enzyme inhibiting peptides were separated from the hydrolysates and sequenced. The homologous sequence of these peptides were found in the main storage proteins of soybean. The ethanol extracts of soybean were orally administrated to ovariectomic mouse once a day for 2 weeks. The markers of bone absorption in urine, Pyridinoline(Pyr) and deoxypirinoline(Dpyr), were quantitated for 9 to 19 weeks. The raised excretion of Pyr and Dpyr due to decreased estrogen level by ovariectomy was significantly improved to the original level 4 to 6 days after administration and that level was kept for 1 to 2 weeks even after the administration stopped. Thus, s food items containing soy products might play a physiologically potent role in preventing life-style related diseases such as hypertension and symptoms after menopause.

Keywords: soybean, ACE inhibitory peptides, bone absorption,

Introduction

Recently, the relationship between food and health has drawn much attention especially from the view point of improvement or prevention of so called life style related disease. Physiologically active peptides can be released by enzymatic proteolysis of food proteins and may act as potent modulators of metabolism. The possible regulatory roles of peptides relating to food proteins, include immune defense, opioid action, antihypertensive activities, etc. Hypertension is a first place in frequency that adults over thirty years

[1] Department of Applied Life Chemistry, Graduate School of Agriculture Kinki University, Japan

old come to hospital, and is the most comon and high risk for arteriosclerosis, stroke, myocardial mulfunction, and renal disease in the developed countries. Angiotensin I-converting enzyme (EC 3. 4. 15. 1; ACE) plays an important role in controlling blood pressure. The synthetic ACE inhibitors such as captopril,and enalaprilare now in the first choices for the clinical treatment of hypertensive patients.[1,2] Recently, many ACE inhibitory peptides have been isolated from various food proteins such as milk whey,[3] casein,[4-6] zein,[7,8] tuna,[9]sardine,[10] wheat gluten,[11]and bovine blood plasma,[12] soy sauce,[13] soybean paste[14] and soymilk.[15]

Another keen interest is the potent and preventive action of soybean products for bone absorption, the elevation of which leads to osteoporosis.

In the present study, ACE inhibitory peptides derived from soy protein, and the hypotensive action by oral administration to SHR are addressed. The improvement of bone absorption by oral administration of soy extracts to ovariectomic mouse is also introduced.

Materials and methods

Materials. SPI (Fuji-pro R) was obtained from Fuji oil Co., Ltd. (Osaka, Japan). Hippuryl-L-histidyl-L-leucine (HHL) was obtained from the Peptide Insutitute (Osaka, Japan). ACE from rabbit lung acetone powder were obtained from Sigma Chemical Co. (U.S.A.). Pepsin (1:10000), TNBS (special grade), and the other reagents were obtained from Wako Chemical Co. (Osaka, Japan). Sephadex G-25 and G-10 for Gel pearmiation chromatograhy, and MONO-S and MONO-Q, for ion-exchange chromatography, were obtained from Pharmacia Fine Chemicals (Sweden).

Assay of ACE inhibitory activity. The activity of ACE inhibition was assayed by a modification of the method of Cushman and Cheung.[16] Briefly, an ACE solution containing 100 mM sodium borate buffer (pH 8.3), 300 mM NaCl, 8 mU ACE, and an appropriate amount of the inhibitor was preincubated for 10 min at 37C. A substrate solution containing 100 mM sodium borate buffer (pH 8.3), 10 mM HHL and 300 mM NaCl was also preincubated at 37 C. The reaction was initiated by mixing the two solutions, and terminated by adding 1N HCl after incubation for 30min. Hippuryl acid liberated was extracted with ethyl acetate, followed by quantitation of it by measuring absorbance at 228 nm .

Determination of molecular weight. Molecular weight of the purified peptide was determined by gel permeation chromatography using a HPLC. The molecular weight makers used were as follows: bovine serum albumin (MW

66,000 Da), Carbonic anhydrase (MW 29,000 Da), cytochrome C (MW 12,900 Da), angiotensin I (MW 1,296.5 Da).

Sequence analysis. Sequence analysis were performed by stepwise Edman degradation using a gas-phase automated sequenator (Applied Biosystems, model 477A), coupled with HPLC identification of the resulting phenyl-thiohydantoin (PTH)-amino acid derivatives.

Measurement of blood pressure

Spontaneous hypertension rat(SHR) were kept individually in steel cage in a room kept at 25C with a 12 h light-dark cycle, and fed a conventional diet. Tap water was feeded ad libitum. Peptide was dissolved in PBS and administered orally using a metal gastric zonde for rat. Control groups were administrated with the same volume of PBS. Systolic blood pressure (SBP) was measured by the tail cuff method using an instrument(Natsume Co. Ltd., Tokyo, Japan) after warming the rat in a chamber maintained at 35 C for 10 min. All results are expressed as means ±S.E.M. The significance of the differences was analyzed using Student's *t* test.

Results and discussion

SPI was hydrolyzed by pepsin, a digestive protease in stomach, to investigate the ACE inhibitory activity of the hydrolysates. ACE inhibitory activity was developed as the hydrolysisOproceeded. OThe inhibitory activity of hydrolysed SPI is much higher than that of non-hydrolyzed SPI. IC50 values of the hydrolysed SPI and non-hydrolysed SPI were 0.35 mM and 2.0 mM, respectively. These results suggest that the peptic hydrolysis produces the potent ACE inhibitory peptides from SPI. Isolation of ACE inhibiting peptides from the peptic hydrolysates.

The hydrolysed SPI was centrifuged to remove undissolved residues, then, fractionated into two major fractions with high molecular weight and low molecular weight (less than 10kDa) by gel permiationchromatography on Sephadex G-25. The low molecular weight fraction was futher fractionated into three fractions, I, II, and III by Sephadex G-10. The fractions were futher purified with a cation-exchange chromatogramphy on MONO-S. ACE inhibitory activity was detected in three large peak . The three fractions (pI, pII, and pIII) with high ACE inhibitory activity were purified by reversed-phase HPLC. By repeating HPLC, 4 peaks (I-B2, II-A1, II-B2, and III) were finally collected as the purified peptide with ACE inhibitory activity. The amino acid sequences of the four peptides determined were heptapeptide for I-B2, II-A1, and II-B2, and hexapeptide for III. Three peptides other than I-B2 contained Phenylalanin at C-terminal of the peptides. IC50 of the

peptides were 60, 150, 40, and 70 uM,for I-B2, II-A1, II-B2, and III, respectively. These values were not so high comparing with those of the inhibitory peptides from various food reported previously. The comparison of homology of the peptide sequence indicated all of them were identified in the main storage proteins of soybean,²-conglycinin and glycinin, which occupied approximately 70% of total protein of SPI. The same sequences as those of I-B2 and II-A1are located in ±- and ±'-subunit of ²¿-conglycinin. The same sequence as that of FII-B2 is located in A4 subunit of glycinin. The sequence for III is found in ±'-subunit of ²-conglycinin. Highly homologous sequence with I-B2 and II-B2 are found in vicilin and legumin of broad bean. These results suggest the possibility that ACE inhibitory peptides are produced by protease digestion from main proteins of broad bean.

In relation to structure and activity of the ACE inhibitory peptides, some authors13-15) reported that the peptides with the strong inhibitory activity have Pro, Phe, or Tyr at the C-terminus and contain hydrophobic amino acids in the sequence. Although action of intestinal proteases on the peptides is not avoided before absorption in intestine, the peptides other than II-A1 may be possible to leave di- or tripeptides with Phe and Tyr at the C-terminus and hydrophobic amino acids in the sequence after the proteolysis ,leading to the increased absorption from the intestine. Thus, soy protein products is considered to have the potential as physiologically functional food with anti-hypertension as well as anti-hyperlipidemia.

Effects of soybean extracts on bone metabolism of ovariectomic mouse

The ethanol extracts of soybean were orally administrated once a day for 2 weeks to ovariectomic mouse, which exerted examperated bone absorption due to the decreased level of estrogen. The markers of bone absorption in urine, Pyridinoline(Pyr) and deoxypirinoline(Dpyr), were quantitated for 9 to 19 weeks. The raised excretion of Pyr and Dpyr was significantly decreased to the original and normal level 4 to 6 days after administration and that level was kept for 1 to 2 weeks even after the administration stopped. Thus, soybean and food items containing soy products might play a physiologically potent role in preventing life-style related diseases such as hypertension and symptoms after menopause.

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Sterols from soybeans and other sources in cholesterol reduction

J. Zawistowski and D. D. Kitts[1]

Introduction

The evolution of value-added food products with specific health benefits has come to the forefront in the last decade as a result of consumer pressure to establish food systems that are designed to satisfy a perception of chronic disease prevention, in addition to meeting high quality standards for taste and nutritional requirements. The food industry sectors has accepted consumer challenges willingly for purposes to revolutionize the wellness factor expected with high quality foods and in turn facilitate a means of differentiating their product from others on the competitive food market. In addition, the creation of a new food category often called Functional Foods and/or Nutraceuticals has occurred as a result of recognizing the need to reduce health care costs of an aging population and fact that the consumer today has an enhanced awareness concerning the link between diet and health. Functional foods and beverages represent significant growth in the global food market. In the US alone (2002), sales of $ 59 billion within the consumer packaged goods health and wellness industry have been reported. This represents 7.3% growth over 2001 and an 11% compound annual growth rate for functional foods. With over 35,000 botanicals known to generate health benefits, functional foods and nutraceuticals is the fastest growing category on the food market.

There is no doubt that health is a major selling point to generate health benefits in the food and dietary supplement industry today, however, health benefit claims are not always based on sound scientific evidence.

Phytosterols represent one of the most intensely studied group of nutraceuticals in the area of cardiovascular diseases. This group of compounds has been clinically proven to reduce blood cholesterol. With nearly 100 million Americans and another 100 million Europeans experiencing elevated cholesterol levels, phytosterols containing functional foods may offer preventive solutions that are safe and effective alternatives or complementary to medical strategies to lower blood cholesterol. Based on numerous clinical studies, the US National Cholesterol Education Program

[1] Forbes Medi-Tech Inc., and Foods Nutrition and Health, University of British Columbia, Vancouver, Canada

(NCEP) has recommended that phytosterol consumption in the range of 2g/day is a contributing factor to the primary prevention of coronary heart disease.

In the US, the Food and Drug Administration (FDA) has adopted the GRAS (Generally Recognized As Safe) rulings as a regulatory venue for the approval of functional foods by approving stanol and sterol esters for use in margarine products such as Benecol ® and Take Control ®. These spreads are products of the Finnish company, Raisio Oy Ltd. and the Anglo-Dutch food giant, Unilever, respectively. Both spreads reached the American market in May of 1999. FDA clearance under the GRAS notification process for use in food products has also been approved for Reducol™produced by Forbes Medi-Tech Inc., a Canadian company, as well as CoroWise™ sterols of Cargill Co. These products have also been approved for use as a dietary supplement under the Dietary Supplement Health and Education Act (DSHEA). The FDA issued an interim ruling that allowed a health claim that links the consumption of plant sterol and stanol esters to reducing risk of coronary heart disease (CHD) in September 2000. Recently, the FDA has issued a letter of "Enforcement Discretion" that allows Reducol™and CoroWise™ to use the same claim that is stipulated in the interim ruling. The stated health claim – "may reduce the risk of heart disease" is typical of what is allowed to be on a label of food products.

The purpose of this paper is to describe the factors that have been proposed to explain the effectiveness of different phytosterols as dietary supplements in food systems as cholesterol lowering agents.

Phytosterols chemistry and occurrence

Phytosterols are lipid-like compounds found in plants. Soybeans, rapeseeds and coniferous trees are three major commercial sources of phytosterols. Soybean sterols and other sterols derived from oilseeds are obtained during oil processing as byproducts of vitamin E manufacturing. In the contrary, coniferous sterols are derived from tall and pitch oil using extraction, distillation and crystallization processes. Phytosterols are essential for stabilizing cell membranes in plants. A similar role is shared by cholesterol in animals. More than 40 plant sterols have been identified, of which ß-sitosterol, stigmasterol and campesterol are the most abundant (1). These particular sterols represent 4-desmethyl sterols that share an identical ring structure with cholesterol, but differ only in respective side chains (Figure 1). For example, the presence of a side chain substitution of a methyl-(campesterol) or an ethyl (sitosterol) group on carbon 24 distinguishes different sterols. Moreover, the additional double bond at position 22 is unique for stigmasterol (2). Phytostanols are a less abundant class of sterols

that are found in oil seeds and wood pulp alternatives and contain fully saturated ring structure as evidenced by the lack of the carbon-carbon double bond found in both cholesterol and phytosterols. The hydrogenation of plant sterols results in the formation of plant stanols; thus the saturation of the 5 position of campesterol produces campestanol and a similar positional saturation of β-sitosterol will yield sitostanol. Soybean phytosterols contain mainly unsaturated sterols with a low concentration (about 2%) of saturated counterparts (stanols). On the other hand, coniferous sterols contain significant amount of stanols, around 10% and more.

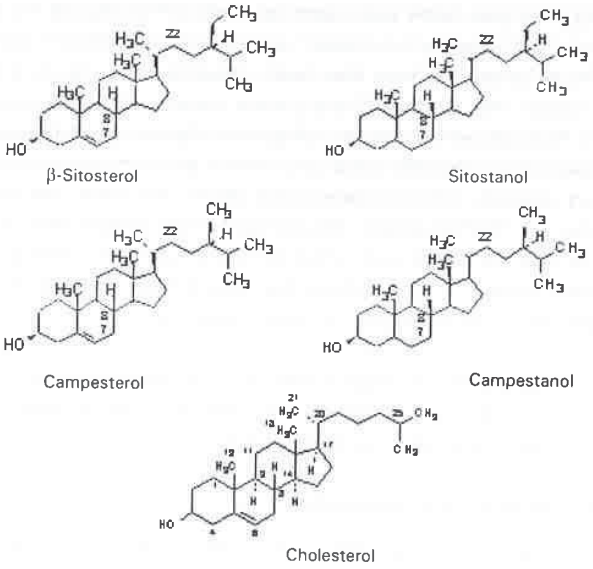

Figure 1. Chemical structure of some phytosterols, stanols and cholesterol

Efficacy of phytosterols to inhibit cholesterol absorption

Plant sterols and stanols are consumed at approximately 100-300 mg/day and 20-50 mg/day, respectively, as part of a typical Western diet. Fortification of conventional foods with plant sterols can significantly increase the daily intake of these particular phytochemicals. For over 50 years, numerous studies have reported a cholesterol lowering property associated with both types of sterols (3-10) and as a consequence may contribute to a reduced the risk of coronary heart disease. Some studies have demonstrated that the ingestion of sitosterol at 3-6 g/day leads to a decrease total serum cholesterol by 7-9%. Most of the published data showed that a daily intake of 2-3g of phytosterols lowers LDL-cholesterol

concentrations by 10-15% (1). Subsequently, consumption of 2g/day of either sterols or stanols may reduce the risk of heart disease by about 25% (1). A dose-response relationship between intake levels of phytosterols and a cholesterol lowering effect indicates ranges of efficacy for total cholesterol, LDL-cholesterol and LDL/HDL cholesterol (7). In adult subjects with a maximal negative cholesterol balance, cholesterol inhibition is achieved with feeding 3 g phytosterol/day (4), although other workers showed similar effectiveness for sterols consumed at 1.8g/day (11). Recently, Weststrate and Meijer (6) showed that sterol and stanol esters are comparably effective if administered to mildly hypercholesterolemic subjects at 3.3 g/day. Furthermore, free sterols are equally efficacious as sterol/stenol esters. Jones at al. (9) showed 15% decrease in the plasma LDL-cholesterol levels were hypercholesterolemic men were fed a diet containing 1.7 g Reducol™ (mixture of free sterols and stanols) per day for 30 days in a parallel study design. No change in cholesterol synthesis rates or HDL-cholesterol and triacylglycerides has been observed (9). Although most of the reported studies have been conducted by feeding phytosterols to subjects three times a day, twice a day consumption of sterols showed similar efficacy (6). Furthermore, Plat et al. (12) have reported that a once a day consumption of 2.5 g plant stanols yield the same efficacy as consumption of the same amount of stanols divided over the three meals. A summary of the number of studies that have reported cholesterol lowering and LDL-cholesterol lowering power from plant sterol containing food systems is given in Table 1.

Table 1. Randomized Double Blind Trials with Margarines containing Sterols

Type of Sterols	No. of Subjects[1]	Age[2]	Duration (weeks)	Daily dose (g)	Reduction in serum LDL (mmol/l)
Stanols	510	33 - 50	2 - 52	1.0 - 4.0	0.28 - 0.64
Sterols	300	33 - 45	2 - 4	0.8 - 3.2	0.20 - 0.44

[1] 14 trials (1); [2] Total number of subjects in 14 trials; [3] Age (years) of subjects

Mechanism for phytosterol lowering serum cholesterol and LDL-cholesterol

There are at least three possible mechanisms of action for phytosterol-induced reduction of serum cholesterol (4). Plant sterols and especially stanols remain relatively insoluble in the intestinal chyme, and can interfere with the micellar solubility of cholesterol. Thus, within the intestinal lumen, the presence of phytosterols reduces the solubility of cholesterol in the oil

phase by excluding it from the mixed micelles prior to absorption. This results in the precipitation of cholesterol monohydrate that is not absorbed. This action can be accelerated if the phytosterol stimulates excretion of bile salts. Phytosterols can also compete with cholesterol for uptake by the intestinal mucosal cell or by interfering with the esterification of the animal sterol in the mucosa (13).

Safety of phytosterols

Only a small proportion of dietary plant sterol is absorbed, thus resulting in the fact that serum concentrations of the major plant sterols, such as campesterol and beta-sitosterol, are very low even with high dietary intakes (14). Previous studies in animals and humans have shown that only 3-5% of sitosterol and stigmasterol are absorbed by the intestine (15). In contrast, absorption of campesterol is about two-fold higher than sitosterol or stigmasterol, but much lower than cholesterol. A plasma plant sterol concentration is dependent on whether sterols or stanols are consumed. Jones at al. (11) showed that consumption of 1.8 g/day of sitosterol ester by hyperlipidemic subjects during 22 days in the crossover design study, increases plasma campesterol and beta-sitosterol levels by about 72% and 33%, respectively. In the contrary, the same subjects after consumption of 1.8 g/day stanol ester showed that plasma campesterol and beta-sitosterol levels decreased by 28% and 23%, respectively. Plasma campesterol and beta-sitosterol concentrations did not change during the study where subjects were fed using similar doses (1.7g/day) of mixture of free sterols/stanols (9).

A numerous clinical studies, safety evaluation studies as well as history of use phytosterols-enriched foods indicate that sterols and stanols are generally recognized as safe. However, one important concern is their potential interference with the absorption of fat-soluble nutrients such as vitamins and antioxidants. Clinical trials conducted with sterol and stanol esters have shown that ±- and ²- carotene (pro-vitamins) levels were reduced 10-25% with no effect on serum retinol levels (6,7,16,17). A similar plant sterol induced reduction of plasma lycopene levels has also been shown, but less uniform for all products test (6). Decreases in plasma vitamin D (25-OH-vitamin D), vitamin K and ±-tocopherol have not been observed (7). Both, ±- and ²- carotene, precursor for vitamin A, is transported mainly in LDL particles, which are reduced in stanol treatment . The clinical importance of reductions in plasma ²- carotene is unknown because the fat-soluble vitamin, retinal, for which ²- carotene is the precursor is seemingly unaffected by plant ester intake (18). Finally, with some exceptions (17), many of the clinical trials that have reported cholesterol-lowering properties of plant sterols as well as reductions in ²- carotene are relatively short-term

studies lasting up to 3.5 weeks. Longer, placebo-controlled clinical studies are required to confirm the potential delayed side effects of dietary fortification of plant sterols. This is particularly important for those subjects that may suffer from phytosterolemia (19).

Conclusion

The cholesterol lowering properties of dietary plant sterols are consistent among most of conducted clinical studies. Daily intakes of about 2 g of plant sterols reduce plasma LDL-cholesterol level by 10-15%. Although there are many factors effect cardiovascular hearth disease, plasma cholesterol is a major factor for coronary artery disease. It has been estimated that lowering the LDL-cholesterol level by 10-15%, may reduce risk of hearth disease by 25% (1). Therefore, phytosterols containing foods are good alternatives for prevention and risk reduction of for people with borderline and higher cholesterol levels. These nutraceuticals may have significant benefit to health care system.

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IV INTERNATIONAL SOYBEAN PROCESSING AND UTILIZATION CONFERENCE

MARCH 3

Global expansion of high value vegetable soybean

S. Shanmugasundaram[1] and M.R. Yan[2]

Abstract

Vegetable soybean is a large-seeded, slightly sweet type of soybean harvested while pods are still green. Japan, China, Korea, and Taiwan have historically been the major producers and consumers of the vegetable. Recently AVRDC has promoted vegetable soybean to nearly 50 countries around the world through 72 cooperators. In many countries such as Mauritius, Bangladesh, Sudan, and Vietnam, vegetable soybean is a new crop and has been accepted by the public. It is a nutritious vegetable with value-added properties that contribute to job opportunities and improved income for the poor. A total of 32 improved vegetable soybean cultivars have been released for farmers and they are commercially produced throughout Asia, Africa and the Americas. Besides generating income for farmers, vegetable soybean is a valuable component to the entire farming system. The total biological yield of vegetable soybean can be as high as 40 t/ha in 75 days, consisting of 10 tons of marketable pods and 30 tons of residue that can enrich the soil or feed animals. The future research of this emerging crop will focus on mechanization of its production and harvest, overcoming seed production and seed storage problems, and improving the quality of its flavor. An aromatic soybean for South Asia and Africa markets is in the pipeline.

Introduction

Vegetable soybean, *Glycine max* (L.) Merrill, is a large-seeded type of soybean harvested at the $R_{6.5}$ growth stage while pods and plants are still green (Fehr et al., 1971). The detached pods and shelled green beans are used as a fresh vegetable in Japan, China, and Korea and to a lesser extent in Thailand and Nepal (Lumpkin and Konovsky, 1991; Shanmugasundaram et al., 1991). The pods and shelled green beans are frozen and marketed (Shanmugasundaram, 2001). Vegetable soybean has a large seed size (>30 g/100 seeds dry weight) and a slightly sweet taste. Vegetable soybean is rich in protein (13%), cholesterol-free fat (5.7%), phosphorus (158 mg/100 g), calcium (78 mg/100 g), vitamin B_1 (0.4 mg/100 g) and B_2 (0.17 mg/100 g). It also contains isoflavone and tocopherol.

[1] Deputy Director General for Research and Legume Breeder
[2] Principal Research Assistant, Legume Unit, AVRDC – The World Vegetable Center, P.O. Box 42, Shanhua, Tainan, Taiwan, sundar@netra.avrdc.org.tw

Japan, China, Korea, and Taiwan were the major producers and consumers of vegetable soybean in the past. Annual production in Japan was 110,000 tons from 1988 to 1992, but production has declined to around 70,000 tons today. An additional 70,000 tons is imported from other countries. Until 1992, Taiwan monopolized the export market to Japan, but now 50% of Japan's exports come from China, followed by 34% from Taiwan, 13% from Thailand, and 3% from other countries including Indonesia and Vietnam. Since Taiwanese farmers have expertise in growing vegetable soybean for export, prices for their product is slightly higher than for farmers in other countries.

Lin and Cheng (2001) described the historical perspectives of vegetable soybean in Taiwan. Initially Taiwan had 27 frozen food manufacturing companies capturing 90% of the Japanese frozen edamame export market. But due to rising labor and raw material costs, only 11 frozen edamame processors have survived. Almost all the companies have factories in China. In addition to exporting vegetable soybean to Japan, Taiwan exports 5000 tons of vegetable soybeans to USA and smaller quantities to Canada, Europe, and Australia. Exports to USA from Taiwan, China, and Thailand are on the rise.

Since vegetable soybean is only a minor crop, very little publicly funded research is focused on its genetic improvement. A few universities in the USA have developed large-seeded (>20 g/100 seeds dry weight) varieties and claimed them to be vegetable soybeans. However, their quality is unacceptable in the discriminating Japanese market. In Japan, private seed companies have developed several vegetable soybean cultivars (Kitamura, 2001). Carter and Shanmugasundaram (1993) have compiled a list of soybean cultivars used for vegetable soybean in Japan and Taiwan.

AVRDC's research focus

AVRDC commenced research to develop improved vegetable soybeans in 1981. AVRDC screened the vegetable soybean and large-seeded soybean germplasm from Japan and USA. From the variety, Taishoshiroge, a single plant selection was made and purified. It was designated as AGS 292 (pure line selection from Taishoshiroge). It has total and graded pod yields of up to 26 t/ha and 10 t/ha, respectively in the spring season. In 1987, AGS 292 was the first vegetable soybean variety officially released for farmers' cultivation in Taiwan. Later, AVRDC found that AGS 292 is relatively less sensitive to photoperiod and temperature. Since 1987, AGS 292 has been officially released for farmers' cultivation in at least six countries around the globe. In total, 12 countries have released a total of 32 vegetable soybean cultivars from AVRDC materials (Table 1, Fig. 1).
In many countries such as Mauritius, Bangladesh, Sudan, and Vietnam, vegetable soybean is a new crop and has been accepted by the public. It is popular and available almost year round. Both farmers and consumers have benefited from the crop. Vegetable soybean is a nutritious vegetable with value-added properties that contribute to job opportunities and improved

income for the poor. The total biological yield of vegetable soybean can be as high as 40 t/ha in 75 days. The total pod yield is about 10 t/ha, which farmers can sell at the market. The residue (leaf + stem) contains about 120 kg N, 18 kg P_2O_5 and 150 kg K_2O; this residue can be either returned to the soil or fed to animals.

Over 20 years, AVRDC has made and evaluated 568 crosses in an effort to develop vegetable soybeans that were adapted to the tropics and subtropics. The number of countries requesting AVRDC vegetable soybean germplasm and improved lines has increased from two in 1979 to 41 in 2000. Similarly the number of cooperators from private and public institutions has increased from two in 1979 to 72 in 2000 (Fig. 1). AVRDC has distributed a total of nearly 9,000 seed packets for evaluation around the world.

Prospects for further expansion

Asia – AVRDC vegetable soybeans have been evaluated and produced in countries throughout East, South, and Southeast Asia. Quality soybeans can be grown in these regions but there is a need to promote them in the market and to organize processing facilities. Vietnam and Indonesia are already exporting. Malaysia is marketing domestically and India is moving in that direction.

Africa – AVRDC's Regional Center for Africa (RCA) is evaluating and promoting AVRDC vegetable soybeans in Africa (see paper by M.L. Chadha and Mel Oluoch in this proceedings). They have distributed vegetable soybean seeds to 26 African countries from 1998–2003. Buker (2001) has introduced AGS 292 to Uganda. There is hope that these beans can generate income, increase jobs, improve nutrition, and improve the sustainability of soils.

Americas – Greater awareness on the benefits of healthy diets is driving the demand for vegetable soybeans in the USA. Universities and private seed companies are developing improved varieties. Jim Lothrop's Company in Hawaii has released 12 varieties from AVRDC breeding lines adapted for Hawaii and North America. Another seed company has signed an agreement to produce and market the seeds of AVRDC selections on a non-exclusive basis. In Latin America, results of evaluation of vegetable soybean showed that the potential is there; however, commercial production is yet to begin.

Europe – A company in England is interested in producing vegetable soybean for domestic marketing. Currently the English are importing the crop from Taiwan and Thailand.

Future Research Goals

Machinery for harvesting and shelling the beans have been introduced in Taiwan to reduce production costs; these technologies may serve as models

for farmers in developing countries to modify (Shanmugasundaram and Yan, 2001). Improved seed production and simple seed storage practices are being developed for farmers and industries in developing countries.

Consumers in South Asia and Africa do not like the beany flavor of vegetable soybean. A group of brown-seeded varieties in Japan, called *Dadacha-mame*, have an aroma similar to fragrant rice (Fushimi and Masuda, 2001). AVRDC has studied the inheritance of this flavor and our breeders are transferring these "fragrance genes" to green, yellow and black-seeded vegetable soybeans. Such flavored vegetable soybeans can be marketed as "Basmati" vegetable soybeans for discriminating consumers.

Local name	AVRDC ID #	Year	Country	Remarks
GC 83005-9	GC 83005-9	1995	Bangladesh	HY, suitable for homestead cultivation
AGS 292	AGS 292	1990	China	
MKS 1	AGS 190	1995	Malaysia	HY
VSS 1	AGS 292	1999	Mauritius	
VSS 2	AGS 339	1999	Mauritius	
Rawal-1	AGS 190	1994	Pakistan	HY
PSB-VS 1	AGS 191	1997	Philippines	HY
PSB-VS 2	AGS 190	1997	Philippines	HY
PSB-VS 3	AGS 186	1997	Philippines	HY
	AGS 190	1992	Sri Lanka	HY, suitable for soymilk and ice cream and soynuts, less beany flavor
	AGS 292	2002	Sudan	
Kaohsiung No. 1	AGS 292	1987	Taiwan	HY, MH, DM, EM
Kaohsiung No. 2	Ryokkoh x KS 8	1991	Taiwan	HY, MH
Kaohsiung No. 3	PI 157424 x KS 8	1991	Taiwan	HY, MH
Kaohsiung No.6**	AG S292	2001	Taiwan	
Kaohsiung No.7**	AGS 292	2001	Taiwan	
KPS 292	AGS 292	1992	Thailand	HY
CM 1	AGS 190	1995	Thailand	HY, suitable for domestic consumption
Mana	AGS 292	1999	Hawaii, USA	
Makani	AGS 334	1999	Hawaii, USA	
Momona	AGS 337	1999	Hawaii, USA	
Nui	AGS 346	1999	Hawaii, USA	
Buker's Favorite	AGS 292		USA	
Koapaka	GC97002 F3	2002	Hawaii, USA	HY, adaptation* MKK, Ohio
Hiluhilu	GC97022 F3	2002	Hawaii, USA	HY, adaptation MKK, Ohio, SC
Kanaloa	GC97002 F3	2002	Hawaii, USA	HY, adaptation Ohio
Kila	GC97022 F3	2002	Hawaii, USA	HY, adaptation SC
Onaona	GC97002 F3	2002	Hawaii, USA	HY, adaptation SC
Mimiki	GC97022 F3	2002	Hawaii, USA	HY, adaptation SC
Palanehu	GC97002 F3	2002	Hawaii, USA	HY, adaptation MKK
Akua	GC97029 F3	2002	Hawaii, USA	HY, adaptation MKK
VRQ 46	AGS 346	1999	Vietnam	EM (65–85 days), HY (11–14 t/ha), 3 crops/year
Total	32		12	

DM = resistant to downy mildew; EM = early maturing; HY = high yielding; MH = suitable for mechanical harvesting; * MKK = Molokai, Hawaii, USA Ohio = Ohio, USA; SC = South Carolina, USA; **Cross between AVRDC line and other cultivar

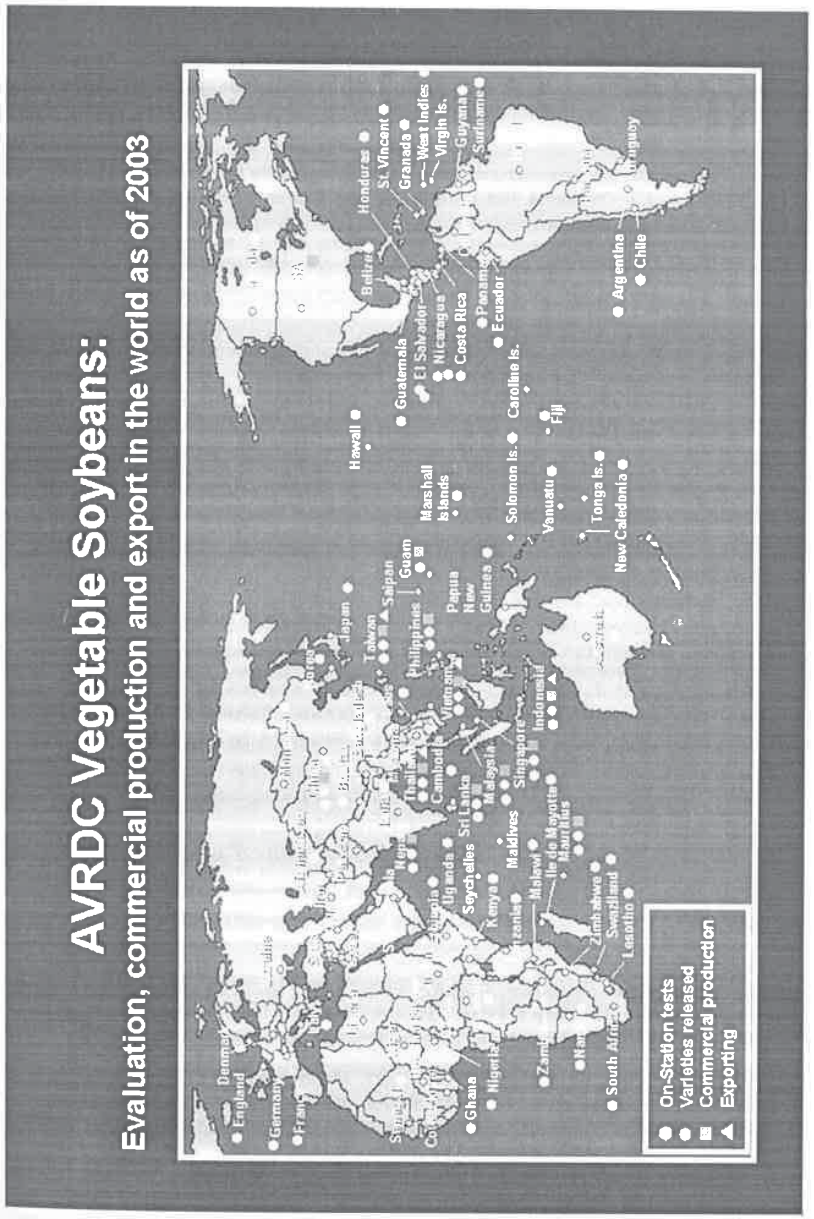

Figure 1. Countries evaluating, commercially producing, and exporting AVRDC vegetable soybeans

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Vegetable soybean research and development in Africa

M. L. Chadha and M. O. Oluoch[1]

Abstract

Vegetable soybean is an old product of East Asia, with great potential for adaptation and utilization as a new food crop in Sub-Saharan Africa. Although field soybean cultivation is well known by many African farmers, there is no tradition for vegetable soybean cultivation and consumption in Africa. Utilization of vegetable soybean not only provide good nutrition but also enhances human health and longevity. It is an important source of high quality but inexpensive protein. It also has a superior amino acid profile compared to other sources of plant proteins and can be a major future source of dietary protein in Africa. Another advantage of vegetable soybean is its potential in improving soil fertility by adding nitrogen to the soil which is most important for nutrient poor soils in Africa. Production and utilization of vegetable soybean can help to link the African Countries to take advantage of this unique opportunity of South South cooperation to develop and contribute novel crops to the African food and Agriculture. AVRDC-The World Vegetable Center is currently promoting research and development work in Africa on this novel crop through its Regional Center for Africa (RCA). Since 1997, AVRDC-RCA has been conducting evaluation trials to determine the performance and to identify suitable lines with good agronomic traits, good pod quality and resistance against prevalent diseases. Results from trials conducted from 1997 to 2001 has helped in identifying four promising lines; namely AGS 292, AGS 329, AGS 338 and AGS 339, which have good taste, and high fresh pod yields of over 7 t/ha and seed yields of 3 to 4 t/ha, respectively. AVRDC has already distributed germplasm of vegetable soybean to more than 26 countries in Africa. The outcome of the vegetable soybean technologies developed has a potential spill over effect to all regions in Africa. Organoleptic taste analysis results for seven years (1997-2003) on the four promising lines identified has shown that the taste and quality is acceptable to diverse groups drawn from more than 23 countries in Africa. On the basis of fresh pod yield characteristics, quality and organoleptic tests by various groups, the four promising lines are now being promoted for utilization and seed production. AVRDC-RCA has distributed seed of the promising vegetable soybean lines to over 10,000 small scale farmers in different countries in Africa.

[1] AVRDC-The World Vegetable Center, Regional Center for Africa, P.O. Box 10, Duluti, Arusha, Tanzania; mlchadha@avrdc-rca.co.tz

Introduction

Vegetable soybean is one of the important vegetables in Japan, Taiwan, China and Korea but could be an attractive option for African farmers if local or foreign markets could be developed. Its importance in human nutrition is increasingly recognized in many countries. Vegetable soybean is harvested after the R_6 and before R_7 growth stage, while the pods are still green and the seeds have developed to fill 80 to 90% of the pod width (*Shanmugasundaram et al 1992*). The seeds of vegetable soybean are large, tender and are considered most delicious and nutritious when eaten while still green. When boiled, the seeds have the highest net protein utilization value among all other soybean proteins (*Wang, 1979*). Compared to other leguminous vegetables, vegetable soybean are rich in protein, fat, phosphorous, calcium, iron, vitamin B, and vitamin B_2. Increase in interest and demand for vegetable soybean in many countries encouraged AVRDC to broaden its research scope to cover adaptation and establishment of this crop in Africa through its Regional Center for Africa. Vegetable soybean can be grown year-round if using well-adapted cultivars. A main constraint, however, is lack of cultivars adapted to local environments and their cultural practices requirement. Since 1997 experiments have been conducted to evaluate the yield potential and quality parameters of different vegetable soybean lines. Research and Development and technology diffusion activities in this direction have already been started and four promising lines; namely AGS 292, AGS 338, AGS 339, AGS 329 have been identified with good potential and scope for vegetable soybean adaptation in Africa. Compared to other protein-rich foods such as meat, fish, and eggs, soybean is by far the cheapest source of protein. Another advantage of soybean is that it improves soil fertility by adding nitrogen to the soil. According to FAO estimates, over 160 million tones of soybean was produced worldwide in 2000. Africa contribution is less than 1%, with about 817 000 hectares under cultivation in sub-Saharan Africa. On the other hand, reports on production of vegetable soybean in Africa is negligible. In Africa, however, soybean has fewer disease and insect problems because the crop was recently introduced to Africa and the extent of cultivation is still relatively small.

Research aspects

Germplasm distribution

Since 1998, AVRDC has distributed over 375 lines of vegetable soybean to Research Institutes and Extension Organizations in 26 countries in Africa (Table 1) for testing, utilization and variety development.

Table 1. AVRDC Vegetable Soybean Germplasm distribution in Africa (1998-2003)

Countries	No. of lines	Countries	No. of lines
Mauritius	30	Senegal	10
Tanzania	71	Botswana	15
South Africa	42	Liberia	10
Zambia	15	Chad	15
Ghana	15	Togo	10
Zimbabwe	20	R.D Congo (Afrique)	4
Ethiopia	17	Sao Tome'e Principe	8
Uganda	28	Mali	6
Swaziland	15	Angola	6
Congo	15	Somalia	2
Lesotho	2	Sudan	4
Kenya	4	Malawi	4
Mozambique	4	Namibia	4

Varietal improvement and seed production

Research and Development programs at AVRDC has resulted in new vegetable soybean lines. These have been evaluated for their superior characteristics and are undergoing adaptation in many countries. The Ministry of Agriculture in Mauritius in 1999 released AVRDC lines AGS 292 and AGS 339 as VSS1 and VSS2, respectively. The yield potential of fresh green pods in this specific lines range from 8-10 t/ha. Whole fresh pods of vegetable soybean is becoming popular among consumers in Mauritius, where it is proving to be a profitable crop for farmers. In Tanzania the seeds of lines AGS 338, 339, 292 and 329 have been disseminated to National Research Institutes for further evaluation and future release. In an experimental trial in Swaziland in 1998-99, AGS 346, AGS 344, AGS 345 and AGS 335 produced fresh pod yields of 16.8, 11.5, 10.5, and 9.75t/ha, respectively. In Malawi, line GC 87020-205-6-5 produced 3.47 t/ha. Soybean rust has been reported to be a serious constraint to production in Southern Africa. Evaluation trials conducted in Zimbabwe during 1999 and 2000 and using twenty AVRDC lines to examine the response of fungicides to select soybean rust tolerant lines showed that lines GC 00138-29B, GC 00138-29A, and GC 84058-184 performs well under rust infestation. Since 1997, AVRDC-RCA has been conducting evaluation trials to determine the horticultural performance and seed yield potential of promising lines. Results from four year experiments identified lines AGS 339, AGS 329, AGS 338, and AGS 292 for good taste and high fresh pod yields averaging about 7.3 t/ha (Table 2). Amongst the four lines tested, AGS 338 gave the highest seed yield and no. of pods per plant (Table 3). These lines are undergoing utilization and promotional programs in Africa.

Table 2. Average performance of promising Vegetable Soybean lines at AVRDC-RCA, Arusha, Tanzania during 1997,1998, 2000 and 2001.

Genotype	Fresh pod yield t/ha)	Plant height at maturity (cm)	No. of pods/plant	Pod length (cm)	Pod width (cm)	Pod No. /500g
AGS 292	7.16	24.4	12.2	5.6	1.34	158
AGS 329	6.95	23.4	14.8	5.8	1.27	146
AGS 338	7.93	26.3	21.3	5.7	1.29	163
AGS 339	7.15	23.4	18.2	5.9	1.34	175
$LSD_{.05}$	59	2.42	3.29	0.55	0.18	61.73
F-test	ns	ns	*	ns	ns	ns
CV(%)	23	3.1	8.0	4.8	4.3	12.1

Means within the column followed by the same letter are not significantly different at 5% level based on DMRT.

Table 3. Average Seed Yield and Seed Characteristics of Vegetable Soybean lines Evaluated at AVRDC-RCA, Arusha, Tanzania; during 2000 to 2001.

Genotypes	Seed Yield (kg/ha)	Seed Yield/ Plant (g)	Dry matter yield/plant (g)	No. of seeds/ plant	No. of pods/ plant	No. of seeds/pod	Pod length (cm)
AGS339	3506.8	20.0	11.7a	28b	21	2.0	2.7c
AGS 329	3068.4	18.5	7.3b	28b	18	2.0	3.4b
AGS 338	4151.9	24.8	10.1a	44a	19	2.3	3.3b
AGS 292	2889.4	17.6	11.1a	28b	19	1.7	4.0a
F-test	ns	ns	*	ns	ns	ns	**
$LSD_{.05}$	1500	9.10	2.63	13.79	3.54	1.10	0.42
CV(%)	24	20	13	21.5	9.2	27.6	6.18

Means within the column followed by the same letter are not significantly different at 5% level based on DMRT.

Quality evaluation of vegetable soybean

Attempts have been made at AVRDC to develop an objective quality evaluation system that could determine the eating quality of vegetable soybean based on sweetness, taste, flavor and texture. The analysis of the nutrient content has shown that vegetable soybean is an excellent source of thiamine and calcium, and also a good source of protein, riboflavin and iron. The high fat and protein content in vegetable soybean also makes it a good diet component in improving the absorption of Vitamin A (Table 4).

Source: Asian Vegetable Research and Development Center (AVRDC), S.C.S. Tsou.

International Institute of Tropical Agriculture (IITA) has come up with a wide range of new food products from soybean. About 150 food products

Table 4. Nutritive value of vegetable soybean.

Component	Percentage
Sugar	3.34
Protein	13.60
Starch	3.36
Oil	6.32
Ash	1.48
Fiber	1.53
Non N-Extract	10.65

with good nutritive value and consumer acceptability have been developed. Many of the new products have been tried out in villages, modified by local people to suit local culinary tastes, and ultimately adopted with increasing popularity. In some communities in Nigeria up to 98% of households have started eating soybean foods. At the same time, more that 90 000 people have been trained on how to produce and use soybean to fortify their diets. Some of these new soybean products have become so popular that they have been upscaled to industrial production, providing income generation opportunities. The benefits of IITA's soybean research are also seen in other African countries, such as Ghana and Côte d'Ivoire. This work will help in popularizing the vegetable soybean and make it more easily acceptable in the area.

Evaluation of several lines of Vegetable Soybeans has shown that line AGS- 292 is high in iso-flavones. Medical scientists report that iso-flavones exert functional properties that may prevent heart disease, some cancers, and other health problems associated with aging. Iso-flavone levels in AGS-292 were three times higher than the mean of a sampling of 88 soybean sources. AVRDC has conducted a survey of iron bio-availability in more than 20 vegetables including vegetable soybean. The research found that cooking increases iron bio-availability to almost double; from 4 to 9%; in vegetable soybean. Cooked vegetable soybean was found to increase the iron availability in other foods in the same meal.

Soil improvement

The potential of vegetable soybean and vegetable cowpea as green manure in conditioning the soil was investigated at AVRDC-RCA in Arusha, Tanzania. Three amaranthus varieties as model crops were sown on sunken beds using split-plot design with three replications; with vegetable soybean and vegetable cowpea incorporated as green manure in the soil as treatments. A control plot was used for comparison in each plot. Vegetable soybean as green manure showed a higher effect on yield of Amaranthus when compared to vegetable cowpea (Table 5).

Table 5. The Effect of vegetable cowpea and vegetable soybean as green manure on yield response of amaranthus

Green Manure Crop	Amaranthus Biological Yield		Amaranthus Economic Yield	
	(g/plant)	(t/ha)	(g/plant)	(t/ha)
No Crop (control)	0.4b	3.2b	0.3b	2.8 c
Cowpea	0.6a	6.9a	0.4b	6.0b
Soybean	0.8a	8.4a	0.7a	7.4a
F-test	*	*	*	***
$LSD_{.05}$	0.28	1.75	0.26	1.23
CV(%)	14	9.5	18.4	7.6

Means within the same column followed by the same letter are not significantly different at 5% level based on DMRT.

Vegetable soybean can also be intercropped with other crops (e.g. okra and maize). An experiment conducted at AVRDC-RCA has shown that growing vegetable soybean with okra can improve productivity without affecting the average yields of both crops.

Development and promotion

Quality taste evaluation

Because of the complexity of analyzing overall quality, test panels are the most frequently used evaluation method (Tsou and Hong, 1991). Major quality requirements of vegetable soybean in terms of palatability are appearance, taste, flavor, and texture. Organoleptic tests have been conducted on several vegetable soybean lines by involving more than 700 representatives from 23 African counties; namely Angola, Botswana, Kenya, Malawi, Mozambique, Namibia, Swaziland, Zambia, Zimbabwe, Tanzania, South Africa, Ethiopia, Lesotho, Sudan, Mauritius, Seychelles, Senegal, Rwanda, Eritrea, Togo, Ghana and Burkina Faso. Results showed that the four promising lines tested are well accepted.

Promotional programs for increase in production and utilization

In the recent years, AVRDC-Regional Center for Africa has disseminated seed of vegetable soybean to more than 10,000 small scale farmers for production and utilization in Africa and over 2 tons of seed of new lines have been produced at AVRDC-RCA and distributed to national Institutions and small scale farmers for adaptation and utilization in several African Countries. The major emphasis has been placed on the following countries:

Zambia: Jointly with UNICEF-Zambia, over 5000 vegetable nutrition kits (*which includes upto 14 different vegetable seeds in one kit*) have been distributed to resource poor households in 20 districts. Vegetable soybean is included among the seeds in the nutrition kit to help address the Protein-Calorie Malnutrition. These households have tasted the vegetable soybean for the first time and more farmers are interested in growing and utilizing this new vegetable.

Sudan: Vegetable Soybean seeds (as part of nutrition kits) has been distributed to over 2000 households in southern part of the country during year 2000-2002. Many households have produced their own seed and are distributing to other fellow farmers.

Tanzania: Over 2500 farmers have been given seed of vegetable soybean for testing and have liked the crop and its simple utilization procedure.
Mozambique: Over 800 households have been given seed of this vegetable soybean as part of the nutrition kit. Three NGOs and National Agriculture Research Institute (INIA) are helping to promote the production and utilization of vegetable soybean in Mozambique.

Mauritius: Vegetable soybean is becoming very popular in the country and many farmers have began growing the newly released varieties. The fresh green pods are sold for upto US$2.00 per kg in the local markets, which is a reasonable price to consumers and has good potential to improve the income of many farmers.

Future research and development prospects:

- Improve the vegetable soybean varieties to increase yield and quality, and to have a more attractive color and tastier pods.
- Advise farmers to follow efficient production methods to improve output and quality.
- Establish diversified mixed intercropping systems with vegetable soybean to improve income and productivity in Africa.
- Diversify uses and improve quality standards for vegetable soybean products.
- Strengthen seed production and storage research to generate quality seeds for dissemination and distribution.
- Incorporate IPM strategies in the production chain to reduce pesticide use and to help add value to improve potential for export.
- Build up capacity to improve the knowledge base for production and utilization.
- Create awareness by setting up demonstrations to showcase production and utilization technologies to help in the promotion of vegetable soybean.

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Vegetable soybean research and industry development in Thailand

S. Srisombun[1]; S. Ratanarat[1]; S. Kaewmeechai[1] and S. Shanmugasundaram[2]

Abstract

Vegetable soybean has been consumed by Thai people for more than hundred years. However, the high quality vegetable soybean production and industry development for export has been promoted only in the past 20 years. The initial stage of vegetable soybean development for export was the establishment of a frozen vegetable soybean factory in the late 1980's. The cooperation between Thailand and AVRDC in research, development, extension and market promotion at the early stage of industry development in 1991 stimulated vegetable soybean production for export and domestic use. The export of a small quantity commenced in 1990 and increased to almost 10,000 t in 2002. Vegetable soybean can be grown in three seasons in a year mainly in the northern region with the planted area of about 2,600 ha in 2003. The graded pod yield averaged about 5.72 t/ha under farmer field conditions. The Thai Department of Agricultural Extension and the Thai Department of Health are responsible for production under good agricultural practice for domestic consumption. The private sectors promote high quality product for export. The Thai Department of Agriculture and universities are responsible for research and development to support production. The future strategy is to promote high quality products for both export and domestic consumption, variety improvement with high pod yield and good pod quality for export and development of appropriate technologies for the new production areas and product diversification for domestic market.

Introduction

From the time the soybean was introduced from China to the northern part of Thailand, the vegetable soybean has been consumed for more than hundred years in Thailand. The medium seed size, 15-20 g/100 seeds, soybean grown for food or oil extraction is also traditionally used as vegetable soybean, called "TAU RAE". Green pods attached to the stem are commonly boiled and sold in the countrywide local markets (Srisombun *et al.*, 2001). However, the quality requirements for export markets are entirely different from those for traditional domestic markets

[1] Department of Agriculture, Bangkok, Thailand
[2] AVRDC, Taiwan, ROC

(Table 1). The vegetable soybean research and industry development in Thailand commenced almost 20 year ago.

History of vegetable soybean development

Vegetable soybean industry development

The initial stage of vegetable soybean development for export was the establishment of a frozen vegetable soybean factory as a joint venture between Thais and Japanese in the late 1980's. Then, export of a small quantity of vegetable soybean from Thailand to Japan commenced in 1990. The export volume dramatically increased to approximately 8,000-9,000 t during the years 2001-2002 (Table 2). Thailand ranked the third country to export vegetable soybean to Japanese market after China and Taiwan. The export quantity is expected to increase due to a strong demand from Japanese and the USA markets. The Japanese market demand for import is around 70,000 t from 1990 onwards, approximately half of the country's total demand (Kitamura, 2001). Since 2000 the USA imported about 10,000 t per annum and the volume of import is expected to increase about threefold in 2003. In 2000, Thailand exported 97 percent of vegetable soybean to Japan and only two percent to the USA.

At present, there are three frozen vegetable soybean factories that play a major role to produce vegetable soybean for export. The factories supply the seeds and necessary inputs to the contract farmers in production areas scattered around the factories. The farmers use the improved technologies mainly from government research institutes both from the Thai Department of Agriculture (THDA) and the universities. The seed supply was mainly from factories' seed multiplication farms. The rest of the seed supply was annually imported from Taiwan, about 50 t during 2000-2002 at a cost of 11 million Baht (40 Baht $\simeq$ US$1).

Vegetable soybean production

Lack of experience in the production of high quality vegetable soybean for export was a major obstacle in the early stages of development of vegetable soybean industry. Due to the cooperation between people concerned in the government, private sector in Thailand, and AVRDC staff, vegetable soybean production was promoted on a scientific footing. A training course on vegetable soybean production was organized for extension experts in Chiang Mai, Thailand in 1991 (THDAE and AVRDC, 1991). Later, the THDA and the Thai Department of Agricultural Extension (THDAE) organized a five-year development program, during 1992-1996, to encourage the production and market promotion (THDA, 1991). As a result, the annual

area planted to vegetable soybean increased to about 2,000 ha in 1999 and the area is expected to reach 2,600 ha in 2003 excluding the area planted to traditional vegetable soybean. In addition, the demand for high quality vegetable soybean for domestic consumption has increased particularly in the big cities. The annual quantity of vegetable soybean consumed by Thais is about 1,000-2,000 t. The domestic consumption is increasing steadily, particularly among the middle class.

The average graded pod yield observed from ten farmers' fields in 2003 was about 5.72 t/ha (Srisombun and Ratanarat, 2003). About 70-80 percent of the vegetable soybean production areas is in the upper north of the country at latitudes of 17-19°N. The crop is grown in three main seasons. The dry season crop (50 percent of the total area) is grown, from mid December to mid January, after rice harvest. The second crop is grown in the early rainy season from April to mid June and the remaining vegetable soybean crop is grown in the late rainy season in August. Due to low daily minimum temperature (8-12°C during the first and the second week of January) the yield of the dry season crop in the upper north of the country is low and pod quality is poor. As a result, the lower northern, lower northeastern and upper central regions are considered as alternative areas for vegetable soybean production.

Vegetable soybean research, extension and market promotion

Vegetable soybean variety improvement

The initial research in collaboration with AVRDC was instrumental for vegetable soybean development in Thailand. A number of varieties/lines were introduced from AVRDC and Japan to Thailand since 1986. Subsequently, two varieties, KPS292 and Chiang Mai 1, were released by Kasetsart University and the THDA in 1992 and 1993, respectively. KPS292 was selected from AGS292 (Kaohsiung # 1), and it is well suited for export. Chiang Mai 1 was selected from Vesoy # 4 (AGS190), it is suitable only for domestic market and gave somewhat higher pod yield than that of KPS292 (Chotiyanwong and Chotiyanwong, 1997). At present, AGS292 and two new varieties, No.75 (Ryokkoh75) and No.2808 are grown for export to Japan. AGS292 produced a graded pod yield of about 5.46 t/ha averaged from 13 environments during 2001-2002 in the Research Centers (Chotiyanwong *et al.*, 2003) and it gave about 35 percent higher pod yield than that of No.75 (Table 3). The agronomic characteristics of the two popular varieties are also shown in Table 3.

At present, the objective of the breeding program is to obtain higher graded pod yield with superior quality for export. A large number of AVRDC materials have been introduced to Thailand. Many crosses were made using parents from AVRDC. A number of promising lines are being tested for graded pod yield, pod quality and adaptability in the main production areas.

Vegetable soybean protection

The major diseases of vegetable soybean are soybean crinkle leaf virus (SCL), anthracnose (*Colletotrichum truncatum* Schw.) and rust (*Phakopsora pachyrhizi* Syd.) (THDA, 2002). First epidemic of SCL occurred during 1984-1988 mainly in the lower north of the country. Subsequently in 1998, the disease was found almost in all the main production areas and in all three growing seasons and since then it has been a constraint to vegetable soybean production. Anthracnose and rust could cause a considerable loss of pod yield and reduce pod quality in early and late rainy seasons, respectively.

The key pests are beanfly (*Melanagromyza sojae, Ophiomyia phaseoli*), whitefly (*Bemisia tabaci*) and pod borer (*Etiella zinckenella*) (THDA, 2002).

Another constraint to vegetable soybean production is weeds particularly in rainy season crops. Pre-emergence herbicide together with hand weeding is recommended to control weeds (THDA, 2002).

Crop management and judicious use of chemicals are recommended to control these diseases, insect pests and weeds. Kinds, rates and times of chemical use are carefully chosen to minimize or to have no residues in the product. Use of chemicals is prohibited at least 15 days prior to pod harvest.

Extension and market promotion

Based on research results, a recommended package of technologies for vegetable soybean production, Good Agricultural Practice (GAP), had been developed and promoted to farmers to ensure good pod yield and superior quality product (THDA, 2002). The production technology is mainly produced from the THDA and the universities. The THDAE is responsible for vegetable soybean production for domestic consumption and local market promotion. Due to the high nutritional value of vegetable soybean and its significance in providing vitamins and minerals (Messina, 2001), Thai Department of Health (THDH) and THDAE has a project to encourage the people particularly children in the rural areas to consume more vegetable soybean. The annual campaign of vegetable soybean production as well as vegetable soybean food and sweet is organized commencing in 2003.

On the other hand, the private sector promotes the production for export. The data observed in ten farmers' fields in 2003 revealed that the cost of vegetable soybean production for export varied from 40,000 to 68,000 Baht/ha. The relationship between profit as income: production cost ratio and graded pod yield was found ($r = 0.91$, $p < 0.001$) (Srisombun and Ratanarat, 2003). The farmers had to produce at least 3.55 t/ha of graded pod in order to break-even. Then the more pod yield produced, the more profit obtained. The graded pods bought from farmers were further sorted and about 10-20 percent was discarded. The raw material is examined for chemical residues in the factories in order to meet the standard quality for export. The net profit of vegetable soybean production is approximately 10 times higher than that for grain soybean. In the future, as the overseas markets demand increases, vegetable soybean will be very competitive compared to other crops in the kingdom and it is hoped that vegetable soybean becomes more popular among the Thai people.

Future prospects

Vegetable soybean production for both domestic consumption and export to earn valuable foreign exchange will continue to be promoted. The THDAE and THDH are responsible for production under GAP for domestic consumption. The private sector promotes high quality product for export. The THDA and the universities are responsible for research and development to support the production. The future of vegetable soybean research and industry development will focus on: (1) Promotion of high quality products for export. (2) Variety improvement for export purpose with 10 percent higher graded pod yield and better quality than AGS292 and No.75. (3) Development of appropriate package of technologies for the new production areas with high pod yield and superior quality and (4) Product diversification and domestic market promotion for the Thai people.

Table 1. Agronomic characteristics and bean quality of traditional vegetable soybean

Characteristic	Traditional vegetable soybean	High quality vegetable soybean
100 seed weight (g)	15-20	25-30
100 bean weight (g)	35-45	60-75
Fresh pod color	Not important	Dark green
Fresh pod type	Pods attached to the stem	Single pod or pods attached to a node
Pubescence color	Brown	Gray
Bean quality		
- Taste	Not important	Sweet
- Hardiness	Quite hard	Soft

Table 2. Vegetable soybean import to Japan

	Year 2001		Year 2002	
Country	Quantity (t)	Value (m yen)	Quantity (t)	Value (m yen)
China	44,958	8,048	34,617	6,315
Taiwan	22,696	4,847	23,588	5,159
Thailand	7,767	1,515	8,837	1,718
Indonesia	1,738	302	2,416	458
India	41	7.8	-	-
Vietnam	-	-	19	2.4
Korea	-	-	10	2.6
USA	-	-	23	4.6
Total	77,200	14,720	69,510	13,659

Source: JETRO, Bangkok

Table 3. Pod yield and major agronomic characteristics of two vegetable soybean varieties

Characteristic	AGS292	No.75
Graded pod yield (t/ha)	5.46	4.03
100 bean weight (g)	62.2	74.3
Days to harvest	66.6	68.4
Days to maturity	93.0	93.0
Flower color	Purple	White
Pubescence color	Gray	Gray
Fresh pod color	Green	Dark green
Market preference	Accepted	Preferred

Days to harvest and maturity from sowing date

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Vegetable soybean research on cultivar development and future market potential in South America with emphasis on Brazil

N. A. Vello; W. F. Bernardi; J. I.H.V. Pinto;
M. C. Nekatschalow; C. A. Didoné and A. R. Cogo[1]

Abstract

Vegetable soybeans or edamame present the tendency of increasing the niches of market in Brazil and South America, mainly among the people of occidental origin. In a breeding program carried out in University of São Paulo, Brazil, large seeded genotypes represented mainly by introductions from other countries were crossed with two adapted cultivars (Doko and FT-2). The selection practiced among $F_{10:15}$ lines was very efficient in increasing the seed yield, leading to a realized genetic gain of 29%, without significant changes in other traits: seed size, protein and oil contents. Several selected lines presented the desirable phenotypes for using as vegetable soybeans: large seeds, yellow seed coat, cream hilum color, yellow cotyledons, and high seed yield. A part of these outstanding lines have been used as parents in a second cycle of recombination and, some of them are showing potential to be reliased as new cultivars for attending the future requirements of the promissing niches of market for vegetable soybeans in Brazil and South America.

The vegetable soybeans are important for the human consumption in the oriental countries, mainly Japan, China, Taiwan, Korea, and Indonesia. The most prominent institution researching and promoting vegetable soybeans worldwide is the Asian Vegetable Research Development Center (AVRDC), in Taiwan. Vegetable soybeans are rich in protein, cholesterol-free fat (useful in absorption of vitamin A), phosphorus, calcium, iron, thiamine, riboflavin, and vitamin E (Shanmugasundaram and Yan, 1999). It is a type of soybean [*Glycine max* (L.) Merrill] with wide pods and large seeds (weight of one hundred seeds = or > 20 grams), cultivated as a vegetable species (sowing in seedbeds, transplanting the seedlings to hills spaced 0.80m x 0.80m in the field with large amount of organic manure, thinning, cutting), and harvesting in the stage of maximum pod filling (stage R_6 of Fehr and Caviness, 1977); in the harvest, the plants still are immature (reason of the name edamame, in Japanese); thus, the leaves are manually eliminated for the formation of bunches of plants (stems, branches and pods) for the market; more recently, it is also used to remove the immature pods, to pack and freeze them, for domestic consumption and or exportation.

1 University of São Paulo, Faculty of Agriculture "Luiz de Queiroz", Department of Genetics, P.O. Box 83, 13 400-970 – Piracicaba, SP, Brazil. *Corresponding author: lgn@esalq.usp.br

The most common system of human consumption is through aperitif, by boiling the pods in pickling brine, placing the boiled pods between teeth and pulling themselves for withdrawal of the grains, that are chewed and ingested. Beyond the use in the edamame form, the large seeded soybeans also presents other types of consumption in the oriental countries. For consumption in the salad form, is used to cook mature grains with different colors of seed coat and hilum. For candy (amanatô) used in special Japanese celebrations, the mature grains with black seed coat (Kuromame in Japanese) are mixtured with sugar and cooked in vapor. For some special foods (p.ex. tofu) it is used mature grains, with yellow seed coat, cream– or buff– or tan–colored hilum and yellow cotyledons.

The large seed size is a requirement for manufacturing and or consuming some types of soybean food, but also it is related to the good flavor and taste. According to Liu (1997), it has been speculated that the hypocotyl axis or germ is the main source of the beany flavor and undesirable taste in soybean products. One explanation is that the soybean hypocotyl has the highest percentage of polyunsaturated fatty acids (1.17 and 2.22 times than in cotyledons, respectively for linoleic (omega-6) and linolenic (omega-3) acid contents) and a concentration of isoflavones 5-6 times higher than in cotyledons. Large seeds have a higher mass of cotyledons relatively to hypocotyl axis, favoring the good flavor and taste in soybean products. The polyunsatured fatty acids and isoflavones have been associated to the beany flavor, undesirable taste, bitterness and astringency of soybean food (Carrão-Panizzi and Kitamura, 1995), but more recently they were considered the main causes of the functional or nutraceutical value of the soybean foods (Worral, 1999) and, thus, they can to contribute for enhancing living quality and preveting chronic diseases. This paradox represents a challenge to the soybean breeders whom, probably, will need to design a special program for each type of soybean use, that is, direct human food (vegetable soybeans) or functional food (manufactured produts).

In South America, the cultivation of vegetable soybeans has been restricted to the colonies of descendants of oriental peoples. In some regions of Brazil, from the North (p.ex. Belém, State of Pará) to the South (p.ex. Curitibanos, State of Santa Catarina), small crops of vegetable soybeans have been cultivated for attending niches of domestic and export markets. After the recognition of the functional value of the soybeans for the Food and Drug Administration of U.S.A., in October of 1999, the use of the soybeans for human consumption has been increased also among the occidental peoples. The growth of organic agriculture is another factor that is contributing for increasing the culture and the use of the soybeans for human consumption in occidental countries; for example, in State of São Paulo, Brazil, organic crops of vegetable soybeans have been included in crop rotation systems having organic sugar-cane as main crop.

In Brazil and other occidental countries, there is a limited number of vegetable soybean cultivars (Embrapa, 2003) available for cultivation: the "Instituto Agronômico de Campinas" in State of São Paulo, has developed some cultivars of vegetable soybeans, excelling 'IAC-Céu Azul' and 'IAC-PL1'; the "EMBRAPA Soja" developed a new cultivar of vegetable soybean that will be available very soon in the seed market; 'FT-Monsanto' is a cultivar developed by "Francisco Terasawa – Pesquisa e Sementes" in association with Monsanto; the Naturalle Company (a joint venture between Terasawa and Axial Bank) is preparing to reliase new cultivars of vegetable soybeans adapted to the Brazilian environmental conditions. In Argentina, the cultivar Agata was reliased by an agreement between "Facultad de Ciencias Agrarias de Zavalla, de la Universidad Nacional de Rosario" and "Innovaciones Tecnológicas Agropecuarias S.A. – INTEA" (Inta, 2002).

Materials and methods

A breeding program of vegetable soybeans is in developing at "Universidade de São Paulo (USP), Escola Superior de Agricultura Luiz de Queiroz (ESALQ), Departamento de Genética", Piracicaba, State of São Paulo, Brazil. This program was initiated with agronomic testing of about 100 large-seeded soybean genotypes, including germplasm introduced from other countries, mainly from AVRDC – Taiwan, and some Brazilian cultivars and landraces cultivated by descendants of oriental peoples. The most promissing 40 genotypes were crossed with two adapted Brazilian cultivars, both having high physiological quality of seeds, that is, maintenance of the germination ability and the seedling vigour for long period of time, under tropical environmental conditions; these two adapted parents also have other desirable traits, as high seed yielding; furthermore, the 'Doko' parent has long juvenile period, and the 'FT-2' parent has high earliness. The progenies derived from about 5000 F_2 plants were tested and selected during the inbreeding generation advances, until to obtain 34 $F_{6:10}$ progenies (Tsutsumi, 2000). About 30 plants were harvested individually in each progeny, originating 1020 $F_{10:11}$ pure lines. New selection cycles were applied during the additional inbreeding generations resulting 196 selected $F_{10:15}$ inbred lines. In 2002/03, the superior 96 $F_{10:16}$ lines were selected for continuing the program. All the experiments included four vegetable soybean cultivars as experimental checks: IAC-Céu Azul, IAC-PL-1, Nimame and Tamba. These checks have been used as reference for applying the selection.

Results and discussion

There is a large variation among crosses for the number of selected $F_{10:16}$ lines (Table 1). The Late Giant x Doko cross excelled with the highest number (19) of selected lines; to follow, a group of ten crosses presented 4-9 selected lines in each cross; finally, a group of seven crosses presented 1-3 selected lines/cross.

Table 1. Number (N) and means of selected lines within cross, for the traits seed yield (SY), one hundred seed weight (SW), protein (PR) and oil (OI) contents.

		Means			
Cross	N	SY Kg.ha^{-1}	SW grams	PR %	OI %
PI 229320 x Doko	7	2550	22.5	42.5	22.6
PI 80441 x Doko	5	2520	23.2	41.7	23.0
(TK # 5 x Unknown) F7 x Doko	6	1150	26.4	42.6	22.2
(TN # 4 x PI 230970) F7-4 x Doko	5	2255	24.6	42.4	21.9
(TN # 4 x PI 230970) F7-4 x FT-2	2	2645	18.8	42.1	22.6
(KS 473 x SJ2 - F7) x Doko	6	3010	22.4	42.8	22.2
TMV x Doko	4	2460	22.5	41.7	22.8
Araçatuba x Doko	1	1050	24.4	41.7	23.4
Late Giant x Doko	19	2955	22.2	42.1	22.1
Japão 1 x Doko	6	2045	27.5	42.8	22.3
Japão 2 x Doko	3	1795	25.5	42.7	22.6
Yamagataken x Doko	3	2015	28.3	42.1	22.6
Aliança Preta x Doko	9	3000	23.8	42.2	22.4
IAC PL-1 x FT-2	4	2165	19.4	42.6	22.6
FC 31-665 x Doko	2	890	21.9	42.7	22.4
Majos x Doko	8	3005	19.8	42.0	22.5
PI 165.672 x Doko	3	1855	27.7	42.8	22.5
Tadacha x Doko	3	2000	24.9	41.4	22.7
Tamba (check)		1900	28.3	42.0	21.1

The selection practiced in 2002/03 was very efficient to increase the seed yield, leading to a general mean of 2455 kg/ha, that is corresponding to a realized genetic gain of 29% in relation to the population mean before selection; coincidently, the same progress of 29% occurred relatively to the mean of the Tamba check. Some crosses distinguished by presenting seed yield means around 200% in relation to the general mean of the checks; also, there is the possibility to practice selection among individual lines within cross, because they have yielded until 300% in comparison to the general mean of the checks. This genetic gain is primarily a consequence of the cumulation of polygenes for high seed yield in the selected lines; certainly, a second factor favoring

seed yield is the superior adaptation of the selected lines to Brazilian environments as a consequence of the actions of genes for long juvenile period (Carpentieri-Pipolo et al., 2000) inherited from the Doko parent.

The other traits were not the main target of the selection and, consequently, they were not modified in a significant way. The following population means were observed, respectively before/after selection and for the Tamba check: 23.1/23.4 and 28.3 (Tamba) grams/100 seeds for seed size; 42.3/42.2 and 42.0% (Tamba) for protein content; 22.3/22.4 and 21.1 (Tamba) for oil content. It would be expected a reduction in the protein content mean as a correlated response to the selection for high seed yield, because the negative genetic correlation (Brim, 1973)between these traits. However, the maintenance of the same level of the oil content before and after the practice of selection for high seed yield could be explained by the low and positive value of the genetic correlation between these two traits.

The crosses also showed variation for the traits related to the color, shine, and shape of the seeds. The most outstanding cross was [(TN#4 x PI 230970) $F_{7:4}$ x Doko] in which all the five selected lines showed the desirable phenotypes for using as vegetable soybeans, that are, large seeds, yellow seed coat, cream hilum color, yellow cotyledons, and high seed yield (126 to 160% in relation to the general mean of the checks.). Other crosses exhibited part of the selected lines with the desirable traits previously reported: PI 229320 x Doko, Late Giant x Doko, Japão-2 x Doko. A part of these outstanding lines have been used as parents in a second cycle of recombination and, some of them are showing potential to be reliased as new cultivars for attending the future requirements of the promissing niches of market for vegetable soybeans in Brazil and South America.

Conclusion

The selection among $F_{10:15}$ lines was very efficient in increasing the seed yield, without significant changes in other traits: seed size, protein and oil contents. Several selected lines presented the desirable phenotypes for using as vegetable soybeans: large seed, yellow seed coat, cream hilum color, yellow cotyledons, and high seed yield. A part of these lines showed potential for using as parents in an additional breeding cycle and as new cultivars; thus, they can to contribute for attending the new requirements of the increasing niches of market of vegetable soybeans in Brazil and South America.

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Production and researches on vegetable soybean [*Glycine max (L.)* Merrill] in Southern China

T. Wu[1]

Abstract

This paper is to review the history and current condition of vegetable soybean (VSB) production in southern China. Cultivation, processing and breeding research are discussed, and distribution also included. Finally the author gives the prospect of VSB production in the future in the south of China.

Vegetable soybeans (VSB) are usually regarded as a delicious, high nutrient food by Chinese people and have already been used for cooking in the southern part of China for a very long time. Some local varieties of VSB have been used for a long time. They are unique resources of soybean germplasm. VSB has been sowed widely in the south of China in recent years. China becomes one of the most important areas of VSB production and consumption nowadays. The total estimated yield of VSB is approximately 1.6 10^6T, and 1.5 10^6T consumed in 2003.

Development of VSB in the South of China

Like other areas of the world, cultivation of VSB is limited by the appetite habit of Chinese people (Junyi G., 2002). *Mu Cao Yan Yi* (1116), one of the earliest books of recording edible soybean, said that "soybeans have three colors: green, brown, and black. Soybean seeds have two sizes: big and small. The big-seed genotypes are planted in Jiangsu, Zhejiang and northern Hunan". Lu You, a famous poet of South Song Dynasty, wrote many poems about harvesting VSB. *Guo Wen Tao* 993 summarized distribution of Chinese VSB in a book, named *Cultivation History of Chinese Soybean*, in which he pointed out that varieties of VSB were abundant in Jiangsu, Zhejiang and Anhui. Fresh soybean was mainly planted in the Yangtze River valley in the historical record, which is also true at present situation.

90% VSB is produced in Zhejiang, Jiangsu, Fujian, Guangdong, Hunan and Shanghai. Before the 1980s, Major cultivation ecotype was Summer-sown soybean. Spring-sown and Autumn-sown soybeans only occupy about 10%. Since 1990, such situation has changed. Spring-sown soybean became

[1] The Department of Plant Science, Shanghai Jiaotong University, Shanghai, China

dominate one and occupied about 70% in production. The area of VSB in Zhejiang was 3.7 10^4ha in 1998, 7.6 10^4 ha in 2001, 8.5 10^4 ha in 2003. The average area of VSB in Shanghai was 9 10^3 ha from 2000 to 2003 (data from Shanghai Vegetables Bureau, 2003). According to author's estimation, it may actually near to 1.5 10^4ha. The area of VSB in Fujian reached 6 10^3 ha in 2000 (Guoqiang L., Shuchuan X, 2001), and approximately 1.0 10^4 ha in 2003. The planting area of VSB in Jiangsu was 4.0 10^4 ha, according to the author's investigation. Nanjing (Jiangsu's capital city) has 1.4 10^4 ha of VSB in 2003 (data from Nanjing vegetables Bureau, 2003). The area of VSB in Guangdong is 1.0 10^4 ha in 2003 (Department of provisions and oils, Guangdong, 2003). It summed up to 1.6 10^5 ha in the south of China in 2003, and the actual area might be larger.

The production of VSB is mainly on the suburb of great cities. The area of planting vegetables in Shanghai is 9.0 10^4 ha, in which about 10% is for VSB. In Jiaxing and Ningbo of Zhejiang province, which are adjacent to Shanghai, the area of VSB reaches 1.5 0^4ha and 1.0 10^4ha, respectively. VSB produced in those areas mainly come into the big market of Shanghai. VSB produced in Nanjing is mainly for the local residents, so the planting area shows a constant number from year to year. In Nanping of Fujian province and Pushan of Zhejiang province (mainly serve to Shanghai and Nanjing markets), production of VSB reachs to 6.0 10^4 T in 2003. In recent years, some places such as Cixi and Xiaoshan of Zhejiang province become export regions for VSB production. Their VSB harvested goes into the processing in the manufactures. The planting area is 8.5 10^3 ha in 2003. Production in these regions depends on export demands.

Market circulation of VSB in the South of China

At present, VSB is sown according to farmer's own opinion or market order. The spontaneous way determines the production. There are three channels to enter markets:

Firstly, individual traders or dealers purchased VSB directly from the farmers. After long distance transport, they sale it in the city market to consumers by means of wholesale business. The circulation is a leading way, but it fluctuates with the price, consequently the total output is unstable, and the profit of farmers is limited by the merchants. For example, in 2003, the catering became slack suddenly due to "SARS", while the area of VSB in Cixi and Xiaoshan of Zhejiang province increased to 8.5 10^3 ha, and the total output exceeded 4.5 10^4 T, but the market only consumed 7.3 10^3 T. In contrast, in 2002, the whole output was 3.5 10^4 T, and 2.2 10^4 T for the market, the price in 2003 dropped to 30% of that in 2002. It is the

lowest price since the 1990s, so the profit of farmers was badly damaged. They had to harvest dry soybean, which occupied 30% of total planting areas.

Secondly, farmers sell or wholesale VSB directly in the market. Due to the transporting limitation, this pattern is only limited to regions on the suburb of great cities. This is only suitable for a few farmers.

Thirdly, farmers grow VSB according to enterprises contracts. This pattern is common for export business, and become a leading way nowadays. Only a few varieties can meet export standards (e.g. Taiwan 75).

Processing of VSB in the South of China

Processed VSB (such as soybean pickles, saline green soybean, bamboo shoot green soybean and smoke-dried green soybean) can be preserved for a long time. Smoke-dried green soybean is cooked in brine, then roasted on the fire. Bamboo shoot green soybean is cooked with bamboo shoot and lima-bean in brine before dried by smoke. One restaurant called Zhuangyuanlou" (which mean existed more than 100 years) in Shanghai processes soybean by mechanization and provides 50T soybean pickles to the market every year.

With the development of freezing equipments, deep-frozen and fresh-keeping green soybean have become the major products in recent years. A lot of mini-type processing enterprises joined into the market. Shanghai Songjiang Nongfeng Food Corporation processed 10T deep-frozen VSB (Jiaoxuan 2 bred by Shanghai Jiangtong University). Average price is as twice as that of unprocessed VSB.

Now there has been mini-type threshing-machine of VSB on the market, manual and electric. The manual one weighs 3kg and it is easy to transit and suitable for individual-process. It can thresh 5 ~ 8kg soybean per hour. The electric one is fitted for vegetable processing enterprises and thresh 15 ~ 30kg per hour.

The cultivation techniques of VSB in the South of China

There are several cultivation ways for VSB production. Monoculture, clearance and planting in ridge of field. The cultivation system is rather complicated. With the changing of farming system, farmers have more fields to plant for VSB while the area for winter wheat decreases. Most VSB is planted in open field, but greenhouse cultivation, small plastic tunnel and plastic culture ways are also exist.

Planting in open field

The technique can be divided into two types. One is early-spring-planting (70% of the whole field). Another is summer-and-fall-planting. In the south of China, VSB are mostly mono-planted in spring, and in summer mainly planted in the ridge of field.

In Fujian and Guangdong, the soybean is sown in January or August and harvested in April or May, respectively. In Zhejiang and Jiangsu it is sowed between March and May and harvested from June to August. Varieties contain Taiwan 75, Tai 1088, etc.

There is a few field for summer-sown VSB. Usually they are planted in ridges of paddy field. Large scale planting is uncommon. In Jiangsu and Zhejiang it is sown in June and harvested from late September to early November. Summer-sown soybeans are sensitive to photoperiod. The yield is affected by the planting time (Tianfu H. et al., 2002). Because blossom and pod-bearing are in high temperature, pods are easy to go bad, turn black and become thin. Planting soybeans in ridges of paddy field with low temperature and ventilation can avoid those problems. Major local varieties such as Niutabian, Lanxidaqingdou, Zhejiangbayueba and newly bred varieties such as Xinliuqing, Chuxiu, Ningshu60, Suneiqing are popular Lei Z. et al. 1998ýÿ Heping G. et al., 2000 Nianyong C. et al. 1998.

The land for autumn-sown VSB is few. In Jiangsu and Zhejiang it is mostly mono-planted in July after harvesting wheat and fruits. Its harvesting time is the same as that of summer-sown VSB, but its quality is worse so that its output value and price are lower too. Local autumn-sown VSB varieties include Jinhuaqiudou, Bayueba, Yunheqiudadou etc. Huanjinwei al et. 1996ýÿ.

The cultivation technique of VSB in greenhouse in the South of China

The technology is mainly used for spring-sown VSB. It includes greenhouse, small plastic tunnel, plastic culture cultivation. (Gu He Ping, 2000). With the rapid development of the cultivation technique in greenhouse, it becomes a new technology and mainly lies in demonstration farms. It aims to occupy the early spring market, while open field can not produce VSB due to the cold weather from March to May Weihong S, 2000. VSB is sown from Dec. to Jan. in the Fujian and Guangdong, harvested in Feb. or Mar., sown from Jan to Feb. in Zhejiang, jiangsu and Shanghai, harvested in April and May. Because the equipment used for raising temperature isn't in greenhouse, small plastic tunnel and plastic culture ways are necessary when it is cold. Jiaoxuan 1 was planted in this way on Feb 14th at Punan Farm and harvested in 20 May. The output is 5160 ~ 6330kg/ha. It has been widely used in Fengxian Distract of Shanghai (Yuping X. 2001).

Cultivation in small plastic tunnel with plastic film covering is a kind of technique with high applied value. It was used by farmers in the south of China, especially large farms. For example, the VSB of Xiaoshan in Zhejiang province entered the market twenty days in advance in 2002 in this way, and it spreads to 62.3% of total area in 2003. VSB should be sown in February and harvested in May or June in Guangdong and Fujian, but in Shanghai, Jiangsu and Zhejiang, sown in February and harvested in June. This technology is characterized by its low cost, convenience in operation.

Plastic culture is a kind of traditional cultivation technique. In order to provide VSB to the market earlier and get better profits, some farmers culture the seedlings in the plastic tunnel, and then transplanted into fields. In Shanghai, Jiangsu and Zhejiang, it needs to nurse seedlings in March, and to harvest in the early June. Lately, with the diversion of labor force, the planting areas for this technology become smaller. But it can also be preserved up to now because it can provide VSB to the market earlier and farmers get good benefit.

VSB breeding in South of China

The breeding works of VSB in China began at the late 1990s. Some varieties such as Xinliuqing, Zhoudou 30, Jiaoxuan 1 and 2 have been bred. But the quality in most cultivars can not meet the standard of the international market.

Study on specific gravity and ecological property of VSB seeds

Ma Xiaohong(2003) demonstrated that differences existed in the specific gravity of soybean seeds in different latitudes and different types of soybean varieties. The results indicated that the specific gravity of spring soybean increased along with the rising of latitude. The specific gravity of summer soybean varieties and autumn soybean varieties were greater than that of spring soybean varieties. However, in the same area the variability coefficient of spring soybean varieties was larger than that of summer and autumn soybean varieties. The specific gravity of the small size soybean was greater than that of large one. The specific gravity of soybean seeds was positive correlated with the growing period from seeding to mature, as well as with the growing period from seeding to pod setting. The specific gravity of soybean seeds was significant negative correlated with the day ratio between blooming and pod setting. Slow seed filling was advantage for soybean specific gravity increasing. The relationship of the specific gravity of soybean seeds and temperature changes from positive to negative correlation. The relationship of the specific gravity of soybean seeds and the range of temperature changes from negative to positive correlation.

Research on the yield trait of VSB

VSB differs from other soybeans on yield traits and it demands merchandise indexes as well. For example, the pods number should be under 340/kg. A pod should set 2-3 seeds, pod waste should be bigger and thinner.

Wu Tianlong (1998) found that VSB is mainly for its pod waste development in the early stage and for seeds development in the middle stage and for seed dry weight in the late stage. Since the pod descend rapidly for dehydration in the late stage, the idea harvesting time is the middle stage.

Han Lide (2003) found the pod's width and length of summer-sown VSB increase quickly in 10-20d after flowering and pod elongation rate reach the maximum in 25-40d after flowering. Development of the weight of 100 seeds fit to quadratic curve, the weight of 100 seeds reached the maximum in 35-55d after flowering for Cultivar, and 55-70d after flowering for cultivars. Content of water-soluble sugar begin to increase gradually in R_5-R_7 growth stage, and it decrease after R_7 growth stage. The author suggested the favorable time for the harvesting of VSB based on these development characteristics: R_6-R_7 growth stage.

Wu Tianlong (2003) divided the VSB pods peel into two parts: inner and leather coat. They have significant positive correlation to fresh weight of pods, the weight of 100 seeds and the yield of single plant, respectively. Path analysis indicates inner coat play a determinate role in the pod yield of single plant and the weight of 100 seeds.

Wu Tianlong (2000) found the length of pods has significant positive relationship with the width of pod. Since both of length and width of pod have significant positive relationship with the weight of 100 seeds, they could be used to forecast the weight of 100 seeds. As the international market standards, 6.4cm length of pods, 1.63cm width of pods, 70g weight of 100 fresh seeds, 34g weight of 100 dry seeds are suggested, which makes the difficulty for the breeding of spring-sown VSB.

By observation of cryo-sections of different soybean cultivars under microscope, Lv Xiaoming (2003) found that there were differences in the cell density of cotyledon and seed coat between vegetable-type and grain-type soybean cultivars. Grain-type soybean has compact cell in the cotyledon, whereas vegetable-soybean has loose. Cell density of the seed coat palisade layer and infundibular layer of VSB is higher than those of grain-type soybeans. VSB become soft after a short period of cooking, whereas grain-type needs a longer cooking.

Breeding of vegetable soybean in the South of China.
In the south of China, many VSB cultivars were landraces. Among those many summer-sown cultivars are actually good in some characteristics. In Zhejiang and Jiangsu province, spring-sown landraces Shanghai Liuyuebai, Wuyueba, Suzhouwuyuehuang were popular, summer-sown landraces such as Lanxi Daqingdou, Niutabian, Zhejiang Bayueba were popular(Heping G, 2000; Nianyong C, 1998).

After the 1980s, production of Chinese VSB increased rapidly, some new cultivars was bred. Summer-sown cultivars used widely are as following: Chuxiu bred by the Huaiyin Agriculture Science Institute of Zhejing province, Xiangchun 18 bred by the Crop research Institute of Huann Province has a high fresh pod rate (Jianfeng M, 2002), Xinliuqing and Andou 3 bred by the Agriculture Science Institute of Anhui province (Chengju X, 2001). Shiyueqing bred by Agriculture Science Institute of Anhui province is a fine autumn-sown VSB (Ouhe D, 2001).

There are lots of varieties of spring-sown VSB in China: Liaoxuan1 bred by Liaoning Academy of Agricultural Science, Jiaoxuan 1, 2 bred by Shanghai Jiaotong University (Tianlong W. et al., 2002), Qingdali 1, 2 bred by Fujian Academy of Agricultural Science (Shuchuan X, 1999), Shennong 951 bred by Shanghai Academy of Agricultural Science, Tezao 1 by the University of Anhui (Zhihao W, 2000), Huijia 2 by Jiangsu Academy of Agricultural Science (Xin C. 1997) and Ningshu 60 bred by Nanjing Vegetable Institute.

Prospects of VSB development in the South of China

It develops rapidly on VSB in the south of China, but there are some problems to be solved.

As long as the current cultivar (Taiwan 75) has good quality already, future breeding works should focused on the improvement of the disease-resistance, earlier maturity (10-15 days earlier), the green cotyledon, and insensitivity to photoperiod.

Planting technique of plastic tunnels should be popularized. The technology on fertilizer application, management and disease control should also be studied. VSB production should be also addressed to meet all-year-around consuming market, so researches for whole year VSB production is still under the way.

VSB processing must be improved. Processing techniques on soybean pickles, saline green soybean, bamboo shoot green soybean and smoke-dried green soybean should be studied further.

Circulation channels need to be widened. Processing manufactures and business companies should cooperate with farmers to fulfill order-production and stable base.
One of the most important bases of VSB is setting up in the south of China. With the needs for VSB increased home and aboard and economic development, VSB in the south of China would become a major vegetable.

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Edamame development in Australia

A.T.James[1]

Abstract

This paper reports the research program with aims to underpin varietal development and quality assurance for the emerging edamame or vegetable soybean industry in Australia.

Introduction

Edamame is a traditional food of Japan and China that is now consumed throughout east-Asia (Yinbo et al., 1997). Traditionally, the whole plant is harvested green at the R6 or R7 stage (Fehr et al., 1971; Mebrahtu et al., 1991; Rao et al., 2002) and transported intact to market to assure customers of the freshness of the product. After purchase, pods are removed from the plant, boiled and consumed as a snack food. In Japan, the common use for edamame is as a snack consumed with beer in commercial establishments. Boiled salted pods should be blemish-free and bright green (Shanmugasundarum et al., 1997; Carter and Shanmugasundarum, 1993). Traditionally, cultivars with green seed coat and cotyledon at maturity have been preferred by growers because the harvest period can be extended closer to maturity of the plant without experiencing the yellowing associated with maturity. Seed pods should have sparse gray pubescence and contain three seeds per pod, though two seeded pods are acceptable in the market (Konovsky et al., 1994). There should be an absolute minimum of one seeded pods because they are disliked by the consumer, requiring greater effort to shell them. Four seeds in a pod are not preferred because the number four is considered unlucky in Japanese culture.

Desirable edamame should have very large seed, high sugar levels, and a smooth texture (Konovsky et al., 1994). Varieties suitable for edamame generally possess greater than 10% dry weight of sucrose from mid pod development until maturity (Masuda and Harada 2000). Young et al. (2000) found that beans that were sweet were also somewhat nutty, less beany flavored, slightly oily, without an unpleasant aftertaste, and generally better in overall eating quality. This is not surprising given that sugar content is positively correlated with oil content and negatively with protein (Krober and Cartter, 1962; Geater et al., 2000; Hartwig *et al.*, 1997; Wilcox and Shibles, 2001). For the fresh-frozen market, uniformity of maturity, a thicker

[1] CSIRO Plant Industry, Qld Bioscience Precinct, 306 Carmody Road, St Lucia Qld 4067 Australia.

pod wall to reduce freezing damage and plant habit to permit mechanized harvest is required in addition to the quality traits required in the fresh product (Chotiyarnwong *et al.*, 1996). Cultivar development for *edamame* for the fresh market should focus on production in multiple sequential planting dates so that the harvest period can be maximized.

In Australia, initial consumption of high-quality locally produced edamame will occur in the traditional setting of Japanese restaurants. However, as interest spreads it is anticipated that as edamame will become a high-value fashionable vegetable and wider use will occur in the bar and restaurant trade and in wealthy households. After all edamame is a highly palatable and attractive food that is enjoyable to consume with beer. Consumption is therefore likely to appeal to Australians. Ultimately, export of fresh edamame to Japan will develop to supply markets out of season to northern hemisphere production.

Limitations to production

The edamame improvement program in Australia seeks to understand and overcome limitations to production of high quality edamame in Australia. Research therefore seeks to define the factors limiting uptake and commercialisation.

Seed production. Production of high quality planting seed was a key constraint to production that needed to be overcome. In many Australian cropping environments, traditional varieties have uneven ripening within a plant and severe shattering of grain. Often pods at the top of the plant shatter while pods lower on the plant were still green. If mechanically harvested, large seed with very low moisture content tends to be mechanically damaged, whilst immature seed with high moisture content tends not to be viable after drying.

When grown in some key Australian environments, many traditional varieties have a range of seed maturity and seed moisture content within a single plant. Varieties of this type are not suitable for mechanised harvest. This key constraint was overcome by the development of genotypes with more synchronous maturity and reduced seed shattering.

Planting and agronomy. Varietal development initially focussed on developing large seeded genotypes with high grain sugar content and resistance to foliar and root diseases. The varieties also had to be adapted to a range of latitudes from 16°N to 28°S, to summer and winter production in the tropics and to a wide range of planting dates in the subtropics.

Harvesting. Two techniques for harvesting have been trailed. Hand harvested intact plants which were lightly trimmed of excess leaves and harvesting using a green-bean picker. Hand harvested product was far superior in quality to the machine harvest. Hand harvesting is however labour-intensive and therefore expensive. It was predicted that efficiency of hand harvest would be improved by having larger plants grown at quite low plant populations.

Harvest window. In order to supply fresh edamame to markets with produce over a sufficiently long production season to permit market development, production needs to occur over at least a three-month period from any region of production. Varietal development therefore needed to focus on broad adaptation to sowing date in a range of production regions.

Distance to market. Australia is a large country, markets for fresh produce are often up to 2000 km by road transport from areas of production. Techniques were needed to transport fresh edamame to market in good condition. In trials in which whole plants were harvested at the R6 or R7 stage and transported intact to market using technology similar to that employed for Broccoli, customers were delighted with the freshness and quality of the product.

Key research themes

Photothermal adaptation. Prior research (James et al. 1997) has shown that it is possible to combine the long juvenile trait, which is a single gene source of non-photoperiod sensitive delayed flowering, with otherwise early maturing genetic backgrounds. In this way genotypes with medium duration, broad adaptation to planting date and wide adaptation to latitude can be produced. The change from temperate to sub tropical adaptation can be done though backcrossing of the long juvenile into otherwise desirable genetic backgrounds in order to convert adaptation from higher to lower latitudes whilst retaining the quality characteristics of the original.

A confounding factor in the edamame breeding program was that many of the recurrent parents had undesirable attributes such as susceptibility to foliar diseases and severe seed shattering at maturity. A range of donors for the long juvenile trait, which also possessed desirable resistance to foliar diseases and agronomic traits, were utilised in a limited backcrossing and selection strategy involving several recurrent parents (Table 1).

Ultimately a genotype possessing the long juvenile trait, large seed size, high sugar content, resistance to foliar diseases, relatively even maturity

and low seed shattering was selected from a complex backcross with the parentage (((Paranagoinana X Akita #2) x Toyomasari) x Saturn). This genotype appears to possess good adaptation to the potential cropping regions in Australia (Figure 1) and may be sown over a wide planting window to enable harvest during both the autumn and spring seasons in north Qld and during the summer period in southern Qld and northern NSW.

Table 1. Parents used in the edamame breeding program Donors of the long juvenile trait Recurrent parents with edamame quality or other desirable traits

Donors of the long juvenile trait	Recurrent parents with edamame quality or other desirable traits
G2120	KS # 1
Melrose	CM #1
Paranagoiana	Vesoy #1
	Vesoy #4
	Saturn
	Toyomasari
	Tanbaguro

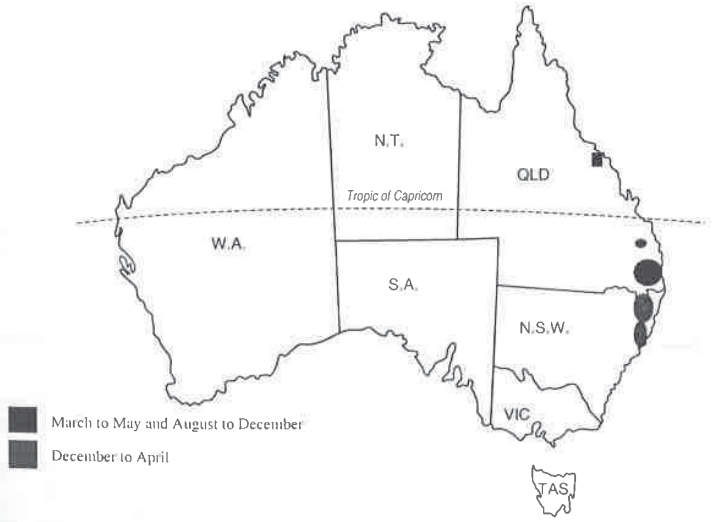

Figure 1. Cropping areas in Australia likely to produce edamame in the first phase of production.

Improving sugar content. The research program aims to document environmental effects on sugar accumulation in grain and in so doing develop advice for growers on how to manage and when to harvest to maximise quality. At this stage, trials have shown that there is genotypic variation for late accumulation of sugars in soybean grain.

Harvest timing for peak consumer quality. This work seeks to develop a critical understanding of the ripening process in CSIRO-bred and elite Japanese cultivars of edamame soybean, and in particular, to understand how changes in seed size and the seed content of protein, oil, sugars (fructose, glucose, sucrose, raffinose and stachyose), vitamins (A, D and E) and isoflavones (genistein, diadzein and glycitein) vary with ripening and maturation environment. This knowledge will be used to develop strategies to ensure consistent supplies of excellent quality edamame to high-value markets initially in Australia and subsequently overseas.

Conclusions

Much work remains to be done in the commercialisation of Edamame in Australia. However, the necessary background technology to enable the production of a high quality product and the transport to market whilst retaining this quality will soon be sufficiently advanced to enable commercial production to commence.

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Selecting the right beans for food processing

K. Liu[1]

Abstract

Natural variation, plant breeding, and, in recent years, biotechnology have made soybeans vary significantly in physical appearance, chemical composition and physicochemical properties among varieties. As a result, food industry now has an array of soybeans for selection. On a broad classification, we have genetically modified soybeans and conventional soybeans. We also have soybeans for direct food uses and soybeans for crushing into oil and feed meal. Based on specific end uses, we have tofu beans, natto beans, edamame (green vegetable) beans, beans with modified oil composition, and beans for better feed uses, etc. Most of these variations are selected or bred for improved quality, value-added utilization, and premium price. Just like different wheat products require different types of wheat as starting material, for best quality, different soy products require different varieties of soybeans. To help food industry select the right beans, in this presentation, I will discuss some of these beans with respect to their features and targeted applications. This is followed by analysis of driving forces and hurdles for development and commercialization.

Introduction

Soybeans are not just soybeans. In fact, they vary significantly in physical appearance, chemical composition, and physicochemical properties among varieties. Variation in appearance includes seed size, shape and color. Seed color is further narrowed down to seed coat color, hilum color and color of cotyledon tissues. Variation in chemical composition includes contents of major constituents (protein, oil, carbohydrate) and minor constituents (lipoxygenase activity, phytic acid, isoflavones, etc.). It also included quality or sub-components of these constituents, such as fatty acid composition of soy oil, amino acid composition of soy protein, ratio of protein factions, and soluble sugar content within the total carbohydrate. Physicochemical properties include cooking quality (texture) and taste. All these differences can affect visual appearance, texture, taste, nutritional value, health benefits, and production yield of finished products. For examples, light colored beans give a whiter soymilk or tofu product. High protein beans tend to produce a soymilk or tofu product with high protein

[1] Adjunct Associate Professor, Dept. of Food Science, University of Missouri, Columbia, MO, 65211, USA

content and improved yield. High isoflavone beans lead to a high isoflavone product. Soybeans with high soluble sugars give a sweeter taste as green vegetable when harvested at 80% maturity. Even the ratio of two major protein fractions, glycinin and beta-conglycinin, known also as 11s and 7s, can affect product quality. As early as in the late 70, researchers found that higher ratio of 11s/7s beans produce a firmer tofu. This is just like different types of wheat for different wheat products. For best quality, different soy products require different varieties of soybeans.

At present, soybeans have three major uses, as protein food, edible oil and feed, although industrial use is gaining momentum. Over the years, many types of beans have been bred to fit each of these uses. In order to meet special needs of food processors, for the past several decades, soybean breeders at public institutions and private companies have set priorities to breed soybeans with improved quality and modified composition. Consequently, many specialty soybeans have been bred, including food grade soybeans, such as natto beans and tofu beans, and soybeans with high protein, high oil, altered fatty acid composition, enhanced amino acid composition, low oligosaccharide content, or low lipoxygenase activity. Some of these beans have successfully been commercialized, while others are waiting for commercialization, depending on economic feasibility.

Oil beans

Since majority of soybean production is crushed for edible oil and defatted meal, not surprising, what we commonly see in the field or market are commodity beans, known also as oil beans. In general, these beans have small to medium seed size, dark hilum, high oil content, high field yield, but low to medium protein content.

Food beans

Protein food made of soybeans mainly refers to various types of traditional soyfoods, including tofu, soymilk, green vegetable soybeans (edamame), soy sprout, soy sauce, miso, etc. Many of them date back to thousands of years ago in China. It also includes modern soy protein products made mainly from defatted soy meal. Although commodity soybeans can be processed into various types of protein food (All soybeans are edible, by the way), for better quality, nutritional value and production yield, manufacturers are increasingly looking for many specialty soybeans that are targeted for different end products. As a result, we now have tofu beans, edamame beans, lipoxygenase-null beans, and low oligosaccharide beans, etc.

Tofu beans are bred mainly for soymilk and tofu production. Compared to the commodity soybeans normally seen in the field, tofu soybeans are

clear in appearance (clear hilum, light yellow to yellow seed coat), whiter cotyledon color, large in seed size, and superior in composition (e.g., higher in protein content, improved ratio of 11S/7S, or lack of lipoxygenase). When soymilk, tofu or other soy products are made of these special beans, improvements in production yield as well as quality (appearance, composition, and taste) are expected. Natto is an ethnic Japanese soyfood made by fermenting whole soybeans with *Bacillus natto,* a bacterium. When properly prepared, natto has a slimy appearance, sweet taste, and a characteristic aroma. For natto making a key factor affecting the quality of natto is selection of right soybeans. Natto beans are featured by very small seed size, clear hilum, higher sugar profile and other traits. Small beans have larger surface and allow faster growth of the natto organism.

Soybeans with altered fatty acid contents

Oftentimes, a vegetable oil is not considered ideal in term of its three quality attributes: nutritional value, oxidative stability, and functionality, all of which are determined mainly by fatty acid composition of the oil. For the past several decades, plant breeding has been a priority approach to alter the fatty acid composition of soybeans. This has led to the development of many new oils in which one or two major fatty acids either diminish or predominate, with each type of oil having its own targeted food application and market value. These include: 1) low linolenic acid oil aimed at increasing oil oxidative stability, 2) low saturated oil aimed at meeting consumer's choice for healthy items, 3) high oleic acid oil aimed at improving both health image and oxidative stability, and 4) high saturate oil aimed at replacing hydrogenated oils for production of low to zero *trans* shortenings and margarine.

GM soybeans

Challenges and prospects

Although many specialty soybeans have been shown to have improved end use values, there are some issues remained. First, many specialty soybeans have a lower field yield compared with commodity beans. Thus, a premium is usually imposed for compensation of the yield loss. Second, production and handling of specialty soybeans require identity preservation, which adds additional cost. Third, many new beans, particularly those with altered fatty acid composition or altered amino acid composition have not successfully been commercialized at this time. High cost is certainly a major reason. Therefore, more efforts are needed to improve filed yield of new beans with a specialty trait. Plant biotechnology has proved to be an effective tool in this regard. Additional efforts in commercializing these new beans are also needed.

Optimism exists for the future of specialty soybeans. This is because there are several driving forces, including a demand from end users for better beans and better oils, a pressure for farmers to increase crop values, advancement in breeding technology, such as molecular breeding, genetic engineering and genomics, and development of high throughput analytical techniques for breeding screen. A phenomenal growth in soyfoods industry and increase in global acceptance of biotech crops serve as additional driving forces. All these forces have led to a new industry trend of transforming from grain commodities to grain attributes.

Conclusions

For better end product quality and yield, soybean processors have prized for various types of specialty soybeans. At the same time, to improve crop values for farmers, breeding for specialty beans has been an integral part of overall breeding programs in the U.S. and elsewhere. As a result, we are seeing an industry trend that transforms grain commodity into grain attributes.

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Industrial quality of soybean in Argentina

M. Cuniberti[1], R. Rossi[2], R. Herrero[1] and B. Ferrari[2]

Abstract

The environment is an important factor in the expression of soybean oil and protein quantity and quality. In areas of lower latitude where the seed filling period matches high temperatures, the oil quantity and quality are increased with better fatty acids composition. The increase in the oleic acid and the decrease in the linolenic one is associated with low oil stability. In years or areas that the grain maturity occurs with cool temperatures, the oil has a different composition and presence of linolenic acid increases. A similar effect occurs when the planting date is delayed, increasing the protein and decreasing the oil content in later dates. Transgenic varieties have a fatty acid composition, and a protein and oil content similar to non transgenic ones. The Argentine oil industry has carried out important investments, increasing the mill capacity, improving the port facilities and enlarging the storage facilities, having the most modern plants in the world with high technology to respond to the demand requirements. Soybean represents the country main production with 25% of the Argentine total exports. Home consumption of oil and flour is minimum, being exported 96% of flour and 95,8% of oil production. It is estimated an increment of its importance and production in next years.

Production importance

The soybean is the crop of quickest adoption and expansion in the history of the agriculture in Argentina. With a national production of 59.000 tons (1970/71), in 30 years the country achieved a production of 25.890.000 tons (2000/01) and the crop has become the main product of the national agriculture for its participation on the total value of production and export. The agricultural production shows a very important technological impact due to the non tillage, the biotechnology, the genetic improvements as varieties of short maturity groups (MG) for early planting dates (PD) and the use of silobags in the grain storage.

Argentina has doubled its agricultural production from 35 million tons of grains, basically wheat, corn, and sunflower in the '80s, to 70 millions of which 35 millions are of soybean during the last year.

[1] Agricultural Experimental Station, INTA, C.C 21-2580- Marcos Juárez, Córdoba, Argentina.
[2] Nidera S.A, Venado Tuerto, Sta Fe. E-mail: mcuniberti@correo.inta.gov.ar

The Mercosur *[South Common Market]* still brings possibilities of greater expansion due to the availability of increasing the planting surface during the next years. It was estimated that in the 2003/04 season, its production would overcome USA, but this leadership had already been overcome in the 2002/03 season.

Between the 1992/93 and the 2002/03 season, the planted surface in Argentina increased 238%, with increases in yields of 28% and in production of 313%. In the last harvest, soybean maintained the leadership in cultivated area increment with 700.000 hectares of a total of 12,8 million hectares. The production was of 35,273 million tons and the average national yield was 2780 kg/ha, which can be compared to the highest of the world. In the North Pampeana Region, average yields were of 3500 kg/ha having a maximum of 5300 kg/ha in soybean of short MG. This new production record was the result of the expansion in the sowed surface, the increment of yields and of very favorable climatic conditions. The modern technology used by producers contributed to increase yields.

Currently, soybean covers a wide ecological area that extends from the 23° (in the north of the country) to the 39° of south latitude, concentrating mainly in the North Pampeana Region (it includes part of the Provinces of Santa Fe, Córdoba, Buenos Aires, Entre Ríos, and La Pampa), with near 94% of the sowed surface and 95% of the total production of the country. The rest is spread between the North Region and the South Pampeana Region.

There are different maturity groups from MG II (in the South Pampeana Region) to MG IX (North Region) being those most planting varieties from MG III to VII.

Since 1982, in Argentina there were registered 343 soybean cultivars of which 51,6% corresponds to national creation and the rest to introduced germoplasm. Between national germoplasm, 69% of the cultivars corresponds to the private sector and 31% to the public sector, while regarding introduced germoplasm, 90% is from U.S origin and the other 10% from Brazil. The genetic gain yield average in Argentina is of 0.73% of increment per year or 20.7 kg/ha/year (Baigorri et al., 2002).

In Argentina, genetic research programs of transgenic soybean were developed from the beginning of the '90s. The most relevant works were the selection of events with gliphosate tolerance (RRÔ). In 1996, the entity that rules agriculture authorized the production and commercialization of the event 40-3-2 and its derived lines, so breeding programs started to work in the creation of varieties that have this event with adaptation to the

main production areas of the country and the development of technologies adapted for the production (Rossi, 2002). The incorporation of transgenic varieties allowed to double the soybean surface in Argentina growing the crop in marginal areas and allowing the expansion of agricultural frontiers and the industry strengthening. The national soybean yield increased more than 15% since the introduction of these varieties. The commercialization of the transgenic grain has been carried out without problems and Argentina still has 95% of the production in several international markets, becoming the first exporter of oil and protein flour in the last years. The transgenic soybean in Argentina has produced one of the biggest technological changes in the history of the modern agriculture.

Industrial quality

The quality of the Argentine soybean is good, it is measured by the protein and oil values contained in the grain that together reach 62% or more (Table 1). In comparison with other producer countries, protein and oil contents are lower than in Brazil and at the level of U.S. In the last years, the main characteristic of the industrial quality of Argentine soybean is that it shows high oil content and relatively low protein (Table 1), mainly in the North and South Pampeana Region. This is due to environmental and genetic factors. The best known varieties have been selected to get high yield, they have high oil and low protein content; showing a positive relationship between high yield and oil content and a negative one with proteins.

Table 1. Protein and oil contents and profat expressed as % of dry matter (dm) of Argentine soybean.

Seasons	Protein (%)	Oil (%)	Profat (%)
1997/98	39.3	22.8	62.1
1998/99	39.1	22.6	61.7
1999/00	39.5	22.3	61.8
2000/01	39.7	23.2	62.9
2001/02	38.4	23.9	62.3
2002/03	38.5	22.7	61.2
Mean	39.1	22.9	62.0

In general, in areas of lower latitudes (north of the country) this relationship is not observed, showing high values of proteins and oil at the same time,

mainly of proteins, being obtained 43%dm of protein and 22.5%dm of oil. In the North Pampeana Region, they vary between 38-42% and 20-24%. The varieties cultivated in the Argentina come from breeding programs which objectives are to get high yields and diseases resistance, without a specific objective to increase protein and oil levels. However, in the soybean germoplasm it is possible to meet genotypes with protein value of more than 47%dm, but with very low oil content. Studies carried out by the INTA Marcos Juárez (Herrero y col., 2003) show that there is a great number of varieties in Argentina with different characteristics in relation to oil and protein contents.

Proteins

Until 2002, there were not enthusiasm to grow specific varieties with higher protein and oil contents because the local market did not consider them. In the last season, a private company developed a system through direct contracts with farmers to promote the use of selected varieties with high Profat (Protein + oil), (Rossi, 2002).

The Argentine oil industry is having problems in the production of flours with a protein content higher than 44%. An important value to consider is the protein that remains in the flour after the oil extraction. In the last years, this value has remained constant about 49.5% dm getting from 43% to 12.5% of humidity in the grain. To achieve 44%, humidity should be diminished to 11-11.5% and this causes a cost and yield loss in the process. A proper value is 50.8% of protein after the extraction process with a grain value of 40.35% dm. The situation is more difficult for the industry when it is necessary to produce "Hypro" flour that have 46.5% of protein to 11% of humidity (Rossi, 2003). In this case, it is necessary more protein content. For this reason, the industry currently needs an increment in protein content in the argentine soybean.

There is genotype-environment interaction in the expression of oil and protein contents; however, the relative values among varieties are in an environment average. For each region or MG there is diversity in protein and oil contents and profat.

High temperatures and drought stress produce lower protein and higher oil content.

The relationship was also observed regarding planting date (PD). The consequence is an increase in the protein content from 39.2% to 41.5% and a decrease in oil content from 22.4 to 19.9% with the delayed PD

from November to January (Cuniberti et al., 2000). In the North Pampeana Region, the oil decreases 0.53% and the protein increases 0.45% for every month of delay in the PD from October to January (Herrero et al., 1999).

The MG is also important, increasing 1.5% the protein average and decreasing 0.98% the oil content for each increase in the MG in the PD of January, not being a significant effect in the dates of November and December. There is a tendency to more protein and less oil content in longer MG.

Oil

The high oil content of the Argentine soybean is good for the oil industry, but in the last years it was observed a higher production of linolenic acid (C18:3) in some export shipments that were questioned. This situation generated discussions on the possible causes and they were attributed to the production and expansion of the transgenic soybean, a reduction of the MG in all regions, the early planting, and the climatic changes of the last times. In order to know if the increment was due to "genetic modification" or other factors, the company Nidera (Muratorio et al., 2001) has developed a study on soybean oils coming from traditional and transgenic varieties in different locations and PD. The study concluded that the increment observed in the last times has not been due to genetically modified varieties known as RR (Tolerant to Gliphosate), since differences are not observed in the average values of their respective oils. Table 2.

Table 2. Fatty acids composition in transgenic, non transgenic and in a blending

	C14:0	C14:1	C16:0	C16	C18	C18:1	C18:2	C18	C20	C20	C22	C22	C24
All	0.1	0.0	11.6	0.1	3.9	21.1	54.3	8.3	0.2	0.1	0.2	0.0	0.0
Non	0.1	0.0	11.5	0.1	3.7	21.6	54.5	8.1	0.2	0.1	0.1	0.0	0.0
Transge	0.1	0.0	11.8	0.1	4.0	20.6	54.2	8.5	0.3	0.1	0.2	0.0	0.0

In relation to the atypical values, these are the same in transgenic or non transgenic soybeans and they can be due to other factors. Regarding the degree of unsaturated oils that is showed by the relationships between the fatty acids themselves and through the Iodine Indexes, they bring the same average value. Table 3. The oil and protein quantities also have similar values (Cuniberti et al., 1999).

Table 3. Total saturated, unsaturated, monounsaturated and polyunsaturated fatty acids contents in transgenic and non transgenic Argentine soybean.

	Total Saturated	Total Unsaturated	Monounsaturated	Polyunsaturate
All	16.0	84.0	21.3	62.7
Non Transgenic	15.6	84.4	21.8	62.6
Transgenic	16.4	83.5	20.8	62.7

It was also observed in the North Argentine locations and the cultivars planted early in which the seed filling period relates to high temperatures produce higher oleic and lower linolenic content. In general, short cycle varieties produce low levels of linolenic and those of long cycle high levels, being partially attributed to the maturity moment that they are exposed.

In order to observe the effect of latitude, PD and MG on the oils quantity and quality, they were carried out studies (Cuniberti et al., 2000) in which it was observed that the saturated fatty acids (palmitic and stearic) are relatively constant in the different regions, while significant variations were observed among environment, PD and MG in the unsaturated acids.

The oleic acid, which is a desirable fatty acid, decreases in latest PD, in longest MG, and in highest latitudes, while the linolenic increases. Fig. 1 and 2 show the average performance in the PD of October, December, and January of the locations of Reconquista, Marcos Juárez, and Balcarce in 2003.

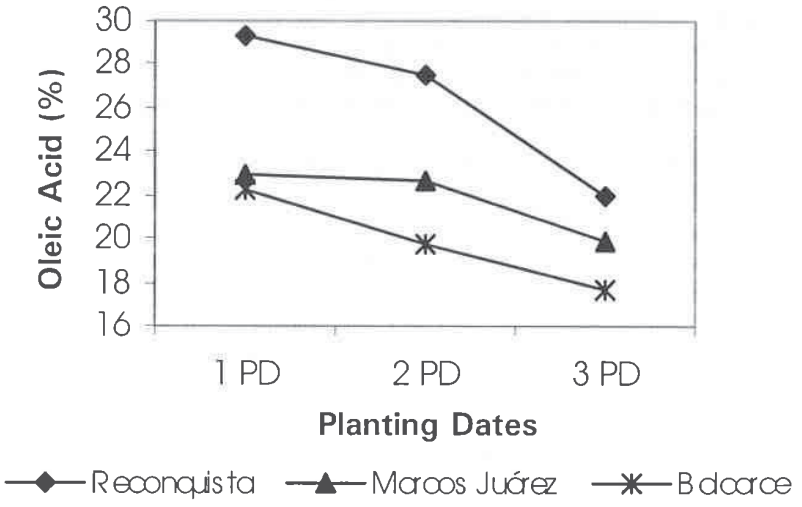

Figure 1 . Oleic acid content expressed in percentage (%) of oil content in three locations and three planting dates mean.

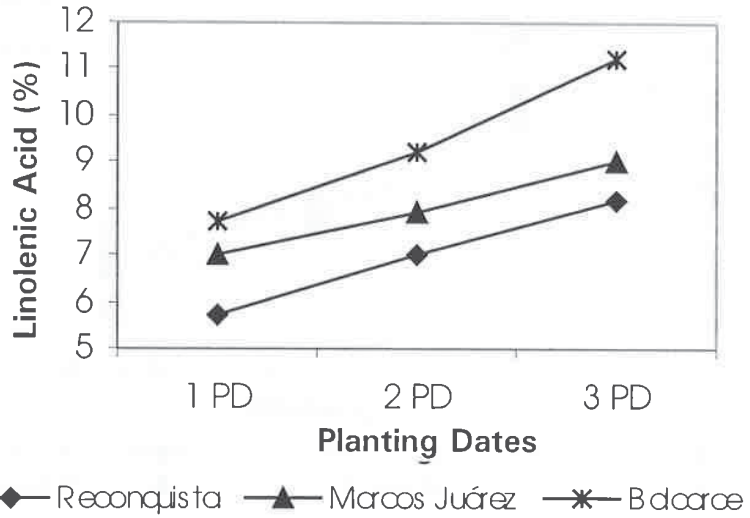

Figure 2. Linolenic acid content expressed in percentage (%) of oil content in three locations and three planting dates mean.

The latitude effect was significant, showing that the oleic acid decreased from 28.8% in the lowest latitude in the north area (29°11'SL) to 19.8% in the highest latitude in the south area (37°45'SL). On the other hand, the linolenic acid increased from 6.5 % to 9.4 %. Table 4. Fig. 3 and 4.

Table 4. Protein and oil contents (% on dry matter) and fatty acids composition (% of total fatty acids) at different latitudes

Latitude	Protein	Oil	FATTY ACIDS (%)				
	(%)	(%)	Palmitic	Stearic	Oleic	Linolenic	Linoleic
29°11'SL	39.7	23.6	10.3	4.6	**28.8**	**6.5**	47.4
32°41'SL	39.4	22.5	10.9	4.4	**22.8**	**7.7**	51.8
37°45'SL	37.8	20.6	10.5	4.2	**19.8**	**9.4**	54.0

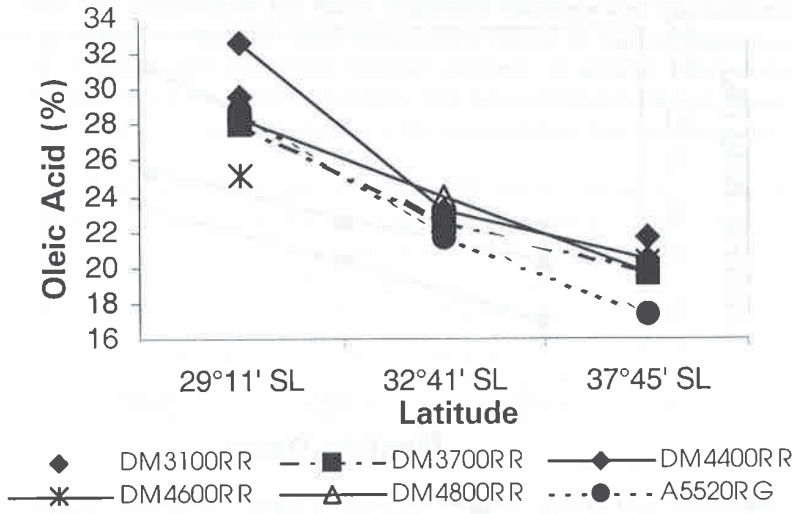

Figure 3. Oleic acid content expressed in percentage (%) of oil content in Reconquista (29°11'SL), Marcos Juárez (32°41'SL) and Balcarce (37°45'SL), three planting dates mean.

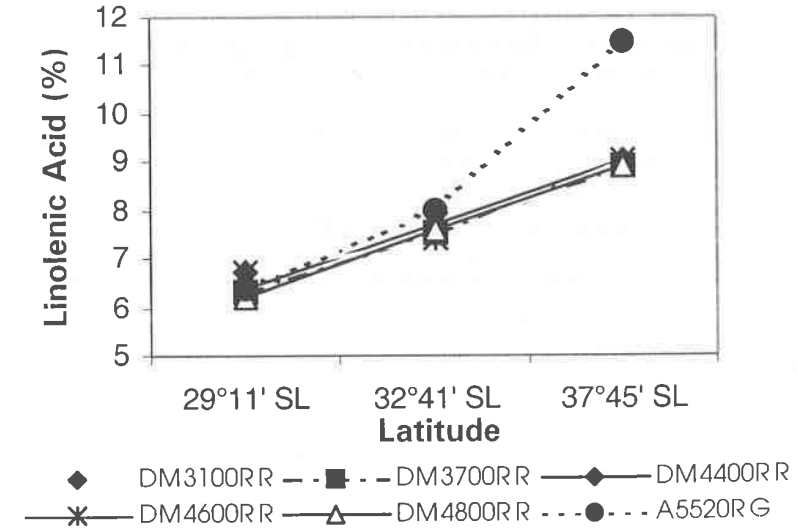

Figure 4. Linolenic acid content expressed in percentage (%) of oil content in Reconquista (29°11'SL), Marcos Juárez (32°41'SL) and Balcarce (37°45'SL), three planting dates mean.

Damaged grain and acidity

Damaged grain and acidity increase due to different causes: the delay in harvest due to rains, what happened in the last crops, the temperature and drought stress during seed development, and the increment of diseases levels in the last times like Purple seed stain, Alternaria pod and seed decay, Fusarium blight and Phomosis seed decay, among others. The consequence is the increase of the acidity in grains more sensitive to heat when processed in the industrial plants. For this reason, industry should low the drying temperature determining lower yields of "crushing." To improve the acidity levels, it is necessary a greater degummed. This increases the industry cost, the reductions in physical refinement in neutralization and changes the profile of fatty acids.

It is important to mention that in spite of the sanitary aspects of the grain, there was not detected any mycotoxin type.

Diseases are increasing due to the extension of the planted area and the well-known "of end cycle" are the main ones. Although breeding has developed better varieties and they do not usually produce important yield losses, they affect the production quality.

Industry

The Argentine oil industry has carried out important investments, increasing the mill capacity, improving the port facilities and enlarging the storage facilities, having the most modern plants in the world with high technology to respond to the demand requirements. Soybean represents the country main production with 25% of the Argentine total exports.

Home consumption

In Argentina, the consumption is promoted with a great variety of products for different applications. It is a country with a strong culinary tradition based on the animal proteins, so soybean is not massively accepted, yet. Currently, it is being promoted the direct consumption of soybean through programs and plans to be incorporated in school dining rooms, hospitals, homes of short resources children, etc. to supplement their nutritional diets with food of good nutritional value and also cheap.

Home consumption of oil and flour is minimum, being exported 96% of flour and 95,8% of oil production.

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Assuring soybean quality: application of high throughput analytical technologies and quality management systems

P. K. Das[1]

Abstract

A technology revolution is quietly taking place in the soybean industry where new advanced analytical technologies, combined with the adoption of international quality management systems, are ushering in a breakthrough in generating high quality analytical data to support the product delivery pipeline of nutritionally enhanced soybeans for the food, feed and processing applications. Intensity and complexity of analytical data are dictated by the needs of growers, grain handlers, processors, food and feed companies, and consumers. These needs are further defined in specificity, accuracy and throughput by the scope and nature of product development stage. Many of the analytical technologies are high throughput, meaning they generate high volume of data on soybean traits and attributes. For example, during the development of a putative high protein soybean variety, hundreds of thousands of soybean single plants may be screened to select seeds for line advancements. These technologies are also being called upon to provide data on numerous soybean quality traits and attributes simultaneously because of the increasing demand for agronomically and nutritionally better beans for the industry. Meeting such analytical needs require the development and use of appropriate high throughput analytical technologies. Additionally, such analytical data generation needs adoption of quality management systems, underpinned by robust information management systems. That way, soybean product delivery pipeline stakeholders can use the millions of analytical data points made available through the high throughput analytical technologies - effectively and reliably - in making their business decisions in a timely manner.

Introduction

With the advent of increasing consumer and market interest in the output trait products, the soybean industry is undergoing a technology revolution in the form of the soybean products of the products being required to possess health and nutritional quality attributes, in addition to the agronomic and production cost features that are basic requirements for all agricultural products.

[1] Director, Crop Analytics; Monsanto Company, St. Louis, MO pradip.k.das@monsanto.com

Key end-use quality traits currently identified in the market place to be of potential benefit include: high oil, high protein and/or protein modified soy; high tocopherol soy; null anti-nutritional soybeans; high omega-3 fatty acid soy; and modified phytochemical-rich soy. For example, the Better Bean Initiative (BBI) by the United Soybean Board of the United States of America, advocated developing compositionally enhanced, quality soy products for the food industry featuring "decreased linolenic acid for better flavor stability; decreased saturated fat for improved human health, and; increased oleic acid to reduce or eliminate the need for hydrogenation." For the feed market, BBI product concepts include a "better balance of amino acids to improve animal digestion efficiencies and lessen the environmental impact of animal waste."[1]

Through the application of plant breeding and biotechnology, nutrition science and functional genomics, the promise of healthy, nutritious quality trait products is becoming increasingly real for farmers, food and feed processors, food companies and consumers. The potential commercial value of these traits is estimated at billions of dollars.

Need for high throughput analytical technologies at every stage in the product delivery pipeline

Agronomic traits can generally be visually assessed, and therefore do not require crop analytics. In contrast, end-use quality traits will be based on modified macro- and/or micro-constituents. Since macro- and micro-constituents changes can only be detected by analytical techniques, crop analytics becomes an essential technology to develop and deliver compositionally enhanced soy products.

Crop analytics are required at all stages of discovery, development and commercialization. For discovery, analytics are required to identify new traits or constituents in existing and exotic germplasm. Analytics are also essential for extracting full value from the genomics database by determining which genes effect the composition of macro- or micro-constituents. With only such phenotype analytics, functional genomics efforts to discover genetically engineered food and feed products yield the desired results in the shortest time and in the most assured manner. Finally, crop analytics makes possible the rapid validation of technical hypotheses and early demonstration of proof-of-concept in quality trait discovery programs.

Once a trait is discovered, rapid commercial development necessitates high-throughput, low-cost, and preferably non-destructive crop analytics. Traditional plant breeding is required to incorporate quality traits into elite

commercial varieties or hybrids. Breeding generates hundreds of thousands of samples that must be analyzed for the trait of interest to rapidly develop commercial lines containing the trait. Marker-assisted breeding and Quality Trait Loci (QTL) can facilitate identification of individuals or populations with the desired trait; however, the primary tool for compositionally enhanced product pipeline remains in its compositional analysis. Crop analytics are also required to understand the genetic and environmental stability of traits.

Finally, after a quality trait has been developed and commercially introduced, analytics are required to capture the maximum value created by the trait. Compositional analysis is necessary to determine identity, maintain integrity of the trait in the distribution chain, and certify that the product quality meets end-user specifications. Crop analytics also helps capture value by reducing the costs of identity preservation and quality assurance.

Compositional analysis capabilities, needed at each stage of discovery, development and commercialization, differ. The accuracy, precision, number and size of samples, turn-around time, techniques and instrumentation best for one stage may not be good, or even applicable, for another. Despite the differences, the analytical expertise required at each stage is similar. Moreover, the techniques needed for discovery will provide the basis for the techniques needed for development, which in turn will provide the basis for the techniques needed to support commercialization. Additionally, techniques and instruments for compositional traits in one crop may be very similar for other crops, resulting in the use of same or similar technologies for multiple crops.

Analytical Technologies and methodologies needed for compositionally enhanced soybean product research, development, commercialization, and market support, range over a wide array of techniques and methods. Choice of technology depends on nature of the trait to analyze, measurement criteria such as need for accuracy & precision, specificity & quantitation, nature and size of sample, and throughput, timing and environment of the needed measurement. Furthermore, selection of technology may also differ based on data application - whether the data will be used, for example, in selecting lines for variety advancement, or, for pricing in grain trading.

Soybean breeding and commercial enterprises have been utilizing secondary screening techniques such as near infrared reflectance or transmittance (NIR/T) for many years to measure protein, oil, moisture, fatty acids, amino acids and other constituents of soybeans. Such instruments are used in screening incoming material at the grain elevators, sometimes even for

paying premium to growers based on grain quality. Even though secondary techniques require calibrations developed and upgraded continuously over time, their precision and ease of use make such instrumentation widely popular. Recent advances in NIR/Imaging and Fourier Transform (FT) NIR have led to high throughput applications for compositional analysis of bulk as well as single plant or single seed soybeans. Nuclear Magnetic Resonance (NMR) and Magnetic Resonance Imaging (MRI) are the other technologies increasingly used for analyzing traits such as oil and fatty acids in whole beans in a high throughput manner.

Calibrations for the secondary techniques are developed based on primary analysis tools such as extraction and gravimetry, combustion and elemental analysis, high performance liquid (HPLC), or, gas chromatography (GC). These techniques, combined with various detection systems such as mass spectrometry (MS) and ultraviolet/visible/fluorescence spectroscopy, yield high throughput methodologies for measuring specific soybean traits – sometimes present at very low levels. Identification and quantitative analysis of microconstituents in soybeans often require such instrumentation of high sophistication and sensitivity.

In recent years, two new tools are emerging as important analytical measurement areas in soybean research – metabolomics and proteomics. Metabolomics requires profiling of metabolites implicated in biochemical pathways active in the soybean product of interest. Proteomics is the study of the full array of proteins expressed by the subset of soybean genome that primarily describes the specific product properties. Instrumentation used in metabolomics includes tools such as quadrupole time-of-flight (Q-TOF), matrix-assisted laser desorption (MALDI/TOF), and triple quadrupole mass spectrometry. Proteomics is carried out through determining identity and profile of proteins via the combination of two-dimensional gel electrophoresis (2D GE), capillary electrophoresis (CE), and various types of mass spectrometry listed above. Most of these techniques can be used in a high throughput mode, thus making them powerful analytical measurement tools for discovery research as well as assuring quality of soybean products.

Finally, to track the high volumes of samples through the analytical process, and, to make effective use of the resultant millions of crop analytical data points, a robust, validated and quality-assured laboratory information management system (LIMS), is a necessity. A competent LIMS, combined with appropriate data mining and bioinformatics tools, is key towards establishing infrastructure of a well-organized, effective crop analytical laboratory engaged in soybean quality research and development.

Need for quality management systems in analytical data generation

Analytical data can obviously be generated in widely differing manners and of varying quality, given the multitude of available analytical technologies, methods and procedures. Even with the best of intent and effort, lack of uniformity and consistency in generating analytical data can invariably create confusion and disarray in acceptance of the analytical data among various constituents in the food and feed product chain. Improving the overall quality of the raw materials needs the use of analytical benchmarks, and thus requires a novel approach to quality assurance in crop analytical data. Recent adoption of the International Organization for Standardization (ISO) [2] quality standards and management system is the result of such approach.

Registration or accreditation to the ISO standards shows that the laboratory or organization generating the quality trait data is adhering to an internationally recognized set of standards defined with a particular scope. Each organization decides which set of standards it should adopt based on its nature of business, function or needs of its customers.

ISO 17025 standard is specifically focused on testing and calibration labs providing explicit guidance on their quality systems. The essence of ISO 17025 is to ensure validity of test data through rigorous analytical controls and technical competence.

A laboratory, seeking ISO 17025 accreditation, needs to implement protocols in its analytical operations ensuring that its analytical procedures include data validation steps with controls through internal or external standards. Such standards must be traceable to universally accepted standards.

Furthermore, analytical operations must meet proficiency requirements in test well as technical competence of the personnel carrying out the tests. Round robin procedures (ring tests) among multiple internal and external labs are a way of establishing test and technical competency. Accreditation to ISO 17025 provides an objective third-party assessment of technical competence related to lab's particular operational scope.

Anticipated benefits of implementing such quality systems are manifold. A lab with such quality assurance programs may enjoy higher credibility of its data. Operations under quality assurance systems may be more streamlined, less error-prone, efficient and ultimately more cost-effective. Businesses making decisions based on data generated with quality assurance may have higher confidence and probability of success in achieving desired business results. In the event of a crisis or regulatory dispute, data

generated through a quality system based on properly executed ISO 17025 will help in defense of legal and regulatory scrutiny.

In general, consistently accurate lab test results are essential to the national and international commerce. ISO 17025 accreditation is becoming an important quality benchmark for foreign and domestic customers as well as regulatory authorities.

Assuring soybean quality: high quality crop analytics and functional genomics are the key to the future

In modern times, genetics is a strong force propelling changes in the grain production and marketing system. While there has been a long and steady stream of significant genetic improvements over the past 50 years, the flow has accelerated during the past decade. As the major investments made in genetics research begin to pay off, most people expect sweeping genetic breakthroughs during the next decade. Additionally, the focus of genetics programs is becoming much broader. Breeding and genetic engineering programs are looking well beyond the traditional traits into input traits that add value to the grain itself. While farmers and processors have shown an incredible ability to adapt and embrace these changes, it is nevertheless necessary that they be provided with adequate information resources to make knowledgeable decisions. Therefore, providing farmers and processors high quality data related to new varieties is essential.

In conclusion, to bring the highest quality soybean products to the market, crop analytical data, generated by the use of appropriate high throughput analytical technologies on seeds and grains, must be underpinned by adoption of quality standards such as ISO 17025. High quality crop analytical data, embraced by all stakeholders of the agricultural product delivery pipeline in making business decisions on soybean quality trait products, will enable us to deliver successfully on the promise of globally abundant, nutritious food and feed - in a sustainable manner for everyone.

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[1] United Soybean Board - QualiSoy website (http://www.talksoy.com/Innovations/Qualisoy.htm)

[2] International Organization of Standards (ISO) website (http://www.iso.ch/)

Influence of storage on the characteristics of soybean, soymilk and tofu

S. K. C. Chang[1], Z.-S. Liu[1], H.-J. Hou[1] and L. A. Wilson[2]

Abstract

Soybeans are subject to storage and shipping after harvest. During this period of time, the physical and biochemical characteristics of soybean may change to various degrees, depending upon the initial soybean moisture and storage/shipping conditions, including various humidities and temperatures. The changes in soybean characteristics may include darkening in surface color, increase in hydration time, reduction in solid and protein solubility, decrease in phytate content, isoflavone structural conversions, changes in protein structures, sugar pattern and lipid oxidation. The coagulant requirement per unit weight of soymilk for tofu making is reduced by improper storage. The yield and quality of soymilk and tofu are also negatively affected by severe storage conditions. Proper storage/shipping management is necessary to keep soybean quality for making high quality soy foods. Therefore, storage research is important to provide useful information for the soybean industry to better manage soybeans during harvest, storage, and transportation to maintain good quality for tofu making.

Introduction

Soybeans are subject to storage and transportation before processing into various soy products. They may be stored up to one year or longer after harvest in a wide variety of environmental circumstances. It is well known during shipping in a boat, the temperature of soybeans that are placed in close proximity of the engine compartment may be heated to higher than 50C. Furthermore, the long hot and humid summer months in Southeastern Asia, where soy foods are regularly consumed, may cause soybean deterioration if they are not refrigerated. The quality of edible soybeans and the viability of soybean seeds decrease gradually with prolonged storage under certain conditions. The magnitude of the quality deterioration depends on storage conditions, including time, temperature, relative humidity (RH), and microbial contamination. Early studies on grain storage have shown that the initial moisture content is an important factor to influence quality

[1] Dept. of Cereal and Food Sciences, North Dakota State University, Fargo, ND 58105.
[2] Department of Food Science and Human Nutrition, Iowa State University, Ames, IA. USA. Kow.chang@ndsu.nodak.edu

stability during storage at 25 °C. Furthermore, it has been reported that temperature and relative humidity of the storage environment affect the chemical compositions of soybeans, but relative humidity is more important than temperature.

Depending on storage conditions, the deterioration of soybeans may be reflected by several changes, including a decrease in hydration rate, an increase in the leakage of solids during soaking, an increase in the acidity of soybeans and darkening in bean color (Hou and Chang, 1998). The reported quality decreases in soymilk and tofu due to storage of soybeans include darkened soymilk/tofu color, low protein extractability (Hou and Chang, 1998), changes in tofu textural properties (Hou and Chang, 1998; Murphy and others, 1997), and less uniform in microstructure of tofu (Thomas and others, 1989). Tofu is a complex food system; it requires a series of processing steps. Tofu is considered as a protein gel and hence proteins in soymilk affect coagulation during tofu making. However, non-protein component, such as phytate has been reported to play an important role in the coagulation process (Saio and others, 1969). The coagulation of soymilk is the most critical step to influence yield and texture qualities, because it involves complex interactions among soy proteins, coagulants (usually Ca^{+2} and/or Mg^{+2}), and non-protein constituents such as phytate, lipids, and carbohydrate. The exact mechanisms of how soybean biochemical compositions change during storage concomitantly to affect tofu yield and quality are not fully understood.

Most previous storage studies use certain accelerated-aging (adverse) conditions (Saio and others, 1980; Thomas and others, 1989; Lambrecht and others, 1996). A refrigerated (cold) storage is considered as the best long-term conditions to keep grain quality (Brooker and others, 1992). Soybeans after harvest are usually stored in uncontrolled ambient temperature and relative humidity conditions, which depend upon the regional environments. Here we would like to report some of our studies on the influence of the storage of soybean under four conditions on soybean quality and coagulant requirement as related to soymilk/tofu making.

Objective

The objectives of this study were (1) to investigate the effects of soybean storage under these four conditions on biochemical compositions and tofu quality and (2) to investigate how storage affect the coagulant requirements for tofu making.

Materials and methods

Influence of storage under four conditions on soybean and tofu quality:

The soybean of the Proto cultivar was stored in four conditions, including adverse conditions (84% RH, 30 °C), mild conditions (57% RH, 20 °C), cold conditions (3-4 °C in a walk-in-cooler), and uncontrolled ambient conditions (in a garage in Fargo, ND). Soybeans in the conditions of uncontrolled ambient garage, cold condition, and 57% RH, 20 °C were stored for up to 18 months and sampled at 3-month intervals; while soybeans in 84% RH, 30 °C was stored for up to 9 months and sampled at 1-month intervals. For the uncontrolled ambient conditions, soybeans were packed in a paper bag and placed in a garage without temperature control. For the cold storage condition, soybeans were packed in a plastic bag and stored in a walk-in cooler (3-4 °C). Saturated salt solutions of sodium bromide and potassium chloride were used for 57% RH at 20C and 84% RH at 30C, respectively, to control humidity in a sealed storage container. Soybeans sealed in a plastic bag and stored at -20 °C were served as the control.

The surface color of soybean was determined using a colorimeter using the Hunter Lab scales. Soybean samples were ground and proximate chemical compositions were analyzed (AOAC 1995). Phytate (Hou and Chang, 2003), isoflavones (Hou and Chang, 2002), titratable acidity (AOAC 935.57, 1995) and sugar contents were also analyzed. Glycinin and b-conglycinin were isolated and their composition and structure were characterized (Hou and Chang, 2002)

Soymilk and soft tofu were prepared using calcium sulfate as a coagulant by the method of Cai and Chang (1997). The curd was pressed. Tofu yield was expressed as g tofu /100 g raw soybeans. Textural properties were calculated from the curves according to Bourne (1978) using an Instron Universal Testing Machine. Solids and protein contents in soymilk and tofu were determined.

Influence of storage on coagulant requirements

The Proto soybean was adjusted to various relative humidity levels (equivalent to Aw 0.60 to 0.81) by spraying distilled water on the beans and equilibrated for three weeks in a cooler (Aw 0.60 to 0.81) and stored for up to 3 months at 30C. At one-month intervals, soybeans were retrieved from the storage and processed into 10.5 Brix soymilk. The optimal coagulant (Ca chloride or Mg chloride) concentration of the soymilk for filled tofu making was measured according to a method reported by Liu

and Chang (2003). The chemical composition, including titratable acidity, pH, phytate content, and 11S/7S ratio were analyzed. Filled tofu was prepared with each respective optimal coagulant concentration according to method described by Liu and Chang (2003). Tofu's breaking stress and apparent Young's modulus were determined. Results were analyzed using statistical methods to determine significant differences and correlation coefficients.

Results and discussion

Influence of storage under four conditions on soybean and tofu quality:

Soybean color significantly changed over time during adverse storage. Generally, the whiteness of soybeans (Hunter L values) decreased along with storage time, except at 2 and 7 months, indicating that soybean became darker. Redness values (Hunter a values) increased up to a maximum at 5 months of storage, however, yellowness (Hunter b values) decreased gradually over the time of storage. Soybean in the adverse environment absorbed significant amount of moisture over time, the moisture content increased from the original 5.4 % to 17.85 % at the end of 9 months. Soybean at the bottom of the containers under the adverse storage conditions began molding after 3 months and generated off-flavor. Those molded soybeans were carefully picked out and discarded to avoid the influence of molds on tofu and chemical compositions. The lipid, proteins, ash and total carbohydrate content in the beans did not change significantly over time of storage. However, sugar content decreased significantly under the adverse storage conditions.

Phytate content in soybean stored under the adverse conditions showed a linear decrease with storage time. At the end of 10 months, phytate content declined to 0.87 %, which was a 35% reduction from the initial amount. TA in soybean stored under the adverse conditions increased linearly with storage time which increased 42% after 3 months, 112% after 7 months, and up to 178% after 9 months. The phytate content of soybean stored under the three other conditions did not change after 12 months and decreased slightly thereafter (8 %to 13% by the18 months). Titratable acidity also increased slightly (approximately 12%) in the three other storage conditions by the end of 18 moths.

The yield of tofu made from soybeans stored under the adverse conditions decreased significantly with soybean storage time from 512 g at the month zero to 71 g at the 7 months. Beyond 7 months, no tofu was produced, which was primarily due to the very low solids and protein contents recovered in soymilk. The tofu hardness increased significantly with soybean

storage time. Beyond 3 months of storage, the tofu yield decreased significantly with a concomitant increase in tofu hardness up to more than 5000 g at 6 months of storage. There was a highly negative correlation ($r = -0.99$) between tofu yield and tofu hardness.

The solids content in soymilk remained above 8% for 4 months, then was reduced to about 7.5% in 6 months and continually decreased to less than 2.4% in 8 months. The protein content in the wet soymilk showed a similar decreasing trend with the solids content of soymilk; it reduced from 4.9% to 4.4% in the first month then remained above 4% until 6 months. By end of the 8th month, the protein content in the wet soymilk was only about 1%. The molecular structures of the glycinin isolated from adversely stored beans were changed, with increases in glycosylation and in the helical structures, and decreases in b-pleated sheet and surface hydrophobicity. Isoflavones were changed from glucosides and malonylglucosides to more aglycone forms during adverse storage. Isoflvaones changed slightly for beans stored at 57%RH and in the ambient conditions. Beans stored at 4C did not change in its isoflavone pattern. Changes to aglycones may impart more bitterness to the soymilk produced from improperly stored beans.

The high titratable acidity after storage might also play an important role in tofu yield decrease, because acid could act as a coagulant to accelerate coagulation. Besides the protein factor, the decreases in tofu yield were due mainly to the loss of the water that was pressed out that should have been imbibed by the proteins inside the curd. The sharp increase in solids content in tofu particularly beyond 5 months of adverse storage indicated the inability of the curd matrix to imbibe water resulting in a significant decrease in moisture and yield. The lower the moisture content, the harder the tofu texture produced.

The yield and textural properties of the tofu made from the soybean stored in the other three conditions for up to 18 months did not change substantially. Compared to the adverse conditions of 84% RH, 30 °C, the solids contents in soymilk for the other three conditions did not change significantly, ranging from 8.40-8.94 %, over the storage time. Similarly, protein content in wet soymilk remained at above 4.5% for these three conditions over the storage time. Solids contents in tofu made from stored soybeans under these three conditions ranged from 11.2% to 11.8%, and did not show substantial changes over time of 18 months.

Influence of storage on coagulant requirements:

With increasing Aw or storage time, the optimal coagulant concentration (OCC) for making tofu decreased significantly. The titratable acidity of the soymilk increased with Aw and storage time over three months. The pH of the soymilk decreased with Aw and storage time. On the other hand, the phytate content did not decrease substantially with the Aw and storage time up to 3 moths. There were no significant differences in 11S/7S ratio of the soymilk among different storage conditions. The decrease in OCC might be due to the increase in soymilk titratable acidity and the decrease in soymilk pH due to soybean storage. There seemed to have some decreases in tofu's breaking stress and apparent Young's modulus. In addition, soymilk and tofu's lightness and yellowness decreased, whereas their redness increased. This was consistent with our previous findings on the changes of soybean color during storage.

Conclusion

In a long-term storage study, soybeans stored under the adverse conditions (84% RH and 30 °C) deteriorated significantly beyond 2 months, which were reflected on off-flavor generation, decrease in tofu yield, and coarser and harder of tofu texture. Soybeans stored in the mild conditions such as 57% RH and 20 °C could remain their qualities for tofu making as good as those stored in the cold conditions (4 °C) for up to 18 months. Soybeans stored in the uncontrolled temperature conditions in the US Northern Plain region for up to 18 months (from March, 1998 to September, 1999, through four seasons and a second summer) also could maintain their qualities for tofu making without substantial differences as compared to those stored in the cold conditions. The coagulant concentration of the soymilk decreased over time and water activity of the storage. The information provided is useful for the industry to manage the storage practice of the soybeans to maximize the quality for tofu making. The study is also important to alert the tofu makers that coagulant concentrations should be modified for stored beans during tofu making if the optimal concentration is significantly affected by storage.

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Challenges and approaches required for identity preservation of specialty soybeans

L. Clarkson[1]

Abstract

Access to niche markets and some larger markets requires "identity preserved" soybeans. Quality goals will be defined by the client. The IP supply system must be designed to meet those goals. Distinctions generally cover variety and physical quality. Most recently, those distinctions often require organic certification and avoidance of transgenic materials. In the future, they may require delivery of a specific transgenic variety. This session examines the challenges and supportive protocols for suppliers wishing to enter a food grade market that requires a bland tasting soybean with organic certification and no more 0.1% transgenic materials.

Long ago, well before "transgenic" issues emerged, sophisticated buyers selected soybeans by characteristic and variety to improve foods and process economies. Today those same motives support strong and growing IP (identity preserved) markets that reward companies for supplying what the client wants, for avoiding what the client does not want and for helping distinguish the client's special products from the commodity flow. Modern IP suppliers distinguish soybeans by variety and preferred characteristic. They typically use contract production and work with seed suppliers, farmers, independent certification agencies, intermediate processors and freight companies to deliver the preferred soybean within very tight tolerances with sufficient documentation to trace it back to the producing farm and seeds supplier. The growing demand for organic soybeans as well as the rapid development of new GMO (transgenic) characteristics will push and pull soybean production even further toward sophisticated, segmented markets.

This presentation highlights segregation protocols that are in place and working, pleasing clients in Asia, North and South America and Europe. The author's experience with such systems comes from managing a company that supplies selected varieties to processors and feeders. It works with clients and researchers to identify and select soybean varieties that optimize process yield, homogeneity, product quality and market acceptance. With few caveats, variety choice contributes more than any other factor in

[1] President Clarkson Grain Inc., USA.

determining value. Clients can choose their entire supply from a single variety or a blend of compatible varieties. They can choose conventional, transgenic, non-transgenic and/or organic materials. To some extent, they can negotiate exclusive control of a particular variety within their market territory. Within certain limitations, they can trace their soybeans back to individual farms and fields, an impossibility under the commodity system. Their choices provide access to selected markets and establish product quality ranking, competitive advantage and consumer price levels.

Variation creates demand for IP systems. Clients range greatly in terms of culture, size and product focus. Products vary from tempeh to oil to soymilk to soy coffee to roasted soy nuts to natto. Market access might require a particular transgenic soybean, might require avoiding all transgenic soybeans, might require a particular flavor, a particular protein level, or might require that the soybean be certified organic. Variation among soybeans is even greater. The US soybean germplasm collection held by the National Soybean Research lab at the University of Illinois offers over 18,000 varieties that vary greatly in color, size, shape, bio-chemical composition and taste. Markets themselves speak with dollars and talk in prices per metric ton. A US#1 yellow commodity soybean might sell in the US at the farm for $202/ton; a non-GMO commodity bean might sell for $212/ton; a good food variety might sell for $293/ton; the same variety with organic certification might sell for $640/ton.

To illustrate protocols at play within a successful IP system, examine the choices and challenges facing a supplier who wishes to connect with a niche or segmented market. Keep in mind that the motive of all the linked players in such a system is to do better economically than they would with a commodity system.

Step one is to determine the market. The basic choices are food, feed and industrial markets. Step two is to determine a client or client sector within that market. Choices include particular characteristics and within those categories – conventional, transgenic or organic. Assume that our new supplier decides to target the soy milk market in the United Kingdom and that he develops a dialogue with one of the UK's leading supermarket chains. His targeted client asks him to supply a food variety with:

- a bland taste
- large size
- rapid water absorption
- good agronomic characteristics that contribute to lower premiums
- IFOAM approved organic certification
- Non-GMO (less than 0.1% transgenic presence as determined by representative sampling and PCR testing)
- Protein minimum: 45%

- Physical quality: better than US#1 yellow soybeans grade
- Foreign material: zero tolerance
- Other varieties: max 1%

The enthused supplier needs to get client approval of a variety and develop his production and supply plan. What varieties should he submit for approval? From which supplier can he get the best seed for the approved variety? Where should he grow his soybeans? How should he spread production to minimize production risks? How many acres should he contract? What premiums will he need to pay to get farmers to support the program? Where will he get a satisfactory supply of seed? What quality control steps are required to guarantee compliance? What will he need to charge to cover his costs and still be competitive? These questions link him to seed suppliers, farmers, conditioners and processors as well as independent certification agencies.

Seed suppliers

Segregation really starts with the seed. Assume that variety X12 pleases the client. Who can supply X12 of acceptable purity with acceptable agronomic properties that meets all known requirements for organic seed under IFOAM rules being applied in the UK? The big challenges are purity of variety and freedom from transgenic factors. Many seed companies cannot meet a 1% tolerance maximum. Many can no longer deliver seed that meets a 0.1% tolerance requirement for transgenic materials. Very few offer organic seed. Doubt and danger require that claims be verified by an approved independent certification agent. Purity will only get worse as the seed moves onto farms.

Contract farmers

Associated farmers bear significant responsibility for segregating the bean. Contamination threats include seed confusion and contamination at time of planting, harvesting, storing and delivering. They need appropriate infrastructure and supportive attitudes to focus on blocking possible threats to product integrity. By accident or neglect and sometimes by factors beyond his control, the farmer might compromise the purity required to please the client. Several points here beg third party quality control attention. They include set backs from neighboring fields and dedication in cleaning machinery and formally tracing the path from seed to bin to truck to delivery point.

Conditioner/1st Processor: Similar concerns carry through to the conditioning plant that will prepare the X12 soybeans for the client to process into soymilk. How does it avoid contaminating a large lot of good soybeans

with accidentally compromised soybeans? What pre-delivery protocols will prevent mistakes? What protocols at the point of delivery will insure that no mistakes have been made between approval and delivery? What protocols are needed after acceptance of delivery to confirm compliance? At what point does the system quit testing and finally approve the proffered X12's? It is good for system allies to minimize system costs and reject non-complying material at the point of least cost.

Quality control

The buyer's and consumer's confidence levels rest on the integrity and quality of the 3rd party certification or verification agents. Are they independent? Are they good at what they do? Is their sampling statistically valid? Is their testing system scientifically valid? Do they and their protocols please the supplier's client? Are their tests layered so that failure of a part does not compromise the integrity of the whole system?

The fully developed IP system is not simple. Done well it pays critical attention to genetics, production zones, selection of contract farmers, farm inputs and evolutions, storage, cleaning and conditioning, segregated deliveries and over-reaching attention to quality control. Done well, the IP system might get the supplier a smile from a happy client. Done well, the system might support the supplier in riding the growth that is coming in demand for identity preserved soybeans and soy foods. The factors supporting growth include genetic engineering, increasingly affluent markets, new abilities to measure, improved storage flexibility, better process yield, food scares and increased interest in optimizing health, greater interest in taste distinction as well as that chronic factor, market competition.

May you IP well!

HACCP and food safety for the soy foods industry

L. A. Wilson[1]

Abstract

We all assume that the food we purchase and prepare is safe, but recent food-borne microbial outbreaks involving hamburger, poultry, fish, seafood, vegetables, fruits, and fruit juices has tested this belief. The General Account Office has estimated that 9,100 people die during 81 million outbreaks annually. The United States Department of Agriculture (USDA) and the U.S. Food and Drug Administration (FDA) have both instituted regulations requiring Hazard Analysis Critical Control Point (HACCP) systems to help prevent *Salmonella, E. Coli* 0157:H7, and other food-borne illness organisms, chemical and physical hazards from reaching consumers via meat, poultry, and seafood. The FDA, which regulates seafood and all non-meat products, has finished implementing HACCP Regulations for the unpasteurized apple and fruit juice industries. It has been suggested that HACCP systems be required for all food industries. The World Trade Organization has emphasized food safety on a global basis with the application of sanitary, phytosanitary measures, the Codex Alimentarius Food Hygiene Guidelines (similar to FDA Food Code). The soyfoods industry must take a proactive position to ensure the safety of their nutritious, functional and flavorful foods. This presentation will inform you about the requirements, applications and interrelationships of Current Good Manufacturing Practices (CGMPs), Standard Operating Procedures (SOPs), Sanitation Standard Operating Procedures (SSOPs), and HACCP Systems to the soyfood industry. Each HACCP system must be applied on a site to site basis, and you will leave with a basic understanding of how these systems can be applied to your situation (farmer, processor, and consumer). Specific soyfood examples (soymilk and tofu) will be used in this presentation.

The soyfoods industry has had relatively few food borne illness outbreaks over the last decade compared to beef, eggs, and fresh juices. However, the potential for a devastating food borne illness outbreak is ever present. Soy products, including tofu, raw seed sprouts, heat treated plant foods and textured proteins have been identified by the U.S. FDA as "Potentially Hazardous Foods (PHFs), which means that they could carry and/or support the growth of a food borne illness microorganism (Salmonella, Clostridium

[1] Dept. of Food Sciences and Human Nutrition, Iowa State University, Ames, IA 50011, USA. lawilson@iastate.edu

botulinum such as Listeria, etc). As a "sensitive" food or ingredient, we must take precautions to prevent our soybeans and soyfoods from making the public sick.

Prerequisite safety program

In order to protect the public and the soybean producers from catastrophic food borne illnesses, several prerequisite methods have been employed by the food companies and federal and international agencies (Table 1).

Table 1. Food Safety Prerequisite Programs Prior to HACCP

Good Agricultural Practices
Good Manufacturing Practices
Standard Operating Procedures
Sanitary Standard Operating Procedures
Recall Programs

Good agricultural practices (GAPs) have been recommended for handling raw commodities on the farm. These include such things as providing sanitary facilities for farm workers and hand washing stations; keep domestic and wild animals that may carry and shed diseases in their feces from entering cultivated acreages, fields and orchards; use of composted animal fertilizers instead of raw manure; etc.

Good manufacturing practices (GMPs) Part 110 of the U.S. Code of Federal Regulations (21 CFR 110). This regulation includes recommendations for the sanitary handling and productions of foods.

Table 2. Current Good Manufacturing Practice in Manufacturing, Processing, Packing, or Holding Human Food (CGMP or GMP) Code of Federal Regulations: Title 21: Part 110

Outside the plant
Receiving
Storage
Processing
Packaging
Food product storage
Personnel and training

These regulations describe the things to do with the plant in the receiving area, in the processing area, in the packaging area, storage facilities (raw and finished product), and how your personnel should be trained to handle

foods in a sanitary manner to help prevent illness from entering. Specific examples include proper hand washing, hair and beard covers, clean clothing, healthy workers handling foods, clean trucks that deliver products preventing rodent pests from entering your facility, proper ventilation, adequate lighting, food grade machinery lubricants, time/temperature control in processing areas, good housekeeping, proper handling of "sensitive" ingredients (products from animals , sprays, produce that can contacts the soil, etc), to name a few.

Standard operating procedures (SOPs) are protocols that should always be followed in and around the food processing area. Specific written documents that tell or show how tests are properly run and waste chemicals disposed of, how to read a thermometer, record the data, how to test for pH, how to run the processing equipment, etc. A very important set of SOPs are Sanitary Standard Operating Procedures. These protocols tell the workers exactly how to clean equipment, floors, ceilings, prepare proper concentrations of sanitizers etc. This provides proper and uniform sanitation of the facility prior and during processing. Personnel training documentation responsibility are critical factors that must be accomplished in SSOPs)

GAPs, GMPs, SOPs and SSOPs are the basis for sanitary handling and processing of foods. More recently these programs have been used as the 'prerequisite' programs that must be followed prior to the implementation of a Hazard Analysis and Critical Control Point System (HACCP) by the U.S.D.A., FSIS and FDA.

Hazard analysis and critical control point identification (HACCP)

The HACCP system originally developed to insure the safety of foods produced for the U.S. Space Program: NASA and Pillsbury, is widely employed in food processing quality assurance programs for the control of food safety. It consists of hazard analysis and the identification of critical control points on the processing lines. Hazard analysis describes those elements of process equipment or environment, which if not maintained and monitored can result in the introduction of hazardous materials into a food product. Critical control points are those elements which if not maintained within certain parameters can result in the production of food which contains pathogenic microorganisms or toxins. In contrast to traditional corrective quality control, HACCP is a preventive system which establishes and defines quality check points as well as accountability for each check point. For HACCP to work, prerequisite programs must be in place, you must know your "real" processing system, and safety must be supported by management. The establishment and maintenance of a preventive system such as HACCP begins with thorough assessment of

hazards. Hazards to the safety of food comprise three classes: a) chemical, b) microbiological, and c) physical (Table 3).

Table 3. HACCP: Hazard Assessment

A.	Chemical hazards	
	1.	Pesticides
	2.	Toxic metals
	3.	Toxic chemicals found in environment
	4.	Cleaning and sanitizing agents
B.	Microbiological hazards	
	1.	Pathogenic organisms – disease causing
	2.	Spoilage organisms
	3.	Indicator organisms (indicating improper sanitation)
C.	Physical hazards	
	1.	Metal
	2.	Glass
	3.	Dirt
	4.	Wood
	5.	Insect
	6.	Animal parts

The number of potential hazards in a food processing situation is very high. To monitor and control all hazards effectively can be expensive. In a HACCP system it is necessary to define the hazards at every step in the processing plant or line, and then establish accountability for monitoring or controlling each one. Which hazards and critical points can be effectively monitored and controlled will then be determined based on product specifications, company philosophy, etc. The main steps in developing HACCP program are listed in Table 4 and 5.

Table 4. Developing a HACCP program

1. Assessing the hazards
2. Determine the critical control points (CCPs)
3. Establish the control limits for each CCP
4. Establish procedures to monitor CCPs
5. Establish corrective action to be taken if a deviation is identified at a CCP.
6. Establish effective record-keeping systems that document the HACCP plan
7. Establish procedures for verification that HACCP system is working correctly

Table 5. Implementation of a HACCP Program

1.	Hazard Analysis
	Flow chart of your 'real' process identity
	Risks and consequences
	Production, distribution, sale, consumption
2.	Identification of critical control points – up to ~76
	Loss of control results in an unacceptable risk to the consumer
3.	Establish of CCP criteria
	Means of testing each attribute (real-time): direct and/or indirect
4.	Monitoring of CCP criteria
	Adequate control – monitor on a continuous basis or technically sound periodic measurements Records critical
5.	Protocols for CCP deviations
	Steps to take to bring under control
	Recommended disposition of product
6.	Verification
	Effectiveness of stated protocols and unanticipated hazards; not normal collected
7.	Record keeping and reporting procedures

HACCP program – Implementation

Even though quality assurance designs and periodically audits the HACCP system, and quality control has the day to day responsibility for implementation of the system, it is evident from the vast array of potential hazards that product quality must be the responsibility of all those involved in processing. The HACCP system enables the distribution of quality accountability. A number of people throughout the production process, both supervisors and key employees, are responsible for monitoring or performing the HACCP control in their specific area. HACCP control points become part of employee job responsibility. This should serve to create a greater awareness of the need for quality and aid in the control of hazards.

Once HACCP is set up, it must be monitored, acted upon, and reviewed to insure that appropriate actions and changes are made when needed. The HACCP system can fit into your company's quality assurance program. Bauman (1991) discussed how HACCP can be established in a QA system by using specifications, GMPs, recall systems, audits, accident reporting, etc. Corlett (1991) has addressed the issues with mandatory critical control points: direct measures testing for the microorganisms, chemical and physical hazards versus indirect measures pH, AW, visual etc.

Applications

With this overview of various food safety programs, let's look at specific applications in the soyfood industry. Soybeans are grown in an open environment, whereby, soil and microorganisms can come in contact with them during the growing season and during harvest. The soybeans arrive at your facility as "potentially hazardous." If we grind the beans into soy flour we have a "potentially hazards" ingredient. If we soak the soybeans 8-12 hours for soymilk production, we are allowing microorganisms to grow. What happens to the soak water when you are through soaking the beans? Can it come in contact to other areas in the plant (cross-contaminant). Have you controlled the time and temperature of your cooking step (CCP) to insure pasteurization of the product? Was the soymilk recontaminated during cooking and/or bottling? If tofu is manufactured from the soymilk, were the curds contaminated during pressing? How cool were the curds during pressing? Was sanitary (potable) water used to package the tofu? Was the tofu re-pasteurized in the containers or heated (Bot cook) to become shelf-stable? Sprout manufacture and food safety has been recognized globally as a real problem. What type of microbial inhibitor/process ('kill step') will you use. FDA recommends that people should not eat raw sprouts.

Conclusion

It must be remembered that a HACCP plan must be specific for each product and each production line within each facility. Building upon these safety programs, we can produce a safe product of high quality and nutritional value for our consumers. It is the soyfoods industry's responsibility from farm to table, to continue to make soyfoods that taste good and to ensure the safety of their nutritious, functional, healthy, and quality foods.

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New uses for refinery byproducts: biodiesel from soybean soapstock

M.J. Haas; K.M. Scott; A. McAloon and T. A. Foglia[1]

Abstract

Semi-refined and refined vegetable oils are the predominant feedstocks for the production of biodiesel. However, their relatively high cost renders the resulting fuels unable to compete economically with petroleum-derived fuel. We have investigated the production of fatty acid methyl esters from soapstock, a byproduct of edible oil refining that is substantially less expensive than edible grade refined oils. The most effective method involved the complete saponification of the soapstock, followed by acidulation using methods similar to those presently employed in industry. This resulted in an acid oil with a free fatty acid content greater than 90%. These fatty acids were efficiently converted to methyl esters by acid-catalyzed esterification, resulting in a product that met the accepted US specifications for biodiesel. Engine emissions and performance during operation on soy soapstock biodiesel were comparable to those on biodiesel produced from soy oil. An economic analysis suggested that the production cost of soapstock biodiesel would be approximately US$ 1.54/gal. This is 25% lower than the estimated cost to produce biodiesel from soy oil.

Introduction

The use of biodiesel fuel, which consists of the simple alkyl esters of fatty acids, is growing rapidly in several parts of the world. Among the desirable features of biodiesel that underly this increase in use are the facts that biodiesel is a renewable fuel, that it reduces the emission of air pollutants by engines, and that it can be a domestically produced energy. However, in addition to meeting engine performance and emissions criteria/specifications, biodiesel will have to compete economically with petroleum-based diesel fuel to survive in the market place. Vegetable oils, partially or fully refined and of edible grade quality, are currently the predominant feedstocks for biodiesel production. However, the cost of such oils is sufficiently high that biodiesel made from them cannot compete economically with petroleum diesel fuel.

[1] United States Department of Agriculture, Agricultural Research Service Easter Regional Research Center. 600 East Mermaid Lane, Wyndmoor, PA 19038 USA

These considerations have led to efforts to identify less expensive lipid-bearing materials that could serve as feedstocks for biodiesel production, and to devise chemical processes for their conversion to fuels. One such inexpensive lipid source is 'soapstock' (SS), a byproduct of the refining of vegetable oils. It consists of a heavy, alkaline, aqueous emulsion of lipids, containing about 50% water with the balance made up of free fatty acids, phosphoacylglycerols, triacylglycerols, pigments and other minor nonpolar soybean components. SS is generated at a rate of about 6% of the volume of crude oil refined, which equates to an annual US production of approximately one billion pounds. Its market value is approximately US$ 0.05 per lb on a dry weight basis, *i.e.* about one-fifth the price of crude soybean oil. We describe here the development of methods for the production of fuel-grade fatty acid esters from SS.

Basic considerations in the production of biodiesel from soapstock

Soapstock contains both free and esterified fatty acids. The alkaline transesterification chemistry typically employed to synthesize simple alkyl esters from acylglycerols is ineffective at esterifying free fatty acids. Thus, a dual reaction approach is required, as in the case of other high-FFA materials such as waste greases. Generally this involves first an acid-catalyzed reaction to esterify free fatty acids, followed by an alkali-catalyzed reaction to transesterify the acylglycerols.

A complicating factor in the case of soapstock is that it contains a substantial amount of water, which is in a heavily emulsified form that prevents its ready removal. This water inhibits both esterification and transesterification reactions.

Enzymatic catalysis for the production of biodiesel from soapstock

Lipases are enzymes that hydrolyze the ester bonds of fats and oils (given a sufficiently high water activity). In systems with very low water activities the reaction is reversed, and lipases catalyze esterification, transesterification and related reactions. Lipases have been explored as esterification/transesterification catalysts for the production of biodiesel.

Compared with conventional acid and base catalysts, lipases offer the advantages of a cleaner product more readily isolated, coupled with a significant reduction in solid and liquid waste streams. Thus we investigated the utility of lipases for the production of biodiesel from soapstock (Haas and Scott, 1996). The approach taken was to first conduct an alkali-catalyzed transesterification of the glycerol-linked fatty acids, and to then employ a lipase to catalyze the esterification of the free fatty acids in the preparation. Freeze drying was employed to remove water from the SS

prior to reaction. It was found that under conditions that supported enzymatic activity the SS mass was nearly solid. This retarded enzyme distribution in the reaction mixture, and restricted overall ester production to only 81% of theoretical maximum. The enzymatic approach was therefore judged unacceptable as means of effective production of fatty acid methyl esters from SS.

Biodiesel production via nonenzymatic catalysis

- via SS saponification and drying

Following the unsatisfactory results with enzymatic catalysis (above), the use of more traditional inorganic catalysts in the production of biodiesel from SS was explored. The approach adopted was to first hydrolyze the lipids, and to then conduct esterification of the resulting free fatty acid product. Alkali-catalyzed hydrolysis (saponification) was conducted to achieve the total hydrolysis. Since SS is already quite alkaline (pH > 9), it was efficient to employ alkaline hydrolysis (saponification) to achieve this goal. In the resulting protocol (Haas, Bloomer and Scott, 2001, 2002) the most rapid route to complete saponification involved the addition of sodium hydroxide to a final total concentration of 4.2% followed by incubation at 100 °C for 2 to 4 hours. These conditions achieved complete hydrolysis of both acylglycerol and phosphoacylglycerol species. Lower amounts of alkali and/or lower temperatures failed to achieve complete saponification in an acceptable time. The water of the sample was subsequently removed by freeze drying, and the dried mass subjected to acid catalyzed esterification at 35 °C to produce fatty acid methyl esters. Optimal conditions for this reaction were identified by implementing a statistically guided approach in experimental design. The minimum reactant concentrations consistent with full esterification within two hours or reaction corresponded to substrate molar ratios (fatty acid/methanol/sulfuric acid) of 1:30:5. The ester product had a free fatty acid composition essentially identical to that of soybean soapstock, and similar to soybean oil. This product met the specifications for biodiesel set by the American Society for Testing and Materials (2002).

- via acid oil as an intermediate

A potential disadvantage of the method just described was that sodium hydroxide added to facilitate saponification combined with sulfuric acid added to catalyze free fatty acid esterification to form solid sodium sulfate, which presented a substantial waste disposal challenge. Therefore alternate routes for the production of biodiesel from SS, were sought.

In contemporary industrial practice, SS is often subjected to a process termed 'acidulation', wherein sulfuric acid and high pressure steam are

employed to acidify, heat, and mix the SS mass. This converts the fatty acids from sodium salts (soaps) to free acids, greatly reducing their emulsifying properties and breaking the heavy emulsion typical of SS. Some hydrolysis of the acylglycerols in the SS also occurs. When steam injection is terminated and the mixture allowed to sit, it separates into an aqueous and an oil phase. The former contains the sodium sulfate generated by reaction of sodium hydroxide in the saponified SS with the added sulfuric acid. However, as opposed to being produced as a solid in the method described above, this is dissolved in the aqueous solution, which facilitates its ready disposal. The other, nonaqueous, phase, termed 'acid oil', is essentially free of water and contains free fatty acids, acylglycerols, pigments and other lipophilic materials. We examined this material as a feedstock for biodiesel production. However, since its fatty acids were present in both free- and lipid-linked forms, commercial acid oil could not be readily converted by a single simple reaction to fatty acid methyl esters.

Subsequently, we investigated the possibility that by complete hydrolysis of the lipids in soapstock prior to acidulation an acid oil rich in free fatty acids, and therefore readily and completely esterified by acid-catalysis could be prepared (Haas *et al.*, 2003). Soybean SS was quickly and completely saponified by the injection of steam at pH values exceeding 11. Acidulation of this material using protocols analogous to those currently employed by industry produced an acid oil with a free fatty acid content in excess of 95 wt%, and lacking intact acylglycerols. This 'high acid' acid oil was readily esterified by acid catalysis in methanol, achieving a nearly quantitative esterification of its fatty acids.

Engine performance and emissions testing

Engine testing was conducted (Haas *et al.*, 2001) to determine the acceptability as a fuel of the fatty acid ester product synthesized from soapstock., using a 1991 calibration, production model heavy duty truck engine that had been specifically fitted to monitor engine emissions and performance. Data was collected for operation on 100% soapstock-based biodiesel and 100% commercial biodiesel prepared from soybean oil, to allow comparison of the performance of these two fuels. A 20% blend of soapstock biodiesel in petroleum fuel was also examined. As a reference standard, data was also collected for certification fuel, which corresponds roughly to industry average petroleum diesel fuel.

Hydrocarbon emissions were low during operation on petroleum diesel, and further reduced by a factor of 25 to 50% on pure biodiesel, irrespective of its source. Emissions of oxides of nitrogen (NO_x) increased by about

10% relative to operation on petroleum diesel fuel, for both biodiesels tested. Carbon monoxide emissions were also comparably affected by both biodiesels, being reduced by 40 to 45% compared to petroleum-derived fuel. Emissions of particulate matter (PM) were reduced by 70% during operation on soy oil biodiesel relative to their levels during the combustion of petroleum diesel. PM emissions were also reduced on soapstock biodiesel, though by only 54%.

When soapstock biodiesel was tested as a B-20 blend, *i.e.* as a 20% v/v mixture in petroleum diesel, hydrocarbon and carbon monoxide emissions were generally similar to those with petroleum diesel, while PM emissions were reduced 20% relative to petroleum fuel. The output of NO_x increased by a small amount (1.5%). These results are comparable to those of soybean oil methyl ester B-20.

Analysis of the economics of production of soapstock biodiesel

We adopted a computer-assisted process modeling approach to assess the economic viability of producing biodiesel from soapstock (Haas and McAloon, 2003). This involved specifying the details of all steps in biodiesel production and purification, the modeling of an industrial facility able to implement these steps at a scale of 5 to 10 million gallons of biodiesel annually, and determination of the product cost. Analysis of the data from this model suggested a production cost of approximately US$ 1.54/gal for biodiesel synthesized from soybean soapstock. A similar model for the production of biodiesel from crude soy oil predicted a production cost of US$ 1.99/gal, which is in general agreement with recent price quotes for soy-oil biodiesel. These results suggest that soapstock-based biodiesel could be a more economically viable product than biodiesel from soy oil, costing nearly 25% less to produce.

Conclusions

By means of simple chemical methods, a low-quality underutilized feedstock has been used to produce biodiesel. This product is comparable in composition, similar in engine performance and emissions, and predicted to be more economical to produce than biodiesel from refined soybean oil.

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Biodiesel in Europe: market growth and opportunities, perspectives for soybean oil

I. Debruyne[1] and J.L. Pinheiro[2]

Abstract

Biodiesel was introduced in Europe in the early 90s. Since then, a rapid expansion has created a large production base of over 2 million MT capacity. The consumption pattern across the EU has been largely determined by fiscal legislation and by technical specifications. In 2002 production reached an estimated volume of 1,065,000 MT, up 15% from 2001. Ten years of technology development created improved production technology and more flexibility with respect to raw materials and outputs. More growth is expected, driven by new incentives to grow the biofuels market on EU level. Volatility of raw material and product prices though, makes biodiesel production in certain periods a risky business, with temporarily poor margins and growth, but also periods with exciting margins. The recent approval of a European Biodiesel standard broadens the road to introduction of other vegetable oils and fats in blends tailor-made for the biodiesel market. This also creates a potential for soybean oil as one of the raw materials to be used.

Paper

In Europe, due to the government subsidies for biodiesel and higher taxes on diesel fuel, the use of biodiesel has gained quite some popularity. Commercial biodiesel-initiatives in Europe started end of the 80s in Austria and France, where the first industrial-scale biodiesel production units went into operation in the early 90s. At that time, two options were still looked at seriously: using pure vegetable oil as fuel for regular Diesel engines, or transesterification with methanol or ethanol giving esters of fatty acids as biodiesel for modern transportation and heating systems. Tremendous progress has been made since, mainly driven by technology developments in France, Germany and Austria. Today, multiple biodiesel plants operate, transforming mainly rapeseed oil and methanol in biodiesel and glycerol as byproduct (FAME fatty acid methyl esters; RME rapeseed methyl esters).

Biodiesel is marketed by petroleum producing and distributing companies, which use it as such or in blends, for transport fuel, and for heating systems in public and private buildings. The consumption pattern across the EU has

[1] Ignace Debruyne & Associates, Izegem, Belgium ignace.debruyne@advalvas.be.
[2] Initium Consultores, Lisbon, Portugal.

been largely determined by national/fiscal legislation and by technical specifications. As shown in table 1, the existing capacity is about the double of the current production, leaving still ample space for growth, especially since more new production plants are planned or under construction.

Table 1. EU-15 biodiesel production capacity (2003)

in '000 MT	Capacity	Production
Germany	1,023	450
France	502	366
Italy	419	210
Austria	95	25
Denmark	38	10
Sweden	8	3
U.K.	5	1
TOTAL	2,090	1,065

A breakthrough in the German market late 1999 has given German manufacturers the lead position in Europe. This market expansion was substantially supported by government initiatives on detaxation and biofuel promotion. In producing biodiesel from vegetable oils, the return on investment and processing cost are essentially compensated by the byproduct credits, making the price of biodiesel approximately the same as the price of vegetable oil. The main success though, has been realized through the intensive cooperation with fuel, car engine and injection pump manufacturers. The joint development of a high quality, standardized biodiesel product created a long-term guarantee for using biodiesel even in the most sophisticated high-pressure Common Rail diesel engines. Although Common Rail technology appeared first in trucks and buses, manufacturers since have adapted it, making it the reference in diesel engines for passenger cars.

The taxes on diesel fuel in Europe have averaged about 400 USD per ton, bringing the pump price of diesel to the same level as the biodiesel price without any tax. To enable this at a zero cost for budget, this has been coupled directly to the production of vegetable oil (rapeseed oil mainly) on set-aside land creating extra income for farmers ("non-food " in Table 2).

Table 2. Oilseed crops in the top 5 countries within the EU-15 (Source: UFOP 1999/2000)

in 1,000 ha	1997/98		1998/99		1999/00		2000/01	
	non-food	food	non-food	food	non-food	food	non-food	food
Denmark	10	94	10	95	27	116	25	90
France	190	1.731	198	1.854	398	1.887	312	1.808
Germany	109	840	149	893	366	865	340	785
Italy	28	729	11	780	24	480	10	480
Spain	16	1.105	12	1.249	0	819	0	888
EU-15	392	5.254	418	5.684	975	4.914	709	4.755
EU-15 total		5.647		6.101		5.889		5.463

On May 8, 2003, the European Commission approved Directive 2003/30/EC on "The promotion of the use of biofuels or other renewable fuels for transport". This Directive aims at promoting the use of all types of biofuels to replace diesel or petrol for transport purposes, with a view to contributing to objectives such as meeting climate change commitments, environmentally friendly security of supply, and promoting renewable energy sources. Member States should ensure that a minimum proportion of renewable biofuels is placed on their markets, and shall set national indicative targets. A reference value for these targets was set at 2%, calculated on the basis of energy content of all fuel for transport purposes by the end of 2005 (5.75 % by end 2010). Member States shall ensure that information is given to the public on the availability of renewable fuels. For example, biodiesel exceeding the limit value of 5 % of FAME in blends with diesel will carry a specific labeling.

Biodiesel use in the EU is very diverse and differs from country to country (Table 3). Two main applications can be identified: use as a specialty fuel at 100%, which is preferred in Germany and Austria; use in blends up to 5% for regular use or up to 20 or 30% for captive fleet use, mainly in France and Italy. Captive fleets are public transport fleets, taxi associations, forestry companies, and yacht clubs (e.g. at Lake Constance). Italy also created a market for biodiesel as heating fuel.

Table 3. EU-15 Biodiesel use (2002)

	Germany	France	Italy	Other	Total
Pure (100 %)	450	-	50	20	520
20/30 % Blend	-	10	20	-	30
5% Blend	-	356	140	19	515
TOTAL	**450**	**366**	**210**	**39**	**1065**
Retail gas stations	150	-	-	20	170
Captive Fleets	300	10	20	-	330
Gas oil blend	-	356	140	19	515
Total (transport)	**450**	**366**	**160**	**39**	**1015**
Heating fuel	-	-	50	-	50
TOTAL	**450**	**366**	**210**	**39**	**1065**

The German application of pure biodiesel requires small changes to be made to the car engine. It also increases the risk of making errors when used by regular consumers. This, together with the extra cost for maintaining a separate distribution network has reached its limits. That's why Germany currently reconsiders its recommendations and will probably opt for "hidden" blending of biodiesel in regular diesel up to a 5% level. France has such legislation in use, and currently applies on average a blend with slightly above 2% biodiesel. This type of blend even brings specific technical advantages: a better lubrication, and consequently less need for additives.

Biofuels are made available as pure biofuels or as a blend in mineral oil derivatives, in accordance with specific quality standards for transport applications. The first countries to establish biodiesel technical specifications were Austria and Germany (DIN 51606) in the early 90s. These specifications were exclusively based on rapeseed oil. Italy and France adopted similar specifications. In 1998, the EU Commission requested CEN, the European Normalization Committee, to produce Europe-wide specifications. CEN comprises a large number of EU and non-EU European countries and includes mostly technical experts associated to the car industry, equipment manufacturers and petroleum industry. After several years of testing, this has resulted in the approval and publication of Biodiesel Standard EN 14214. This standard is endorsed by all involved interest groups, and accepted by the World-Wide Fuel Charter (WWFC). WWFC indicates that biodiesel with specifications following EN 14214 (or the US equivalent standard ASTM D-6751) can be used in diesel blends of diesel type I, II or III at up to 5%.

Although it seems tailor-made for rapeseed oil based biodiesel, it actually allows biodiesel blends made of any mixture of methyl esters that fit on the standard. The CEN standard differs from the American Standard ASTM D-6751 in some specifications only (given in brackets below). Main requirements specific for biodiesel under EN 14214 are the iodine value set

at max. 120 (no limit in ASTM), oxidation stability of minimum 6 hours at 110°C in a Rancimat or OSI test (none in ASTM), kinematic viscosity between 3.50 and 5.00 mm²/s at 40°C (1.90-6.00 in ASTM), Cetane number of minimum 51 (min. 45 in ASTM), and, last but not least, the cold filter plugging point CFPP between +5°C and -20°C for temperate climate zones as found in Europe. The CFPP was mainly introduced for biodiesel to be used without mixing to mineral diesel and is of less importance in fine-tuned blends. CFPP, cloud point, and oxidation stability remain important though, when it comes to storage stability and transportation. More detail on oxidation and storage stability was published recently by the BIOSTAB research project. Specifications on FAME content, residual free and total glycerin, mono-, di- and triglycerides, methanol and water, are more or less in line in both standards and relatively easy to obtain with most biodiesel blends, when properly processed.

Another important property of biodiesel can be found in both standards: biodiesel should have less than 10 ppm S (15 ppm in ASTM) The maximum sulfur level is 3000, 300 and 30 ppm in diesel type I, II or II according to the WWFC. As biodiesel is practically sulfur free and has good ignition and fuel characteristics it emits an exhaust, which is less harmful to health. The subsequent treatment of the exhaust is simpler than with fossil fuels. The low sulfur value and ability to significantly reduce particulate, HC and CO emissions, and increased NOx and aldehyde emissions compared with petroleum-based diesel fuel, are the most important advantages of using biodiesel. This does not even take into account the almost neutral carbon dioxide balance they can guarantee. Biodiesel fuels have generally been found to be nontoxic and are biodegradable, which promotes their use in applications where biodegradability is desired. Biodiesel lowers the risk of cancer and damage to the genetic make up.

The difficulties to find equilibrium between the different markets on which a biodiesel operator is working are mainly created by the high volatility of the raw material and product price (Figure 1). High vegetable oil prices and low diesel prices lead to poor profitability of a biodiesel operation (in 1995-1998), or vice versa (1999-2000). The glycerin price (80% glycerin) shows an even bigger variation during the same period. First, the glycerin price was extremely good and made up a very important part of the total revenues of a biodiesel plant. By the end of 1998, however, the level was down at one third. Prices appreciated again in 1999-2000, further increasing the excellent margins of this period. Since then, the glycerin price collapsed to levels, which were never seen before. During the same period, the methanol price came down from more than 500 EUR/T early 1995, to just above 120 EUR/T early 1999. Methanol went back to 270 EUR/T in 2001, to fall again later.

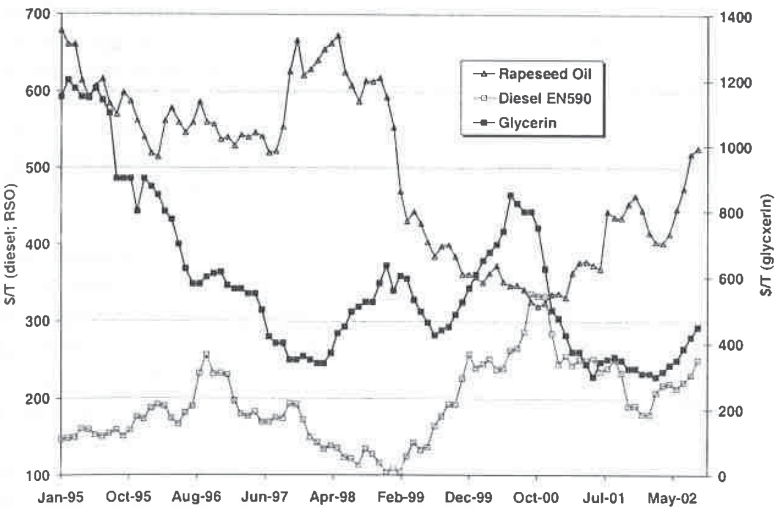

Figure 1. Diesel, vegoil and glycerin prices throughout recent years

This price volatility directly affects the biodiesel margin calculation. In Table 4, the margin is the price difference between the revenue generated by the major products (1000kg of biodiesel and 110 kg of glycerin, or 135kg at 80%), and the cost of the basic ingredients (1000kg of rapeseed oil and 110 kg of methanol), corrected for a bonus of 330€/T detaxation and for EUR/USD fluctuations.

Table 4. Biodiesel Materials Margin (see text)

	Feb-95	Feb-00	Feb-02
1000 kg diesel	149	244	179
135 kg 80% glycerin	162	87	41
1000 kg rapeseed oil	-661	-350	-415
110 kg methanol	-49	-19	-18
Detaxation	416	333	289
Result	17	295	76

Consequently, the availability of raw materials, and a profitable market for the end products is of great importance. A reduced subsidy system for oilseed growing in Europe has already reduced the availability of set-aside rapeseed oil at a competitive price. Producers in Southern Europe also

don't have access to such oil, since it is only produced in the northern half of Europe. Already today, Italy is using soybean oil in blends for making biodiesel. Other raw materials, such as sunflower seed oil and recycled oils and fats also find their way to biodiesel. Main restriction on the use of recycled oils and fats, which are sometimes called UFOs (unidentified oils and fats), is their variable composition and high risk of presence of polymers and other more polar contaminants, which will limit the quality of the biodiesel produced, but also the quality, hence value of the glycerin by-product.

This is where soybean oil can catch a share of the market. Soybean oil is readily available worldwide and in Europe, at competitive price and at high quality level. The necessary refining can be done on very large scale with very high efficiency. The restrictions on iodine value and maybe oxidation stability as a pure biodiesel component as defined by EN 14214 can easily be overcome with intelligent blending combined with proper processing. Any type of vegetable oil or fat (rapeseed, sunflower, soybean, palm, palm kernel oil; recycled UFOs) can be transformed into FAME. As most biodiesel specifications are based on rapeseed oil, it is often needed to make an adapted blend. From practice it is known that several blends will meet the EN 14214 characteristics. Some examples of blends already used in the past: 85% rapeseed + 15% sunflower seed oil, 80% rapeseed + 20% soybean oil, 60%rapeseed + 30%soybean + 10% palm oil. More matches with up to 70% soybean oil can be made when combining with shorter chain fatty acids from palm oil or lauric fats. It is not important where the blending is done: at vegetable oil level or at bio-diesel level. It is all the same and it just depends on the technical possibilities of the plant. Under ASTM D-6751 even pure soybean oil methyl esters can be used under the WWFC. As usual, the mineral oil companies will do the final blending with petroleum-based diesel and additives.

The potential raw materials are available worldwide to develop biodiesel not only in Europe, but also in North and South America and Asia to a notable alternative fuel. Biodiesel will therefore also help to conserve resources and to reduce greenhouse gases - the EU commission has drawn particular attention to this aspect in the justification of its proposals. This creates a potential market for soybean oil as one of the raw materials to be used.

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Biodiesel fuel properties of soybean oil fatty acid esters

G. Knothe[1]

Abstract

Biodiesel is an alternative diesel fuel defined as the mono-alkyl esters of vegetable oils or animal fats, which are obtained by the transesterification of the oil or fat with a monohydric alcohol, usually methanol. Soybean oil is the major feedstock for the production of biodiesel in the United States. As virtually all vegetable oils, soybean oil is mainly composed of the triacylglycerols of several fatty acids. The five main fatty acids found in soybean oil - palmitic, stearic, oleic, linoleic and linolenic acids - differ in chain length and degree of (un)saturation. These structural differences impart different physical properties, many of which are of significance for fuel applications. Several other typical fuel properties are also affected. These properties can also be affected by the kind of ester, i.e., the alcohol used for production of the mono-alkyl esters. This work summarizes some important fuel properties of biodiesel and how they are affected by the properties of the individual esters comprising it.

Introduction

Biodiesel, an alternative diesel fuel (DF) derived from vegetable oils or animal fats (Knothe and Dunn 2001), is being produced commercially in many countries around the world. Transesterifying the oil or fat with a monohydric alcohol, usually methanol, yields the corresponding mono-alkyl esters of the oil or fat, which are defined as biodiesel. Vegetable oil feedstocks for biodiesel depend on which vegetable oil is the most common in the area or country in which the biodiesel is being produced. Therefore, soybean oil is the most commonly used vegetable oil for biodiesel production in the United States and it is a potential biodiesel source in other major soybean oil-producing countries.

Biodiesel standards such as ASTM (American Society for Testing and Materials) D6751 and the European standard EN 14214 have been or are being established around the world. The specifications in these standards include many which can be traced to the major fuel components, i.e., the fatty acid esters. The major properties affecting fuel use of such esters will be outlined here. Other specifications result from the need of biodiesel

1 USDA / ARS / NCAUR; Peoria, IL 61604; U.S.A.

fuel to be free of harmful contaminants, such as those resulting from production or (improper) storage and will not be dealt with here.

The major fatty acids in soybean oil are palmitic (C16:0; hexadecanoic), stearic (C18:0; octadecanoic), oleic (C18:1; 9(*Z*)-octadecenoic), linoleic (C18:2; 9(*Z*),12(*Z*)-octadecadienoic), and linolenic (9(*Z*),12(*Z*),15(*Z*)-octadecatrienoic) acids, which are probably the most common fatty acids found in the realm of vegetable oils. Soybean oil may also contain small amounts of other fatty acids such as palmitoleic (C16:1; 9(*Z*)-hexadecenoic) or arachic (C20:0; eicosanoic) acids. Other oils can contain high amounts of saturated shorter-chain fatty acids (lauric oils) or longer-chain fatty acids such as erucic acid (high-erucic rapeseed oil).

Five important fuel properties, cetane number, low-temperature behavior, oxidative stability, viscosity and lubricity will be discussed here. All of these properties are affected by the fatty acid profile of the biodiesel. They also are important for the properties of blends of biodiesel with conventional DF.

Discussion

General aspects. Any biodiesel fuel that meets standards consists almost exclusively of the mono-alkyl esters of the fatty acids found in the triacylglycerols of the parent oil or fat. Thus, the structures of these esters determine the fuel properties. The following structural differences are usually observed in most common fatty acids and their alkyl esters: a) chain length, i.e., the number of carbon atoms in the fatty acid chain, b) degree of (un)saturation, i.e., the number of double bonds in the fatty acid chain with the position of the double bonds in the chain and the position relative to each other being important aspects, c) the nature of the alcohol used to produce the esters, especially straight-chain *vs.* branched esters. The following discussion focuses on important fuel properties determined by compound structure.

Cetane number (CN). The CN is a dimensionless descriptor related to DF combustion. It is determined by the ignition delay (ID) time a fuel experiences upon injection into the combustion chamber, i.e., the time between injection of the fuel and onset of ignition. The shorter the ID time, the higher the CN and vice versa. The CN is conceptually similar to the octane number used for gasoline. Generally, a material that has a high octane number tends to have a low CN and vice versa.

Some standards for CN determination are ASTM D613 in the United States and ISO 5165 of the International Organization for Standardization (ISO). For the cetane scale, hexadecane (cetane) as high quality standard has

been assigned a CN of 100. Highly branched 2,2,4,4,6,8,8,-heptamethylnonane, is the low-quality standard with an assigned CN of 15. Thus CN decreases with decreasing chain length and increasing branching. The standard ASTM D975 for conventional DF requires a minimum CN of 40 while standards for biodiesel prescribe a minimum of 47 (ASTM D6751; the CN of methyl soyate meets this requirement) or 51 (EN14214). Recently, a new standard, ASTM D6890, which determines a derived cetane number (DCN) in a constant volume chamber with less material than required by ASTM D613, was established. It covers the range of 40.5 to 55.3 DCN.

The influence of compound structure on CNs of fatty compounds was discussed in the literature (Harrington 1986) and the predictions in that paper were confirmed by practical tests (Knothe et al. 2003). CNs of saturated compounds such as esters of palmitic or stearic acids are usually > 70 and sometimes even > 100, while those of oleic acid esters are usually in the range of 50-60 and those of linolenic acid esters may even be < 30. CNs of fatty compounds decrease with increasing unsaturation and decreasing chain length. Branched esters derived from alcohols such as *iso*-propanol, which are of interest because of improved low-temperature properties, have CNs competitive with methyl or other straight-chain esters (Knothe et al. 2003). Thus, one long, straight chain suffices to impart a high CN even if the other moiety is branched. Some of these cetane tests were conducted using an Ignition Quality Tester™(IQT™ (Knothe et al. 2003), which is the basis of ASTM D6890 mentioned above. The IQT™is an automated development of a constant volume combustion apparatus (CVCA). The CVCA was originally developed for determining CNs more rapidly and easily, with reduced fuel use and better reproducibility and thus less cost than with ASTM D613. Results from the IQT™ method were competitive with or more reliable than those derived from ASTM D613. Generally, the results of cetane testing from the literature for compounds with low CN agree better than the results for compounds with high CN. The reason is the non-linear relationship between ID and CN (Knothe et al. 2003). Small changes at shorter ID result in greater changes in CN than at longer ID. This would indicate a leveling-off effect on emissions such as NO_x as discussed above once a certain ID with corresponding CN has been reached as the formation of certain species depends on the ID. However, for newer engines this aspect must be modified as mentioned above.

Higher CNs were correlated with reduced nitrogen oxides (NO_x) exhaust emissions (Ladommatos et al. 1996), leading to efforts to improve the CN of biodiesel by means of additives termed cetane improvers (Knothe et al. 1997). Despite the relatively high CNs of fatty compounds, NO_x exhaust emissions usually increase slightly when operating a diesel engine on

biodiesel. The connection between structure of fatty esters and exhaust emissions was investigated (McCormick et al. 2001) by studying the exhaust emissions caused by enriched fatty acid alkyl esters as fuel. NO_x exhaust emissions increased with increasing unsaturation and decreasing chain length, which can lead to a connection with CN. Particulate emissions were hardly influenced by the aforementioned structural factors. However, the relationship between the CN and emissions is complicated by many factors including the technology level of the engine with more modern engines being less sensitive to CN.

Low-temperature properties. The problematic low-temperature properties of biodiesel are reflected in relatively high cloud points (CP) and pour points (PP) (Knothe and Dunn 2001). The CP, which usually occurs at a higher temperature than the PP, is the temperature at which a fatty material becomes cloudy due to formation of crystals and solidification of saturates. The solids and crystals clog fuel lines and filters, causing major operability problems. With decreasing temperature, ever more components solidify and the material approaches the PP, the lowest temperature at which it will flow. Saturated fatty compounds have significantly higher melting points than unsaturated fatty compounds and in a mixture they crystallize at higher temperature than the unsaturates. Thus, the greater the amount of saturated compounds in biodiesel, the higher the CP and PP.

Besides CP (ASTM D2500) and PP (ASTM D97), other tests for the low-temperature properties of conventional DF are the low-temperature flow test (LTFT; used in North America; ASTM D4539) and cold filter plugging point (CFPP; used outside North America; European standard EN 116). Low-temperature filterability tests reportedly correlate better with operability tests than CP or PP (Owen and Coley 1995) and were applied to biodiesel and its blends with conventional DF. However, for fuel formulations containing at least 10 vol.-% methyl esters, LTFT and CFPP are linear functions of CP and statistical analysis showed a strong 1:1 correlation between LTFT and CP (Dunn and Bagby 1995).

Approaches to solving the low-temperature problems of esters include blending with conventional DF, winterization, additives, branched-chain esters, and bulky substituents in the chain. The latter two approaches may be considered variations of the additive approach. Blending will not be discussed here.

Numerous, usually polymeric, additives were reported mainly in the patent literature to lower PP or sometimes CP (Knothe and Dunn 2001). The effect of some additives appears to be limited because they more strongly

affect the PP than the CP or only slightly influence CP. The CP, however, is more important than the PP for improving low-temperature flow properties (Dunn et al. 1996).

The use of branched esters such as iso-propyl, iso-butyl and 2-butyl instead of methyl esters (Lee et al. 1995, Foglia et al. 1997) improves the low-temperature properties of biodiesel. Neat branched esters have lower melting points, for example that of *iso*-propyl palmitate is 13-14EðC while that of methyl palmitate is 30EðC. The CP of *iso*-propyl soyate was given as -9EðC (Zhang and Van Gerpen 1996) and that of 2-butyl soyate as -12EðC (Lee et al. 1995) while the CP of methyl soyate is 0EðC (Dunn and Bagby 1995). Branching in the ester chain does not have any negative effect on the CNs of these materials as discussed above. In economic terms only *iso*-propyl esters appear attractive as branched esters although they also are more expensive than methyl esters.

Winterization (Dunn et al. 1996, Lee et al. 1996, Dunn et al. 1997), based on the lower melting points of unsaturated fatty compounds *vs.* those of saturated ones, removes by filtration solids formed by cooling the vegetable oil esters, leaving a mixture with higher content of unsaturated compounds and thus with lower CP and PP. The procedure can be repeated to further lower CP and PP. Saturated fatty compounds, which have higher CNs, are the major compounds removed, thus decreasing the CN. Less material is lost by winterization when cold-flow improvers or solvents such as hexane or *iso*-propanol are present (Dunn et al. 1997).

Oxidative stability. This issue affects biodiesel primarily during storage through parameters such as presence of air, heat, traces of metal, antioxidants, peroxides as well as nature of the storage container and has been the subject of considerable research (Frankel 1998). Elevated temperatures or presence of air or metals facilitate oxidation. Oil stability index (OSI) studies confirmed the catalytic effect of metals on oxidation, however, the influence of compound structure, especially unsaturation, was even greater (Knothe and Dunn 2003).

A European standard (EN 14112) was established for including an oxidative stability parameter in the standard EN 14214. EN 14214 calls for determining oxidative stability at 110EðC with a minimum induction time of 6h by the Rancimat method. The Rancimat method is nearly identical to the OSI method, which is an AOCS (American Oil Chemists' Society) method. Other methods, including wet-chemical ones such as acid value and peroxide value, pressurized differential scanning calorimetry, etc., have been applied in oxidation studies of biodiesel.

The reason for autoxidation is the presence of double bonds in the chains of many fatty compounds with the species formed during this process eventually causing the fuel to deteriorate. Autoxidation proceeds with different rates depending on the number and position of double bonds (Frankel 1998), with positions allylic to double bonds being susceptible to oxidation. The *bis*-allylic positions in polyunsaturated fatty acids such as linoleic acid (double bonds at)ð9 and)ð12, one *bis*-allylic position at C-11) and linolenic acid (double bonds at)ð9,)ð12, and)ð15, two *bis*-allylic positions at C-11 and C-14), are even more prone to autoxidation than allylic positions. Relative rates of oxidation given in the literature (Frankel 1998) are 1 for oleates, 41 for linoleates, and 98 for linolenates.

Viscosity. Viscosity affects the atomization of a fuel upon injection into the combustion chamber and ultimately the formation of engine deposits. The higher the viscosity, the greater the tendency of the fuel to cause such problems. The viscosities of transesterified oils, i.e. biodiesel, are reduced by about an order of magnitude compared to the parent oils (Knothe and Dunn 2001). High viscosity is the major reason why neat vegetable oils have been largely abandoned as alternative DF. The kinematic viscosity of diesel fuels can be assessed by standards such as ASTM D445 or ISO 3104. Prescribed viscosity ranges in biodiesel standards are 1.9-6.0 mm^2/sec (ASTM D6751) and 3.5-5.0 mm^2/sec (EN14214). The effect on viscosity of blending biodiesel and conventional DF was investigated (Tat and Van Gerpen 1999) and an equation derived which allows the calculation of the viscosity of blends.

Viscosity increases with chain length (fatty acid or alcohol moiety) and increasing saturation. Double bonds with *cis* configuration have lower viscosity than *trans* double bonds while double bond position affects viscosity less (Knothe et al., manuscript in preparation). Branching in the ester moiety has little or no influence on viscosity, again showing that this is a technically promising approach for improving low-temperature properties without significantly affecting other fuel properties. Some examples of kinematic viscosity at 40EðC are: methyl palmitate 4.38 mm^2/sec, methyl oleate 4.51 mm^2/sec, methyl linoleate 3.65 mm^2/sec, methyl linolenate 3.14 mm^2/sec, ethyl palmitate 4.57 mm^2/sec.

Lubricity. With the advent of low-sulfur DFs, the issue of lubricity is becoming increasingly important. Desulfurization of conventional DF reduces or eliminates its inherent lubricity, which is essential for proper functioning of engine components such as fuel pumps and injectors. Adding biodiesel at low levels (1-2%) restores lubricity to low-sulfur petroleum-derived DF. An advantage of biodiesel compared to lubricity-enhancing additives is that

biodiesel inherently has fuel properties competitive and compatible with conventional DF. Lubricity-enhancing additives usually do not exhibit such inherent fuel properties. No major effects of biodiesel fatty acid composition on lubricity were reported, except for castor oil which had better lubricity than other vegetable oil esters (Drown et al. 2001). Ethyl esters had greater lubricity compared to methyl esters (Drown et al. 2001). Standards for lubricity testing of DFs are the scuffing load ball-on-cylinder lubricity evaluator (SLBOCLE; ASTM D6078) or the high-frequency reciprocating rig (HFRR; ASTM D6079; ISO 12156). Lubricity is not included in biodiesel standards despite the advantage of biodiesel with respect to this fuel property.

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Current research on biodiesel fuel in Japan

H. Nabetani; S. Iwamoto and M. Nakajima[1]

Introduction

There is a limit to estimated amount of petroleum, coal and natural gas, and they will be exhausted in the future. On the other hand, biomass resources are renewable and can be produced continuously by photosynthesis. So, biomass resources can be utilized as renewable energy without disturbing carbon balance of ecosystem on the earth. The amount of biomass is estimated about 830 billion ton as carbon. It accounts for the total amount of petroleum, coal and natural gas, and 80 times as much as the amount of the energy consumed in a year in the world.

In the "Kyoto Protocol" at the 3rd Session of the Conference of the Parties to the United Nations Framework Convention on Climate Change (COP3), Japan decided to reduce the amount of discharge of CO_2 by 6% in the average amount between 2008 and 2012 compared to the amount in 1990. "Improvement in energy efficiency", "absorption by forest" and "promotion of utilization of new energy and renewable energy" are thought to be important measures to achieve the goal. That is, in order to reduce the amount of discharge of CO_2 and prevent global warming, it is required to reduce consumption of fossil resources by utilizing biomass resources as energy and materials.

In order to promote utilization of biomass resources and contribute to prevention of global warming, creation of recycling society and activation of rural area, a comprehensive strategy called "Biomass Japan" was drawn up by six ministries in Japan including the Ministry of Agriculture, Forestry and Fisheries (MAFF), and endorsed by the Japanese Cabinet in last December.

In this paper, outline of the strategy and sate of biomass energy utilization including biodiesel fuel in Japan are introduced. Then, research activities on biodiesel utilization implemented by authors' group and other groups in Japan will be presented.

[1] National Food Research Institute, Kan-nondai, Tsukuba, Ibaraki 305-8642, Japan

Comprehensive strategy "Biomass Japan"

It is possible to produce energy such as electricity, heat and liquid fuel, and products such as bio-degradable plastics and industrial materials from biomass resources. The possibility will further increase along with technology development. Now, it is strongly required to take advantage of biomass resources based on technology development and realize a society which can develop in a sustainable way.

In the society, CO_2 discharge can be reduced and the goal in "Kyoto Protocol" can be achieved by replacing energy and materials from fossil resources with those from biomass which is "carbon neutral". Furthermore, by promoting biomass related industries as strategic industries, competitive power of Japanese industries is expected to be reinforced. The size of biomass industries is supposed to grow up to about 260 billion yen by 2010, and further grow. And, by utilizing abundant biomass resources which grow on the blessing of nature, new industries and additional employment will be brought to rural areas, and Japanese agriculture, forestry and fisheries will be activated. In order to realize such society called "Biomass Japan", a comprehensive strategy was drawn up.

In the strategy, amount of biomass resources in Japan is estimated first. Waste materials such as waste paper, animal excreta, food waste, waste building materials, liquid waste from pulp mill and sewage sludge are supposed to be utilized in earlier stage. Their amount is estimated to be 22 million tons as carbon. Untapped resources such as rice straw and rice husk might be utilized in considerable degree by 2010. Their amount is estimated about 5.3 million tons as carbon. Industrial crops which are cultivated as materials for energy and industrial products will increase by 2020 because the conversion efficiency will be improved and the industries will be able to pay for such materials. The estimated amount of industrial crops is 5.2 million tons as carbon. The total amount of biomass resources in Japan is estimated about 33 million tons as carbon, which accounts for 3.3 times as much as the amount of carbon contained in plastics produced in Japan in a year.

It is important to set a goal in order to promote biomass utilization efficiently. Therefore, in the strategy, several goals are set from technological, regional and nationwide viewpoints. By 2010, from regional viewpoint, about 500 villages will utilize more than 90% of waste materials and more than 40% of untapped resources. From nationwide viewpoint, waste materials will be utilized more than 80%, untapped resources will be utilized more than 25%, and about 100,000 tons of industrial crops will be utilized by then,

based on technological development such as improvement in energy conversion efficiency, reduction in production cost and so on.

Seventy-eight concrete measures toward realization of "Biomass Japan" are planned in the strategy. Main measures are establishment of a council for promotion of "Biomass Japan" under collaboration among ministries, establishment of "Biomass Information Headquarters" as a base of information, establishment of efficient system for collection and transportation of biomass resources, construction of demonstration facilities for utilization of biomass, promotion of development and practical use of innovative conversion technologies, trial use of plastics derived from biomass resources, evaluation of vehicle fuel derived from biomass resources and so on.

State of biomass energy utilization in Japan

Situation of biomass energy in Japanese society is different from that in western countries in some degree. In western countries, biomass energy is regarded as measures to utilize surplus agricultural products. On the other hand, in Japan, local utilization of organic waste is brought into focus, because of difficulty in intensive production of energy crops.

In Japan, the ratio of biomass energy to total energy is only 0.8%, now. The most common resource of biomass energy in Japan is organic liquid waste from pulp production process so called black liquor. It is burned in boiler after concentration. All the liquid waste from pulp mill in Japan is utilized as energy. The amount is about 14 million tons on dry base and accounts for 4.5 million kL of oil. About 30% of total energy consumed in the pulp industry in Japan is supplied by liquid waste from pulp mill.

Pellet fuels such as woody pellet and refuse derived fuel (RDF) are used in Japan in some degree. They are easy to handle in transportation and storage, and to control burning. About 2000 tons of woody pellet are produced in Japan in a year. The price is about 40 yen/kg. About 2,300 tons of RDF is produced in Japan in a year. However, possibility of generation of dioxin is pointed out.

Amount of residue from lumber mill is almost 3 million tons. More than 200 boilers are utilizing residue from lumber mill as fuel. The energy is used for drying process of lumber and heating. And, more than 90% of bagasse which is residue from squeezing process of sugar cane is utilized as fuel of boiler in the factories.

About 15 power generation plants are in operation using woody waste mainly in large scale lumber mill. In a laminated lumber factory in Okayama

prefecture, residue from the process is burned in a boiler and a generator is run using steam from the boiler. Heat from the steam is used for lumber drying process, hot-water supply system and heating system of the factory. All the electricity required in the factory is supplied by the generator.

Demand on appropriate processing of animal excreta is increasing in Japan, and many kinds of processing systems are in the development stage. Some are already practically used. At "Bio-ecology Center" in Yagi town in Kyoto prefecture, animal excreta is mixed with "okara", residue from "tofu" processing, and fermented. A gas engine is run by using bio-gas (methane) from the fermentation process, and electrical power is generated. In addition, residue from the fermentation process is mixed with other animal excrement and used as compost, or barnyard manure.

State of biodiesel fuel utilization in Japan

In Japan, about 2,000,000 tons of edible oil is consumed in a year, and about 450,000 tons of waste edible oil is discarded from food factories and as household garbage. About 200,000 tons out of it is collected and utilized mainly as materials for soap and animal feed. Some of the collected waste edible oil is converted into methyl ester and used as biodiesel fuel.

"Someya-shoten", a collection trader company in Metropolis of Tokyo, produces about 1,500 L of methyl ester from waster edible oil in a day by use of alkaline catalyst method, and sells it at a price of 80 yen/L. This company had been collecting 20 - 30 tons of waste edible oil each day and selling it as animal feed. By selling a part of the waste edible oil as biodiesel fuel, the company could increase its profit by one-million yen per month.

In Kyoto city where COP3 was held, the city government has been collecting waste edible oil from households and restaurants in the city to convert it into methyl ester since 1997. The city government asks a company to produce methyl ester from the waste edible oil by use of alkaline catalyst method. The methyl ester is used as fuel for garbage trucks and buses of the city government. About 220 garbage trucks are using 100% methyl ester as their fuel. In the case of the city buses, B20 which contains 20% methyl ester in ordinary diesel fuel is used because 100% methyl ester can not give them enough power. About 80 city buses are running with B20. Now, amount of biodiesel fuel used in Kyoto city is about 1,500 kL in a year. In Japan, the prefectural governments do not charge any tax on biodiesel fuel which contains100% methyl ester. However, in the case of B20, prefectural governments charge the same tax as ordinary diesel oil. The tax rate is 32 yen/L. Price of the methyl ester produced from waste

edible oil is about 68 yen/L exclusive of tax. Price of ordinary diesel fuel in Japan is about 62 – 63 yen/L including tax. If the tax is added, price of biodiesel fuel becomes almost equal to the price of gasoline. This tax system is pointed out to be one factor which inhibits wide spread of biodiesel fuel in Japanese society.

Total amount of biodiesel fuel used in Japan in a year is estimated to be about 2,000 kL while about 46 million kL of diesel fuel is consumed.

Research activities on biodiesel utilization in Japan

In order to promote utilization of biodiesel fuel, cost for biodiesel fuel production has to be reduced by improving efficiency of the process. In a methyl ester production process with alkaline catalyst method, the catalyst has to be removed from products after the reaction. Otherwise, glycerol which is by-product from the process can not be utilized in other industries. However, removal of alkaline catalyst increases total cost for biodiesel fuel production. When waste edible oil is converted into methyl ester by alkaline catalyst, free fatty acid has to be removed prior to the reaction in order to maintain activity of the catalyst. Removal of free fatty acid reduces yield of the process. In order to solve these problems, some Japanese groups are working on development of new processes for biodiesel fuel production. Saka and Kusdiana[1, 2, 3] investigated methyl-esterification reaction in supercritical methanol without using any catalyst. Experiment was carried out in a batch-wise reaction vessel preheated at 350 and 400°C and at a pressure of 45 – 65 MPa. It was consequently demonstrated, in a preheating temperature of 350°C, 240 s of supercritical treatment of methanol was sufficient to convert the rapeseed oil to methyl esters, and that, although the prepared methyl esters were basically the same as those of common method with a alkaline catalyst, the yield of methyl esters by the former was found to be higher than that by the latter. It was found that the supercritical methanol process requires the shorter reaction time and simpler purification procedure. In addition, by using the supercritical methanol method, methyl esters were produced not only from triglycerides but also from free fatty acids.

Microemulsification is another method to reduce viscosity of triglyceride and make it substitute for diesel fuel. Authors investigated property of microemulsion system which consisted of triolein and ethanol by using monoolein as surfactant[4]. Although monoolein acted as surfactant and sufficiently reduced viscosity of triolein, it was difficult to find out appropriate composition. Therefore, characteristics of microemulsion made of triolein, ethanol and monoolein was further investigated by using a dynamic light

scattering spectrophotometer (DLS) and a small angle X-ray scattering equipment for solution (SAXES) in order to introduce basic knowledge to microemulsion fuel design. Analysis with DLS and SAXES showed that the ethanol phase was continuous when the content of ethanol was high. On the other hand, triolein phase was continuous when the ethanol content was low. Monoolein was found to form concentric spherical shell which covered dispersed phase when the continuous phase was triolein. Outer diameter and thickness of the shell were 32 nm and 6.5 nm, respectively, and hardly depended on the composition of the system.

When waste edible oils are used as materials for biodiesel fuel, an efficient method to remove impurities in the oils might be required. Authors conducted some membrane separation studies with used frying oils in a membrane batch cell using several different types of polymeric membranes to decrease the soluble degradation products[5]. During membrane processing, triglycerides permeated preferentially compared to the majority of the polar compounds including oxidation products, polymers, and color compounds. The viscosity of the used frying oil was reduced to the extent of 22%. The membranes were effective in reducing the soluble impurities, as well as insoluble particulates, without causing any undesirable changes to the oil. However, for commercial application the permeate flux needs to be improved.

Now authors are evaluating efficiency of a reactor for methyl esterification of triglycerides without alkaline catalyst, in order to design a efficient reactor for production of biodiesel fuel from waste edible oil.

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Biodiesel in Brazil: history and perspectives

P. A. Z. Suarez[1]

Abstract

Periodically, during petroleum shortage, vegetable oils and their derivatives have been proposed as alternative to petroleum diesel fuel. Since 1930, different approaches have been proposed in Brazilian's universities and research institutes, including the use of net vegetable oils (pure or in blends) or their derivatives, such us hydrocarbons obtained by thermo-catalytic cracking or fatty-acids methyl or ethyl esthers (usually known as "biodiesel") produced by alcoholysis. Recently, the external dependence in imported diesel fuel and the last petroleum crisis have increased the discussion in Brazil to start using alternatives to diesel fuel, being the biodiesel the alternative of choice for a large petroleum diesel substitution program.

Renewable fuels in Brazil

The use of vegetable oils as fuels was firstly tested in 1896 when the inventor of compression ignition engines (diesel engine), Rudolf Diesel, used peanut oil as an engine fuel (Shay, 1993). However, as petroleum became the dominant energy source, petroleum diesel was developed as the primary fuel for diesel engines. Nonetheless, petroleum and its derived fuels have periodically being in short supply and, as consequence, incentives to search alternatives energy sources have emerged. Thus, in the 1930's and 1940's neat vegetable oils were used in diesel engines from time to time, but usually in emergency situation (Ma, 1999). At this time, the pyrolysis of different triglycerides was also used for fuel supply in different countries. For instance, hydrocarbons were produced in China by a Tung oil pyrolysis batch system and used as gasoline and diesel like fuel (Chang, 1947). Further, the petroleum international crises in the 1970's and 1990's, as well as an increasing concern about the depletion of the world's non-renewable resources and environmental awareness, provided new enthusiasm in the search for renewable fuel sources.

Since the 1930´s, several efforts have also been done in Brazilian's universities and research institutes in order to find renewable fuel sources. In this context, our largest experience includes the use of ethanol from sugar cane as alternative fuel for Otto engines. Thus, in the 1980's a

[1] Instituto de Química, Universidade de Brasília, CP 4478, 70919-970, Brasília-DF, Brazil. psuarez@unb.br

federal program called PROALCOOL implemented and regulated the use of hydrated ethanol (engine's adaptations were needed to use this fuel) and dry ethanol blended with petroleum gasoline. It is worth mentioning that, after 1980 no pure petroleum gasoline was used as fuel in Brazil. The ethanol content in these blends has been augmented during the three decades of PROALCOOL and actually varies from 20 to 25%. On the other hand, vegetable oils (pure or blended with petroleum diesel) or its derivatives have been considered as alternative fuels for Diesel engines. In the 1930's and 1940's lots of studies about the use of neat vegetable oil, such as babassu, coconut, castor seed and cotton seed. (for instance, see: Borges, G.P., 1944) or hydrocarbons produced by its thermo-catalytic cracking (Otto, 1945) were carried out. It's interesting to note that the government prohibited the exportation of cottonseed oil during the 2nd World War in order to force its use in substitution of the imported petroleum diesel as fuel in trains (Chemical & Metallurgical Engineering, May 1943).

More recently, in the 1970´s and 1980´ the transesterification of vegetable oils into methyl (ME) or ethylesthers (EE) was proposed and extensively studied as alternative to petroleum diesel. Before being wide-world known as "BIODIESEL", these esthers mixtures were at first called "PRODIESEL" in Brazil. These studies have been started in the Universidade Federal do Ceará (Fortaleza, Ceará – Brazil) using different vegetable oils sources as soybean, babassu, peanut, cottonseed, sunflower, and others (Parente, 2003). It is important to highlight that the first patent in the world claiming the production of fuels by the alcoholysis of vegetable oils (PI BR 8007957) came from this research in 1980 (Parente, 2003). After that, many research institutes and universities in Brazil started to study and to produce ME and EE. In the last two decades not only academic researches have been done but also pilot plants were started up in several cities and large-scale on-the-road tests have also been done. Recently, the biodiesel moved forward of being purely an experimental fuel to the initial stages of commercialization. Thus, the first industry of fatty acids esthers has started up in the Mato Grosso State (November, 2000), with a production of 1,400 tons/month of soybean ethyl esther (Sant'anna, 2003). Nowadays, two other industries are being implemented in states of Piaui and Ceara, using castor-seed oil as raw-material, (Vigliano, 2003).

Biodiesel perspectives in Brazil

Figure 1 shows the Brazilian's demand and production of petroleum, diesel, gasoline and ethanol in the last ten years. It became clear from this figure that, although an increasing in the consumption, a decline in the petroleum importation was caused by a significant growth in the internal production.

Indeed, the Brazilian external average dependence in petroleum and its derivatives fell down from 48.6% in 1993 to 9.4% in 2001 (ANP, 2003). In the last years a decline in the gasoline consumption allowed an augmentation in its exportation. Note that the decrease of the gasoline demands is probably due to an increase in the ethanol content of the commercialized blends. In opposite, the diesel consumption increased considerably and this increasing demand is currently being partially supplied by a growing direct importation of diesel fuel. As far as 80% of diesel consumption is due to transport (BEB/MME, 2002), the increasing external dependence of this fuel is dramatic to Brazilian economy.

The increasing external dependence in imported fossil diesel and the last petroleum crisis increased the discussion in Brazil to find alternatives to this fuel. In fact, several ministries, research institutes and universities have join affords to make feasible the use of vegetable oil and its derivatives as alternative to diesel fuel. In this context, the main approach proposed was the alcoholysis of vegetable oils. Nonetheless, the use of neat vegetable oil (pure or in blends with petroleum diesel) and hydrocarbons obtained by their thermo-catalytic cracking were proposed and are currently being studied as alternative fuel for diesel engines or stationary engines for electricity production in isolated communities in the Amazonian region and in farms.

In fact, the alcoholysis (with methanol or ethanol) of vegetable oils to produce methyl or ethylesthers is being considered as the main route for a large program (called PROBIODIESEL) of petroleum diesel substitution. It is important to note that ethanolysis, although its technological limitations, is the favorite route because of the Brazilian's potential and know-how in the production of ethanol. This ambitious program, initially proposed to substitute until 2005 all diesel fuel consumed in Brazil initially by B5 (5% biodiesel and 95% petroleum diesel blend) and in fifteen years by B20 (20% biodiesel and 80% petroleum diesel blend), using ethanol esthers (Vigliano, 2003).

Due to its great biodiversity and diversified climate and soil conditions, Brazil has different vegetable oils sources, including soybean, coconut, castor seed, cottonseed, palm-trees, and others. Undoubtedly, inasmuch as Brazil is today the second largest soybean producer in the world (probably will be soon the first) and has a well developed processing industry, this source occupies a prominent position in the development of vegetable oil based fuels. Indeed, the Ministry of Mines and Energy plans to use soybean oil as raw material for biodiesel production in the PROBIODIESEL in main Brazilian regions (Vigliano, 2003).

However, in the semi-arid Northeast and Amazonian States, castor seed oil and palm-tree oil, respectively, seems to be the alternatives of choice.

Indeed, castor culture appears to be excellent adaptable to the semi-arid lands, which will promote a sustainable agriculture in the poorest Brazilian's region. On the other hand, the palm-tree plantations in Amazon rainforest degraded areas will probably represent a good alternative to its sustainable occupation and social and economic development. In this sense, Brazilian's government aims to use the PROBIODIESEL also to develop familiar and sustainable agriculture where underdevelopment is critical.

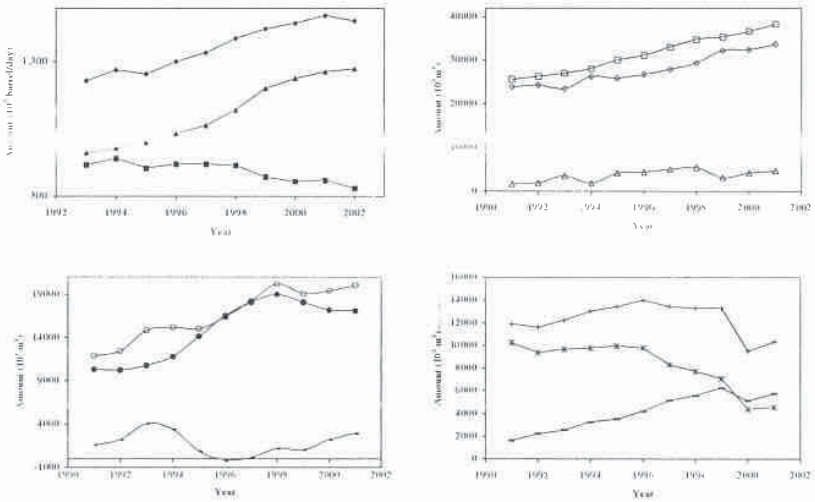

Figure 1. Petroleum and fuels in Brazil: (¨) Petroleum total consumption; (²%) petroleum production; (%) imported petroleum; (£) diesel consumption; (¸) diesel production; (r) imported diesel; (™) gasoline production; (Ï%) gasoline consumption; (-) gasoline exportation; (*) dry ethanol consumption; (-) hydrated ethanol consumption; (+) total ethanol consumption; (Source: BEB/MME, 2002).

In order to implement PROBIODIESEL as a federal policy, different actions are currently being done. It is worth mentioning that two regulatory bills are currently being discussed in the Brazilian's Federal Parliament. Also, the standards for biodiesel and its blends were recently regulated by National Petroleum Agency (Act ANP N° 255; 15 September, 2003). In this act twenty-three parameters were specified for pure biodiesel, including chemical contaminants, physical properties and chemical properties, as depicted in Table 1. It is important to note that, differently to the other ones, iodine number, viscosity and density parameters have no specified values, being required only to "take note". The act specifies also that for biodiesel and petroleum diesel blends, for instance B5, the density and viscosity parameters must be in agreement with petroleum diesel corresponding limits.

Final remarks

"Although experimental studies have demonstrated the feasibility of the project, still now the costs remains as an important limitation to the use of vegetable oils derivatives as fuels" (Otto, 1945). However, the social and environmental arguments, as well as the possibility of getting energy independence, probably will take an important role in the political decision for the PROBIODIESEL implementation in Brazil. On the other hand, our successful experience developing the large use of biomass based fuel (ethanol) will probably be useful in this new venture.

Table 1. Parameters and standards methods specified for pure biodiesel by National Petroleum Agency in the Act ANP Nº 255 (15 September, 2003).

Property	Unit	Limit	Method	
			ASTM	EN/ISO
Flash point, min.	°C	100	D 93	ISO / CD 3679
Water and sediments, max.	% Vol.	0.050	D 2709	-
Cinematic viscosity 40°C	mm^2/s	Take note	D 445	EN ISO 3104
Sulfated ash, max.	mass %	0.02	D 874	ISO 3987
Sulfur, max.	mass %	0.001	D 5453	EN ISO 14596
Cupper corrosion 3h a 50°C, max.	-	1	D 130	EN ISO 2160
Cetane number, min.	-	45	D 613	EN ISO 5165
Cold-filter plugging point, max.	°C	(2)	D 6371	-
Carbon residue, max.	mass %	0.05	D 4530	EN ISO 10370
Acid number, max.	mg KOH/g	0.80	D 664	Pr EN 14104
Free glycerin, max.	mass %	0.02	D 6584	prEN 14105/14106
Total glycerin, max.	mass %	0.38	D 6584	prEN 14105
Appearance	-	LII (3)	-	-
Distillation 95% V/V, max.	°C	360	D 1160	-
Density at 20°C	kg/m^3	Take note	D 1298 D 4052	-
Methanol or Ethanol, max.	mass %	0.5	-	pr EN 14110
Iodine number	mass %	Take note	-	prEN 14111
Mono-glycerides, max.	mass %	1.00	D 6584	prEN 14105
Di-glycerides, max.	mass %	0.25	D 6584	prEN 14105
Tri glycerides, max	mass %	0.25	D 6584	prEN 14105
Alkaline metals (Na + K), max.	mg/kg	10	-	prEN 14108 prEN 14109
Phosphorous, max.	Mg/Kg	10	D 4951	prEN 14107
Stability to oxidation at 110°C, min.	h	6	-	prEN 14112

Acknowledgments

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Is soybean oil itself an acceptable diesel fuel?

D. Pioch; N. Chirat; A. Liennard;
P. Lozano and G.Vaitilingom[1]

Abstract

The quality of vegetable oils as fuel is difficult to assess because of interferences between properties of the triacylglycerols –the main components- and those of the many minor components because their content may varies significantly from sample to sample. A methodology was set up, consisting of working with pure triacylglycerols obtained by flash chromatography of refined oils, and then adding minor components. Soybean oil shows interesting properties, not far from the best oil of the series investigated as diesel fuel –coconut oil-, and in the range of other biofuels Cirad is now accustomed to run diesel engines with.

Introduction

Our World must face more and more the challenge of its development according to requirements and new standard of sustainability. Among other political topics –preservation of environment, rational use of natural resources and equal access to these for all citizens, demographic changes, food for eveybody- energy is an important one because directly linked to the possibilities of technological development and to improving living conditions.

Since the beginning of humanity in relation to lighting or food, and for more than a century now for generation of mechanical power, vegetable and animal oils have been known as very useful resources. In this view, during the last two decades lot of scientific reports deal with the following question: how to use these natural oils for feeding Diesel engines, either *in natura* and alone (and to which extent should they be refined) or as mixtures with petroleum based diesel fuel, or after derivatization into their methyl or ethyl esters, or even after cracking into petroleum-like hydrocarbons. Out of the political issues, these questions involve an important technical side. Cirad has been working in this field for more than 20 years and the present paper deals with results concerning soybean oil, the main oleaginous production in the whole American continent, in view of using it under its native chemical form i.e. not transformed into a monoester, known as Biodiesel.

[1] Physico-chemistry of Processes and Bioenergy Laboratory; CIRAD, Montpellier, France; Daniel.pioch@cirad.fr

From the chemist stand point vegetable oils contain hundreds of individual chemical species –triacylglycerols and minor components- and it is difficult to know the influence of each of them, a useful information indeed because directly linked to the choice of an oil when several opportunities are available and to their best use, in particular about the extent of refining to be advised or the required adaptation of engines.

From literature data it was not possible to understand the behavior of the main lipid species in an engine because (i) most of the engine trials where performed by mechanical engineers, with little attention to chemical composition (ii) the trials often deal with one or two oils and thus it is then difficult to compare data (iii) refining acts on several lipid species simultaneously. Thus we developed a strategy dedicated to this topic. It allowed us to travel on two axis, the composition of the starting oil and the many phenomena or steps occurring in a diesel engine.

Materials and methods

Model oils containing only natural lipids were obtained by flash chromatography of refined oils over a composite bed of Silica Gel LC and Trisyl 300 to remove virtually all non triacylglycerol components. This was operated to a scale up to 10 kg of oil, enough for performing the series of testes, including with an engine at bench. Because of their high content of diacylglycerols, coconut and palm oils were subjected to extraction with methanol prior to flash chromatography. Starting from about 93-98%, the purity of the triacylglycerol class was then 99.9% or better.

The main minor components were obtained by known physical, chemical or enzymatic methods and added to purified triacylglycerols (TAG) either each component alone or together upon the case: phospholipids (1%w), free fatty acids (5%w), diacylglycerols (7%w). These account for the main minor components found in vegetable oils and their percentages are within the range measured in crude oils.

The chemical composition of the model oils were determined by standard methods: mono- and diacylglycerols, free fatty acids, phosphorus, sodium (for soap content), peroxide value, fatty acid composition, TAG composition, water.

The oils were then subjected to series of testes (Table 1):

- for assessing the physicochemical properties (cloud point, solid content vs temperature, viscosity, surface tension),
- or corresponding to one phenomenon occurring almost simultaneously with others in an engine (spray area and granulometry,

"evapostalagmometry" (rate of desapearance of a small drop by vaporization-pyrolysis measured by a lab made device), thermogravimetry, ignition delay and precombustion chemistry in dedicated combustion chambers)
- or for global assessment of the behavior as fuel in a diesel engine (ignition delay, combustion)

Table 1. Methodology adopted for investigating properties of vegetable oils as fuels

Stage or Phenomenon in engine	Test
Storage	Cloud point, Solid content
Injection	Surface tension Viscosity Spray, drop size distribution and specific area
"Evaporation" of biofuel	Evapostalagmometry (droplet evaporation) Thermogravimetry
Air-fuel mixture	Pre-combustional chemistry
Ignition	Ignition delay
Combustion	Exhaust gases, Heat release Power, Carbon deposit

Results and discussion

Owing to the aim of the present meeting, dedicated to soybean oil, the result presented were selected accordingly; other oils wer chosen for comparison. The purity of model soybean oil triglycerides is given in Table 2. Table 3 shows that there is no change in fatty acid (or glyceride) composition. Thus it may be reasonably stated that these model TAG reflect the properties of the TAG class of the oils, freed from the influence of residual free fatty acids, phospholipids, and, much more important, of diacylglycerols which should not be considered as minor components because this class is in position two on the percent weight basis, right after the TAG. Because (i) of the well known surfactant properties of the last which are food emulsifiers, but also of the two other classes and (ii) of their change from one oil sample to the next due to different origins, seed storage conditions and refining processes, it was justified to investigate separately the TAG class alone and then go to model oils by adding those minor components.

About the behavior under cold temperature, an important physical property of a fuel, linseed triacylglycerols (L-TG) and rape (R-TG) show the best

properties of the series (cloud point -13 and -14°C); soybean triacylglycerols (So-TG) stay very close owing to their high Iodine value, and content of tri-unsaturated TAG: cloud point at -9°C, (same for sunflower, Su-TG). The solid content follows the same pattern, with only 5.5% at -20°C So-TG are even better than Su-TG and of course far below the more saturated cotton, palm and coconut (Ct-TG, P-TG, Cc-TG respectively) (Table 4). On the side of viscosity, one of the most critical points of the vegetable oils as fuels, So-TG are the closest to L-TG (87 and 67 cPo respectively), thus better than Su-TG and R-TG.

Table 2. Chemical composition of refined soybean oil and purified TAG

	TAG w%	DAG w%	MAG	Phospholip. ppm	Free fatty acid w%	Iodine val.	Peroxide val. meqO2/kg
Refined oil	~ 99.0	0.9	traces	125	0.02	135	2.1
TAG Flash hromato.	~ 99.9	0.1	Not detect.	<25	0.01	135	1.5

Table 3. Fatty acid composition of refined soybean oil and derived TAG

	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:0	20:1	22:0
Refined oil	0.05	10.2	0.2	2.8	21.9	57.1	7.3	0.2	0.2	0.1
TAG Flash Chromato.	0.05	11.0	0.1	3.3	21.8	55.6	7.4	0.2	0.2	0.2

These physical properties allow the storage and use of soybean oil as fuel in most places where soya is grown, especially in Brazil, and can be easily improved with additives.

Surface tension is also of interest to us because of its direct link to spray properties in the engine: although the variation within the series investigated stays in a small range of magnitude (25.5 to 28.8 $mN.m^{-1}$ for Cc-TG and L-TG respectively), the surface tension of So-TG (28.5 $mN.m^{-1}$) is high and close to L-TG. In spite of this the spray data -average drop diameter; specific spray area- are good and they stay right after Cc-TG, the best oil, and close to P-TG (Table 5). This is due to the fact that spraying is a function of other physical properties, namely viscosity and specific gravity. Worst to mention that this feature concerning the pure TAG class is even improved by addition of minor components because of their surface tension lowering effect as expected, especially for diacylglycerols which lead to the most significant improvement (-15%). It is important to point out that

the effect of minor components on spray specific area is in the range of the effect of the TAG composition itself (from coconut to linseed), thus the content of minor components is an important parameter and the refining stage as well ans this justifies our methodology.

Table 4. Cloud point and solid content vs Iodine value and unsaturated TAG content

TAG (Flash chromato.)	L	R	So	Su	Ct	P	Cc
Cloud point (°C)	-13	-14	-9	-9	-3	27	18
Solid content at -20°C (%w)	1.3	0.9	5.5	6.6	35.8	87.6	98.1
Iodine value	196	111	135	127	110	50	12
Unsaturated TAG U3 (%w)	83	77	61	68	37	1	0

Next parameter investigated comes from the logical sequence in the engine; after spraying, the oil is subjected to vaporization. For the purpose we designed a special device allowing to measure the size of a droplet of oil in a chamber vs time at temperature normally observed in an engine (up to 600°C). The technique was named evapostalagmometry and the parameter K ($mm^2.s^{-1}$) used, independent of drop size, is related to the rate of decrease of the drop area projected on a plane. The main difference with petroleum diesel fuel lies in the increase of the area for oils during the first part of experiment, while within the same time a drop of diesel fuel disappear completely: hydrocarbons do vaporize while TAG cannot. When the temperature is high enough then the drop area starts decreasing owing to thermo-chemical reactions. There are still significant differences for K between oils (Table 5): at 450°C for example, Cc-TG and P-TG have the better score while So-TG are the best of the "C18 unsaturated oils" tested, although quite distant to the first two oils. Thus here again So-TG are in good position.

Table 5. Main fuel properties of soybean TAG and other unsaturated oils

	Soybean	Sunflower	Rapeseed
Spray specific area ($mm^2.ml$)	950	890	910
K Evaporation (10^{-5} $mm^2.s^{-1}$)	74	70	70
Ignition delay (Crank angle degree)	8.6	8.6	10.1

Now looking at the combustion itself, through trials with an engine at bench. For the ignition delay for example, which characterizes good or bad working conditions, Table 5 shows that here again So-TG (and Su-TG) stay right after the best oils, namely Cc-TG and P-TG owing to their shorter average chain length and lower Iodine value, while the relative good score for So-TG remains unexpected from this broad rule. However this comes logically from the properties checked individually at the beginning of the study. Other combustion parameters fall into the same pattern; under identical conditions So-TG produce more CO, hydrocarbons and Nox for example than Cc-TG; same for Conradson residue.

Conclusion

From this systematic study we come to the conclusion that vegetable oils as a whole are not as bad as the classical parameters dedicated to hydrocarbons, like Cetane number or viscosity, could let us conclude (Table 6). Among the series investigated, soybean oil shows interesting properties, favorable to its use *in natura* in diesel engines.

Table 6. Properties of Diesel fuel, Soybean oil and Soybean oil methyl ester

	Diesel fuel	Soybean oil	Soybean methyl ester
Hydrocarbons (w%)	100	0	0
Glycerides (w%)	0	100	< 1
Distillation 10%-90 (°C)	230 - 310	none	325 - 350
Flash point (°C)	92	219	171
Heat value (MJ/kg)	42	38	40
Viscosity 40°C (cS)	2.8	32	4.3
Cetane number	50	38	46

From our expertise, after having checked many types of engines and vegetable oils, we advise to select standard diesel engines fitted with a precombustion chamber (Indirect injection) and to apply light adaptations in order to overcome unsuitable effects of physical and chemical properties of soybean oil. On this basis we did set up dozens of diesel engines ran with coconut, cotton or rapeseed oils, either for electric power production, irrigation pumps or cars, which are still working in factories or isolated communities for example.

While esters (biodiesel) bring a convenient solution whenever large volumes of fuel are consumed in a relatively small area and when both oil and alcohol are available, the use of vegetable oils in natura is suitable for example in small communities spread in remote areas or in case of in-farm

consumption. Of course any substitution of petroleum fuels needs a strong political commitment and incentives, in addition to locally favorable conditions either on the financial, social, environment or geographical sides. No doubt that farmer lobbies in soybean oil producing areas, and in the special case of Brazil owing to its unique expertise under the Proalcol programme, that soybean oil will be used *in natura* owing to its interesting properties as Diesel fuel.

Acknowledgements

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Incorporating soy foods in the diets of HIV-Positive patients in Southern Africa – the WISHH program

K.Weingartner and P. Khanna[1]

Introduction

The World Initiative for Soy in Human Health (WISHH) program of the American Soybean Association seeks to increase the consumption of soy protein by populations in developing countries. The National Soybean Research Laboratory (NSRL) at the University of Illinois at Urbana-Champaign has provided leadership in several areas for this project, including providing soy-specific technical expertise and information, participating in the development and implementation of specific projects, enhancing Internet-based tools for information dissemination, providing strategic planning and targeted operational support, assisting with policy work on macronutrient fortification, assisting private voluntary organizations (PVOs) in conducting cost-benefit analysis, and recipe development.

In southern Africa, Acquired Immune Deficiency Syndrome (AIDS) is recognized as a serious problem, both in terms of health and economics. Globally, about 16,000 new cases are diagnosed each day. With the exception of those few people receiving retroviral therapy, the disease is fatal. It occurs most commonly in adults in their 20s and 30s and, in addition to death of the affected person, results in the removal of the breadwinner and/or the mother. Therefore it is not uncommon for families who have a family member with AIDS to suffer great economic hardship and to have many orphans.

AIDS has been classified as an epidemic in many countries of southern Africa, including Botswana, Kenya, Malawi, South Africa, Zambia, and Zimbabwe. Although the current recommended treatment — a combination of antiviral drugs — has been demonstrated to be effective in significantly extending life, its use has little application in southern Africa. The drugs are expensive and none of the governments in that area, with the exception of Botswana, can afford to buy them. In addition, the drugs themselves pose special problems. Some require refrigeration; others must be taken with large quantities of water. If the drugs are not taken in the correct sequence or if the patients skip taking them, they can develop a form of AIDS that is resistant to the drug therapy. Therefore, all things considered,

[1] National Soybean Research Laboratory; University of Illinois; Urbana-Champaign, Illinois, USA

the governments of southern Africa are not relying on expensive treatments for AIDS. Instead, groups such as the one discussed in this paper are focusing their resources on preventing HIV infection and improving nutrition among those people who may be HIV-positive. It is hoped that these actions will increase the productive working life of HIV-positive people.

Case study: Zimbabwe

Humana People to People is a European non-governmental organization operating in more than 32 countries worldwide. Humana raises funds to finance its core activities; about 60 percent of this money comes from the sale of donated clothes in Europe and North America. Since Humana does not have to rely on donor support for its core operations, it feels that it has greater freedom and flexibility to select projects that it wants to do.

In the last 25 years, Humana has developed a close and sustained relationship with the government and people of Zimbabwe. Humana's first project, in 1977, was the establishment of a training center in Mozambique for Zimbabwean refugees. In 1995, Humana relocated its global headquarters from Denmark to the Murgwi farm in the rural Shamva province of Zimbabwe. These examples of Humana's past and present degree of involvement illustrate how, through its efforts, it has earned the respect and trust of the Zimbabwean population.

As a part of its new beginning, TCE has included activities that will improve the nutrition of people with HIV/AIDS. Although AIDS is at epidemic levels in Zimbabwe, it is not appropriate to single out this group for improved nutrition. It is estimated that more than 20 percent of the Zimbabwean population is HIV-positive. Those people who have HIV are dispersed throughout the population and for the most part are unidentified for several reasons: First, people in Zimbabwe choose to not get the HIV test. In addition, those people who know they are HIV-positive are reluctant to admit this because of the social stigma attached to this disease.

In March 2000, Humana started the TCE (Total Control of the Epidemic) program against AIDS. Humana acts as a facilitator, mobilizing the Zimbabwean people themselves to take control and deal with their situation regarding HIV/AIDS. TCE divided Zimbabwe into geographical "areas." Currently, TCE is operating in three of these areas in Zimbabwe †Bindura, Shamva, and Kwekwe. It will expand its activities into additional areas as donor support for this activity increases. Fifty field officers are trained and assigned to each area. "Passionates," as the TCE volunteers are called, work alongside the field workers.

The TCE field officers and passionates are assigned to an area for three years. The goal for each field officer is to contact or mobilize 2,000 people during that time. They promote "making a new start," which includes motivating people to get tested for HIV, teaching how to avoid getting infected and how to live a healthy life if infected, establishing micro-enterprises to increase income generation, and promoting improved nutrition. Soy restaurants were established as a way of improving nutrition of the population in general and of AIDS patients in particular.

The TCE medical unit feels that the people in Zimbabwe with HIV/AIDS would benefit significantly by consuming food that is more nutritious than what they are currently consuming. The unit discussed the significance of the CD4 count, a blood measurement used to quantify the rate of immunity suppression among HIV-positive people. In people who are HIV-positive, the CD4 count drops from about 1,000 units (healthy) to about 250 units, at which point their immune system is suppressed so much that they are unable to fight the disease. The medical unit said that in North America, the CD4 count in HIV-positive people drops at a rate of about 7 units per month. However, in Zimbabwe, the decrease is about 7 units per week. Therefore, people in Zimbabwe tend to develop AIDS much more rapidly than people living in North America. The medical unit feels that part of the reason for this is that the people in Zimbabwe have poorer nutrition status and thus are more likely to contract diseases. They also have less access to medicine to treat these diseases. The unit feels that improved nutrition could contribute to increasing the lifespan of HIV-positive people in Zimbabwe.

In July 2001, as part of its fight against AIDS, TCE started operating restaurants. Restaurants are popular in Zimbabwe and are frequented by people most often at lunch time, between 11:30 a.m. and 2:00 p.m. These restaurants provide nutritious meals to anyone and do not target any specific group. TCE reasons that in this way, the restaurants will not have any stigma attached to them. A meal costs about Z$30 to Z$40, a price affordable to many Zimbabweans. The meal consists of sudza (made by cooking white maize in boiling water), a vegetable relish, and a side dish containing soy. Currently, TCE is operating a total of 48 restaurants.

I have visited soy restaurants Bindura and Shamva provinces. The restaurants were located in private residences, schools, on the street, and in community centers. The food was prepared on a variety of cooking devices †indoors, on electric and wood-burning stoves and outdoors, on wood-burning grills. The cooks prepared the meal in the morning. One soy restaurant offered "scrambled soy," which consists of textured soy sautéed

with eggs. The meals were served on a plate, without eating utensils, to customers who sat outside on benches or on a porch of the house. The customers use their right hand for eating. A portion of the sudza is scooped and formed into a ball. Sudza has a consistency similar to very firm mashed potatoes. It is eaten with a portion of the soy dish, which is also scooped by hand.

WISHH has conducted several training activities in Zimbabwe with Humana people to people. The first session was held for about 28 "passionates." These passionates are volunteers who either assist field agents working with people in the community, or are operating, or will soon be operating, their own soy kitchen. For the training we worked outside, using wood-burning grills. The participants worked in groups of five to six people. Each group prepared two to three soy dishes including baked goods, main courses, snacks, and side dishes. Eleven soy foods were prepared. The participants tasted and evaluated the products at the end of the afternoon training. Following the training, I worked with the TCE kitchen staff, preparing soy vegetarian patties, sudza and soy, and a soy-vegetable relish. The relish consisted of onions, tomatoes, and rape leaves, to which soy was added. This relish is a very popular side dish and is often prepared and eaten once or twice each day. This food was served for the evening meal to all the TCE staff and their families, about 80 people.

Follow-up activities

We have had very positive responses from those people who consumed the soy foods. In 2003, WISHH started projects in Honduras and Senegal. In 2004, WISHH plans to start projects in Haiti, Ivory Coast, and Mozambique. The recipients of these projects will be either school children or HIV positive individuals.

The decrease mechanism of undesirable astringent taste in soymilk during tofu curd formation

A. Al Mahfuz; C. Tsukamoto and T. Ono

Abstract

Soymilk, which is used in the initial preparation for tofu curd, usually has an undesirable astringent taste. That astringency more or less disappears after the tofu curd has formed. Regard to the protein gelling concept of tofu curd formation, proteins and oil-bodies in soymilk form a curd network that results in the containment of water inside the curd. Different coagulants have different interactions with them. Phytic acid plays an important role in tofu curd formation, interacting with a Mg or Ca coagulant. However the GDL coagulant has less effect with phytic acid during coagulation. It was found that GDL tofu curd released more amounts of free phytates than Ca or Mg coagulated curd and it is expected that almost all of the soluble phytates should exist as K-phytate form because of the abundancy of K in soybean seed. Panel members reported that K-phytate in a very low concentration has the same astringent sensation as soymilk. Moreover, the perception of undesirable astringency decreased when the phytate concentration was lowered in tofu.

Introduction

The undesirable astringent sensation of soymilk disappeared after tofu curd formation as a result of the addition of coagulants. Tofu curds usually have little to no objectionable taste but there are some tofus showing undesirable astringent sensation, even though the same variety of soybean is used. It is particularly difficult to control the consistency of the tofu taste even when made under the same circumstances. Therefore, many tofu manufacturers are putting forth strong efforts to make delicious tofu, but the control of taste characteristics of tofu has yet to be well studied. Ono (2000) has given an attractive mechanism of how soymilk coagulates into tofu. This concept claims that proteins and oil-bodies in soymilk form a curd network that holds water inside. Different coagulants have different interactions with them. Phytic acid plays an important role in tofu curd formation, interacting with a Mg or Ca coagulant. However, the GDL coagulant has less affect with phytic acid during coagulation. Grynspan et al. (1989) and Saio et al. (1969) stated that during the combination of

[1] The United Graduate School of Agricultural Sciences, Iwate University, Ueda 3-18-8, Morioka 020-8550, Japan.E-mail: u0203017@iwate-u.ac.jp

protein and calcium (or magnesium) during tofu curd formation, phytic acid plays an important role in formation the curd network. So, phytate may affect the trapping of the astringent causing certain components to prevent them from interacting with taste cells of the mouth and throat. Moreover, according recent research, "the undesirable astringent taste disappears after tofu curd formation", or "tofu usually has good taste but some tofu shows astringency", seem to a possibility that there is a relation between the new theory and the undesirable taste. The theory of tofu curd formation with its associate components can explain how tofu loses its astringency, as has been seen.

In this study, we are attempting to make clear the factor of influence on the taste characteristics of tofu through experiment data analysis.

Materials and methods

Raw soymilk was prepared with 6-fold weight of de-ionized water using soybean variety Fukuyutaka seeds followed by soaking, grinding, and filtration. It was heated at 100°C for 5 min and cooled down quickly to about 5°C, which is designed as soymilk.

Tofu curd was prepared by adding a certain amount of a coagulant; GDL, $MgCl_2$ or $CaCl_2/CaSO_4$ into cooled soymilk, that was then put in a hot water bath at 85°C for 1hr.

Tofu whey (supernatant) was obtained through the centrifugation of tofu curds at 1000xg for 10min.

Dialysis of the samples was performed by using cellulose membrane (molecular weight cutoff 10,000) immersed in least 50 fold of de-ionized water for 24 hours.

Phytic acid content was determined by the iron binding method reported by Makower (1970) with some modifications.

Proteins, lipids, and sugars were determined by FT-IR with ATR accessory followed by Nakasato et el. (2003).

Evaluation of the astringent intensity was performed by a triangle test using the trained panel members.

Concept of triangle test

The three point discriminating method, namely triangle test with a randomized selection, is very effective to evaluate the taste of foods. One

sample is different among three samples. When a panel member selects one sample randomly, the probability to select the correct answer is one third (1/3). If a panel member could select correct answer three times in three different combinations, the probability of a correct answer is expected to be one twenty seventh (1/27), thus at lower than 5% level of significant. In this study, we used this method and presented the percentage of panel members who could answer at 5% significant.

Results and discussion

As mentioned in the introduction, phytates in soymilk are supposed to mainly exist as K-phytate. However, during tofu curd formation, K- phytate changes to Ca-phytate with the addition of the Ca coagulant and taste characteristics also will be changed. Therefore, first the sensation of K and Ca phytates were investigated. Table 1 shows the taste characteristics of K and Ca-phyate solutions at the same concentration and pH.

Panel members expressed strong astringency in a 1% K-phytate solution whereas there was no bad taste reported in Ca phytate solution of the same concentration. Thus, it can be said that K-phyate has an astringent sensation but Ca-phyate has no undesirable taste.

Table 1. Taste characteristics of K-phytate and Ca-phytate (1% solution, pH-7.0)

Panels	K-phytate	Ca-phytate
1	astringent	smooth
2	astringent	not bad taste
3	undesirable	smooth
4	astringent	no undesirable
5	not smooth	not bad taste
6	astringent	no taste
7	astringent	smooth
8	astringent	not undesirable
9	astringent	not bad taste
10	astringent	smooth

The behavior of the undesirable taste causing component in soymilk

The relation between phytate concentration in soymilk and astringency was investigated. It is expected that most phytates in soymilk exist as K-

phytate form, because the K content in soymilk is much higher than the other minerals. Then, the taste characteristic of K-phyate in soymilk was performed by a sensory evaluation. Each concentration of K phytate was prepared by adding a KOH solution to phytic acid and neutralized then diluted to different concentrations. The sensory evaluation was performed by ten panel members with the three-point discrimination method (Triangle test) against water. Regarding the results of sensory evaluation (Figure 1), all panel members noticed that 0.1% K phytate solution has a very similar astringency to that of soymilk. Moreover, four people out of ten recognized the difference of a 0.05% and two people of 0.03% from water. However, nobody discriminated the difference of a 0.02% solution from water. Moreover, the results of the sensory evaluation value was evaluated at pH domain (before or after [pH 6]) of tofu, where the results was the same as pH 7. From the above result, it is thought that, if the free phytate concentration in tofu becomes high by a certain factor, it is supposed to cause to feeling of astringency in tofu. Then, one of the factors that may increase free phytate contents was supposed to be coagulants. A sort of changes regarding tofu curd formation was investigated against phytate.

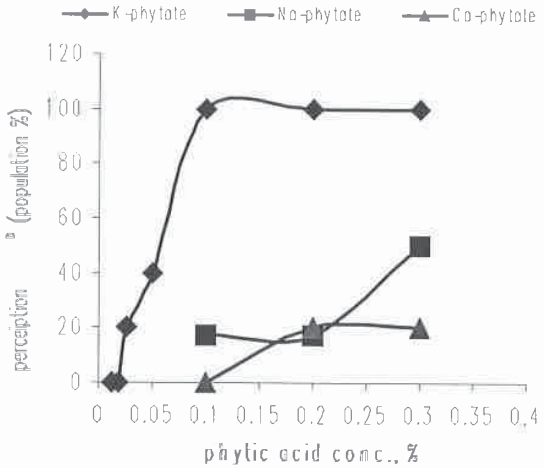


Figure 1. Taste characteristics of phytate with different salt ions in various concentrations at pH 7.0

As the astringency felt in soymilk disappears after tofu curdling, it is considered that an astringency component must be taken in a tofu card network. As shown in Figure 1, the results indicate that the conversion of K-phytate to Ca-phytate during tofu curdling may mask the cause of the

astringent sensation of K-phytate. If, however, K-phyate exists even at a low concentration as a free form in the tofu curd, such tofu curd may show astringent taste. Figure 2 shows phytate amounts remaining in different coagulum tofu curds after dialysis. GDL, Ca and Mg tofu curds released 13%, 6% and 9% of their phytates from the tofu curds respectively. The concentrations of free phytates of GDL, Ca and Mg tofu curds has been calculated at about 0.03, 0.02 and 0.01% respectively. This range of K-phytate is very delicate, because the 0.05% K-phytate solution showed astringency but the 0.02% solution did not (Fig.1). GDL tofu curd released 0.026% of its phytate from the curd by dialysis, which is very near to 0.03% K-phytate solution where 20% panels perceived at 5% level of significant. Therefore, these results create an image that more panels might feel the astringency in tofu if the tofu contains a large quantity of free K-phytates.

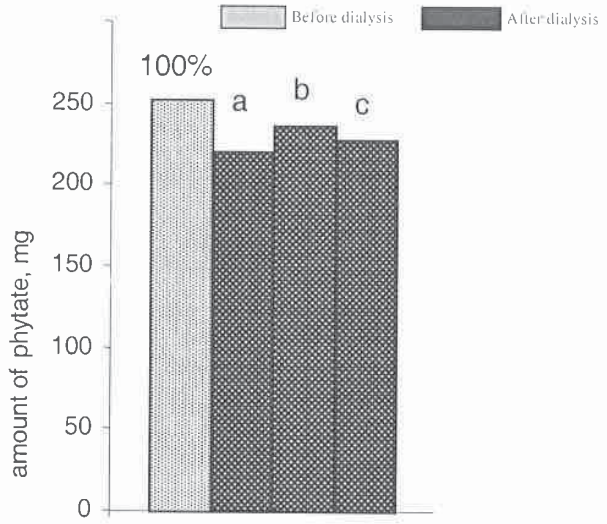


Figure 2. Amount of phytate in (a) GDL, (b) Ca, (c) Mg coagulated tofu curds before and after dialysis.

Preparation of tofu having different concentrations of free phytates

To obtain different concentrations of phytates as a soluble form in the curd, tofu curds were prepared by using mixed coagulants. In this case, a mixture of GDL and $CaSO_4$ was used in a certain concentration shown in Figure 3. Phytic acid concentrations in the whey fraction of tofu curds ranged from 0.052 to 0.005%.

By using these three different concentrations, 0.052, 0.038 and 0.005% of whey fractions, three combinations of triangle tests were performed. Thus, one sample was 0.052% fraction and the other two samples were 0.005%. Then, a 0.052% sample against two 0.038% samples, and one 0.038% against two 0.005% samples. If the panel members could recognize the difference between the two different concentrations, there is a possibility that phytate concentration in tofu whey may affect the difference of taste

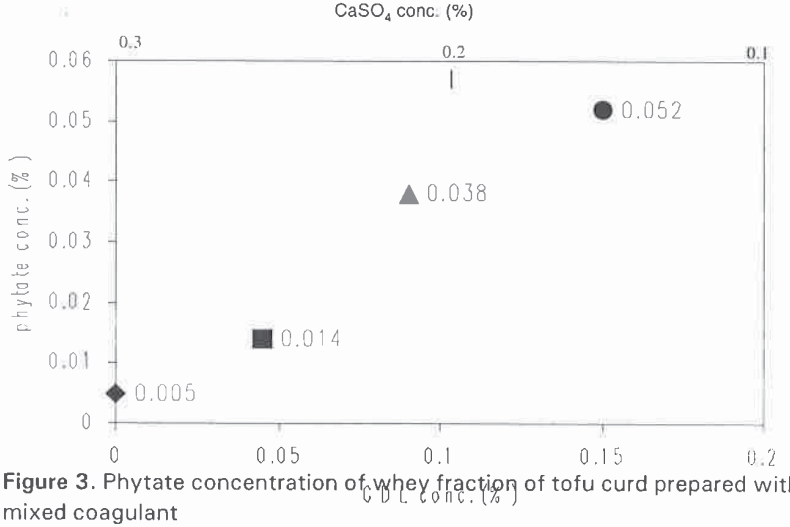
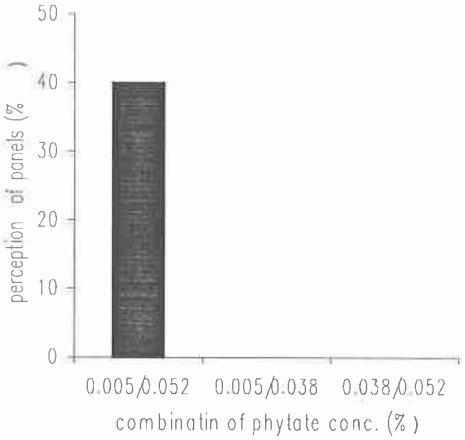


Figure 3. Phytate concentration of whey fraction of tofu curd prepared with mixed coagulant



* probability of difference at 5% level of significance

Figure 4. Triangle tests by the combination of different phytate concentrations in whey fractions

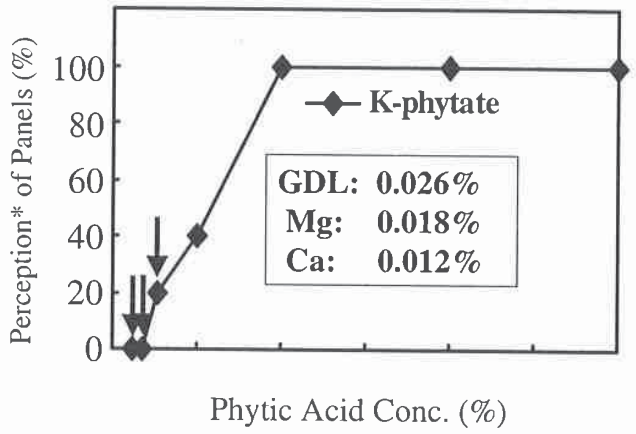


Figure 5. Taste characteristics of K-phytate solution at pH 7.0

characteristics of tofu, especially the appearance of undesirable tastes.

Taste characteristics of the tofu whey

To justify the taste of astringency in tofu, whey fraction was selected because most of the soluble phytates exist in this fraction in a certain concentration. Triangle tests on the combination of different phytate concentrations in whey fractions are shown in the Figure 4. 40% panel members could recognize the difference between 0.052 and 0.005% of phytate concentration at a 5% significant level. However, the difference between the 0.038 and 0.005 samples, as well as between the 0.052 and 0.038 samples were not identified. Figure 5 shows the same data as previously presented in (Figure 1), where the 0.05% K-phytate solution was recognized as different taste compared to water.

Thus, these results indicate that the undesirable taste characteristics of K-phytate in water probably show the same undesirable taste in tofu curd, too.

Major component contents in the whey fractions

There is the possibility that the composition of the other major components of the tofu whey, such as proteins, lipids, and sugars were different. However, no large differences could be found them. The concentrations were approximately 0.85% protein, 0.23 % lipids, and 1.61% sugars.

Conclusion

From the above results, astringency in soymilk disappears after the tofu is curdled and some tofu contains undesirable taste, the cause presumed is as follows. K-phytate exists in soymilk in a large quantity and serves as a key factor of astringency. During the addition of a coagulant into soymilk to form a curd, most of the phytate becomes insoluble and astringency disappeared. However, under certain conditions phytate still exists as a free form at high concentrations after curdling which may create tofu with astringency. Perception of astringent taste decreased with lowering the free phytate concentration.

Various researches proceeding to control phytate should remain under consideration.

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Relationships of the contents of protein and phytic acid in soybeans to the consistency of tofu

K. Toda[1]; T. Ono[2]; K. Kitamura[3]; M. Hajika[1]; K. Takahashi[1] and Y. Nakamura[1]

Abstract

The relationship between the protein content of soybean seeds and the consistency of tofu was examined for Japanese soybean varieties. The seed protein content was estimated by determining the nitrogen content using the Dumas method. Tofu was prepared from a raw homogenate of water-soaked soybeans by heating and by the addition of $MgCl_2$ of various concentrations as a coagulant. The tofu consistency was evaluated by measuring the breaking stress of tofu curd using a Creep meter. The breaking stress of tofu increased when the concentrations of $MgCl_2$ in soymilk increased and reached a maximum value at concentrations of around 0.40%. There was a significant positive correlation between the maximum breaking stress of tofu and the seed protein content. In contrast, the breaking stress of tofu prepared with 0.25% $MgCl_2$ showed less correlation with the seed protein content. Six Japanese soybean varieties showed different $MgCl_2$ concentrations for the maximum breaking stress on the average. Among them, Sachiyutaka showed the highest value of 0.45 and the lowest breaking stress of tofu prepared with 0.25% $MgCl_2$. Those results suggest that coagulation is buffered at the concentration of such as 0.25%, but at the maximum breaking stress, buffering effect is reduced and the seed protein content mainly affects the breaking stress. To reveal the component which buffers the coagulation reaction, we focused on phytic acid, which has six phosphate group to act as a chelating agent for coagulants. The phytic acid content was estimated by measuring the phosphate, which was released from phytic acid by precipitation and degradation, using a modification of the Fiske and Subbarow methods. The phytic acid content showed a significant negative correlation with the breaking stress of tofu prepared with 0.25%, which suggests the buffering effect of phytic acid. At the same phytic acid content, the breaking stress showed high value for Fukuyutaka, Enrei and Sachiyutaka, in that order. There was no significant correlation between the phytic acid content and the $MgCl_2$ concentration for the maximum breaking stress. These results suggest the buffering effect of other components than phytic acid.

[1] National Institute of Crop Science, Ibaraki, Japan.
[2]The United Graduate School of Agricultural Sciences, Iwate University, Iwate, Japan
[3] Graduate School of Agriculture, Hokkaido University, Hokkaido, Japan

Keywords

soybean, tofu, protein content, breaking stress, magnesium chloride concentration, phytic acid.

Introduction

Tofu is the main traditional food derived from soybeans in Japan. It is still difficult to mass-produce tofu of uniform quality from Japanese domestic soybeans, because of the variations in soybean quality due to the small-scale production, the use of different varieties or differences in the cultivation environments. Since consistency of tofu can be varied even among the same variety, it is important to reveal seed components that affect the consistency of tofu. It is generally considered that soybean seeds with a high protein content are suitable for producing enough consistent tofu. Wang *et al.* (1983) and Shen *et al.* (1991) reported that varieties with a higher protein content produced a firmer tofu texture. However, Lim *et al.* (1990) who studied the relationship of consistency of tofu to the protein content of beans and tofu using nine soybean varieties, failed to detect any significant correlation between these parameters. Tofu manufacturers sometimes can not produce consistent tofu-gel in spite of using a soybean variety with a high protein content. It is still required to determine whether the seed protein content is related to the consistency of tofu. In contrast to protein, it is proposed that phytic acid reduces tofu consistency by acting as a chelating agent to a coagulant. Saio *et al.* reported that softer tofu was produced from soybeans containing larger amounts of phytic acid (1969). But some researchers failed to detect any significant correlation between phytic acid content and tofu consistency (Schaefer and Love 1991). Our aim in this study is to clarify whether the contents of protein and phytic acid are related to the consistency of tofu.

Materials and methods

Soybeans (50g each batch) were washed three times with distilled water and soaked in distilled water at 20 °C for 18 h. Hydrated seeds were drained and ground into homogenates with distilled water equivalent to six times the weight of dry seed. Raw soymilk was separated from the homogenate by centrifugation using a centrifugal filter (120 mesh). Soymilk was prepared by incubating raw soymilk in boiling water for 6 min and was cooled in flowing water at 20°C. $MgCl_2$ was added as a coagulant after soymilk was chilled in ice cold water. The coagulated soymilk was incubated at 80 °C for 1 h and cooled in flowing water at 20°C. Cylindrical cakes of tofu 26mm in diameter and 80 mm in depth were prepared and were incubated at 20°C until the measurement of the tofu texture was performed.

The texture profiles of the tofu samples were investigated using a RHEONER (YAMADEN company Ltd., Japan) with a cylindrical plunger 11 mm in diameter at a compression rate of 1mm sec^{-1}, according to the method of Kohyama and Nishinari (1992).

Soybean seeds were ground into flour using an ultra centrifugal mill (ZM100, F. Kurt Retsch GmbH & Co. KG, Germany). Moisture content of seed flour for the calculation on a dry matter basis was determined by heating the flour samples for 3 h at 130 °C. Nitrogen content of seed flour was determined by the Dumas combustion method using rapidN (Elementar Analysensysteme GmbH, Germany) and aspartate as a standard. Protein values of soybeans on a dry matter basis were calculated by multiplying the nitrogen content by 6.25.

Phytic acid in soymilk was precipitated by adding 20 mM calcium chloride, pH 11.5 according to the method of Ishiguro *et al.* (2003). Precipitated phytic acid was degraded to phosphate by heating with sulfate and hydrogen peroxide, followed by phosphorous assay using a modification of the Fiske and Subbarow methods (Bartlett 1959).

Results

Concentration of a coagulant and tofu breaking stress

We determined the breaking stress of tofu prepared by the addition of $MgCl_2$ at different concentrations. The dependency of the breaking stress of tofu on the concentrations of $MgCl_2$ varied with the soybean samples. For example, tofu made from Fukuyutaka produced in Asakura, Fukuoka in 2000 showed the maximum breaking stress at a concentration of 0.35% $MgCl_2$ (Fig. 1). In contrast, tofu made from Sachiyutaka produced in Akaiwa, Okayama in 2000 showed a lower breaking stress than that of tofu made from Fukuyutaka at concentrations of 0.20% through 0.35% $MgCl_2$, but showed a higher value for the maximum breaking stress at a concentration of 0.40% $MgCl_2$ than that of Fukuyutaka.

Correlation between the seed protein content and tofu breaking stress

The correlation between the seed protein content and the tofu breaking stress was examined. We selected the maximum breaking stress and the breaking stress of tofu prepared with 0.25% $MgCl_2$, which is commonly used to produce tofu by both researchers and tofu manufacturers (deMan *et al.* 1986 and personal communication from a tofu manufacturer). The seed protein content was not correlated with the breaking stress at 0.25% $MgCl_2$ ($r = 0.27$) while it exhibited a strong positive correlation ($p < 0.001$, $r = 0.87$) with the maximum breaking stress (Fig. 2).

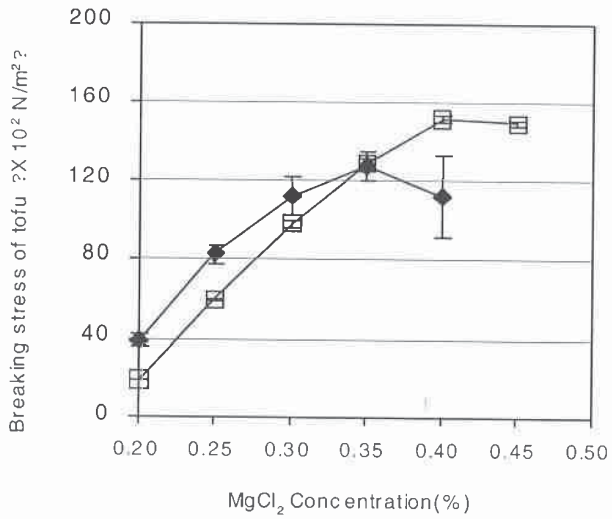

Figure 1. Relationship between the $MgCl_2$ concentrations and the breaking stress of tofu. ◆ denotes the plots of Fukuyutaka and ⊞ denotes the plots of Sachiyutaka.

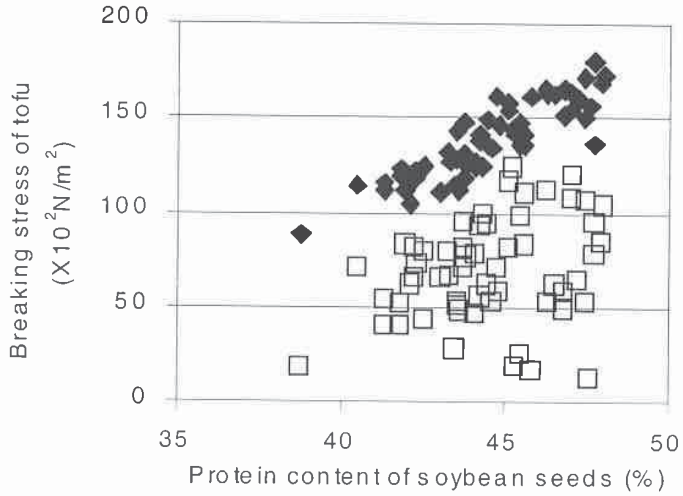

Figure 2. Correlation between the protein content of soybean seeds and the maximum breaking stress of tofu (◆)and the breaking stress of tofu made with 0.25% $MgCl_2$ (⊞.

Comparing tofu breaking stress and $MgCl_2$ concentrations for the maximum breaking stress

We compared the averages of seed protein contents, breaking stress of tofu and $MgCl_2$ concentrations for the maximum breaking stress among six varieties (Table 1). Fukuyutaka required the lowest concentration while Sachiyutaka required the highest concentration for the maximum breaking stress. As for tofu prepared with 0.25% $MgCl_2$, tofu made from Sachiyutaka showed the lowest breaking stress, although its protein content and its maximum breaking stress were high, while tofu made from Fukuyutaka showed the highest breaking stress.

Table 1. Seed protein content, tofu breaking stress and magnesium chloride concentrations for the maximum breaking stress for six soybean varieties

	Enrei Tachinagaha	Fukuyutaka	Sachiyutaka	Ayakogane	Hatayutaka
Protein content of soybean seeds (%)	45.0 ± 1.5 42.3 ± 1.2	44.9 ± 1.6	46.4 ± 1.2	44.3 ± 2.7	43.6 ± 1.2
Maximum breaking stress (X 10^2 N/m²)	153 ± 17 121 ± 7	135 ± 13	156 ± 11	132 ± 27	131 ± 12
Breaking stress of tofu at 0.25% $MgCl_2$ (X10^2N/m²)	72 ± 22	97 ± 21	45 ± 31	72 ± 31	74 ± 13
$MgCl_2$ concentrations for the maximum breaking stress (%) 0.42 ± 0.04	0.42 ± 0.04	0.34 ± 0.03	0.45 ± 0.04	0.38 ± 0.04	0.41 ± 0.04

The values express means ± S.D.

Relationships of the phytic acid content to tofu breaking stress and $MgCl_2$ concentration for the maximum breaking stress

We examined the relationships of the phytic acid content in soymilk to the maximum breaking stress of tofu and the breaking stress of tofu prepared with 0.25% $MgCl_2$ (Fig. 3). There was a significant negative correlation between the phytic acid content and the breaking stress of tofu prepared with 0.25% $MgCl_2$ ($p < 0.001$, $r = -0.64$). No significant correlation was observed between the phytic acid content and the maximum breaking stress. Each variety showed a negative correlation between the phytic acid content and the breaking stress of tofu prepared with 0.25% $MgCl_2$ (Fig. 4, $r = -0.73$, -0.39 and -0.80 for Fukuyutaka, Enrei and Sachiyutaka respectively). At the same phytic acid content, the breaking stress of tofu tends to be higher for Fukuyutaka, Enrei and Sachiyutaka in that order. An approximate linear equation between the phytic acid content and the breaking stress of tofu also shows that trends. There was no significant correlation between the phytic acid content and the $MgCl_2$ concentration for the maximum breaking stress (data not shown).

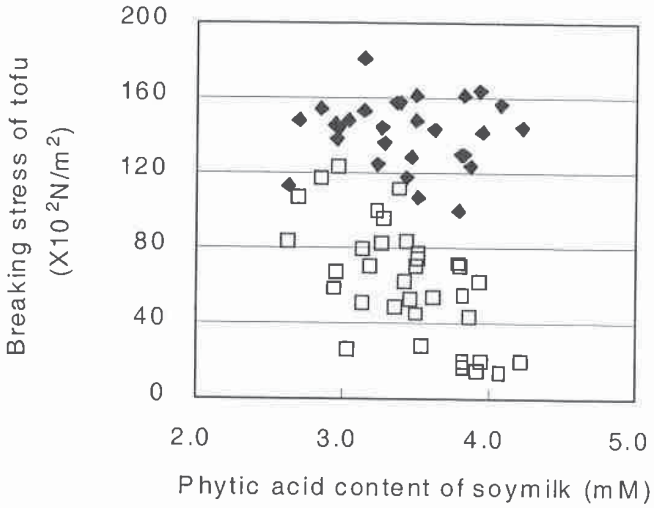

Figure 3. Correlation between the phytic acid content of soymilk and the maximum breaking stress of tofu (♦), and the breaking stress of tofu made with 0.25% $MgCl_2$ (€.

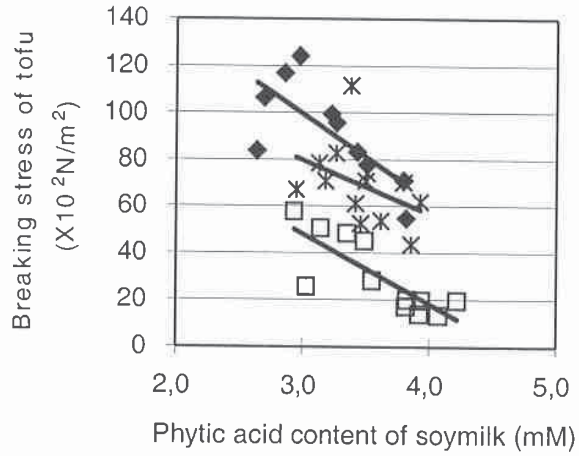

Figure 4. Correlation between the phytic acid content of soymilk and the breaking stress of tofu made with 0.25% $MgCl_2$ for each soybean variety. ♦, ✳ and €denote the plots of Fukuyutaka, Enrei and Sachiyutaka, respectively. Each line shows an approximate linear equation for each variety.

Discussion

Our study reassured the correlation between the seed protein content and the consistency of tofu. But the concentration of a coagulant is important in this case. When the tofu was prepared with various concentrations of a coagulant, the maximum breaking stress of tofu showed a high significant correlation with the breaking stress of tofu (Fig. 2). However, at a lower concentration of a coagulant such as 0.25% $MgCl_2$, there is less correlation between the seed protein content and the breaking stress of tofu. It is due to other components of soybean which buffer coagulation reaction. At a lower concentration of a coagulant, some components in soymilk may buffer coagulation reaction but at the maximum breaking stress, buffering effect is reduced and the seed protein content mainly affects the breaking stress. Since tofu manufactures prefer to use 0.25% $MgCl_2$ mainly for tastes (personal communication with tofu manufacturers), it is important to reveal the components acting as a buffering agent to coagulation. Our study suggests that phytic acid is one of such components. Phytic acid contents in soymilk showed a significant negative correlation with the breaking stress of tofu prepared with 0.25% $MgCl_2$ (Fig. 3). There was no significant correlation between the phytic acid content and the maximum breaking stress, which shows that at the maximum breaking stress, phytic acid hardly affects the tofu consistency as a chelating agent. At the same phytic acid content, tofu prepared from Fukuyutaka, Enrei and Sachiyutaka showed higher breaking stress in that order (Fig. 4). Since Sachiyutaka, Enrei and Fukuyutaka showed higher protein content in that order, such difference in the breaking stress is due to other than the contents of phytic acid and protein. Those results and the fact that the phytic acid content showed no significant correlation with the $MgCl_2$ concentration for the maximum breaking stress propose the existence of other components to buffer the coagulation reaction. Since soybean varieties with the high $MgCl_2$ concentration for the maximum breaking stress of tofu, such as Sachiyutaka, showed low breaking stress of tofu prepared with 0.25% $MgCl_2$, the components concerning to the $MgCl_2$ concentration for the maximum breaking stress is another key factor for the consistency of tofu.

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Provisional soya intake recommendations

M. Messina[1]

Abstract

There is a need for health professionals to provide some guidance regarding appropriate intake levels of soya protein and isoflavones, the two components of the soyabean thought to be primarily responsible for the hypothesized health benefits associated with soyafood consumption. This paper provides a rationale for recommending on the basis of Asian soya intake and the clinical and epidemiologic data, the daily consumption of 15 g soya protein and 50 mg isoflavones. These amounts of soya protein and isoflavones appear to be sufficient to derive the hypothesized health benefits from soya intake and to be safe. Also, since 15 g protein and 50 mg isoflavones are provided by 1 ½ - 2 servings of traditional soyfoods, these intake recommendations are practical and position the soyabean as one other healthy food to add to the diet.

Introduction

The hypothesized health benefits of soya include but are not limited to protection against osteoporosis, coronary heart disease (CHD), and certain forms of cancer. The two soyabean components most experts consider responsible for disease prevention are protein and isoflavones. Consequently, there is a need for health professionals to provide some guidance regarding appropriate intake levels of these two components.

In 1999, the U.S. Food and Drug Administration (FDA) approved a health claim for the cholesterol lowering properties of soya protein and set 25 g per day as the amount necessary for cholesterol reduction. However, the health claim does not address effects of soya protein other than cholesterol reduction; nor does it address health effects of isoflavones unrelated to serum cholesterol. Therefore, there is a void regarding an overall recommendation for soya consumption. Thus, the purpose of this paper is to briefly present a rationale for recommending that healthy adults strive to consume approximately 15 g (range, 10 – 25) soys protein and 50 mg isoflavones (range, 30 – 50) per day. These recommendations are based on two primary considerations; Asian soy intake and efficacy.

[1] Loma Linda University, USA.

Asian soy intake

Much of the initial enthusiasm for the health benefits of soy was based on the lower rates of breast and prostate cancer and CHD mortality in countries with relatively high soyafood consumption. If soya contributes to these lower disease rates then Asian soya intake provides a one possible guideline for establishing Western intake recommendations.

Several recently published large surveys provide quite detailed information on the soya intake of Japanese adults. These surveys suggest soya protein intake ranges from about 8-13 grams per day (Nagata et al., 1998; Nagata et al., 2000; Wakai et al., 1999). A recent prospective epidemiologic study that involved nearly 65,000 women in Shanghai found that average soya protein intake was 7.4 g/day (Zhang et al., 2003). In Japan, soya protein contributes approximately 10% of total protein intake.

One g of protein from traditional soyafoods provides approximately 3.5 mg (aglycone weight) isoflavones. This suggests Asian isoflavone intake is ~25-40 mg/d, which is consistent with the results of several surveys (Nagata, 2000; Wakai et al., 1999).

In addition to average intakes, it is important to have some understanding of the upper range of soya intake in Asia. In this regard, a large survey involving over 3,000 Japanese adults, found the energy-adjusted means for total soya protein intake for the highest quartile for men and women was 13.01 g and 11.04 g, respectively (Nagata et al., 1998). After adjusting for possible underestimation of the questionnaire, average soy protein intake in the fourth quartile for men and women was 18.2 g (18% of total protein intake) and 13.2 g (15% of total protein intake), respectively. Also, in the large prospective study by Zhang et al referred to above, the average soya protein intake among women in the fourth quartile was 16.6 (personal communication).

Efficacy

Although the health effects of soya in many areas have been investigated, most research has focused on breast and prostate cancer, CHD, and osteoporosis. Thus, only these areas will be considered as a basis for making recommendations.

Coronary heart disease

Although there are limited epidemiologic data a recently published prospective study involving nearly 65,000 Chinese women provides important information regarding soy intake and CHD risk. In this study, women in the fourth quartile (cutoff, >11.19 g; mean intake 16.6 g/d) of soya protein

intake were 86% less likely to experience a non-fatal heart attack than were women in the first quartile (Zhang et al., 2003).

Clearly, the intake level in this study associated with coronary benefits is much below the 25 g/day established by the U.S. FDA for cholesterol reduction. However, few dose-response studies have been conducted and on the basis of the clinical and epidemiologic data, Messina has argued that fewer than 25 g/d is efficacious (Messina, 2003a). Also, the health claim is intended primarily for hypercholesterolemics and thus is not necessarily an appropriate reference for the generally healthy adult. Finally, the health claim does not address the possible coronary benefits of soya aside from cholesterol reduction.

Findings from several studies suggest that isoflavones and isoflavone-rich soy protein favorably affect the coronary vessels and a variety of risk factors for CHD. For example, isoflavone supplements (40 mg/day) enhance systemic arterial compliance (an measure of arterial flexibility considered by some experts as an independent marker of CHD risk) (Nestel et al., 1997). Also, the consumption of 56 mg (Wiseman et al., 2000) and 60 mg (Tikkanen and Adlercreutz, 2000) isoflavones from soya protein reduced serum isoprostane levels (an in vivo marker of oxidation) and inhibited in vitro LDL-C oxidation, respectively. Other work suggests that isoflavones may enhance flow-mediated dilation although the literature is inconsistent (Cuevas et al., 2003; Squadrito et al., 2002).

Cancer

The epidemiologic data provide little support for the suggestion that adult consumption of soy reduces postmenopausal breast cancer and only modestly suggest that it is protective against premenopausal breast cancer (Trock et al., 2000). Nevertheless, several clinical trials have shown soya favorable affects a number of markers of breast cancer risk. For example, isoflavone supplements (40 mg/day) were found to decrease breast tissue density in postmenopausal women (Atkinson et al., 2002) and the consumption of soya protein providing 65 mg isoflavones/d and 45 mg isoflavones/d favorably affected estrogen metabolism and increased the length of the menstrual cycle, respectively (Kurzer, 2002). The doses used in these studies are consistent with the current soy intake recommendation.

Although the current soya intake recommendations are intended for adults, intriguing evidence suggest soya consumption early in life reduces adult breast cancer risk. For example, a large case-control study found Chinese women who consumed approximately >11 g soy protein/d during the

teenage years were 50% less likely to develop breast cancer as were Chinese women who consumed little (<2 g/d) soy (Shu et al., 2001). These findings are consistent with the proposed recommendations.

There is an emerging body of evidence suggesting that isoflavone intake is protective against the development, and perhaps even useful for the treatment, of prostate cancer (Griffiths, 2000; Messina, 2003b). However, the epidemiologic data are quite limited and almost no useful clinical data exist. Worthy of note, are the results from two prospective studies, one involving Japanese men in Hawaii and the other Seventh-day Adventists in California (Jacobsen et al., 1998; Severson et al., 1989). Both studies found that the intake of one serving per day of a traditional soyafood (tofu and soymilk in the former and latter studies) was associated with a marked reduction in prostate cancer risk. One serving/d provides less soy than currently recommended but neither of these studies reported total soya intake.

Osteoporosis

The Asian epidemiologic data generally show isoflavone intake to be positively associated with bone mineral density (BMD) (Messina et al., 2001; Setchell and Lydeking-Olsen, 2003). This suggests above average Asian isoflavone intakes are protective relative to below average intakes. In one study, isoflavone intake among premenopausal women in Hong Kong was associated with higher bone mineral density even though average intake was only 21 mg/day (Ho et al., 2001). A similar isoflavone intake was associated with higher bone mineral density among early postmenopausal women in Hong Kong (Ho et al., 2003). In contrast however, in a cross-sectional study involving Japanese postmenopausal women, Somekawa et al found at least 50+ mg isoflavones per day was associated with higher spinal BMD (Somekawa et al., 2001). Still, overall, the epidemiologic data are supportive of the recommendation to consume 50 mg isoflavones/day.

In contrast, although the results are somewhat inconsistent, and very limited dose response work has been conducted, the intervention studies suggest approximately 80 mg isoflavones per day are needed to promote bone health (Chen et al., 2003; Messina et al., 2001; Setchell and Lydeking-Olsen, 2003) It is possible though that short-term clinical studies overestimate the amount of soy actually needed for benefits when consumed over the course of decades.

Finally, several studies show that substituting soy protein for animal protein decreases urinary calcium excretion, likely as a result of the lower sulfur

amino acid content of soy. Evidence suggests substituting 15 g soy protein for animal protein may decrease dietary calcium needs by as much as 150 mg/day which at the population level would marked reduce fracture risk (Messina et al., 2001; Nordin, 2000; Spence et al., 2002).

Conclusions

Fifteen g of soya protein is the amount found in about 1½ - 2 servings of traditional soyfoods. Consuming 15 g soya protein from traditional soyafoods will provide approximately 50 mg isoflavones. These amounts appear to be sufficient to derive the hypothesized health benefits of consuming soyafoods although the recommendation to consume these amounts should be considered provisional until more definitive data are available. Most populations consume approximately 80 g total protein per day. Thus, substituting 15 g of soy protein for other protein sources in the diet indicates that soyafoods would provide less than 20% of the total protein intake and thus the soybean would serve function as just other good source of high quality protein but with multiple potential health benefits. Thus, the current recommendations are consistent with the dietary principle of moderation and variety.

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Beyond nutrition medicinal soybean: an Indian view

V. Kawalkar[1]

Abstract

The concept that natural components in plant foods could have a beneficial effect on human health is not new. In India, it forms the basis of the practice of traditional system of medicine " Ayurveda", while in A.D. 390 Hippocrates the most celebrated Greek physician said " Let food be your medicine and medicine be your food".

The scientific research has now focused on phytochemicals–phytonutrients, natural compounds found in plant foods - soybean that speak directly to our body chemistry with profound effects on our health. When phyto-nutrients enter our body they join our network of messenger molecules.

The medicinal effects of soybean is mostly due to its bio-active components-phytochemicals , which exerts their action beyond nutritional benefits. Soybeans are the concentrated sources of isoflavons, weak-phytoestrogens, binds to estrogen receptors and block the bindings of more potent estrogens, indicating lower risk of breast cancer (WCRF,1997). Consumption of phytochemicals including soy isoflavons may explain the lower incidence of chronic diseases like coronary heart disease and cancer etc.

Introduction

What WHO says? : The World Health Organization believes that : "Health is not merely the absence of disease but a presence of mental, physical, social and spiritual wellbeing". Soybean is a vegetarian food and has a spiritual value. The creed of vegetarianism is gaining ground over the world.
Basically 700 million Indians (70%) are vegetarians consuming plant foods in Indian cousins, which are rich sources of phytochemicals with several biological plausibilities of decreasing the risk of chronic diseases and cancer and cardiovascular problems.These are capable of attacking and neutralizing the free radicals and offsetting their many pathogenic manifestations in the body.

The American heart association endorsed the use of soy foods for cholesterol reduction and soy protein consumption to reduce the risk of heart disease.

[1] Heath,-Food & Nutrition Technology Institution; E-mail:kawalkar@sancharnet.in

Indian traditional system of medicine "Ayurveda" which is based on the body types as per elemental constituents Vata, Pitta and kaph suggest some pacifying effect of soybean on the body prakriti.

Soy: the functional – medicinal food

Some time back I have read in Lancet titled " Functional Food" : The health claim question?

The term "Functional Food" hints specially developed foods will protects consumers from a variety of .diseases and discomforts. How realistic is this expectation? A recent consensus document states that a food can be regarded as "functional" if it has beneficial effects on "target" functions in the body, beyond adequate nutritional effects, in a way that is relevant to health and well-being and/or reduction of disease (Martijn B Katn 1999).

According to British Nutrition Foundation, "Functional foods" are foods that have health giving properties over and above their nutritional value. In China the health promoting foods are usually called medicinal foods. The term medicinal food appeared frequently in the literature of the East Han dynasty (approximately 100 BC.). One of the unique concepts in traditional Chinese medicine is that food and medicine come from the same source. Medicinal food has the usual characteristic of food. (Huo Junsheng, 2003). In Japan, the term "functional food" is to denote "a food imparting health benefits beyond that of basic nutrition and making a claim about this benefit". They are also refered to as "bioactives".

Soy phytoestrogens: Have numerous biological effects, They are antiviral, antiproliferative , and growth inhibiting. Phytoestrogens are weakly oestrogenic and compete with steroid hormones for various enzymes and receptors. They also stimulate production of sex-hormone binding globulin in the liver. In these ways, they may alter steroid hormone metabolism and, by inhibiting growth and proliferation of hormone-dependant cancer cells, may alter cancer risk(WCRF,1997). The commonly found saponin in soybean hold promise because of their potential role in lowering serum cholesterol.

The US FDA has allowed some soy products to carry a label indicating that they may reduce heart disease risk by taking 25 g/day of soy protein (as a source of 60 mg. of soy phyto-estrogens).

World market for functional – medicinal foods has grown by about 60% over the period 1995-2000 and was valued at US $ 50000 million in 2000.

The studies

The Indians mostly are vegetarians and take advantage of the plant foods soybeans for consumption. The soya isoflavones is a weak phytoestrogen, which binds to estrogen receptor is associated with hormonal and reproductive functions. Most of the evidence on phytoestrogens and cancer risk relates to breast cancer. Breast cancer is the third most common cancer in the world. Mortality attributable to breast cancer in 1996, was estimated 5.5% of all cancer deaths (WHO,1997). Apart from free radicals, dietary and non-dietary risk factors, the breast cancer factors are those associated with hormonal and reproductive factor. It is thought that the hormones play a promotional role in breast carcinogenesis stimulating division of initiated cells. Isoflavones are heterocyclic phenols with structural similarities to oestrogens. Other bioactives components of fibre rich foods in addition to isoflavones and lignans may partly explain the association between fibre and the risk of breast cancer (Kawalkar,1998). Evidence for isoflavones and possibly lignans, comes from a human trial on oestrogen metabolism, from ecological observations of vegetarians.

The probable role of life time oestrogen exposure in the risk of breast cancer suggest that isoflavons and lignans may protect against breast cancer (WCRF,1997). The known risk exposure for breast cancer are mainly related to exposure to unopposed oestrogen which may have more of a promotional effect (Potter,1997). In a controlled human feeding trial found an increase in the length of menstrual cycle, in women fed moderate amount of soy bean products containing 45mg. isoflavone per day(Cassidy et al (1993) indicating lower risk of breast cancer. Cancer protective effects of isoflavones have been supported by human ecological observation. Women who consume more soy foods in Japan, have lower rates of breast cancer (Herman et al,1995).Traditional Japanese women with their high soy intake – a rich source of phytoestrogen – have a low incidence of breast and other oestrogen related cancers and few menopausal symptoms (Watanabe et al,1993). The Average Japanese consume about 30mg. of isoflavons per day (Kimira et al, 1998; Arai et al, 2000).

Soy: the medicine beyond nutrition

After consumption of lignan precursors and of the isoflavones themselves many metabolic conversions occur in the gut, which results in the formation of hormone like compounds which may act as phytoestrogens (WCRF,1997). Phytoestrgens have also been shown to lead to an increase in the synthesis of serum hormone-binding globulin in the liver and therby to decrease the relative amount of free oestradiol and redce the uptake and biological activity (Adlercreutz et al,1992). One postulated anticarcinogenic mechanism involves the weak phytoestrogen activity of isoflavones and lignans. The

phytoestrogen may bind to oestrogen receptors and block the binding of more potent oestrogens without eliciting a major response (WCRF,1997). In addition to possessing weak oestrogenic activity , genistein has been shown to inhibit protein-tyrosine kinase (Herman et al,1995) and to have antioxidant activity (Wei et al,1995). Natural edible antioxidant nutrients can effectively supplement the endogenous defenses, which add significantly to the anti free radical forces (Kawalkar,1996). Growth supporting effect of genistein and stimulating the differentiation of a wide variety of cancer cells have been shown in vitro study. It has also further been shown to induce apoptosis in tumour cell by genistein and an isoflavon precursor, biochanin A (Yanagihara et al,1993). The oestrogen like properties of isoflavones created interest as an alternative to conventional hormone replacement therapy (HRT). The beneficial effects of oestrgen on the skeletal system and the reported low incidence of hot flashes in Japan were major incentives for this line of investigation (Look M, 1994).

Publication of meta analysis in 1995 showed that soy protein lowered serum cholesterol in hypercholesterolenic individuals. In 1999, the FDA in the USA approved the claim for reducing heart disease using 25 g/day of soy protein. Later the American heart association endorsed the use of soy foods for cholesterol reduction. The soybean emerged as healthy food and also proved having its role as a medicinal soybean which exerts its action beyond nutrition in addition to preserving its nutritional claim.

An Indian view

In India Soybean production is about six million tonnes worth Rs.60000 million. The soybean is cultivated on about 65 million hectars.

Within next 25 years one in five diabetic patients in the world will be an Indian and coronary heart disease will be the leading cause of death in India (Yajnik,2003). Soybean stands as a better source to provide adequate nutrition and health benefits to millions of Indians. In a study it is observed that in a population 72% families were producing soybean and their domestic consumption was assessed to be only 4 %. The routine consumption of soybean in India by the producers is negligible. This might be due to lack of knowledge about soybeans health benefits and its nutritional values. We are imparting health and nutrition education to the soybean producers to utilize their own produce for their own benefits and improving the community health.

Some health foods are manufactured in India by incorporating soy protein concentrate and Isolates. The utilization of soy grits in traditional Indian recipes enhances their nutritional value and utility for incorporation in the

diets of certain disease condition. In a study the recipes developed from this were highly acceptable and had enhanced nutritive value over their traditional counterparts. Soy grit/meat has the advantage beyond its high nutrition value, of higher therapeutic value, especially for diabetes and hypertensives. In a study it is found that soy foods bring in value addition apart of being a good source of protein, micronutrients and antioxidants like soy isoflvones and help for improvement in degenerative diseases. Soy Proteins rich in isoflavones have shown to be beneficial in relieving menopause related problems. In another study 25gm of soy protein isoate (SPI) mixed with 200 ml. milk was supplemented to menopausal women for six months. All the symptoms of hot flushes ,night sweat and dizziness etc.disappeared after sixth months. A significant change was observed in the total cholesterol level of menopausal women.

Vegetarianism

The vegetarian soy : Based on cultural and religious beliefs, 700 million population in India are vegetarian (70%). Our essentially vegetarian food culture has now come to be widely endorsed in terms of optimal management of health and disease. The fact that Americans have now come to regard Asian dietary practices, more pointedly the Indian. Our ancient rishis depended on plant foods, not only for their bodily sustenance, but also for nourishment of their minds and souls today stand amply testified as truly wholesome foods for our holistic health.

An increasing preference for vegetarian foods has come from the occurance of a wide range of bioactive constituents which have been shown to be endowed with hypocholesterolemic, hypoglycemic, anti-angiogenic, anticarcinogenic and several other positive health effects.

Self imposed vegetarianism in traditionally non- vegetarian areas of the world is on the increase and is expected to be 20-25% of the population. The creed of vegetarianism is gaining ground the world over. In a recent survey commissioned by TIME-CNN and conducted by Harris poll among several hundred adults in the United States, three fourths of the respondents said that they feel healthier since become a vegetarian. Increasing popularity of vegetarianism soybean products are likely to gain favor world over. WCRF also strongly recommends the maximum use of plant foods including soybean as a protective factor for the prevention of various cancers. With this we can say " Let your soy-veg food be your medicine and not medicine be your food ".

Holistic health : the spiritual soy : This has brought a realization of a new dimension to this holistic approach to understand and comprehend the

standard WHO prescribes which is spiritual. Our Rishis, Saints, and Yogis, who are our spiritual scientist, have always advocated a holistic approach to life as a whole. The purpose of food is to merge the spiritual aspirations of the person eating the food with the higher purpose of living. In Gita foods are classified as Satvik, Rajasik and Tamasik food as per their qualities. All these three basic (quality) gunas of foods produces the same quality of gunas in the person and personified in the body, mind and spirit of the 'whole' man, are profoundly influenced by the food consumed by him, so the state of health is said to be "Man is what he eats".

	Three Gunas of the Food		
	Satvik	Rajsik	Tamsik
Soybean	+	+	+

Indian system of medicine: the ayurveda

The ayurvedic soy :

Ayurveda system of medicine is concerned with the well being of the body, mind and spirit by bringing them into harmony. Diet is the most important way of doing this and "Charak" with profound wisdom states "Without a proper diet, Medicine are of no use, with a proper diet, Medicines are unnecessary". In Ayurved the body types are classified as per the elemental con nstituents - Vata, Pitta and Kapha principles (Prakriti). The five elements (Panchmahabhoot) - ether(space), air, earth, fire and water go to make the body-Prakriti.'. The characteristic of people with different Prakriti is as below :

1. Vata Prakriti : Quick, moving fast, always in a hurry and restless. Vata dosha governs movement of the peson and movements such as breathing and blood circulation.
2. Pitta Prakriti : Aggressive and ambitious. Pitta governs heat, metabolism, energy production. Excess of Pitta leads to anger and hostility. Pitta body people tend to be type 'A' personality.
3. Kapha Prakriti : Calm and quiet and does not get affected easily with small tensions. Kaph governs physical, fluid and balance, Kaph body people tend to be type 'B' personality. For thousands of years, Ayurveda has taught that what we eat plays a big part in determining health. The gunas (qualities) of the foods -Satvik, Rajsik and Tamsik influences the Vata, pitta and kapha doshas (Prakriti).

Vat		Pitta		Kaph	
No	Yes	No	Yes	No	Yes
Soybeans	*Soy cheese	Soy sauce	Soybeans	Soybeans	Soy milk
Soy flour	*Soy milk	Soy sausages	Soy cheese	Soy cheese	Soy sausages
Soy powder	*Soy sauce		*Soy Flour	Soy powder	Tempeh
Tofu	Soy sausages		Soy milk	Soy sauce	*Tofu(hot)
Tempeh			*Soy powder	Tofu(cold)	

This chart shows the influence of soybean foods on Vata,Pitta and Kapha doshas-Prakriti. No : Aggravate/ increase the dosha. **Yes** : Pacify/ decrease the dosha.Above are guidelines for basic constitutional types. * OK in moderation (Judith Morrison,2001).

Tofu, soy nuts,soya milk and tempeh are all rich in isoflavones. Isoflvones help prevents prostate enlargement, because they help protect from xenoestrogen damage. They may also help to detoxify dehydrotestosteron (DHT) which may be responsible for prostate enlargement. Many doctors in natural medicine recommend eating 25 to 50 gm of powdered soya protein each day.

The Ayurvedic classification help to explain modern discoveries about psychophysiological type (Schneider R,et al, 1985). The Doshas are the basic controllers of the psychophysiology when they are in balance- then health is optimal. All must work smoothly together to produce optimal health.

Balance in doshas

Doshic balance (in each person) enhances homeostastatic balance in body's biochemistry and favors self-repair mechanisms over free radical damage. Each dosha should maintain its optimum level when doshas become aggravated or excessive, this especially creates imbalance, and that imbalance can lead to disease.

Conclusion

The creed of vegetarianism is gaining ground the world over. To quote the American Dietetic Association "Appropriately planned vegetarian diets are healthful, are nutritionally adequate and provide healthful benefits in the prevention and treatment of certain diseases." Increasing popularity of vegetarianism soybean products are likely to gain favor world over.Indian system of medicine also suggest some pacifying effects of soybean on the body prakriti. Broadly, it can be said that medicinal foods are one step ahead of healthy natural foods in assisting the therapeutic process of the body towards substitution of medicines. So the remark is "let your soy-veg food be your medicine and not medicine be your food."

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Analytical and biological assessment of phytochemicals in soybeans and processed soy products

M. A. Berhow[1]; S. M. Duval[1];
S. F. Vaughn[1] and R. S. MacDonald[2]

Abstract

Interest in the nutraceutical value of soybeans has increased over the past few years as research studies have shown soy foods and soy extracts to be effective in the prevention or mediation of a number of chronic diseases. Yet, in most cases the exact phytochemical agents, or combination of agents, responsible for the observed biological activities have not been fully characterized. While most of the phytochemicals present in soy have been identified, purified individual phytochemicals from soy are not generally available in sufficient quantities for comprehensive nutritional studies. In cooperation with industrial research partners, methodologies are being developed for the production of research quantities of purified phytochemicals, such as the isoflavone glucosides and the saponin glycosides. These in turn are being supplied to cooperative nutritional research programs for their evaluation.

Phytochemical analysis

Complete characterization of the biological/chemical activities of plant phytochemicals will depend on the availability of phytochemicals in a pure form and in quantities sufficient for both bioassay studies and whole organism studies. Sorting out which (or a combination of which) phytochemicals provide the biological activity is going to be a task that will occupy the next era of phytochemical research. This is a common theme found in nearly all current research articles and reviews on phytochemical research (Dillard and German 2000; Francis, Kerem et al. 2002). Many phytochemicals have been identified but are either not commercially available or if available only in small quantities at prices that make them impractical for anything but use as analytical standards. The goals of this research project are to develop rapid and efficient methodology to prepare sufficient quantities of pure phytochemicals from plant materials.

[1] United States Department of Agriculture, Agricultural Research Service, National Center for Agricultural Utilization Research, 1815 N. University Street, Peoria, Illinois 61604 USA.
[2] Food Science Program, University of Missouri, 256 Stringer Wing, Columbia, MO 65211.
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Since phytochemicals are found in relatively low concentrations and often in specialized tissues, two approaches can be used to enhance yields: utilization of commercial agricultural processing streams and the enhancement of production of the phytochemicals in developing plant tissues. Working in collaboration with agricultural and industrial collaborators, we are examining processed products and co-products for enhanced levels of phytochemicals from existing commercial crops, such as soy. Once sufficient quantities of purified phytochemicals are obtained, this material will be sent to collaborators to perform the complicated whole organism studies, such as nutritional and anti-cancer studies. Further analytical chemistry will be required to elucidate the metabolic fates of the phytochemicals in whole organism studies.

Phytochemicals from legumes

The phytochemicals found in legumes, such as those from the soybean, are the focus of many studies today (Hoikkala, Schiavoni et al. 2003; Rowland, Faughnan et al. 2003). The isoflavones are only one class of phytochemicals found in the legumes, other classes include the triterpene saponins, the phytosterols, simple and poly phenolics, in addition to biologically active peptides and proteins (Garcia et al, 1997). Much of the research on health effects in soy has centered on the isoflavones, but problems include lack of reproducible analytical methods for foods, lack of consistent information on the biological activity of the pure isoflavones, debate over the form and bioavailability of the biologically-active metabolites, and lack of knowledge on synergistic effects with other phytochemicals. Isoflavones can be up to 6% of the seed by dry weight. Three aglycone structures are found in soybeans: daidzein, glycitein and genistein. In the plant these are found in a glucosylated form and in a malonyl-glucosyl form. During isolation and processing, these can be hydrolyzed giving rise to the acetyl-glucosyl and aglycone forms. Hence, there are potentially 12 isoflavone chemical forms that can be quantified and isolated (see discussion in Berhow 2002). Significant amounts of pure isoflavones are still not commercially available and much of the research is being conducted with extracts, which contain a number of other components such as the saponins.

Saponins are secondary plant metabolites containing a steroid or triterpenoid aglycone with a number of different carbohydrate moieties, which are linked through either an ether or an ester linkage at one or more glycosylation sites. Seeds of soybean contain from 0.6% to as much as 6.2% dry weight of triterpenoid saponins depending on the variety, cultivation year, location grown and degree of maturity, although typical concentrations appear to be around 1%. The soy saponins have been divided into group A, B, and E saponins on the basis of their aglycone structure. Group B (and possibly E)

saponins appear to exist in the intact plant tissue as conjugates of 2,3-dihydro-2,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP) at the 22 hydroxyl position (see references in Berhow et al, 2002). These conjugates are relatively labile and are easily degraded during most commercial extraction and processing methods to form the group B and E saponins. Group A saponins appear to be a naturally occurring form. The various forms and degradation products formed during processing and extraction have resulted in the characterization of over 20 different saponins from soy, making detailed quantitative analysis difficult. Saponins have been reputed to have important biological activities in humans, including hypocholesterolemic, haemolytic, immunostimulatory, and anti-tumourigenic activities, as well as chemoprotective activities. An industrial purification process has been developed to produce kg quantities of highly refined saponins from soy processing co-products. Using this material, we have been able to prepare fractions of highly purified and fully characterized soy saponins suitable for use in biological studies and as analytical standards for quantification (Berhow et al 2002).

Analytical methodology: Our standard analytical method usually begins with a simple one-step extraction of powdered, defatted soy product using methanol or methanol:DMSO (4:1), sonication, and heat. These extracts can be analyzed using thin layer chromatography (TLC) and high performance liquid chromatography (HPLC) to separate the phytochemicals in the extracts. Peak indentification can be confirmed using HPLC systems coupled to photodiode array detectors (PDA) and mass spectrometers (LC-MS). Most of our HPLC analyses are run using C18 ODS reverse phase columns with either isocratic or gradient elution. Isocratic systems include simple acetonitrile-water systems enhanced by the addition of a small amount of acid. Identification and quantification is usually achieved by monitoring light absorbance at a maximal wavelength and comparing to runs of known standards, but retention times of phytochemicals in complex mixture can vary considerably from those of separately run standards. LC-MS is rapidly becoming the method of choice for natural product analysis. The advantages of LC-MS over other MS methods include the fact that the phytochemicals can be analyzed without chemical derivitization, and the sensitivity and specificity of the MS is superior to that of UV-VIS spectrometry. The two main atmospheric ionization techniques that emerged (and now found on most LC-MS systems today) are electrospray ionization (ESI) (Zhou and Hamberger 1996) and atmospheric pressure chemical ionization (APCI) (Wolfender et al. 1995). The positive mode provides characteristic spectra which can provide structural information, while the use of the negative mode allows for more sensitivity and can be used to quantitate the compounds, with minimal or even no separation. The application of LC-MS

has been extensively applied to the analysis of phytochemicals in soybeans and related legumes and has the potential to be used for the MW determination of nearly all nonvolatile phytochemicals.

Preparation of pure isoflavones

Larger scale isolations can be done with preparative HPLC. Using a commercially prepared isoflavonoid concentrate, we were able to separate the isoflavones from contaminating carbohydrates and saponins on open C18 reverse phase column developed with increasing amounts of organic solvent. The fractions were monitored by HPLC and TLC to determine the best separation of isoflavones from the saponins. The dried isoflavone fractions were resuspended in a small amount of DMSO:MeOH, diluted to 25% organic solvent with water, and loaded on a preparative HPLC system with a Waters radial compression PrepLC assembly using a Waters Bondapak C18 column cartridge (15-20 um, 125Å, 47 x 300 mm). The column was developed isocratically with 25% methanol in water and 1% acetic acid at a flow rate of 50 ml/min. The isoflavonoids eluted with sufficient separation to allow for the isolation of daidzin, glycitin, genistin, and some of the acetylated forms. The fractions were rotoevaporated to remove the methanol and passed through a C18 SPE, washed with water to remove the acid, and eluted with MeOH. Water was added to the eluate and the purified fractions were allowed to evaporate in the hood. If sufficient compound is present, pure compound will crystallize out of the solution. Care must be taken to avoid cross contamination in the case of glycitin and daidzin as these two compounds will co-crystallize. Aglycone standards can be made by hydrolyzing the glucosides with 2M HCl in MeOH under refluxing conditions for 24 hours, and removing the acid and carbohydrates via a C18 SPE.

Isolation of saponin fractions from whole soybeans

A modified method of Tani *et al.* (1985) is used. De-hulled soybeans were ground to a fine powder and extracted with hexane in a Soxhlet extractor overnight. The dried solid residue was then extracted with methanol in a Soxhlet extractor for 48 h, the methanol extract evaporated, re-suspended in 80% aqueous methanol and any remaining solid residue removed by filtration. The supernatant was diluted to 20% methanol by the addition of water, and loaded onto an equilibrated preparative C_{18} reverse phase column (125 Å; 55-105 μ; Waters, Milford, MA, USA). The column was washed extensively with 70% aqueous methanol to remove the isoflavonoids and the saponins were then eluted with 100% methanol. Finally, the column was cleaned with isopropanol. The resulting eluate was diluted to 10% methanol with water. Under certain conditions (if the concentration of saponins was high enough) a saponin precipitate formed which could be

collected by centrifugation. Re-suspension and repeated precipitation in this manner resulted in the further separation of the saponins from the isoflavones. In either case, the fractions were reintroduced to a regenerated preparative C_{18} reverse phase column, washed with 70% aqueous methanol and eluted with 100% methanol. The process was repeated as required to remove any contaminating isoflavones. This process yielded a fraction that was enriched in the group B soyasaponins.

Preparative HPLC separation of the soyasaponins

Separation was achieved using a Shimadzu (Kyoto, Japan) preparative HPLC system consisting of two LC-8A preparative pumps, an SCL-10A VP controller, an SPD-M10A PDA, and an SIL-10A auto-injector running under the Class VP software system. The separation was carried out on a C_{18} reverse phase Inertsil ODS-3 (Metachem Technologies, Torrance, CA, USA) column (250 x 30 mm i.d.; 5 μ). The isocratic mobile phase was 40% aqueous acetonitrile with 0.025% trifluoroacetic acid (TFA) employed at a flow rate of 30 mL/min: 30 mL fractions were collected. The purified soyasaponin fraction (OTC70) was re-suspended in 80% aqueous methanol to a concentration of 25 mg/mL and an 1.5 mL aliquot was injected. Five peaks were resolved and the appropriate fractions pooled and evaporated to dryness. The procedure was repeated fifteen times to yield approximately 160 mg of soyasaponin I, 44 mg of soyasaponin II, 8 mg of soyasaponin III, 5 mg of soyasaponin IV, and 18 mg of a compound initially identified as soyasaponin V, as confirmed on a molecular weight basis by LC-MS.

Analytical methods for assaying phytochemicals in biological systems

The increased sensitivity of the new analytical equipment has pushed the detection limits to very low levels making possible the analysis of low levels of phytochemicals in biological fluids and tissues, such as blood sera, urine, and cultured cells. Use of LC-MS has greatly facilitated the identification of phytochemical metabolites, which will be greatly enhanced by the availability of isotopic-labeled phytochemicals. While a great deal of work has been done on the identification and quantitation of phenolic metabolites in animal sera and tissues, little has been done on the saponin metabolites.

It is now clear that a varied and healthy diet contains many foods that possess components or factors other than the traditional nutrients (i.e. vitamins, minerals, fatty acids), which affect health in ways not previously appreciated by most nutritionists or healthcare providers. Now we know these food components have the potential of affecting the course of fetal/child development and aging by modulating body function, which in turn

have the potential to improve general health and prevent certain diseases. Literally thousands of recently recognized dietary factors commonly referred to as phytochemicals have been identified in fruits, vegetables and grains, but only a few have been carefully studied for their effects on health. Furthermore, other organic factors are created upon processing plant and animal foods. Identification of the beneficial and/or adverse biological effects of these dietary factors is the first step in understanding the potential impact they will have on health. With this knowledge, further studies can be conducted to determine which components in these foods are bioactive, the mechanisms of actions and other important information such as the required intake levels, bioavailability, and nutrient/phytochemical interactions. Documentation of these health effects will pave the way for developing strategies to enhance intake of these dietary components in the general population. Since studies have demonstrated that Americans currently do not consume adequate levels of several known nutrients, one strategy to assure increased intake of health promoting food components is to enhance or enrich food crops with the desired component. However, information is incomplete on the biological and environmental factors that determine the content and composition of plant- and animal-derived foods. Thus, research is needed in these areas, such that strategies can be developed for the nutritional enhancement of commonly consumed foods.

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Metabolic and physiologic effects of *trans* fatty acids

N. Acar[1], N. Combe[2], C. Boué[2] and J.-L. Sébédio[1]

Abstract

Trans fatty acids can be found in ruminant fats, in partially hydrogenated vegetable oils and in heat-treated vegetable oils. Following some data on the worldwide consumption of *trans* fatty acids, this paper reviews the studies carried out on the metabolic effects of *trans* fatty acids. Since *trans* fatty acids were shown to interfere with the metabolism of essential fatty acids and the oxidative metabolism of arachidonic acid, their physiological effects on platelet aggregation, lipoprotein metabolism and neuronal function are discussed.

Introduction

The two primary sources of *trans* fatty acids in the diet are ruminant fats and partially hydrogenated vegetable oils. In meat and dairy products, *trans* fatty acids represent from 1 to 6% of total fatty acids. The products of industrial hydrogenation, such margarine can contain variable amounts of *trans* fatty acids up to 40-50% of total fatty acids.

Trans monounsaturated fatty acids are mainly formed during the hydrogenation of oils to produce margarine and shortenings but are also present in ruminant products such as milk and meat as a result of biohydrogenation in the rumen [1]. However, the isomer distribution presents some major differences since vaccenic acid or 11t-18:1 is the major trans monoethylenic isomer in milk lipids while the *trans* ethylenic bond is more delocalized in the case of partially hydrogenated oils where Dδ8, Dδ9 and Dδ10 are present in important quantities [2].

Trans polyunsaturated fatty acids especially those arising from the two essential fatty acids, linoleic (18:2 n-6) and linolenic (18:3 n-3) acids have shown to be formed during the last step or refining, which is the deodorization process [3] but also during frying of vegetable oils [4]. The two major isomers of 18:3 n-3 are 9c,12c,15t-18:3 and 9t,12c,15c-18:3, while the di*trans* isomers, especially the 9t,12c,15t-18:3 can be observed when the oil has been submitted to higher temperatures (above 220°C). Similarly, the two major isomers of linoleic acid are the mono*trans* isomers (9c,12t

[1] INRA, Lipid Nutrition Unit, 17 rue Sully, 21065 Dijon Cedex, France
[2] ITERG, Department of Biochemistry and Nutrition, 33405 Talence Cedex, France

and 9t,12c-18:2), the di*trans* isomer being only present when the oil has been submitted to high temperatures. Determination of the degree of isomerization (DI) of linoleic (DI 18:2) and of linolenic (DI 18:3) acids indicates that 18:3 is much more sensitive to isomerization than 18:2. Refined oils with DI 18:2 and DI 18:3 ranging from 6.0 to 37.0 respectively, have been found in the market [5].

Consumption of *trans* fatty acids

For France, based on contribution of ruminant fats (RF) and partially hydrogenated vegetable oils (PHVO) to total *trans* fatty acids intake (60/40; RF/PHVO), the mean intake of 11t-18:1 was 0.8g/d and 0.4g for 9t-18:1 (*Fig. 1*) [6]. So, vaccenic acid represented approximately one-third of total isomers. The pattern of Germany is similar: 1g/d for 11t-18:1 and 0.5g/d for 9t-18:1. In contrast to these European countries, the RF/PHVO ratio was of 20/80 in north America with a half-half distribution for both isomers (1.5g/d for 11t-18:1 and 1.7g/d for 9t-18:1) [6]. Nowadays, in Europe, most margarines currently contain less than 1% TFA (compared to 30% in American Margarines). Further, about 70% of TFA are coming from baked, process and prepared foods.

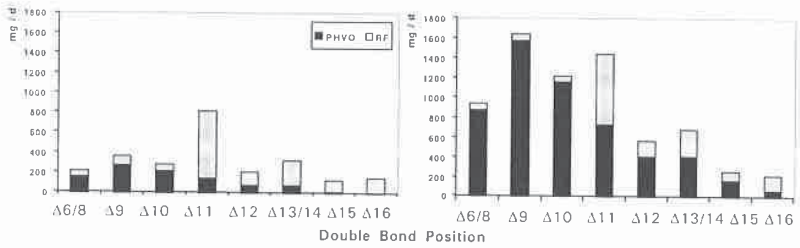

Figure 1. Daily intake of individual trans-18:1 acids in France (left) and North America (right) (Black bars: contribution by partially hydrogenated vegetable oils, PHVO; grey bars: contribution by ruminants fat, RF).

Placental transfer of *trans* fatty acids

The placental transfer of *trans* fatty acids in 31 mother-foetus couples was studied [7]. *Trans* fatty acid level in cord blood was 0.58% of total fatty acids. Preferential transfer of the 9t,12c- 18:2 isomer was observed from mother to cord blood. After mutifactorial corrections, any correlation between weight or head circumference of term newborn and *trans* fatty acids level in either adipose tissue or plasma lipids of mother was observed.
Metabolism and effect on essential fatty acid metabolism.

The metabolism of *trans* isomeric fatty acids has been studied intensively mainly in the early 80's both *"in vitro"* and *"in vivo"* using very often

essential fatty acid (EFA) deficient systems, which are not representative of the *"in vivo"* situation. *"In vitro"* it has been shown for example that most of the *trans* fatty acids could be desaturated by the Dδ9-desaturase which is acting on stearic acid [8]. Consequently, number of *trans* 18:1 isomers present in partially hydrogenated oils are potential precursors of a number of 18:2 isomers and therefore of long chain polyunsaturated fatty acids (PUFA). In a study conducted by Turpeinen [9], it was shown that feeding 3g/d of vaccenic acid induced an increase of rumenic acid (9c,11t-18:2) in serum triacylglycerols (TAG). The rumenic acid content doubled within a few days and a steady state was achieved in 4-6 d. These data support the hypothesis that vaccenic acid may be desaturated into rumenic acid in humans as well as in rodents [10]. *"In vivo"* experiments showed that dietary *trans* fatty acids could be incorporated into nearly all lipid classes of various organs but the concentration is highest in TAG. A decreased deposition of long chain PUFA after a *trans* 18:1 intake, which could be explained by the inhibitory effect of *trans* isomers toward EFA conversions, has been shown in a number of study [8]. However, some conflicting results on the effects of *trans* fatty acid on the conversion of linoleic acid and the deposition of long chain PUFA appeared in the literature. May be the presence of adequate amount of linoleic acid as well as the presence or not of linolenic acid would be a major factor, which has been overlooked in many studies.
For a detailed description of the metabolism of *trans* PUFA, one could refer to the article of Sébédio and Chardigny [11] where all the earlier experiments mostly carried out in USA have been described. We will mostly review here studies carried out in the last decade.

Both our group and others [12, 13] have demonstrated that some 18:2 and 18:3 isomers can be desaturated and elongated in isomers of arachidonic, eicosapentaenoic and docosahexaenoic acids. For example, the two mono*trans* isomers of linoleic acid can be converted in 5c,8c,11t,14c (11t-20:4) and 5c,8c,11c,14t-20:4 (14t-20:4). However the 9t,12c-18:2 is rather elongated into 11t,14c-20:2 than desaturated and elongated into 11t-20:4. Similarly, the 9c,12c,15t-18:3 can be desaturated and elongated into 17t-20:5 and 19t-22:6 [14]. Early studies [11] (for review) on the effect of *trans* PUFA on the metabolism of EFA were dealing with the 9t,12t-18:2 which is only found as trace in human food. More recently, Berdeaux [15] showed that the 9c,12t-18:2 could inhibit the Dδ6 desaturase of linoleic in liver microsomes of rat fed a fat free diet. Blond et al. [16, 17] also showed a decrease of n-3 fatty acids (18:3 n-3, 20:5 n-3, 22:5 n-3 and 22:6 n-3) and an increase in 20:4 n-6 in hepatic phospholipids of animals fed a diet containing *trans* isomers of linolenic acid. While the Dδ6 desaturase activity was not modified by *trans* isomers of 18:3 n-3, the Dδ5 desaturation of dihomo-gamma-linolenic acid in arachidonic acid was increased [17].

Effects of *trans* PUFA on arachidonic acid metabolism

As described above, mono*trans* isomers of 18:2 n-6 and 18:3 n-3 are converted into higher metabolites, including C20 and C22 PUFA and the effects of these molecules were studied using either isolated metabolites from rat liver or fully synthesized isomers.

The effects of 14t-20:4 on the metabolism of arachidonic acid in eicosanoids was studied recently by Berdeaux et al. [18] using rat platelets. It was shown that this structural analogue of 20:3 n-9 induced an inhibition of the conversion of 20:4 n-6 into thromboxane B2 or HHT (cyclooxygenase pathway) and increased the production of 12- hydroxyeicosatetraenoic acid (12-HETE) through the 12-lipoxygenase pathway. These data were well correlated to platelet aggregation. Moreover, using radiolabelled 14t-20:4, it was shown that it was metabolized by platelets into two metabolites [19]. The effects of 17t-20:5 and 19t-22:6 were studied after incorporation into platelet lipids. Thrombin and collagen stimulation of platelets enriched in 20:5 or 22:6 PUFA showed that the occurrence of a *trans* double bond at the (n-3) position decreased the antiaggregant effects of both 20:5 and 22:6 fatty acids. Similarly, the stimulation of these platelets with U46619, a stable analogue of thromboxane A2 showed that platelets enriched in *trans* PUFA were more sensitive than when enriched with *cis* PUFA. These data suggest that the incorporation of *trans* PUFA may modify the sensitivity of the TxA2, receptor of the platelet membranes. This hypothesis is enhanced by the lack of effect on the production of eicosanoids when platelets were triggered by collagen [20].

As several *trans*-18:3 isomers are converted *in vivo* into 20:5 isomers, the effects of 11t-, 14t- and 17t-20:5 on platelet aggregation were studied. However, this study was performed using washed rat platelets as human platelets are not devoid of long-chain *trans* PUFA [21]. Washed rat platelets were stimulated by arachidonic acid in the presence of increasing quantities of EPA or one of its *trans* isomers [22]. It appeared that 20:5 isomers with a *trans* double bond at the Dð11 position were inhibitors of the cyclooxygenase activity and more antiaggregant than EPA or 17t 20:5 [22].

Besides the production of thromboxane by platelets, arachidonic acid is metabolized by endothelial cells into different eicosanoids. The most important one is prostacyclin (PGI2) which has strong anti-aggregant properties. Endothelial cells are also able to produce prostaglandin I3 (PGI3) from EPA, but the question of their ability to metabolize *trans* EPA has only recently been considered. Incorporation of 17*trans*-EPA in cell phospholipids, the major *trans*-18:3 metabolite resulted in a reduction of PGI_2 as measured

by its metabolite 6keto-PGF1að when compared with control cells. However this decrease was of a similar magnitude as that after incorporation of EPA in these cells. The metabolism of labelled 17*trans*-EPA in endothelial cells was also studied. This was of particular interest since platelets, which interact with endothelial cells, were able to produce several metabolites from this *trans* fatty acid. 17*trans*-EPA is converted into at least two metabolites [22]. One of these has the same retention volume as 6-keto prostaglandin F1að when analyzed by high-performance liquid chromatography. Unfortunately, the identification of this metabolite was not possible, due to the limited quantities obtained. However, its production was inhibited when cells were preincubated with indomethacin. This suggests that it results from the cyclooxygenase pathway and may be an isomer of 6 keto- PGF1að.

Lipoprotein metabolism and risk of coronary heart disease in human

Epidemiological studies on associations between *trans* fatty acid intake and risk of coronary heart disease are not conclusive. Moreover, methods of assessing *trans* fatty acid intake have certain weak points. It would be desirable to combine dietary survey methods with biochemical indicators of long term fatty acid intake, such as the adipose tissue. The relationship between the content of *trans* fatty acid in adipose tissue and blood plasma, and LDL- and HDL- cholesterol concentrations was examined in a French population, between 1997 and 1998 [23]. The mean total *trans* fatty acid content of adipose tissue was 2.32 % of total fatty acids in 71 women. Among the *trans* 18:1 positional isomers, the Dð11*trans* was the most prevalent like in ruminant fat. The profile of these isomers in adipose tissue confirmed survey data on *trans* fatty acid dietary origins (60% from ruminant fat and 40% from partially hydrogenated vegetable oils). The plasmatic level of *trans* fatty acids was 0.64% of total fatty acids (range: 0.26-1.03%). As shown in eight European countries (TRANSFAIR study [24]), there were no significant relations between concentration of LDL-cholesterol (1.07g/l) (and HDL-cholesterol (0.47g/l)) and *trans* fatty acid content in either adipose tissue or plasma of this French population [25].

To calculate the quantitative effects of *trans* 18:1 isomers on lipoprotein profile, the data of different intervention studies were combined by multiple regression analysis and carbohydrates were chosen as reference [26]. It was found that 1% of dietary energy as *trans*-18:1 isomers at the expense of carbohydrates results in an increase in LDL cholesterol of 0.034mmol/L while the effects on HDL are very similar to those of carbohydrates. On the contrary, very little has been published on the major "natural" *trans* 18:1 isomers, vaccenic acid. It was recently concluded [27] from an animal study that the ratio of LDL/HDL cholesterol in plasma was significantly higher in hamsters fed vaccenic acid than in those fed elaïdic acid. This was not in agreement with the conclusion drawn from an epidemiological study [28].

To our knowledge, only one human nutritional intervention study (TRANSLINE) has been carried out to assess the health impact of *trans*-18:3 isomers. The volunteers consumed an experimental diet in which the intake of carbohydrate, protein and lipid is detailed elsewhere [29]. After a run-in period, the subjects were randomly allocated to one of two groups eating a diet low or high in *trans* ad-linolenic acid for 6 weeks (0.6% of energy, i.e. about 50% of ad-linolenic acid as *trans* isomers.)

As already discussed, *trans* isomers of oleic acid may raise low-density lipoprotein LDL-cholesterol, it was therefore important to check whether *trans* isomers of ad-linolenic acid could interfere with cholesterol and lipoprotein metabolism in humans. The consumption of *trans* ad-linolenic acid did not change plasma total cholesterol (treatment effect high *vs.* low 1.7% (-2.2 to 5.7), p = 0.41). HDL-cholesterol increased by 3.8% when the subjects continued to consume the low *trans* diet, but did not change on the high *trans* ad-linolenic acid diet (treatment effect -2.7% (-6.8 to 1.6), p = 0.22). As a result the *trans* ad-linolenic acid isomers rich diet raised the LDL- to HDL-cholesterol and the total- to HDL cholesterol ratios by 5 and 8% respectively, while the inverse was observed on the low *trans* diet [30].

Visual and cerebral function

Some recent studies were carried out to assess the effect of *trans*-18:3 isomers consumption on functional aspects of neuronal tissues. Rats consumed for 21 months one control diet containing only *cis* 18:3 n-3 (1.7% of total fatty acids) and one diet in which a part of 18:3 n-3 was

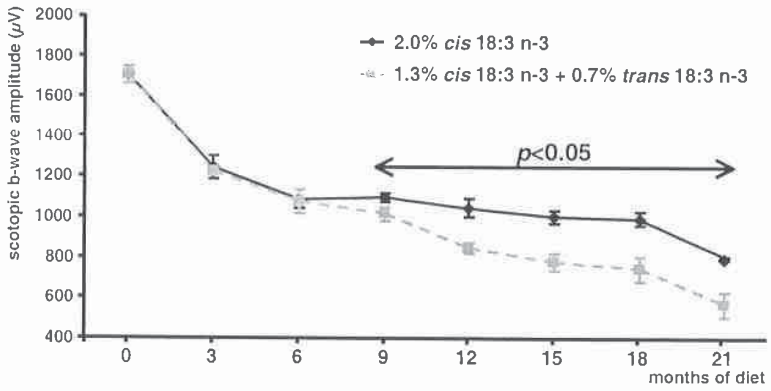

Figure 2. b-wave amplitude of the electroretinogram of rats fed for 21 months with 2.0% of cis 18:3 n-3 (black curve) and 1.7% of cis 18:3 n-3 + 0.7% of trans 18:3 n-3 (grey curve).

given as *trans* 18:3 n-3 (1.0% of *cis* 18:3 n-3 and 0.7% of *trans* 18:3 n-3). After 9 months, the retinal function assessed by electroretinography displayed some defects through reduced b-wave amplitude [31].

The dopamine contents in the striatum and the hippocampus were also affected with higher levels in animals consuming *trans*-18:3 isomers [32]. Comparable results were also obtained within a shorter period on newborn piglets [33]. However, the mechanisms involved need to be fully elucidated.

Conclusion

Fatty acid isomers of oleic, linoleic and linolenic acids may be of natural origin and/or formed during technological treatments of fats and oils. Some of these fatty acid isomers especially the dienoic and trienoic fatty acids may be good substrates for desaturation and elongation as their natural *cis* isomers, linoleic and linolenic acids. They can therefore interfere not only in the metabolism of essential fatty acids but also on the oxidative metabolism of arachidonic acid and play a role in platelet aggregation and visual function.

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Research and development of soybean processing in Japan

K. Saio[1]

Abstract

Total consumption of soybeans in Japan is about 5000,000 tons, one fifth of which are consumed as food uses. Soybean has always been an essential core of Japanese dietary habit. Fuji Foundation for Protein Research, which is an association constructed by multidisciplinary scientists with financial support of Fuji Oil Co. Ltd., has been active since 1980. The research subjects of the Foundation (published in *Soy Protein Research, Japan*) are classified into Quality bleeding, Food science and processing, Biochemistry and nutrition and Health and clinical nutrition. First, a few topics in the category of food science and processing were selected from the reports and briefly introduced. Secondly, two products and a technology developed recently such as Soyafive-S, Unicell protein and Activa supercurd will be explained. At last, the climate in Japanese market are referred to, reflecting a keen attention to health effects and diversification of food commodities.

A few research subjects selected from *Soy Protein Research*, Japan[1)]

The research subjects by Fuji Foundation for Protein Research are shown in Table 1.

Table 1. Classification and numbers of research subjects in 1998 to 2002

	SRP	QB	FC&FP	BC&N	H&CN	Total
2002	1	4	5	13	4	26
2001		3	4	14	2	23
2000		3	3	17	3	26
1999		3	7	15	3	28
1998		2	10	12	4	28
	1	15	29	71	15	131

SRP:Special Research Project, QB:Quality Breeding, FC&FD:Food Chemistry and Food Processing, BC&N:Biochemistry and Nutrition, H&CN:Health and Clinical Nutrition. Total numbers of Research from 1980 to 2002 are 514 subjects.

[1] Senior volunteer, JICA Cambodia Office

In this present paper, a few topics in the category of food science and processing were briefly introduced.

Preparation of transparent gel [2]

This paper was reported by N. Kitabatake et al. in 1999. Defatted soybean extract was dialyzed against distilled water at pH 7.5, changing 3 times every 3 hr. The dialysate (desalted soybean solution) preheated at more than 90C was heated again in the presence of NaCl (0.2M). The solution gave translucent gel which was melted by the heating and gelified again cooling such as agar-gel or gelatin. The phenomena are shown by use of a differential thermal analyzer (Fig.1). The desalted soybean solution has less beany flavor and such a possibility to make reversible translucent gel.

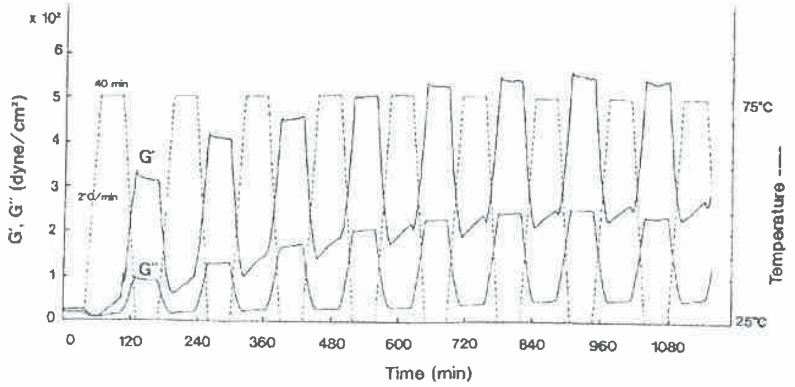

Figure 1. Change in the storage modulus, G' and the loss modulus, G", of the desalted soybean protein isolate solutions preheated at 90C in the repetition ofheating and cooling. The protein concentration was 75 mg/mL and NaCl concentration was 0.2M

Removal of phytic acid from soybean isolates [3]

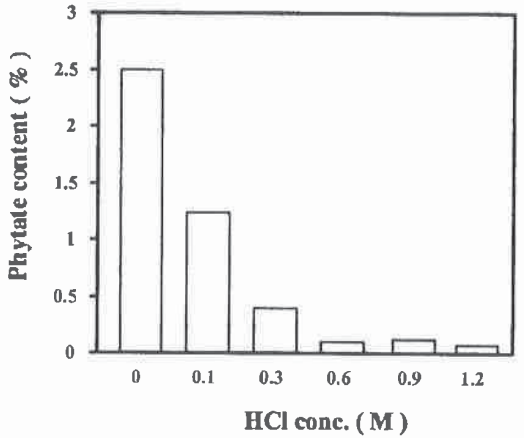

Figure 2. Effects of HCl concentration on the extraction of phytic acid from soy proteins. The extraction was carried out with various concentrations of aqueous HCl for 30 min at room temperature

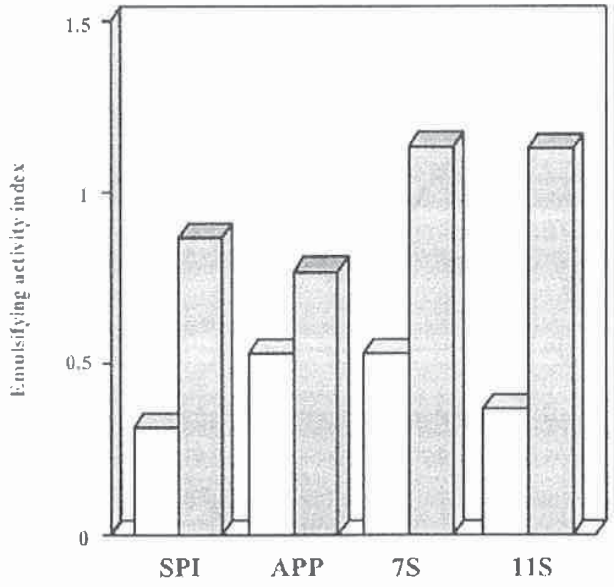

Figure 3. Emulsifying activity of soybean protein before (left bar) and after (right bar) 0.6M HCl extraction.

This paper was described by M.Katoh et al. in 2002. Their method was very simple, using 0.6M HCl and removing 96% phytic acid (Fig.2). Digestibility of SPI with pepsin was increased but not with trypsin. Surface hydrophobicity was increased by the removal of phytic acid and higher surface hydrophobicity resulted in higher emulsifying capacity (Fig.3). Moreover, the hydrolysates derived from phytate-removed SPI accelerated the calcium transport across the Caco-2 cell monolayer.

Utilization of phytase in Koji-natto [4]

This paper was reported by K. Ohtsuki et al. in 2001. They noticed that traditional fermented soyfoods have less phytic acid, especially in the Koji-natto and that phytic acid is hydrolyzed during the fermentation. The contents of phytic acid in various soyfoods and other foods are analyzed. The results showed that soyfoods used koji (*Asp. oryzae*) have less in phytic acid than natto fermented with Bacillus and other foods. They tried to make shio-natto in their laboratory. Consequently, the content of phytic acid decreased to 50% in 2 weeks and to 10% in 4 weeks during the fermentation (Fig.4). Moreover, the ratio of free to total minerals in the shio-natto was higher than that in kinako (Table 2). It suggests that fermentation with koji is a good process to reduce phytic acid.

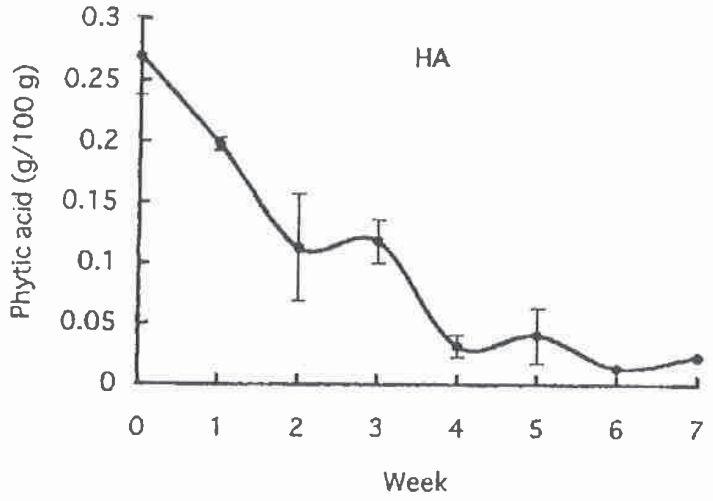

Figure 5. Changes in phytic acid content in Sio-natto (Hikiwari natto). The aging was carried at 4C

Table 2. Contents of minerals in shio-natto and Kinako

	Shio-natto*			Kinako		
	Free	Total	F/T	Free	Total	F/T
	mg%			mg%		
Ca	8.8	32.8	0.27	16.5	167.2	0.10
Mg	12.5	56.3	0.22	52.5	328.8	0.16
Fe	11.0	58.8	0.19	0.95	17.2	0.06
Zn	1.38	1.56	0.88	2.0	4.38	0.46
Cu	0.23	0.63	0.37	0.1	1.56	0.06

*** Four week fermented sample, 454, in Figure 6.**

Deodorization of soybean proteins by enzymatic and physicochemical processing [5]

This paper was reported by K. Inouye in 1999. He tried to develop a process for deodorization of SPI, using various solid absorbents such as polystylene, polymetacrylate and zeorite, as compared with charcoal. Hexanol contents decreased with all absorbents, showing the highest decrease in polystyrene (SP207, 825) and Zeorite (HSZ360) (Fig.6). Since a model experiment done in other hand showed that hexanol-abosorption ability of these absorbents was much higher than the hexanol content of SPI, they suggested that more than one third of total hexanol is presented in bound form (bound form-hexanol was not sensitive in sensory test). The digestion with chymotrypsin of SPI did not effect on the removal of hexanol.

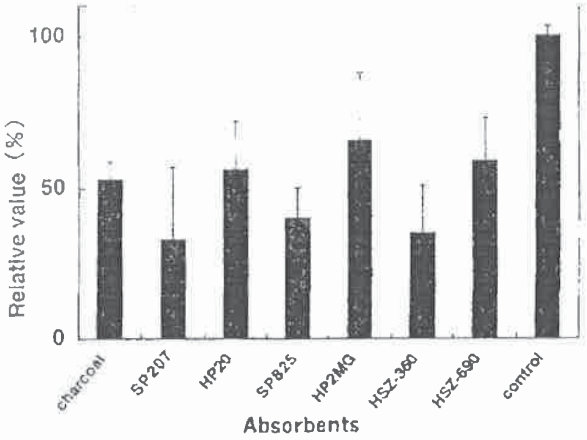

Figure 6. Removal of hexanal from the SPI solution by absorbents. Two grams of each absorbents were added to 40ml of the SPI solution(0.05g/ml). After stirring for 15 min at room temperature 2 ml of the mixture was centrifuged by 5,000xg for 10 min. Hexnal in the supernatant was determined

New food ingredients related to soybean processing

SOYAFIVE-S [6]

SOYAFIVE-S was prepared by Fuji Oil Co. Ltd. This ingredient mainly composed of dietary fiber of soybean was produced from soybean residue by heating in weak acidic conditions, extracting, refining, pasteurizing and spry-drying. The molecular weight of the main fraction of SOYAFIVE-S is about 55,000 consisting of long chain rhamnoglucan and short chain of homoglucans. The characteristics as ingredient are soluble in both cold and hot water with relatively low viscosity. The viscosity of this ingredient is lower than guar gum and pectin (Fig.7). Moreover, it has a strong adhesive property and antioxidative property, which prevents peroxidation of soyben oil by adding and the effects on antioxidation is higher than addition of Arabic gum (Fig 8).

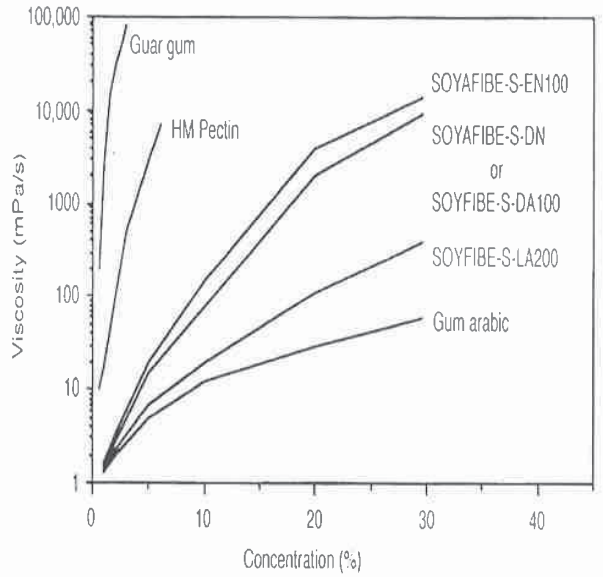

Figure 7. Viscosity of SOYAFIVE-S at 25

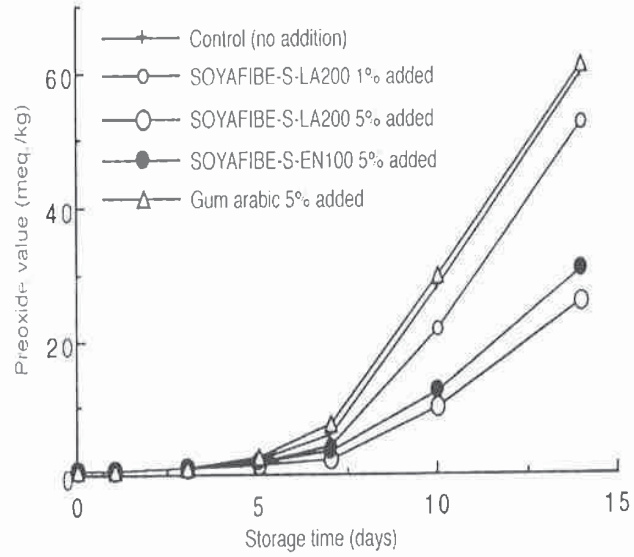

Figure 8. Antioxidative effect on soybean oil

The functional properties of SOYAFIVE-S such as soluble, stabilizing under acidic condition, emulsifying and emulsion stability, adhesion and film-making, dispersing and foam stability, anti- sticking, softening etc. are applied to many purpose.

Unicell protein

So called Unicell protein was prepared by Japan Cellfoods Co. Ltd. It was prepared by soaking soybeans in water, macerating, steaming and removing pectin substances between cells with enzymes. The products are composed of soybean cotyledonary cells. Chemical composition of the protein was almost similar to original soybean except a little less in ash content and extremely less in off-flavor such as hexanal or hexanol (Table 4). The products can successfully use for bread-making. Y.Yamori has used it in an epidemic search under Monaliza Research Programs[7].

Table 4. Chemical composition and flavor of Unicell Protein

	Moisture %	Protein %	Fat %	Carbohydrate %	Hexanal area	Hexanol area
Soybeans	10.9	35.3	21	29.2	5596	6392
%/g in dry		39.6	23.6	31.7		
Unicell(puree)	76.7	9.2	5.5	7.5	trace	trace
%/g in dry		39.5	23.6	32.2		

Activa supercurd

Activa supercurd was prepared by Ajinomoto Co. Ltd. as an enzymatic additive for tofu-making. Trans-glutaminase derived from microorganism was found and isolated by Ajinomoto Co Ltd. and Amano Co. Ltd. in 1989 [8),9)]. The enzyme enforces the stable gel-forming in hardness, springiness and consistency by making bridges in or between protein molecules. This special additive for tofu-making has been popularizing in these years and taking a role in diversification of tofu items. Moreover, the addition of Activa supercurd could improve the decrease in tofu gel-forming capacity of old crop.

Recent climate in Japanese market

A keen attention to healthy effects

With the background of a keen attention for health by Japanese, foods for special health use have been regulated under the Nutrition Law since 1993. The number of foods regulated including soyfoods. These foods have certified scientifically to improve intestinal disorders and to lower high

cholesterol levels. Isoflavon of soybean is not authorized in the Law, however tea analogues derived from soybean hypocotyls have been in Japanese market.

Diversification of soyfood items

Along with marked changes in life style, the major of home-cooking have been transferred to industrial preparation, where ambitious industries are trying to sell a wide variety of food items. Traditional or local foods are one of target and good health effect is another target. For instance, yuba (heat-induced film of soymilk) is recently becoming popular in Japanese market. The many food items are processed and sold as kinds of delicatessen.

The development of soybean processing in Japan has depended upon practical technologies by soybean industry. Our soybean industry has progressed and will continue industrialization and harmonization with Japanese tradition under the extreme changes in food system

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[10] http://www.health-station.co.jp/tokuho.htm

Potential of Ohmic heating and high pressure cooking for practical use in soy protein processing

A. Noguchi[1]

Abstract

Soybeans are used for manifold purposes; soybeans are not only a major source of edible oil, but also serve as a traditional food, and as food components such as TVP, meat extenders, and food modifiers, and as a source of peptides and fiber. Although the technologies to produce these products have been improved over time and at present have been nearly perfected, the demand of consumers for better products requires the development of sophisticated technology based on new principles. In Japan, soybean food manufacturers expect new technology to provide them with soybeans having better texture, smoother surface and the potential for more effective sterilization.

The recently developed methodologies of Ohmic heating and high pressure cooking have been the focus of strong interest on the manufacturers because of their broad range of applications in food processing. This paper focuses on the ability of Ohmic heating to achieve quick and linear heat increases and the possible versatile usages of high pressure cooking.

Introduction

Approximately 5 million metric tons (MT) of soybeans are imported into Japan annually. However, 4 million MT are consumed for purpose of edible oil production, resulting in the yearly accumulation of more than 3 million MT of defatted soybean. About 90% of this defatted soybean is used in livestock feed because of shortages of feed resources in Japan. According to the Food Balance Sheet (FY 2000, MAFF), domestic soybean production was less than 235 kilotons whereas the quantity of imported soybean was 4,829 kilotons. More specifically, the domestic consumption of soybeans in 2000 was 4.9 million MT in total and its breakdown was as follows (in kilotons): animal feed, 100; planting, 6; oil production, 3,721; waste products, 125; gross food, 814 and miso and soy sauce production, 196. In general, the consumption of soybean-based foods are considered to be linked to the principle foods (e.g., cooked rice) in Japan. The continuous

[1] Director of Research Planning and Coordination Division, Japan International Research Center for Agricultural Sciences (JIRCAS), 1-1, Ohwashi, Tsukuba, Ibaraki 305-8686/JAPAN, nog@jircas.affrc.go.jp

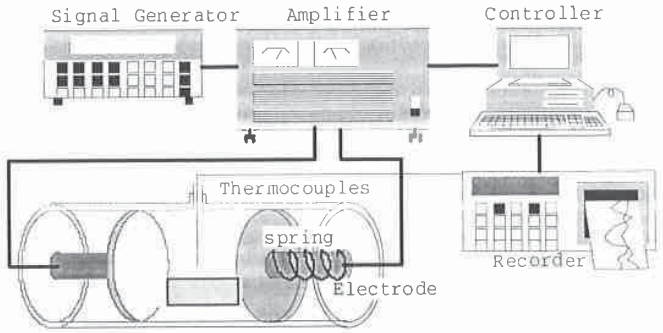
decline in rice consumption has thus resulted in a reduced demand for soybean foods. This trend is especially seen among young Japanese, due to wide-ranging changes in eating habits. Since the early 1980s, Japanese food markets have experienced a glut and food companies have expected efforts to diversify soybean products by formulating new ingredients such as meat protein substitutes and extenders and by improving the quality of final products. Soybean processing companies are eager to open new markets, and are thus putting a great deal of effort into food research on soybean-related foods. Recent research on the functionality of soybean foods and processing technology offers food companies encouragement to continue their efforts.

As aspects of this functionality will be taken up in the relevant session of this conference, this paper will focus on two recently developed technologies - Ohmic heating and high pressure cooking - and will discuss their potential for applications in the near future.

Ohmic heating

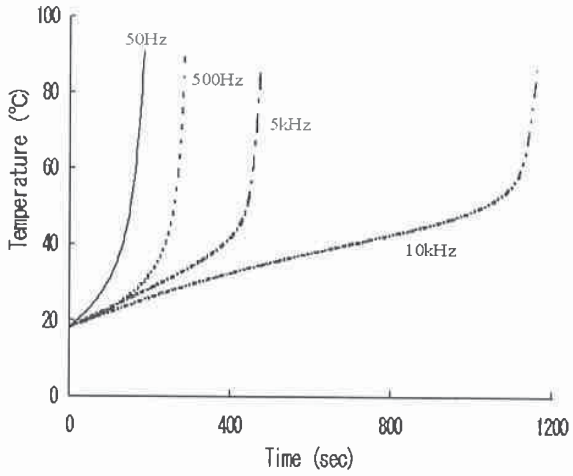
In general terms, the thermal processing of food products involves heat transfer into the food from hot surroundings, the driving force being the temperature gradient between the food products and its surroundings. Heat transfer in liquid foods mainly takes place by convection provided that the liquid is not too viscous. However, in processing particulate foods, the solid phase is heated only by conduction which is a much slower process than convection due to the lower thermal conductivity of most foods[1]. The quality deterioration of food products during thermal processing can be minimized by the High Temperature Short Time (HTST) process. However, there appears to be an upper temperature limit necessary to avoid

overcooking of the food product's surface. When food products contain sufficient water and Figure 1 Bread bodies prepared by electrolytes to pass electric current, Ohmic heating can be Ohmic heating for crumb making used to generate heat within the food products via the passage of an alternating current. The method enables the solid phase or viscous liquids to be heated as fast as thin liquids, thus making it possible to use HTST techniques on solid or viscous foods. One of the industrial achievements of this technology in Japan is the production of bread crumbs (Fig. 1). Companies claim that this method saves ob production costs by reducing the necessary heat energy and simplifying the overall system.



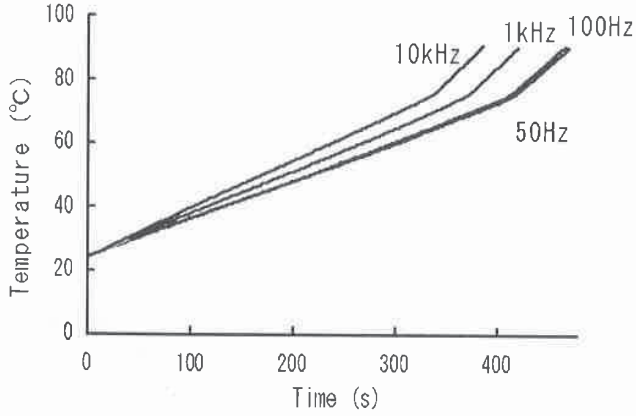
Solid food such as plant tissue is composed of individual cell units separated by cell walls and membranes. The major component of the cell membrane is phospholipids, and is regarded as an electrical condenser. Therefore, it is likely that the frequency of the alternating current becomes one of the key parameters for quick heating by reducing the impedance of plant tissue. On the other hand, the fish protein gel 'Kamaboko' prepared from Alaska pollack is reported to have a heating rate which depends on the applied frequency of alternating current and shows 7.5 times the heating rate at 10 kHz compared with that at 50 Hz, in spite of the fact that the gel does not have any membrane structure. These results suggest that the state of liquid components in the matrix will be also one of the key points on which quick Ohmic heating is based.

Imai et al.[2] extracted a cylindrical sample (30x30mm) from the middle of the radish, which was perpendicular to its vascular bundle, and applied a sine wave of variable frequency (50Hz-10kHZ) of 20-40 V/cm through a titanium electrode (Fig. 2). Of the frequencies examined, 50Hz yielded the sharpest initial rise of temperature and the shortest time for raising temperature at the mid-part of the radish to 80 C Figure 2. Schematic diagram of heating set-up Fig. 3). The heating rate above 60 C was linear



at all frequencies. Pressurization (400MPa, 25 C for 100min) of the radish eliminated the sharp initial increase of temperature observed at 50Hz, and yielded an almost linear rise of temperature similar to that seen above 60 C.

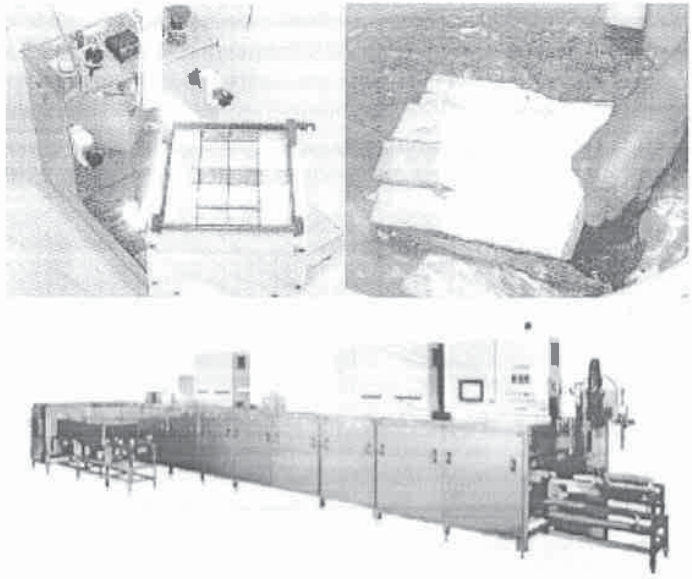
When the radish was treated with a square wave (10 ms with 10 ms interval) of 45 V/cm for 30s, its impedance decreased and never recovered even after storage at room temperature for 20 h. Upon treatment at 50Hz and 40 V/cm until its mid-part reached 30 C or was heated to 80 C in hot water and then cooled to 30 C, ^{1}H-NMR imaging analysis revealed more free movement of liquid components in the treated radish compared to the untreated radish. Figure 3. Center temperature profile These results



suggested that initial rapid heating during Ohmic heating at 40V/cm at low frequency is caused by the electroporation of the tissue membrane of radish, resulting in reduction of its impedance. The breaking strength of the ohmic-heated radish revealed that its original rigidity was maintained at its outer part even after heating. However, the radish cooked in hot water was wholly softened, especially in its outer parts.

Ohmic heating of an egg albumin solution (10%w/vol) was also examined by Imai et al. at 50Hz-10kHz under constant 10V/cm (Fig. 4). The heating rate of the solution was almost constant and increased slightly as the frequency increased. Gel formation was observed at about 75 C and the heating rate was rather increased above this temperature irrespective of frequency used. The solution and gel showed almost same impedance at the examined temperature (20-90 C) and frequency (10Hz-100kHz). When the concentration of egg albumin was reduced to 2%w/vol, this solution yielded no gel formation Figure 4. Heating rate of egg albumin solution and showed a constant heating rate at over 75 C during Ohmic heating at various frequencies.

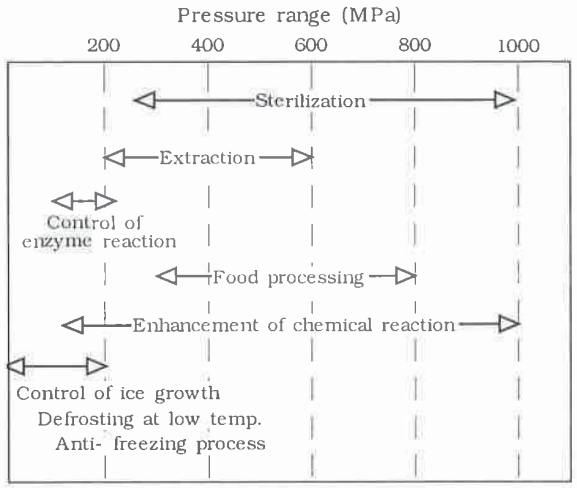
The breaking strength of the gels showed little difference between gels prepared by boiling water and those prepared by Ohmic heating. These results suggest that the liquid components are not compartmentalized in the gel and that the sudden increase of heating rate above 75 C is caused



by the reduction of heat transfer in the gel at the point of phase change into gel. Imai et al. also examined the effects of Ohmic heating on fresh egg white at the same conditions as that of the egg albumin solution. The fresh egg white did not show any sudden increase of heating rate until it reached 90 C. However, the homogenized fresh egg white and its soluble part separated beforehand, showing a slightly reduced heating rate and a sudden increase at about 60 C. It was concluded that that the gelatinous component of fresh egg white such as ovomucin repressed the transfer of heat generated during Ohmic heating.

These results clearly indicate that Ohmic heating brings about a quick temperature rise in plants and protein solutions. The major benefits of Ohmic heating can be summarized as follows: (1) is a continuous process; (2) allows rapid and uniform treatment of liquid and particulates, with minimal heat damage and retention time differences; (3) allows ease of process control with instant shutdown; (4) provides reduced maintenance costs; (5) is an environmentally friendly system, and (6) serves as an ideal process for shear0-sensitive products. Some Japanese tofu manufacturers have become interested in these characteristics of Ohmic heating and are starting to examine its potential for tofu making. Yanagiya Machinery Co., Ltd has commenced sales of a tofu making machine (Fig. 5) utilizing Ohmic heating, emphasizing its easy control and quick and homogenous cooking abilities. Hokuriku Electric Power Company has also started selling a continuous system having commercial-level putput.

Lijun et al.[3] confirmed by DSC that ²-conglycinin (7S) and glycinin (11S) exhibit denaturation temperatures of (70 ± 2) C and (90 ± 2)C respectively with solid contend of soymilk (5.76%^ÿ10.37%). They applied Figure 5. Ohmic heating apparatus and the resultant two-step Ohmic heating (70 C for 10 min and tofu (above); continuous system (below) then 100 C for 5 min) to soymilk and coagulated the soymilk with $CaSO_4$ to obtain tofu. They then examined its hardness, firmness, yield and water- holding capacity compared with tofu prepared by single-step heating at 100 C for 5 min. They found that two-step heating always yielded better values for all of these parameters than did single heating. This phenomenon is explained as follows; The dissociation of 7S and 11S globulin can occur at the same time under one-stage heating and then associated with each other in a random fashion. In the case of two-stage heating, 7S globulin dissociated into its subunits at the first heating stage, and at the second heating stage, these 7S sub-units and newly-dissociated 11S sub-units associated cooperatively. These resultant moderately-associated protein units result in a more firm and uniform tofu.



High pressure cooking

Methodologies utilizing pressure in food processing have received less attention than thermal processing technologies. This is because the necessary test apparatuses for food research were developed at a relatively late point in time. In addition, until present, high pressure ranges have hardly any relevance to food processing. However, recent advances in equipment are expected to make this technology more feasible for food companies' purpose, with the background that pressure can be momentary and equally applied to any form and composition of food without leaving any physical damage or any pressure energy after its release. The methodology applies hydrostatic Figure 6 Pressure range for the applications pressure of several hundred MPa to foods for purposes of sterilization, protein denaturation, control of enzymatic and chemical reactions, homogeneous defrosting at low temperature, and so forth. High pressure cooking is based on cold isostatic pressing (CIP) originally developed for ceramics process and is achieved by applying high pressure to the foods via the surrounding water or other liquids.

Sterilization accomplished through heat treatment is one of the most important processes utilized in food manufacturing. Depending on the components of the materials handled, however, all possible means must be tried in order to avoid undesirable changes and loss of essential components during thermal processing. For example, there are high-grade types jams that contain a fairly high proportion of strawberry meat which retain their original form. Because of high viscosity, homogeneous and

quick heat transfer in the jam could not be achieved, and thus such jams must be stirred well for long periods during thermal sterilization. However, longer stirring and heat treatment causes damage to strawberry meat, especially in terms of color and flavor.

High pressure sterilization does not cause thermal degradation and losses of heat sensitive components (vitamins, color pigments and aromatic components). High pressure equally sterilizes the inside and the surface of foods, regardless of shape, and also reduces to one-tenth the energy necessary in usual heat treatments. Many researchers believe that high pressure affects proteins and cell membranes of microorganisms. It is likely that proteins are transformed at $\geq$ 300MPa and cell membranes are forced to change in terms of physical state from liquid to solid at $\geq$100 MPa due to the increase of the melting point of phospholipids under higher pressure. Generally, it is thought that the primary site that undergoes high pressure damage is the cell membrane of microorganisms.[4]

It should be noted that the necessary level of high pressure varies depending on the composition and pH of the target food. Higher concentrations of sugars, salts and amino acids reduce the effects of high pressure on foods. Furthermore, the amounts and distribution of free water are key for achieving proper sterilization using high pressure. Recent research revealed that microorganisms become more sensitive to high pressure at relatively higher (60-80 C) or lower (>-20 C) temperatures. Some spores could germinate under high pressure of about 100 MPa and are inactivated at intermittent pressure of about 600 MPa. Hayakawa et al.[5] found that the oscillatory pressurization was more effective against *B. stearothermophilus* spores than was the continuous process. However, the germination process is still unclear and not all spores germinate by applying pressure.

It was stated that Bridgman[6] was the first to carry out research on protein denaturation using high pressure. In Japan, Suzuki and Suzuki[7,8] have performed a number of studies on proteins, demonstrating that protein denaturation occurs at 300 MPa, and that at above 700 MPa, the changes became irreversible. Recent studies on high pressure cooking have been expanded to cover fish protein[9], sea foods[10] and egg protein[11]. Honma examined the binding of fresh meats with egg white at 500 MPa at 20 C for 10 min and found out that the prepared meats did not separate from each other even after cooking at 200 C. Pork meat could be processed by high pressure in order to formulate new way of making products such as raw ham. Raw ham prepared in this way was highly evaluated in taste tests.

Kajiyama et al[12]. applied high pressure (<500 MPa) to soymilk and examined how its proteins change. The soymilk remained liquid within the range of

the examined pressure and its viscosity increased when the time of pressurization was less than 10 min. However, treatment at 500 MPa for 30 min solidified the soymilk. Soymilk showed better emulsifying and stability properties but had poor capacity. Additionally the content of SH residue in soymilk increased slightly under anaerobic pressurization. Hard-type tofu could be made from the pressurized soymilk using $CaCl_2$. Electrophoresis and isoelectrofocusing techniques revealed that soy proteins dissociated while some of them coagulated by high pressure. Fluorescence analysis also showed that soy proteins were modified by high pressure, having larger hydrophobic regions. After pressure treatment, soymilk showed higher affinity to beany flavor components and saponin which would lead to improved usages of soymilk in soy foods.

High pressure cooking can be characterized as follows: (1) the transfer of high pressure is spontaneous and does not depend on the shape of materials; (2) high pressure cooking alleviates the problem of mechanical deterioration of materials caused by agitation required to achieve homogeneous and quick heat transfer; (3) high pressure can be maintained by the mechanical methods and economizes energy required for cooking; and (4) the release of high pressure can be achieved instantly, which makes the cooking process controllable.

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Soybean isoflavones and food processing

M. I. Genovese[1]

Abstract

Isoflavones are present in significant amounts in soybeans. Three different isoflavones were identified in soybeans and soy products: daidzein, genistein and glycitein, and these are normally present as conjugates (7-O-β-glycosides): malonylglycosides, acetylglycosides and desesterified glycosides (daidzin, genistin and glycitin). The isoflavone content of soybeans differs between varieties and also for the same variety cultivated in different regions. Soybeans are mainly used for obtention of the vegetable oil, and the resulting defatted flakes or flours are mainly destined for animal feeds but also for the production of soy protein products (defatted flours, soy isolates, soy concentrates and textured products), whose functionality makes them important ingredients for several food products. Isoflavone content varies between them and can even vary for the same product as it depends on the isoflavone content present in raw soybeans and on the kind of processing used for its obtention. The differences observed on isoflavone distribution among soy protein products seem to be a result of the kind and degree of thermal heating to which they are subjected during processing and also of the action of exogenous and/or endogenous β glycosidases. There are no reports in the literature about the occurrence of thermal degradation of isoflavones.

Soybean isoflavones and food processing

Plants have compounds which are able to cause an oestrogenic response in animals, and consequently these compounds are denominated phytoestrogens. The most common phytoestrogens are isoflavones, lignans and coumestans (Price & Fenwick, 1985).

The isoflavones can be found in legumes, but only in soybeans are present in significant amounts. Three isoflavones were identified in soy seeds and derived products: daidzein, genistein and glycitein. Their oestrogenic activity is associated to the stilbene-like diaryl core containing a minimum of one hydroxyl group on each of these aryl rings, as those present as substituents in the 4' and 7 positions (Dodge, 1998). Isoflavones are normally found as glycosyl-conjugates (7-O-β-glycosides): malonylglycosides, acetylglycosides

[1] Prof. Dr. do Departamento de Alimentos e Nutrição Experimental Fac. Ciências Farmacêuticas - Universidade de São Paulo genovese@usp.br

and desesterified glycosides (daidzin, genistin and glycitin) (Liener, 1994), and their structures are shown in *Figure 1*.

Isoflavones levels are variable across the seed. They are found in high concentrations in soy hypocotyls (1.4 to 1.8 g/100 g), but, although in significant lower amounts, they are also present in soy cotyledons (0.16 to 0.32 g/100 g) and soy hulls (0.01 a 0.02 g/100 g). Considering the seed as a whole, as the cotyledon corresponds to 90% of the seed and the hypocotyl to only 2%, the main contribution in terms of isoflavones is from the cotyledon. The distribution of the isoflavones found in cotyledons and hypocotyls is also different. In soy hypocotyls there is a predominance of daidzin and glycitin, while in cotyledons there is a genistin amount 20 times higher than that of hypocotyls and absence of glycitin (Eldridge & Kwolek, 1983).

The increasing interest in these compounds is the result of several epidemiological studies showing a lower incidence of breast, prostate and colon cancer in Asian populations, where soy consumption *per capita* is 20 to 50 times higher than in occidental populations. In addition, studies in cell cultures, animal models, and some clinical assays in humans indicated that isoflavones would have also effects related to prevention and diminution of tumors not hormone-dependents, protection against cardiovascular diseases, lowering of blood cholesterol levels, benefits on osteoporosis treatment and reduction of menopausal symptoms (Setchell & Cassidy, 1999; Tham *et al.*, 1998; Anderson & Garner, 1997; Knight & Eden, 1996).

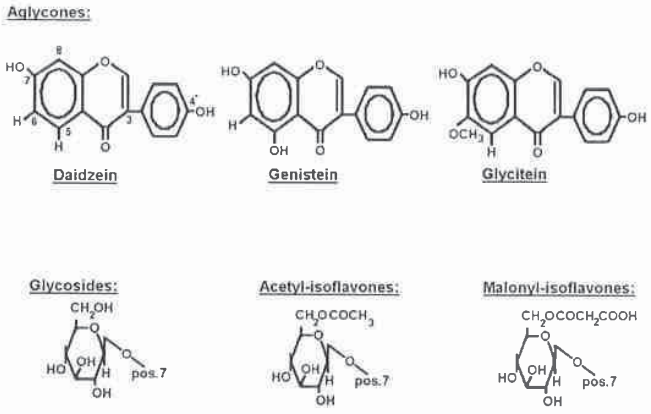

Figure 1. Chemical structure of soy isoflavones.

Soybeans are present in our diet through consumption of the seeds and of derived foods made from them, such as tofu, miso and tempeh. However, Brazilian people traditionally do not consume soy seeds and these foods are more characteristic of oriental diets. Soybean oil, which is largely used in Brazilian dishes, does not have isoflavones in its composition.

The isoflavone content of soybeans differs between varieties and also for the same variety cultivated in different regions. In addition, the same variety cultivated in the same region may present variations in isoflavone contents according to the crop year, due to climatic and environmental factors (Eldridge & Kwolek, 1983).

The determination of the isoflavone content of 15 different cultivars of soy seeds from the Parana State showed that cultivars from Ponta Grossa presented a 31% higher isoflavone concentration (average 120 mg/100 g) in comparison to the cultivars from Londrina (average 82 mg/100 g). These differences were attributed to differences in soil composition and temperature between the two regions (Carrão-Panizzi *et al.*, 1998). It was observed a high variation in isoflavone levels among the varieties analyzed, from 54 to 147 mg/100 g.

Soybeans are mainly used for extraction of the vegetable oil, and the resulting defatted flakes or flours are mainly destined for animal feeds but a minor part is also used for the production of soy protein products (defatted flours, soy isolates, soy concentrates and textured products), whose functionality makes them important ingredients for several food products. Among functional properties of soy proteins we can mention the water holding capacity, emulsification properties and gelation.

Soy protein products are prepared from the flour or flakes remaining after oil extraction and are represented by the defatted flours/flakes, concentrates, isolates and textured proteins, these last resulting from the extrusion of anyone of the three protein products but most commonly from the defatted flour. Soy protein products are used in several industrialized foods, such as meat products, dressings, soups, and bakery products. Each one of the soy protein products has specific applications which can be even increased by partial hydrolysis.

The protein content of these products is of about 50% for the flours, 75% for concentrates and 95% for protein isolates.

Isoflavone content varies between them and can even vary for the same product as it depends on the isoflavone content present in raw soybeans and on the kind of processing used for its obtention (Genovese & Lajolo, 2001b).

Seo & Morr (1984) found the following isoflavone levels in soy defatted flours: 596 µg/g of daidzin, 2150 µg/g of genistin, 56 µg/g of daidzein and 67 µg/g of genistein. For soy protein isolate they reported 134 µg/g of daidzin, 672 µg/g of genistin, 56 µg/g of daidzein and 103 µg/g of genistein.

Wang *et al* (1990) determined isoflavones only in the form of aglycones and reported their results as free and total isoflavone contents (after acid hydrolysis of the glycosylated forms). The values found were (in µg/g, DW):

- soy seeds 1: daidzein 26, total daidzein 331, genistein 28, total genistein 462.
- soy seeds 2: daidzein 14, total daidzein 507, genistein 17, total genistein 680.
- defatted soy flour: daidzein 125, total daidzein 625, genistein 59, total genistein 744.
- tofu: daidzein 84-94 , total daidzein 168-256, genistein 60-93, total genistein 288-392.
- soy milk: daidzein 22, total daidzein 130, genistein 29, total genistein 92.
- shoyu: daidzein 24, total daidzein 24, genistein 18, total genistein 14.
- soy sprout: daidzein 24, total daidzein 1334, genistein 0, total genistein 1458.

Coward *et al* (1993) reported free and glycosylated isoflavone content for several soy products (in µg/g, DW):

- defatted soy flour: daidzein 33-44, daidzin 1110-1160, genistein 34-53, genistin 1318-1448.
- soy protein isolate: daidzein 73-102, daidzin 232-278, genistein 114-189, genistin 430-589.
- alcohol-extracted soy concentrate: daidzein 4-45, daidzin 64-102, genistein 4-69, genistin 87-227.
- water-extracted soy concentrate: daidzein 39, daidzin 1180, genistein 33, genistin 1404.
- soy fiber: daidzein 85, daidzin 141, genistein 114, genistin 154.
- tofu: daidzein 77-113, daidzin 591-1513, genistein 151-116, genistin 1215-2087.
- soy milk: daidzein 141, daidzin 1337, genistein 98, genistin 1680.
- shoyu: daidzein 54, daidzin 0, genistein 36, genistin 0.
- soy cheese: daidzein 1, daidzin 43, genistein 5, genistin 57.

The conditions used in soy processing can cause alterations both in isoflavone level and distribution. It was reported that 97 to 98 % of the total content of isoflavones in soy protein products are in the esterified form. The distribution between these forms, however, varies from product to product (Wang & Murphy, 1994). Results obtained in our laboratory showed that in soy protein isolates there is a drastic reduction of the percentage of β-glycosides in parallel to an increase in aglycones, when compared to defatted flours.

Soy protein concentrates present very low isoflavone contents, as they normally correspond to the residue resulting from alcohol extraction of the defatted flours. When the concentrates are prepared through water extraction in acid pH, however, much higher isoflavone contents are found (Coward *et al*, 1993).

Textured soy protein is commonly prepared through the extrusion of the defatted flour. This kind of processing can lead to a higher concentration of acetyl-conjugates and desesterified glycosides, in relation to aglycones and malonyl-conjugates (Wang & Murphy, 1994; Coward *et al*, 1998).
A *Figura 2* mostra as reações de interconversão das diversas formas das isoflavonas encontradas na soja e nos derivados protéicos. No hipocótilo e cotilédone predominam malonilconjugados e β-glicosídeos desesterificados, sendo que durante o processamento para a obtenção dos derivados protéicos de soja formam-se acetilconjugados e agliconas.

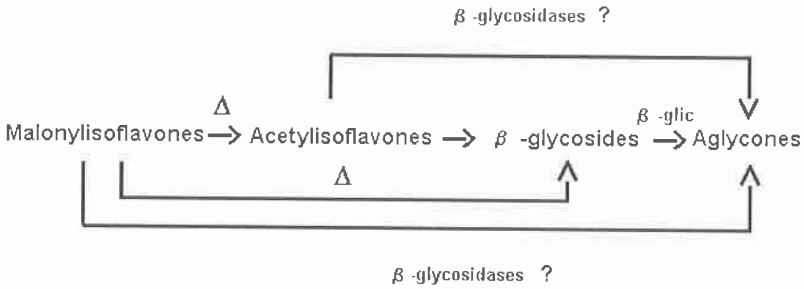

Figure 2. Interconversion reactions of soy isoflavones.

Thermal treatment can cause decarboxylation and deesterification of malonyl-conjugates with formation of acetyl-conjugates and β-glycosides, respectively. Moist heat seems to favor the conversion to βglycosides, while dry heat would cause loss of carbon dioxide from malonyl-conjugates leading to formation of acetyl-conjugates, as it is observed during extrusion, frying and toasting (Coward *et al.*, 1998). Fermentation used to produce miso and tempeh causes a loss of the glycosides to form the aglycones by action of fungal β-glycosidases.

In conclusion, the differences observed in isoflavone isomers distribution in soy products seem to be the result of the kind and extension of heating to which the food is submitted during its preparation and of the favoring of the action of endogenous or exogenous β-glycosidases. There are no conclusive reports in the literature about thermal destruction of isoflavones. The decrease of isoflavone contents observed for some extruded products can be associated to a loss in solubility caused by the process instead to

thermal degradation. The decreased solubility would result in a lower extraction efficiency and as a consequence in a subestimation of isoflavone amounts.

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IV INTERNATIONAL SOYBEAN PROCESSING AND UTILIZATION CONFERENCE

MARCH 4

Utilization-applications of by-products from processing soybeans

J. P. Cherry[1]

It is well known in the United States, and for that matter, worldwide, the goals for the agricultural commodity, soybean, are to strengthen development of new food and non-food or industrial products, including ingredients or co-products, for specialty applications. In addition to increasing use in nutritious food products, soybean ingredients are gaining importance as sources of phytochemicals, or nutraceuticals, for reduced risk of coronary heart disease, selected cancers and osteoporosis. The non-food products being developed are biodegradable, environmentally friendly, and defined in the realm of "green chemistry." This is being done to reduce/eliminate developing surpluses of soybeans occurring because of expanding world production of this agricultural commodity. For example, production in South America, specifically, Brazil, Paraguay and Argentina, is expected to be equivalent to that in the U.S. in the next few years. Hence, emphasized is that each newly discovered use, considered as a significant breakthrough should increase utilization of U.S. soybeans by requiring 10 million bushels or more per year. It is an accepted fact that the potential for discovering and implementing new uses for soybeans is virtually unlimited. This presentation, although a main focus on the advances of non-food or industrial uses of soy products and co-products, also emphasizes new developments for by-products from soybean processing as feedstocks, especially functional foods or nutraceuticals.

The U.S. soybean check-off program, whereby farmers pay a portion of their earnings to provide incentives to identify and develop new soybean products and ingredients for selected commercial applications, and creates research and technology transfer partnerships among Federal and State governments, academia and big and small industries, their support agencies and entrepreneurs, is proving to be very successful. The emphasis in the non-food or industrial products market is on biodegradable adhesives, plastics, coatings, inks, lubricants, biofuels and specialty products, such as solvents. Strategies to competing in the domestic and world markets include analyzing markets for potential needs, developing and testing new products to fit those needs, identifying opportunities and potential partners

[1] Eastern Regional Research Center – Agricultural Research Service – U.S. Department of Agriculture – 600 E. Mermaid Lane– Wyndmoor, Pennsylvania 19038

and monitoring industry trends and regulations that may affect market areas. Research efforts and development of the resulting technologies are expanding processing of quality soybean products and ingredients and moving them into the marketplace.

Especially noted is that advances in characterizing the physicochemical properties of molecular components of soybean with the aid of sophisticated research instruments and processing equipment are leading to new structure-function properties of ingredient and product performances. This includes characterizing the chemical properties of soy oils and proteins and relating them to changes that occur because of breeding programs or genetic development, growing-seasonal variations, agronomic factors, and processing parameters. The restructuring of separated and modified triglycerides of oils and polypeptides of proteins with unique properties and chemical-enzymatic modifications of components to enhance functionalities, such as foaming, emulsification, film and adhesive properties, are enhancing continued development of new uses.

Soy products and co-products

The composition of commercial soybean, on a moisture-free basis is 40% protein, 21% oil and 34% carbohydrate. Figure 1 presents the products, co-products and by-products processed from soybean. The main commercial products from soybean processing, via solvent extraction, include oil and meal (44-48% protein). From the meal, a variety of high-protein co-products are made including chips, grits, full-fat flakes, fat-free flakes, fat-free flour, texturized flour, protein concentrates, texturized protein concentrates, protein isolates and texturized protein isolates. The meal, because of its excellent amino acid composition, ease of digestion and competitive price, is mainly used as an animal (food animals, pets), poultry and aquaculture feed supplement. All of the other protein co-products are used in the food and non-food industries as functional ingredients. As food ingredients, the added incentive is the favorable nutrient profile, phytochemicals or nutraceutical content, for heart health, lowering symptoms of menopause and osteoporosis, reducing cancer, allergies and diabetes and kidney disease. The role of selected nutrients, e.g., isoflavones, vitamin E and essential amino acids are contributing to these health benefits. The result has been a "Daily Soybean Guide Pyramid."

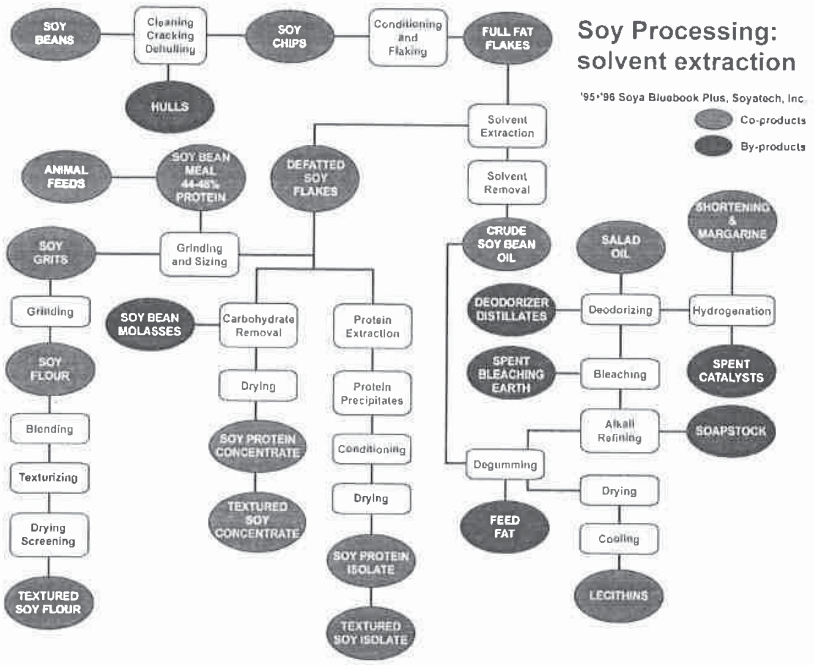

Figure 1. Products, co-products and by-products processed from soybean.

Much of the oil is used for edible applications such as salad oils, dressings, shortenings and margarines. The oil is increasingly being used as biofuels or biodiesel and in lubricants and cleaning agents, etc., because production is greater than use, causing surpluses. Lecithin, once defined as a by-product of crude oil processing, has grown in use and value to become a co-product of soybean processing. It is a valuable ingredient primarily a mixture of phospholipids, including phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol and phosphatidic acid. These components have outstanding functional properties when applied to food, non-food and feed products. The pharmaceutical and cosmetic industries have benefited greatly from this ingredient. As one company advertises, we don't make the many products so important to our daily lives, we just make them better. What makes the lecithin components so useful are their unique lipophilic and hydrophilic properties.

The success of research developments and transfer of technologies has led to a vast array of soybean products and ingredients used by the non-food or industrial uses stakeholders. This has proven to be so great that a

catalog and website are now available presenting soy products. In 2001, these references had over 300 non-food products and non-food products with soy ingredients, sold by over 110 companies. Today, these counts are over 400 and 250, respectively. The products are placed into three groupings as follows:

1. Consumer Products – used everyday by industries, around the home and yard, in the car, etc.; the products, sold by companies in stores, are generally packaged in sizes suited for everyday use. They are:

- All Purpose Lubricants and Greases – environmentally safe, biodegradable for industrial, shop and home uses, to penetrate and loosen rusted or frozen parts; provide coatings on metals and plastics and on auto, truck and farm machinery; and greases for wheel axel bearings.
- Alternative Fuels – methylesters of fatty acids, biodiesel, for diesel powered engines, including for sail boats, trawlers, dredges, commercial ships, research vessels, farm tractors and equipment, mining equipment, and inner-city transportation vehicles. The agencies of the U.S. government, e.g., Agricultural Research Service and the U.S. Coast Guard Fleet, are using biodiesel blended diesel fuels in diesel-operated vehicles.
- Auto Polishes – ingredients for vehicle waxes.
- Bar Chain Oils – biotechbased bar and chain saw lubricants, especially forest cutting operations.
- Building Materials, Composites – decorative interior wood and plastic for housing and offices, biocomposites with unique and elegant appearances combined with the warmth, feel and look of exotic hardwood panels.
- Candles – cleaner-burning than traditional candles with no sooty residue and a lower melting point, burning cooler.
- Carpets – Soy-urethane-backed carpeting, used by federal government offices.
- Cleaning Products, Polymers and Dispersants – all purpose cleaners or cleaning solvents for machinery, carpet and upholstery shampoo; fruits and vegetable washes, dishwashing liquid, laundry additive/detergent/stain remover; anti-allergen spray against household dust; car, truck and tractor washes; craft paint and adhesive removers; soy oil-based surfactants with natural enzymes.
- Coatings – concrete, brick, wood and fiber cement siding stains.
- Crayons – brighter with no waxy build-up, flaking or smearing products.
- Diesel Additives – boosts fuel economy, cleans injectors, increases lubricity, extends engine life and reduces emissions.
- Furniture – biocomposites made with soy resins.
- Hair Care – provides nutrients to hair without coatings.

- Hand Cleaners – cleans without abusing skin, and waterless grease-cutting hand cleaner.
- Hydraulic Fluids – farming, mining and construction equipment.
- Motor Oils – gives qualities of high performance motor oil and biodegradability properties.
- Paint Strippers and Gels – removes paints, shellacs, varnishes, inks, crayons, magic marker and asphalt tar from a variety of surfaces including brick, wood, metal and cement.
- Personal Care – hand and body wash/lotion, skin cleanser, moisturizing lotion, sun screen and shower gel.
- Soy-based Adhesives – finger-jointing adhesive system creates finger-jointed wood products for dimensional lumber, I-joints, and window and door parts.
- Soy Polyol – used in both rigid and flexible foam applications in every day plastic applications from insulation and flotation to furniture padding and carpet backing.
- Two-Cycle Engine Oils – home yard tools such as mowers, weed wackers, garden plows, etc.
- Wax – seals container surfaces and used for scented candles and wax fire starters, renewable fire logs for fireplaces, camp or wood stoves and lubricant wax esters for transmissions.

2. Ingredients/Intermediates – as not finished products, these are chemicals and ingredients used by manufacturers in making finished products, sold by companies in bulk quantities.

- Industrial Oils – refined, bleached and deodorized oils for paints, varnishes, alkyd resins, epoxidized oils, pharmaceuticals, printing inks, pesticide carriers and methyl esters.
- Industrial Proteins – gives functionality to skin, hair and sun care products; used as rheology modifiers, co-binders and coating agents in papers and paperboard.
- Industrial Solvents – cleaning agents and degreasing processes.
- Lubricant Base stocks – >80% oleic acid and unique proprietary fatty acids as specialty ingredients.
- Surfactants/Emulsifiers – lecithin and emulsifiers for specialty functional properties.
- Wax – hydrogenated methyl esters for specialty applications.

3. Institutional/Industrial Products – used by companies in construction and by institutions like schools, hospitals and farms, packaged in large quantities for multi-purpose applications depending on the user's needs.

- Agricultural Adjuvants – sticker/spreader and encapsulant for herbicide/insecticide/fungicide applications.
- Concrete Supplies – concrete sealer, soap and water clean-up, release agent for concrete forms.
- Dielectric Fluids – fluids with fire resistant properties for transformers.
- Dust Suppressants – dust control agents.
- Fuel Oil Emulsifiers – for easy burning.
- Hydraulic Fluids – lubricants.
- Industrial Cleaners – oil-based asphalt release agent, shoreline clean-up agents, grease removers, laundry detergents, dish soaps and household cleaners.
- Industrial Lubricants – pump oil, gasoline and diesel engine lubricants, railroad switches, gear head oils, turbine lubricants, drilling lubricants and wire rope lubricants.
- Metalworking Fluids – friction control during hard metal cutting such as stainless steel.
- Odor Reduction – manure pits – cattle, poultry and swine housing/contaminant, water/sewer treatment and paper pulp processing.
- Paint Strippers – graffiti remover.
- Printing Inks – newspapers, magazines, etc.
- Printing Supplies – removes ink haze, scuz and ghost images.
- Saw Guide Oils – lubricants for smooth even cuttings in carpentry work.

Soy processing by-products – new resources

The products and co-products, including ingredients and their applications are the major moneymakers of processing soybean for the marketplace. During processing of products and co-products, by-products are produced. They are hulls from the cleaning, cracking and dehulling step, molasses during preparation of protein concentrates, and refining by-product lipid, soapstock as a result of the degumming of crude oil. Also produced as by-products from deodorizing and bleaching steps of salad oil processing are deodorizer distillates and spent bleaching earth, and materials from the hydrogenation step for shortening and margarine, spent catalysts. These by-products are natural compounds and their use or disposal has been a challenge to the oilseed processing industries. A number of these by-products are returned to the land as fertilizers or soil modifiers, fed to animals, poultry or fish as nutrients, burnt for energy or applied to value-added conversions. Noted is that they are rich in carbohydrates, mainly fiber, proteins, lipids, lignins and, especially phytochemicals. Research studies are ongoing, and more is needed, to utilize these by-products as value-added co-products, similar to what was successfully done with the once called by-product, now a co-product, lecithin.

Soybean hulls, a by-product of the seed crushing process and made up of cellulose, hemicellulose, lignin, protein and ash, can be converted to valuable co-products. They can be used as a replacement (up to 10%) for forage fiber (hay) in feed, especially dairy cow rations to increase milk yields. Other work showed that soy hulls contain 86% complex carbohydrates, including 30% pectin, 50% hemicellulose and 30% cellulose, making them a source of dietary fiber. When soy hulls are included as a fiber supplement in bakery products in human nutrition feeding studies, blood serum cholesterol is lowered. Other efforts showed that grinding soy hulls to fine particulate matter, and treating them with citric acid makes them excellent metal absorbents, especially copper ions, for cleaning waste streams. Another component of soy hulls is the enzyme, peroxidase. This enzyme has been shown to be heat stable at 80°C, pH 5.0. It may be effective in organic solvents for industrial applications.

An ethanol extract, defined as a phytochemical concentrate (PCC) of the soy molasses by-product was shown to repress genomic DNA elastogenic damage and point mutations in mammalian cells. Identified in the PCC were isoflavones, genistein, genisten, daidzein and daidzin. These compounds expressed a wide range of growth suppression of selected human colon cancer cells. For example, epidemiological and biological studies support the role for genistein in prevention of prostate cancer. The research is leading to the novel isolation of chemoprotectants from another of the commercial soy processing byproducts.

Because there is so much processing of soybean oil, high quantities of the byproduct, deodorizer distillate or sludge are produced. This product, also referred to as scum oil, comes from the refining steps of deodorizing, bleaching and refining. The sludge is composed of a complex mixture of aggregated compounds, tocopherol (vitamin E), sterol esters (campestene, ^{2}-sitosterol), squalene and free fatty acids. The tocopherol, the basis for the demand to process this byproduct, can be used by the vitamin market and as nutraceuticals, sterols, as feedstock for hormone production, squalene, a high carbon source for cosmetics and cholesterol biosynthesis and fatty acids, for biodiesel. Supercritical CO_2 extraction methods have been shown to be economically feasible for preparation of tocopherol from the deodorizer distillate.

Spent bleaching clay from oil refining contains substantial amounts of absorbed oil. This by-product is both a problem, being prone to spontaneous combustion, and a source of recoverable oils. The oil can be recovered by four treatments, steam, aqueous, solvent or pressure extraction. The spent bleaching clay has been tested as a low level (0.5-2.0%) feed supplement, pellet binder and metabolizable energy) for poultry diets.

The crude oil is refined for edible oils (salad oil; and from which shortenings and margarines are produced) by a degumming process. One by-product produced is fat used for animal feed. Further refining produces the byproduct, soapstock. Soapstock, or refining by-product lipid results from crude oil mixed with dilute sodium hydroxide solution. This limited value by-product is a mixture of free fatty acids, phosphatides and unsaponifiable materials. It is mixed with fertilizers and can be returned to the meal to increase weight and fat content for animal feed. Additionally, the soapstock is added to feed for dust control, appearance, ease of handling and improved pelletability. In recent years, work has developed new approaches to processing soapstock with acid and steam to separate it into two phases, an aqueous and an oil layer. The latter contains free fatty acids, acylglycerols, pigments and lipophilic materials, and is termed acid oil. It is used as an animal feed ingredient and as a new feedstock for biodiesel production.

Spent catalysts result from the use of nickel and clay support in the hydrogenation of fats and oils. The recovered material contains nickel, clay and residual oil, and filtration media used to aid in separation of the physically fine spent catalyst. Depositing nickel in landfills, where most of it goes, is becoming an environmental concern. Incineration is being used as one way to recycle nickel into the stainless steel industry while generating electricity from the released energy. Research studies are needed to characterize spent catalysts, especially the organic fraction to further the value of this by-product.

The soybean continues to earn its worldwide importance and names as "yellow jewel," "great treasure," "nature's miracle protein," and "meat of the field." This agricultural commodity is an accepted worldwide food and feed resource. The ability to use processed soybean products and ingredients in many applications as biodegradable substitutes for petroleum-based products is a rapidly growing industry. The ability to use the by-products as biodegradable substitutes further expands this already excellent opportunity now developed. Moreover, selected soybean ingredients rapidly growing in importance, now found to be rich in by-products, are gaining recognition as major nutritional sources of nutraceuticals or functional foods.

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Processing and modification of soy proteins for non-food applications

M.P. Hojilla-Evangelista; D.J. Sessa and J.L. Willett[1]

Introduction

The United States is the world's leading soybean producer and exporter. In 2001-2002, soybean production in the U.S. was approximately 74 million metric tons, with a farm value of $15.0 billion (Economic Research Service, 2003). Approximately 90 percent of total oilseed production in the U.S. is based on soybeans. After crushing for oil production, some 35 million metric tons of soybean meal are generated annually in the U.S. The primary use of soybean meal is animal feed. With a protein content of approximately 50 percent, soybean meal provides a relatively low-cost raw material ($0.30/kg) for the development of new biobased products.

At the U.S. Department of Agriculture's National Center for Agricultural Utilization Research in Peoria, IL, as part of our biobased products research program, we have been developing new uses for soybeans, focusing primarily on protein-based materials. Project areas include use of soy proteins in foamed plywood adhesives and protein-based metal-binding agents.

Soybean protein-based plywood adhesives

Various approaches to soybean-based plywood adhesives have been reported. None of these methods involve foam application of glue. Foam extrusion represents 10% to 20%, or 90 to 180 million kg/yr, of total U.S. domestic consumption of phenol-formaldehyde resin for plywood use (Sellers 1985). Of this, protein extender accounts for approximately 5% of the total weight. Therefore, total substitution of soybean protein for animal blood protein would use approximately 10 million kg/yr.

Foam extrusion is a method of applying glue to plywood. The glue is foamed with air and extruded as strands onto the plywood such that the entire veneer surface is covered when pressed. One advantage of the foam application process is the significant reduction in water content – 20% compared to 60% in typical plywood glues. Other advantages include reduced sensitivity to veneer moisture, more uniform coverage, and lower gluing costs. There are also fewer variables in the glue mixing process, so production is more consistent.

[1] Plant Polymer Research Unit, U.S. Department of Agriculture*, Agricultural Research Service National Center for Agricultural Utilization Research 1815 North University Peoria, IL 61604 USA.

Typically, animal blood proteins have been used as extenders in foamed plywood glues. Increasing concerns have been raised over the handling of animal blood particulates, especially as they may contain disease agents or pathogens. There is also evidence that animal blood degrades rapidly. In addition, the number of suppliers in the U.S. is limited. Soy protein provides an alternative to animal blood proteins in plywood glues. Soy protein is available in a wide range of forms with different protein levels, and is often cheaper than animal blood at present ($0.48/kg for soy flour vs $0.88/kg for spray-dried animal blood protein). Soy proteins are also known to have excellent foaming properties.

Soy protein sources reported here include Honeysoy 90 (soy flour, Cenex Harvest States, Mankato, MN), ISU-CCUR (soy flour, Iowa State University Center for Crop Utilization Research, Ames, IA), Nutrisoy 7B (soy flour, Archer Daniels Midland, Decatur, IL), Soyafluff (soy flour, Central Soya Co., Fort Wayne, IN), Arcon F (protein concentrate, Archer Daniels Midland, Decatur, IL), and Procon 2000 (protein concentrate, Central Soya Co. Fort Wayne, IN).

Foaming properties of glue mixes were evaluated using the whipping method (Hojilla-Evangelista and Dunn, 2001). Standard glue formulations were prepared by slow mixing in a KitchenAid mixer. After standing overnight, the mixes were whipped at maximum speed for 5 minutes with the same mixer, and the foams were transferred to tared graduated beakers. Foam densities were determined at the beginning of the stability test. After 1 and 3 hours, foam volumes were recorded to determine foam stability. Properties of foams prepared with various soybean protein sources are given in Table 1.

Table 1. Foaming properties of plywood glues mixes with soy flour or protein concentrate

Protein Source	Initial foam volume (mL)	Foam volume (1 hour)	Foam volume (3 hours)
Animal blood (control)	588 (13)	93 (1)	81 (3)
Honeysoy 90	563 (38)	89 (1)	85 (2)
ISU-CCUR	550 (0)	91 (0)	82 (0)
Nutrisoy 7B	475 (0)	95 (0)	83 (4)
Soyafluff	425 (0)	76 (6)	11 (1)
Arcon F	380 (20)	91 (3)	81 (4)
Procon 2000	370 (20)	92 (5)	88 (3)

Values are mean (standard deviation) of two replicates.

The control glue mix with animal blood protein produced significantly greater foam volume than all of the soybean protein mixes except the Honeysoy 90 and ISU-CCUR flour. Soy flour-based glue mixes produced significantly greater foam volumes than those using protein concentrate or isolate. Several of the foams prepared with soy flour or concentrate had stabilities comparable to the animal blood mix.

The adhesive properties were evaluated using three-ply Southern pine samples (Hojilla-Evangelista, 2002). Standard glues mixes consisted of 22.0 g water, 6.0 g Glu-X (a wheat flour for plywood glues, The Robertson Corp., Brownstown, IN), 3.5 g protein source, 65.1 g phenol-formaldehyde resin, 3.0 g 50% NaOH, and 0.3 g foaming additive (Carsonol SHS, Lonza Inc., Fairlawn, NJ). Refoaming properties were improved by using a modified glue mix in which the quantity of soy was increased to provide 3.5 g protein/ 100 g glue mix. As shown in Table 2, glues prepared using soybean proteins in most cases gave wet tensile strengths comparable to the animal blood protein glues. Because of the variance in the data, the tensile strengths with soy protein glues were not significantly different from those obtained with animal blood protein. Use of the modified glue mix did not significantly alter the tensile strengths.

Table 2. Tensile strengths of plywood

Protein source	Standard glue mix	Modified glue mix
Animal blood	232 (53)	235 (46)
Honeysoy 90	216 (50)	242 (36)
ISU-CCUR	267 (13)	231 (40)
Nutrisoy 7B	231 (36)	247 (30)
Soyafluff	225 (25)	231 (48)
Arcon F	218 (31)	215 (18)
Procon 2000	241 (36)	240 (36)

Values are mean (standard deviation) for a minimum of 16 specimens

Soy flours have significant potential to replace animal blood protein as the extender in foamed plywood glues. The soy flour-based glues are cost-competitive and possess mixing, foaming, and adhesive properties equal to the animal blood protein glues commonly used. Plant trials at several plywood manufacturers in the U.S. have shown that soy flour glues can provide an economical alternative to glues made with animal blood protein.

Metal-binding properties of soy protein modified with citric acid

Citric acid has been studied as a crosslinking agent for cotton textiles and to improve wet performance of paper. When heated in the absence of moisture, citric acid can generate acid anhydrides, which will react with nucleophiles to yield crosslinked products. Under appropriate conditions, citric acid derivatives are formed with metal binding or ion-exchange capabilities (Sessa and Wing, 1998). The nucleophilic groups present in soy protein offer the potential to produce metal-binding products by modification with citric acid.

Citric acid was dissolved in deionized water and dispersed with soy protein isolate (SPI, Ralston-Purina Company, St. Louis, MO). Batters were then

dried in a forced-air oven at 60 C for 24 hours to reduce the water content, followed by reaction at 100-140 C for up to 24 hours. Reaction products were slurried in water, filtered, and rinsed. Carboxyl content was determined by titration, and copper-binding capacity was measured. All reaction products were dark brown in color.

The effect of citric acid content on reaction efficiency, carboxyl content, and Cu^{2+} binding is given in Table 3. There is a gradual decrease in reaction efficiency as the citric acid content is increased from 20 grams to 100 grams per 100 grams SPI. The carboxyl content increases with increasing citric acid, as did the Cu^{2+} binding capacity.

Table 3. Effect of citric acid content on properties of modified soy protein isolate

Citric acid (grams)	Reaction efficiency (%)	COOH (mmol/g)	Cu^{2+} (mmol/g)
0	-	0.67	0.43
20	89.6	1.83	0.66
40	88.6	2.74	0.71
60	83.4	3.56	0.78
80	80.4	4.05	0.89
100	74.7	4.13	1.18

Citric acid per 100 g soy protein isolate, reacted at 120 C for 24 hours

The effect of reaction temperature was determined by reacting 1:1 mixtures of SPI and citric acid for 24 hours at various temperatures. Results are given in Table 4. The maximum reaction efficiency is obtained at 130 C, followed by a decrease at 140 C. Maximum carboxyl content and Cu^{2+} binding are obtained with a reaction temperature of 120 C. Citric acid may form either monoesters which do not crosslink, as well as di- or triesters which can act as crosslinks. The observed decreases in carboxyl content and Cu^{2+} binding for reaction temperatures above 120 C suggest that greater crosslinking occurs at these temperatures.

Table 4. Effect of reaction temperature on properties of modified soy protein isolate.

Reaction temperature (C)	Reaction efficiency (%)	COOH (mmol/g)	Cu^{2+} (mmol/g)
100	49.4	2.71	0.78
110	61.4	3.48	1.01
120	74.7	4.13	1.18
130	97.8	3.58	0.56
140	88.9	3.52	0.38

100 grams each of citric acid and soy protein isolate

Conclusion

Soybean proteins offer a low-cost renewable resource for the development of biobased products. Soy proteins can substitute at reduced cost for animal blood proteins in foamed plywood glues without sacrificing processing characteristics or strength. Soy protein isolates can be modified with citric acid to provide crosslinked materials with metal-binding capacity.

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* Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

Industrial uses of soy proteins

D. J. Myers[1]; M. Kuo[2] and D. Stokke[2]

Soybeans, originated as an industrial crop in the United States, and experienced considerable progress in providing consumer goods prior to World War II. Many of the industrial uses included the protein that was used in a variety of industrial products such as wood adhesives, plastics and paper coatings until it was replaced by less expensive, superior-performing petrochemicals. In the United States today, an estimated 98% of all the soy protein produced is used in animal feeds with the balance used primarily in edible food applications. A very small percentage of the protein is used in non-food industrial uses. There is an effort today to increase the use of soy proteins in applications other than food and feeds as a way to add value to the protein products. There may now be opportunities for soy protein to compete in some of these markets because of the inevitable increase in petroleum prices and the increased emphasis on using "environmentally friendly" products. Although time will not allow a complete overview of all the nonfood uses of soy protein, this presentation will provide a brief history of the industrial uses of soy protein in the United States and discuss some of the ongoing research in wood adhesives and plastics.

History of industrial use of soy proteins

The use of soy proteins in industrial applications is not a new idea. One ancient Chinese text notes that soybeans were crushed to extract oil for use in a variety of different food and nonfood applications such as a protein "cake" used for food and as a fertilizer. The crudest form of soy protein, namely ground cake or meal, had limited industrial uses in adhesives and paper sizings, for binding charcoal briquettes, and as an additive in wall plaster.

In 1883, the German scientists E. Meissl and F. Boecker published the first compositional analysis of soybeans and introduced the terms "soy casein" and "soy albumin" to describe soy protein fractions. The general approach of his protein isolation procedure became the basis for today's commercial soy protein isolate production. This soy casein was white and quite pure, and was used to replace milk casein in paper sizing. Other early uses for soy casein were in the preparation of silk and artificial textiles, rubber, leathers, plastic materials, films, photographic emulsions, and paints. Recent

[1] Department of Food Science and Human Nutrition
[2] Natural Resource Ecology and Management, Center for Crops Utilization Research, Iowa State University Ames, Iowa, USA

history of soy protein use in industrial applications began in the early 1900's, with research demonstrating potential applications in both plastics and adhesives. In the 1913 and 1917, patents were issued for preparing plastics from soybeans. Satow obtained a U.S. patent for immersing coagulated (acid treated) or "glutenized" soy proteins in a formaldehyde solution to produce a moldable plastic. In 1923, a patent was issued to O. Johnson for a glue based on soy meal, and in 1926, the I. F. Laucks Co. began marketing a "soybean-oilmeal-based" glue for the Douglas fir plywood industry. This application was the first major industrial use of soy protein.

The early successes of soy-based plastics, adhesives and other industrial products made from soybeans and the economic stresses in the agricultural sector during the Great Depression (1930's) promoted the establishment of various organizations to foster new industrial uses for agricultural products. The primary purpose of this movement, called Chemurgy, was to create industrial raw materials from traditional agricultural farm products. Nearly 200 industrial uses for soybeans have been attributed to this movement including plastics, regenerated protein textile fibers, fire extinguishing foams, plywood adhesives and paper coatings. For more information on the history of industrial uses of soy protein, please see reviews by Johnson, et.al, (1992), Myers (1993) and Johnson and Myers (1995).

On the national level, the chemurgic movement had largely ended shortly after WWII and certainly by the mid-1950s. Work continued in the development of these applications up until other materials, primarily those that were petroleum-based, replaced many of the products formulated with soy-protein. Unfortunately, very few of these products formulated with soy protein were ever commercialized in significant quantities relative to the amount of soy protein produced and did not use quantities of soy protein that could approach that used in animal feeds.

Today, an even smaller percentage of the soy protein produced is used in industrial non-food applications. Estimates for the amount of protein used in industrial products have ranged from 0.5% to 3.0% (Myers, 1993).

Industrial uses of soybean protein

Wood Adhesives

One of the largest nonfood markets for soy flour was as a plywood adhesive. The reported advantages of soy-based adhesives (Lambuth, 1977) were that: 1) soy flour was cheap and plentiful compared to other protein-based adhesives; 2) the adhesive formed a relatively strong, moderately water-resistant bond; 3) the adhesive was not tacky which made the glued veneer

easy to handle thereby increasing manufacturing efficiency; 4) high moisture (20-35%) veneer could be bonded without splitting; and 5) adhesive could be applied hot or cold, a property that helped soy adhesives remain competitive until the early 1960s.

Although the use of soybean adhesives for Douglas Fir plywood was fairly successful, there were several factors that limited its use in broader applications and still restricts its competitiveness today. Probably the number one issue that has limited the growth of soy adhesives in wood products has been its lack of water resistance compared to phenol formaldehyde (PF). For this reason, soy adhesives have been limited only to interior applications. Although there have been many attempts to improve moisture resistance including cross-linking agents, tanning agents, the formation of calcium proteinates and blending with other proteins, none have been totally successful in preventing the weakening of the bond in humid or wet conditions (Conner, 1989).

Research efforts to develop soy protein-based adhesives for wood and fiber-based composite products have increased in recent years. The impetus for increased research is the need to find value-added uses for soybeans and to develop more environmentally friendly adhesives that are not based totally on petroleum and natural gas-based (synthetic) chemicals. Furthermore, concerns regarding future crude oil supplies have compelled the wood industry to develop adhesives from renewable materials. Research of protein-based adhesives during the past decade has been mainly made with soy proteins primarily because of their availability and low costs. In the early stages of development, researchers found that many of the same issues that limited the use of soy protein-based adhesives with the advent of better performing synthetic are still true today, namely lower bonding strength and water resistance.

One of the current concepts to achieve the goal of improving the performance soy protein-based adhesives is to use these renewable materials as copolymers with synthetic resins. A US patent (Reibel et. al., 1997) describes methods of preparing a soybean-based molding compound by cross-linking soy flour with methyl diphenyl isocyanate (MDI). Research by Dr. Roland Kreibich showed that by combining hydrolyzed soy protein with an adhesive currently used in end jointed lumber, phenol resorcinol formaldehyde (PRF), an adhesive could be developed that was cost competitive with PRF. Furthermore, the adhesive had a faster cure time and could bond wood with higher moisture contents than PRF alone. Both of the aforementioned research accomplishments have been commercialized and are currently being used to make wood and fiber-based products in the United States.

These aforementioned discoveries provided the impetus to increase research efforts in many research laboratories to investigate ways to combine soy protein with other synthetic resins to develop new value-added adhesive systems. Researchers at Iowa State University replaced up to 70% of the phenol formaldehyde in adhesives with soy protein flour (Kuo, et. al., 2001). The adhesives developed can be used to bond a variety wood and fiber-based composite products. There are additional efforts ongoing at other research laboratories in the United States to develop soy protein-based adhesives including work by researchers at an USDA laboratory (Hse, et. al., 2000) who developed a soy flour and PF copolymer resin comprising 30% soy flour and 70% PF also that can be used for oriented strandboard.

In spite of the new technologies developed for soy protein-based adhesives, there has been limited commercial success. The use soy protein in the wood adhesive industry has always been primarily driven by the price of soy flour, relative to the synthetics. There is still a significant challenge to manufacture wood adhesives from soy protein at reasonable costs to meet stringent performance requirements. The future of soy protein in this market will be determined by price, the ability to capitalize on its unique properties such as cold setting and rapid water loss during bonding, and the improvement of the adhesive bond relative to water resistance. As these challenges are met through further research, more commercial success can be expected.

Plastics

One of the earliest recognized potential industrial applications for soy protein was in plastics. There have been numerous efforts to develop plastics formulated with soybean meal or soybean isolate without much success. The most famous attempt to develop this technology was by the Ford Motor Co. Ford developed a process using a phenol-formaldehyde resin composed of approximately 30% soybean meal in the production of plastics for their automobiles. Distributor heads, gear control knobs, horn buttons, and window frames were made from the plastic, and the company had planned to use 4,000,000 lbs. of meal for every 1,000,000 cars produced. This work produced the famous picture of Henry Ford in 1941 hitting the plastic trunk lid of a vehicle using the blunt end of an ax demonstrating the toughness of the plastic. This research effort, however, eventually was abandoned during World War II. The major problem with these plastics was their propensity to absorb water.

The effort started by Ford was continued by the Soybean Research Group in Urbana, Ill. These plastics had good color and strength, and were relatively low in cost; but, they also showed poor water resistance, lacked permanence

when subjected to water, and experienced forming difficulties except with water plasticization. Researchers also noted that a suitable plasticizer was never found for the soy isolate to work in injector dies and not much was understood about the protein-formaldehyde complex to reduce the chance of making any further work successful. Work with soy protein isolate was discontinued in favor of modified soybean meals as modifiers and extenders in phenol-formaldehyde plastics. This soy meal combined with phenolic resins in a 50:50 ratio produced a plastic that had higher water absorption rates and longer cure times than the resin alone; however, when blended with 25% wood filler, the plastic had excellent strength, good cure time, and fine finish. In addition to saving valuable phenolic resin, the plastic had improved dyeing properties, however, it cured more slowly than phenolic resin and wood flour alone, therefore, the plastic was never successfully commercialized.

Environmental concerns and increased polymer prices relative to agricultural products are renewing interest in soy plastics. Many experts believe that the solution to many of our waste problems lies in composting and recycling. Despite the recent emphasis on conventional recycling, less than 2% of our plastic wastes are recycled because the sorting and reclamation costs are quite high compared with the low values of reclaimed plastics. New soy protein plastics offer new possibilities in both composting strategies and strategies to reclaim resources as fertilizer and livestock feed. The first soy plastics developed during the modern era (early 1990's) varied from 40 to 90% soy protein isolate, the balance being corn starch depending on the desired properties (Jane, et.al., 1993). Although these plastics met the some of the requirements for biodegradability, they tended to be very brittle and were not cost competitive. The soy plastics that are being developed today use a combination of soy flour and a variety of plasticizers that solve the aforementioned problems with products ranging from injection and compression molded sheets and rigid products as well as extruded sheets and foams (Mungara and Jane, 2002).

Potential to increase utilization of soy proteins in industrial markets

Changing market forces could provide an opportunity for soy protein to compete more successfully in the nonfood industrial markets. Petroleum prices may soon reach a level where the cost of soy-protein-derived products will be able to compete with petroleum-derived products. Secondly, environmental concerns are forcing the industry to consider the use of more "environmentally friendly" products and processes. For soy protein to be adopted as an industrial ingredient however, the performance of the proteins will have to meet the performance standards of the chemicals currently being used, regardless of price and the "environmental

friendliness". Furthermore, the environmental advantage of the protein in the formulation and/or process will have to be established before such claims can be considered.

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SoyScreen™: a sunscreen active agent from soybean oil

J. A. Laszlo[1]; D. L. Compton[1] and K. Warner[2]

Concerns have been raised about the potental adverse health and ecological effects of the commonly used sunscreen active ingredients. A sunscreen active ingredient can be derived from two natural plant components, ferulic acid and triglycerides. Transesterification of ferulic acid ethyl ester with soybean oil produces a mixture of feruloylated monoacyl- and diacylglycerols that have a strong UVA/B absorbance. The reaction is catalyzed by immobilized *Candida antarctica* lipase B. We examined the influence of operating conditions on the service life of the immobilized enzyme in packed beds. Unreacted ferulic acid ethyl ester and ethyl esters of fatty acids can be separated from the product by molecular distillation. The recovered materials are converted to sunscreen product by a second round of reaction with the biocatalyst, demonstrating a synthesis of a plant-derived, environmentally benign sunscreen. Fields of use for the derivatized oil beyond skincare applications will be discussed.

[1] New Crops and Processing Technology
[2] Food and Industrial Oils, U.S. Department of Agriculture, Agricultural Research Service, National Center for Agricultural Utilization Research, Peoria, IL 61604 USA, laszloja@ncaur.usda.gov

Soybean based waterproof biodegradable products by injection molding

S. Isobe[1]; Q. Wu[1]; K. Suzuki[1]; T. Yanai[2]; T. Tomita[2] and M. Nagai[3]

Abstract

One of major by-products from soybean process, soybean curd residue (Okara) has many useful ingredient. Recycling of Okara is very important subject. Many trial has been done to reuse the Okara, however, almost trial has not been to install the commercial process due to their treatment cost and their qualities. To convert biodegradable stuff is one of their trail fields. However, their product has disadvantage of high cost and low properties against water-resistance. To minimize costs and to improve waterproof property, we used zein-containing corn gluten meal and succeeded in making solid materials by injection molding. We turned the materials into pellets with an extruder, and then molded the pellets into seedling culture pots with an injection molder. This study project was carried out jointly with Showa Sangyo Co. and The Japan Steel Works. In this project, we were able to successfully reduce costs and to obtain solid molded products for practical use by adopting the injection molding method, which has many advantages in productivity (low costs, high moldability, flexibility to make various shapes of molds). At present, we are working to assess the biodegradable molded material actually applied and to improve materials for different purposes.

Introduction

Almost one million tons soy bean residue (Okara) is produced from the soybean curd and other traditional soybean process and isolate soy protein process annually in Japan. Okara has many useful components (Tabale 1). Therefore, almost Okara were used to animal feed. However, currently Japanese livestock formeres prefer to feed pellets due to control feeding condition. Additionally, Japanese soybean products processors come together and increase each factory production amount. The capacity of receiving Okara to the surrounding livestock form are limited, so the company have to treat the exceeded Okara. As shown as table 2, many trail for usage of Okara are done, however, the best solution did not find yet. One of key point for usage of Okara is how to reduce the water in Okara or how

[1] National Food Research Institute, Japan: -HYPERLINK "mailto:seiichi@nfri.affrc.go.jp" – seiichi@nfri.affrc.go.jp
[2] Showa Sangyo Co, Japan
[3] Japan Steel Works Ltd. Japan

to dry the Okara with low cost treatment. The mechanical dehydration process had been development in our laboratory. We reported the effects of dehydration from Okara with the screw pressing and the electro-osmoses treatments [1]. After dehydration, the handling of Okara is improved for usage. One of the advanced usages is to convert the biodegradable stuff like food packing material.

Packing materials are widely used to keep the quality of farm products and foods and to make their distribution and sale convenient, and include expanded polystyrene wrap for vegetables, meat and fish and containers for cup needles. A variety of packing materials have been developed to meet the purpose. These materials can be said to be indispensable things to our dietary life. Synthetic materials have many advantages: they have a good workability, their gas permeability is easy to control, and they are very stable. On the other hand, these materials are difficult to be degraded by microorganisms and others unlike natural materials, and generate much heat and a large volume of exhaust gases when burned, thus posing a global issue of environmental pollution at the time of disposal. Because of this, efforts have been made to avoid problems of synthetic substances by turning high polymers, the raw materials, into biodegradable forms. Our laboratory has also been working to develop biodegradable packing materials. To make these materials from farm products, the problem is the technology of modifying and processing these products, i.e., how to reproduce the properties of existing synthetic packaging by using the completely biodegradable substances (e.g., starch, protein) contained in the products. At present, biodegradable packing materials have some problems: for example, they cannot fully exhibit their characteristics according to the purpose of use compared to synthetic materials, and their costs are very high. But as some of the chemicals used for synthetic packing materials have recently been registered as endocrine disrupters and as there have been moves to reduce carbon dioxide (CO_2) emissions, demand for wrappings friendly to people and the environment is more and more increasing. In addition, using farm product-based materials to make biodegradable packaging would lead to a more efficient use of farm products because the parts that are not edible and have little utility as well as food processing by-product materials would be used more effectively. This paper reports the biodegradable materials made from soybean residue and other natural high polymers.

Table 1. Content of Soy bean curd residue (Okara)

	Water(%)	Protein(%)	Fat(%)	Fiber(%)	Ash(%)	Ca(mg/100g)	K(mg/100g)
Wet base	80.2	4.9	2.6	3.7	0.7	66.2	316.3
Dry base	-	24.5	13.0	18.4	3.5	334	1594

Table 2. Usage of Okara

Direct usage		Food ingredients
		Feed
		Fertilizer
Pretreatment	Enzyme treatment	Food ingredients
	Fermentation	Methane gas for fuel
	Drying	Food ingredient
		Feed (Including Aqua-feed)
		Mushroom cultivate medium
		Fertilizer

Materials and methods (development of biodegradable products by injection molding)

To minimize costs and increase water-resistant property, we used zein-containing corn gluten meal as the main raw material and Okara (soybean-curd (tofu) residue) as strength increasing agents, and succeeded in making solid materials by injection molding. Zein is an alcohol-soluble protein from corn starch industry. This by-product is water-insoluble and shows a promising water resistance. Therefore, zein was studied as a biodegradable polymer in several reports[2-4]. However, pure zein is too expensive for most applications, which limits its usage[5]. Corn gluten meal (CGM) mainly contains zein (60-70%). In Japan, the recent price of CGM directly dried without any treatment after extraction is 80 Japanese yen/kg (0.66 US$/kg). In USA, commercial CGM containing at least 60% protein can be currently available at the price of 20-27 US$/65 pounds (0.68-0.92 US$/kg) depending on quantity. Therefore, CGM is cheaper than PE. But the CGM study is limited in compression-molding because this material has poor processibility.

Materials

CGM containing 9.1% oil and 8.9% moisture (supplied from Showa Sangyo Co., LTD, Japan) was dried at 110 °C for 12h before use. Dried CGM mainly contains 69.8 ± 0.1% zein protein, measured with a nitrogen/protein instrument (FP-528, LECO corporation, MI, USA). Ethanol (extra pure, 99.5%) , glycerol (first grade) , Urea (first grade) and minerals($ZnSO_4$û$07H_2O$, $CaCl_2$û$07H_2O$, $MaSO_4$û$07H_2O$) were purchased from Wako Pure Chemical Ind., Ltd., Japan, and used as received. Okara was received from small soybean curd manufacture and dried before use.

Methods

The production process is shown in Fig. 1. First, we turned the materials into pellets with an extruder(TEX30FC, Japan Steel Works (JSW), Japan), and then molded the pellets into seedling culture pots with an injection molding machine(J85MEL, JSW, Japan) (Fig. 2).

Table 3 showed the combination of the ingredients for pellets. Molded sample were tested the mechanical properties and the biodegradability.

Table 3. Combination of the ingredients for pellet sample

Material(%)	Case 1	Case 2	Case 3
Corn gluten meal	66.7	56.3	53.1
Okara(dry)	-	6.3*	6.3*
Glycerol	20.0	18.8	17.7
Water	-	9.4*	8.8*
Ethanol	13.3	9.4	8.8
Urea	-	-	2.4
$ZnSO_4 \cdot 7H_2O$	-	-	0.3
$CaCl_2 \cdot 7H_2O$	-	-	0.3
$MaSO_4 \cdot 7H_2O$	-	-	0.3

* Wet Okara (60% moisture) can be added in place of Dry Okara and water

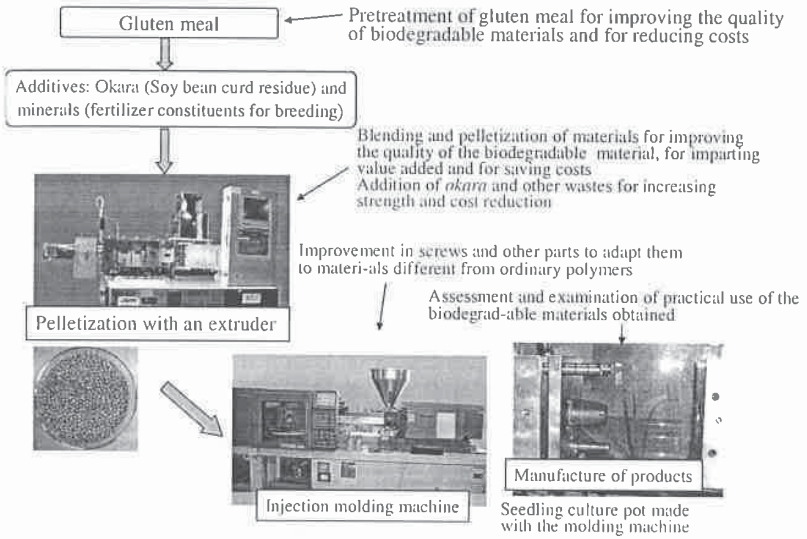


Figure1. Production method of biodegradable solid materials by the injection molding

Results and discussion

In this experiment, we were able to successfully reduce costs and to obtain good waterproof molded products for practical use by adopting the injection molding method, which has many advantages in productivity (low costs,

high moldability, flexibility to make various shapes of molds). Okara has a role of improvement of the mechanical properties molded products(Fig.3) and cost down. Although it was known that protein has thermoplasticity equivalent to high polymers, the injection molding method was not applied to food-based materials because, when melted by heat, their physical properties are easy to change by the temperature. In the present study, we devised a stable injection molding system by improving the conventional high polymer treatment method, by using a higher pressure and stricter temperature control, and by redesigning the injection screw. In addition, we tested seedling culture pots and other products made of the solid material as to biodegradability in the soil, and confirmed that these products were degraded in about one month in the moist soil (Fig. 4).

In this technology, costs can be saved because such inexpensive raw materials as corn gluten meal, glycerin (plasticizer) and food processing by-products (Okara) are used. When the solid material is used to make seedling culture pots or the like, if microelements or other useful substances for plant growth are added to the material at the pelletization stage, these substances will be released and stably supplied to crops as the pots or other products are gradually biodegraded in the soil. The solid material is also expected to have some secondary effects, including more efficient cultivation management and prevention of excessive fertilization.

At present, we are working to assess the biodegradable molded material actually applied and to improve materials for different purposes.

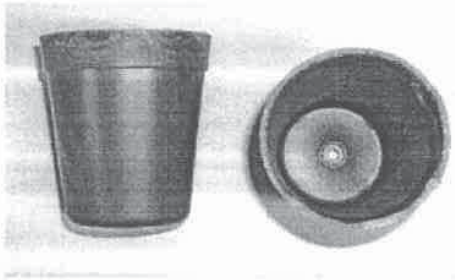
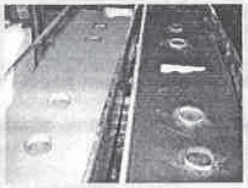


Figure 2. Biodegradable seedling culture pots made by the injection molding method

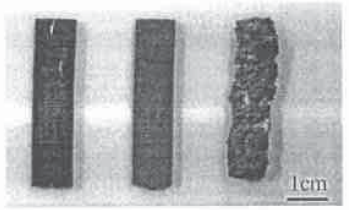
	Break strength[MPa]	Extension ratio [%]
Okara	2.56 ± 0.16	4.25 ± 0.43
Okara & Mineral	1.28 ± 0.22	2.00 ± 0.00
Control	2.29 ± 0.23	3.80 ± 1.23

Figure 3. Physical properties of mold product

On the test stands of our own making, the soil for upland fields was used:
Soil moisture adjusted to 10± 5% and 30± 5%
Test period: 30 days

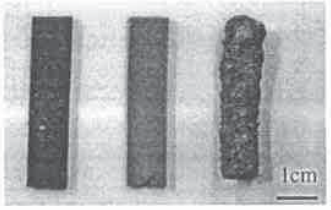


Okara additives (outer appearance)



Before the test 10% 30%

Okara and mineral additives (outer appearance)



Before the test 10% 30%

Figure 4. Degradation condition of biodegradable pots in the soil with different moisture (10%, 30%) one month after

Final words

In the future, we should strive to improve the quality of biodegradable materials and also to study the possibility of reducing the costs of both raw materials and processing work. In addition, it will be important for those engaged in production and distribution as well as the consumer to recognize the necessity of switching to more eco-friendly systems in the 21st century. Thus far, new technologies of making food products from farm crops have been established through development of various food processing techniques. In the future, it will be needed for us to reinforce studies on the technology of converting farm products and other natural

resources into non-food materials as well, regarding these efforts as similarly important tasks.

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How to get functional ingredients from soybeans

M. N. Riaz[1]

Abstract

Most commercial soybeans are processed in crushing plant where extraction separate them into oil and meal. The high protein content of soy meal make it a valuable component of formulated animal feeds. Some of it is processed for human consumption. Soybean comprise about 8% hull, 90% cotyledon and 2% hypocotyl (germ). At the crushing plant the beans are cleaned, then dehulled. After conditioning the cotyledon is broken into four to eight pieces, the pieces are flaked and the oil is extracted using hexane. If these flaked are intended for animal use, they are heated with live steam. But if these flaked are intended for human uses, they are desolventized by exposure to super-heated hexane. These flakes become the starting material for further processing into soy flour, concentrates, and isolates. Isoflavones can be extracted by physical method or by chemical method for food application.

Keywords: Soybean flour; Concentrate; Isolate; Isoflavones; soyfoods; textured soy protein;

Soybeans are the most important crops in the world. Soybeans are grown for variety of agriculture and industrial uses. Soybeans and soybean products have been used extensively in the Orient as a source of protein and energy in human diets for at least 4,000 years in one form or another. Soybeans are by far the most important crop in the world. They are grown for a variety of agriculture and industrial uses. The United States ranked number one after Brazil, China, Argentina and India.

Traditional soy foods such as tofu, soy milk, miso and temph have been consumed for centuries by Asian populations. Modern soy products such as soy flour, concentrates, isolates and textured soy protein, have been used for several decades as a functional ingredient by the food industries in the U.S. and Europe. Both isolated and concentrated soy proteins are easily digested by humans, and equal the protein quality of milk, meat and eggs. Moreover, soy proteins are acceptable in almost all diets containing virtually no cholesterol and being lactose-free. The nutritional value of soy protein provides the consumer with a cholesterol free, lower fat alternative to animal protein. There are two categories of functional foods; 1) potential

[1] Ph.D., Food Protein R&D Center, Texas A&M University College Station, TX 77843-2476 USA

functional foods, and 2) established functional foods. Soybeans are in the category of established functional foods.

Just recently, in October 1999, the US Food and Drug Administration (FDA) has approved the use of a soy protein health claim on food labels in the US. Based on scientific evidence from 43 human intervention studies, including 14 clinical trails, one epidemiological study and one meta-analysis, the FDA has concluded that the consumption of 25 gm of soy protein a day as part of a diet low in saturated fat and cholesterol, may reduce the risk of heart disease.

The increased acceptance of soy protein is due to its versatile qualities, and good functional properties in food application. Several food industries are working to bring soy foods to the consumers rather than depend upon the consumer to seek them out. Conventional breads, snacks/cracker, and breakfast cereals to which soy has been added, are likely to be particularly attractive (several breads containing soy have already been successful).

Raw soybeans cannot be used successfully in human foods unless processed properly. There are several anti-nutritional factors which have been identified in raw soybeans; i.e. Trypsin and Chymotrypsin Inhibitors; Phytohaemagglutiniins (Lectin); Allergenic Factors: Glycinin and beta-conglycinin. We must eliminate, destroy or reduce these factors in order to utilize the soybeans.

How do we get functional ingredients from soybeans?

Over the last two decades, researchers and scientists from all over the world have documented the health benefits of soy protein. Soy protein has been found helpful in reducing cholesterol levels, menopause and reduction in the risk for several diseases, i.e. cancer, heart diseases, osteoporosis and several others. Soy protein supplies all nine essential amino acids and provides many functional benefits to the food processors. Soy proteins are acceptable in almost all diets, containing virtually no cholesterol and being lactose free.

The interest in soybeans has reached an all-time high since the FDA approved the ruling for soy health claim. For the nutraceutical and functional point of view, soy flour, soy concentrates, and soy isolates are the best way to use soy protein in the diets. In general, soy flour contains 50-54% protein, whereas soy concentrates contain 65-70% protein, and soy isolates contain above 90% protein. Most of the nutraceutical industries are using these three forms of soy products in their formulation. Therefore, in this paper, different processing methods to produce soy flour, soy concentrates and soy isolates from whole soybeans will be discussed.

Processing of soybeans

Today's modern soybean processing plant will process between three and four million pounds of soybeans daily, or roughly 75,000 bushels or 2, 500 acres of soybean each day. Furthermore it is not uncommon to have in excess of one million bushels of bean stored at the processing plant (Fulmer, 1988).

Cleaning:

Soybean cleaning is important for producing high quality end products as well as protection of equipment. This is the first step in processing soybeans. Cleaning of soybeans is done on sieves under air aspiration so that dust, plant tissue, pebbles and light contaminating material, as well as bigger impurities, (stones, stems, nails, etc) are separated. The larger impurities are mostly separated step by step in a destoner and a magnetic iron separator. Cleaned beans are weighed with automatic hopper scales, which provides a means to control the rate of feed and the total amount of raw material for accounting purposes.

Drying:

Soybeans are dried in grain driers to a moisture level of 10% which is needed prior to dehulling. The temperature of the dryer should be in between 70-76 °C to achieve the desired moisture level. Uniform drying of the soybeans is very important for removal of hulls, since we need to remove the hulls from every individual bean and not an average. To do a good job of hull removal, it is essential to subject the beans to some kind of thermal impact. This is necessary because the hulls are attached to the bean with a proteinatious material, that when exposed to heat, affects the release of the hulls. After drying the beans, they are stored for tempering approximately for 72 hours to stabilized the moisture contents. Dried beans are usually cleaned to remove as much loose hulls, pods, sticks and other foreign material as possible. This step will increase the efficiency of the cracking rolls and aspirator.

Dehulling of soybeans

The objective of the cracking is to break the soybean into suitable pieces for dehulling and flaking. Dried soybeans are cracked by using cracking mills where seeds are broken into smaller pieces. After cracking, the hulls are separated under air-flow and the lighter hulls are withdrawn. It is important at this point to understand exactly how air separation works, since it is presently the basis for all soybean dehulling. With properly dried and cracked soybeans, the cotyledons should separate easily from the hulls. Soybeans contain about 8% of hulls by weight. As a practical matter, it is not possible to get an absolute separation of hulls from the cotyledons.

Conditioning:

Cracked and dehulled soybeans are conditioned in cookers to approximately 70 °C with steam. Small amounts of water can be added to adjust the moisture to approximately 11%, which makes the beans ideal for flaking. The main function of the conditioning is to facilitate the flaking step.

Flaking:

In order to extract the oil from the beans it is important to destroy the cell structure of the beans . To acheive this, beans are flaked in the flaking machines, since solvent can flow much more readily through a bed of flakes than through a bed of soy meats or fine particles. The ideal thickness of a flake is in between 0.25 to 0.35 mm. Thickness of these flakes depends upon the size of the cracked beans, conditioning and the adjustment of the flaking rolls.

Extraction of flakes:

Soy flours, soy concentrates and soy isolates typically are made from flakes (also called white flakes). Soy flakes are fed into a solvent extractor, and after sufficient contact time with the solvent (hexane) these flakes are desolventized to remove the residual hexane. This process reduces the residual oil to low level in flakes. These flakes can be desolventized in standard desolventizer-toaster (DT) which uses live steam to drive the solvent off. Or another system called flashed desolventizer or vapor desolventizer can be used. This system produces less heat-treated soy products. Soy flakes processed by flash solvent- removing systems have protein dispersibility index (PDI) as high as 95.

In the flash desolventizer, solvent wet flakes go directly from the extractor into super-heated vapor blown at high velocity through a long tube. Solvent is heated under pressure to 115-138 °C. As this vapor is blown through the tube, flakes are conveyed in and picked up by the vapor stream. In short seconds, the super heated solvent will flash off the residual liquid solvent leaving desolventized flakes to be collected in the cyclone (Fulmer, 1989). High PDI soy ingredients are more soluble, but at the same time, they also have highly active enzymes and antinutritional factors, which should be deactivated by heat before the final product is consumed.

Expanding of flakes:

Recently, several soy flour, soy concentrates and isolates producing companies started using an expander-extruder after the flaking step, and before the extraction. The use of expanders was originally developed in

Brazil and was introduced in the US in early 1980. According to Watkins et al (1988), the use of expander offer several advantages in processing soybeans for extraction.

1. The seed is finely homogenized in the expander, freeing the oil for rapid extraction.
2. Dense collets are formed, (weigh more per cubic foot).
3. The collets are porous, and do not restrict percolation of solvent through the extractor bed, as it may occur with fragile flakes.
4. More oil is recovered by solvent from collets as compared to flakes.
5. The solvent drains more completely from the extracted collets, resulting in more complete removal of oil.
6. Less energy is required for desolventizing the collets compared to flakes.

Production of soy flour:

Defatted flakes are milled using hammer mill to produce soy flour. At least 97% of the flour must pass through a U.S. Standard No. 100 sieve. This flour contains 52-54% protein as is. Soy flour, concentrates and isolates contain varying levels of isoflavones, depending on their origin and the protein-concentration method. Soy flour usually retains more isoflavones than soy concentrates.

Production of soy protein concentrates:

Soy protein concentrates are processed selectively by removing the soluble carbohydrates from soy protein flour by either aqueous alcohol or isoelectric leaching. Defatted flakes or soy flour are used as starting material. Soy protein concentrates contain at least 65% protein. During processing of soy concentrates, objectives are to immobilize the protein while leaching away the soluble, removing the strong flavor components and the flatulence sugars (sucrose, stachyose and raffinose). In turn, both protein and dietary fiber contents are increased.

There are several different methods to produce soy concentrates (Lusas and Rhee,1995).
1. Extraction of flakes with aqueous 20 to 80% ethyl alcohol
2. Acid leaching of flakes or flour
3. Denaturing the protein with moist heat and extraction with water (Ohren, 1981).

Alcohol extraction is considered to produce the blandest products. Mild heat drying conditions are used in an acidic water extraction process to retain high PDIs. (Lusas and Riaz, 1995). Isoflavones content in soy concentrate depends on whether it has been water-washed or alcohol-

washed, as isoflavones are soluble in alcohol. Because of its blander flavor, soy concentrates is often preferred over soy flour.

Production of soy protein isolates:

There are several ways that have been described for manufacturing isolated soy protein. But the only commercial procedure currently being used is extraction of defatted soy flakes or soy flour with water, followed by centrifugation. In this process, the protein is solubilized at pH 6.8-10 at 27-66 °C by using sodium hydroxide and other alkaline agents approved for food uses. The protein solution is then separated from the flakes or flour by centrifugation. The solids are recovered as a by-products, containing 16-36% protein, 9-13% crude fiber and 45-75% total dietary fiber when dried to 6-7% moisture content and have been marketed for food use. The solution is then acidified to pH 4.5 by using hydrochloric or phosphoric acid, and the protein is precipitated as a curd. The curd is then washed with water and concentrated by centrifugation, and can be neutralized to pH 6.5-7.0 or spray dried in its acidic form (Johnson and Kikuchi, 1989).

Soy isolates, the most concentrated form of soy protein available, generally contain about half as many isoflavones as soy flour as a result of losses during processing. Functionally, isolated soy protein has many of the same attributes as soy flour and concentrates, and is used in different meat applications.

Isoflavones:

Isoflavones currently receive much attention from the scientific world, so consequently, the industry takes up the opportunity to develop unique products that offer consumers an alternative to consuming traditional amounts of soy. Isoflavones are found in high amounts in soybeans. Genistein and daidzein are the major isoflavones found in soybeans. In whole soybeans, the isoflavones are present in a concentration of 0.2-1.5 mg/g. Soy experts recommend between 30 to 50 mg of isoflavones per day. One would need to consume 20-150 g of soy foods; 200 grams or almost 7 ounces of tofu, or 3 cups of soy milk daily, to get the recommended amount of isoflavones. Isoflavones are available in several different forms, crunchy granules, flour and fine powder, can be labeled as "soy isoflavones concentrate", which can be incorporated in cereal, snack, cookie, bread, health bars, drinks and meat replacements.

Processing of isoflavones:

The concentration of isoflavones in soy foods and soy ingredients depends upon: 1) genetics of soybeans; 2) environment where soybeans are grown; and, 3) processing technologies. According to Dr. Murphy, Iowa State

University; the level of isoflavones in soybeans, even from the same field, grown all in the same year, may vary in concentration by a factor of five. There are two methods to extract isoflavones from soybeans;1) chemical extraction; and, 2) mechanical extraction.

Chemical extraction:

Material from the solvent extraction process (discussed earlier), when converted into soy concentrates and isolates (discussed earlier), generates by-product called soy molasses. These molasses are a rich source of isoflavones. These isoflavones are chemically extracted by using patented processes by different food companies.

Mechanical extraction:

In this process, soybean germs are mechanically separated from the cotyledons. These soy germs have 20 times more isoflavones than the rest of the seed. On an average, 400 pounds of soybeans will produce one pound of germ. These germs are then roasted, milled and converted into different forms, like flour and granules of different sizes. These germs are considered a very rich source of isoflavones and other nutrients. On an average, these germs will have 40% protein, essential fatty acids, omega 3 and 6, lecithin, vitamin E, and saponin.

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Non solvent technology in soybean processing

W. B. Wijeratne[1]

Abstract

Alternatives to solvent extraction are desirable for soybean processing on small scale. Dry extrusion of soybeans, coupled with continuous screw pressing offers a low cost, environmentally friendly alternative. A process based on this concept has been developed and commercialized. Soy flour produced by this process has excellent flavor, good nutritional properties and functionality. It was shown that up to 30% of this flour can be used in bakery, pasta, and snack and confectionery products without adversely affecting the flavor. The flour can also be re-processed into textured soy protein. Commercial operations of 10-150 tons of soybeans per day capacity are using this technology.

Key Words: soybean, extrusion, dry extrusion, ExPress® System, identity preservation, textured soy.

Introduction

Soybeans occupy a unique position among edible legumes. Traditional food legumes (pulses) are rich in protein but contain limited amounts of oil (energy). Soybeans contain more protein than the pulses and also a relatively higher content of edible oil. The quality of soy protein is now recognized as being similar to that of meat protein. The edible oil in soybeans is approximately 85% unsaturated and contains the essential fatty acids. In addition to the nutritional advantages, soybeans are also recognized for their benefit to human health. Recent scientific evidence points to the positive effect of soybeans for cardiovascular health, cancer, bone density and alleviation of postmenopausal discomfort. Above all, the greatest potential of soybeans lies in increasing the available food supply for the rapidly increasing population in the 21st century. While the supply of soybeans is abundant, its direct consumption is estimated at less than 10% of total production, much of this being restricted to the Far East where soy food has been consumed for centuries. The spread of soybean consumption to the food deficit parts of the world is contingent upon development of processing technologies and products appropriate to the agricultural, economic and social circumstances in those regions.

[1] Director of Research & Food Technology, Insta-Pro International, 10104 Douglas Avenue, Des Moines, Iowa, USA 515-254-1267 wilmot@insta-pro.com

Need and opportunity

The bulk of the world soybean crop is processed into oil and meal by solvent extraction involving the use of organic solvents. Solvent extraction technology thrives on economies of scale. It requires heavy capital investment and well-developed infrastructure for collection, storage and distribution of raw material and finished goods. The major advantage of solvent extraction is the high efficiency of oil extraction. However, there are certain circumstances under which solvent extraction presents constraints. The emission of solvent vapors into the atmosphere has become a significant environmental concern relative to solvent extraction of oilseeds. Indeed, the solvent extraction industry is under pressure to limit the level of emissions to prescribed levels. New processing technologies that do not use solvents would find favorable consideration under these circumstances.

Many countries that were not traditional soybean producers have embarked on national level programs for soybean production. Such newly developing soybean industries do not have the production volume, infrastructure and capital resources required to establish and sustain a viable solvent extraction industry. Soybean production units are relatively small and scattered over large geographic areas. Hence, de-centralized processing on the small scale becomes a more viable option. This approach is also conducive to value-adding at the rural level with the associated benefits of generating income and employment in rural communities.

Identity preservation is another factor that is becoming important in soybean processing. This may be due to the need of varietal traits for specific nutritional or functional properties. It may also stem from the need to preserve identity of varieties that are organically grown or those that are free of genetic modification. The large scale and fully automated structure of the solvent extraction industry does not lend itself to segregation of varieties through the processing cycle. Alternative processing technologies that can ensure identity preservation could take advantage of the developing market for identity preserved soybean products.

Although solvent extraction dominates the oilseed crushing industry, direct screw pressing (full press operations) is still practiced to some extent. The main drawbacks of this technique are low extraction efficiency and fluctuation in the quality of protein meal and oil. Prolonged heat treatment of oilseed prior to pressing, non-uniform heating due to variation of seed size within seed lots and the need for multi-pass pressing have adverse effects on meal and oil quality. Screw pressing is generally applied to high oil bearing seeds. In the case of soybeans, where the oil content is relatively

low, screw pressing has not been applied to any appreciable extent. This paper describes the development and commercialization of an extrusion-based technique for processing soybeans for food use without the use of organic solvents.

The concept

The origin of this work dates back to 1987 when Nelson et al. (1987) at the University of Illinois were using Insta-Pro dry extruders to process full-fat soybeans for human consumption. When whole or de-hulled soybeans at field moisture content were cracked and extruded, the extrudate discharged in semi-fluid consistency. The material reverted to a dry and mealy consistency soon after exiting the extruder. Microscopic examination of the extrudate showed that the extrusion process disrupted the cell structure of the soybean cotyledon. Consequently, the oil was released from the naturally protected environment within the spherosomes into the matrix. It was proposed that the short window before the oil gets re-absorbed into the matrix offers opportunity to press out the oil by mechanical means. Bench level and pilot plant level studies were carried out to determine the feasibility of extracting oil by hydraulic pressing and screw pressing immediately after extrusion. It was demonstrated that approximately 70% oil recovery was feasible in a single pass through a screw press when the soybean extrudate was pressed immediately after extrusion. However, the extraction rate fell drastically when the extrudate was allowed to cool down before pressing.

The manufacturer of the dry extruder (Insta-Pro International) has since developed the Extrusion/Pressing concept through process scale up and field-testing to commercial application. The process has been registered under the name "ExPress®" system.

The technology

Whole or de-hulled soybeans at field moisture content are fed continuously to the dry extruder. The extruder is set up in appropriate configuration to achieve the desired degree of cooking. Within the extruder barrel, the material is subjected to friction, shear and pressure whereby heat is generated through viscous dissipation of mechanical energy. No external heat source such as steam is required for the process when using dry (autogenous) extruders. The temperature profile within the extruder barrel can be varied depending upon the intended use of the processed protein meal. Typically, the top temperature at the exit of the extruder barrel is about 150° C. Lower temperature profiles are used when the meal is intended for use as a functional ingredient in food applications. Higher temperature profiles are

used when the meal is used directly in feed formulation for various animal species. The average residence time of the material within the extruder is under 30 seconds. This high-temperature-short-time cooking cycle makes it possible to process soybeans at the field moisture content while protecting their nutritional value. The frictional forces within the extruder barrel and the sudden decompression of the material as it exits the extruder cause the rupture of the cell structure and release of oil from the sub-cellular sites.

Soybeans exit the extruder and are immediately and continuously conveyed into a continuous horizontal screw press where the oil and meal are separated. The process of extrusion changes the physical characteristics of the soybeans from solid particles to an oily meal. The bulk density and compressibility are drastically altered. Hence, a conventional screw press designed to press whole oilseeds will not handle the extruded soybeans. The pressing worm and the barrel cage of the press must be re-configured to handle the extruded product. The process of extrusion results in a significant increase in the throughput rate of the press over its rated capacity for whole oilseeds. The oil and meal exiting the press are at elevated temperature. They are further processed or cooled to near room temperature before storage. The residual oil content in the pressed soybean meal is reduced to 5-7% in a single pass through the Extruder/Press system. In spite of the residual oil content, the meal has been found to be remarkably stable. The reason may be that the process of extrusion results in protecting the oil by micro-encapsulation.

Process scale up

Process scale up included optimization of the extruder configurations in commercial scale models. Four extruders that have throughput capacities of 300 kg/hr, 750 kg/hr, 1200 kg/hr, and 4,000 kg/hr respectively have been brought on line for the process. Similarly, continuous oil presses have been developed to match the capacities of the extruders so that they can be appropriately coupled for continuous operation. The screw and barrel configurations and other important engineering parameters of the press construction were modified to handle extruded soybeans. Auxiliary equipment such as conveyors to interface between the extruder and the press, and coolers to reduce the temperature of the protein meal coming off the press, milling systems to convert the meal into flour, have been sourced to provide complete processing systems of various capacities.

Food applications

The greatest significance of this process is that it provides a relatively simple means to produce soy flour of remarkably superior flavor compared to other products on the market. The main reason for good flavor is that the lipoxygenase enzyme systems responsible for off-flavor development are

deactivated in the extrusion process before they have chance to react with unsaturated fatty acids. Unlike in solvent extraction, moisture conditioning and tempering is not required. Hence, chance of providing a favorable environment for lipoxygenase catalyzed reactions is avoided. Furthermore, the extrusion cooking parameters can be adjusted so as to impart a pleasant nutty flavor to the meal if such is desirable.

By careful control of the process, the functional properties of soy flour produced by this process can be greatly influenced. The protein dispersibility index (PDI) can be controlled in the range of 14-60%. Soy flour of 50% PDI and higher has been re-processed with dry extruders into texturized products that have texture profiles similar to conventional products made from solvent de-fatted soy flour. The textured products have similar water absorption rates and faster rehydration rates relative to conventional products made from solvent defatted soy flour. Up to 30% of soy flour made by the new process has been incorporated in unleavened bakery products and pasta that is the staple of a vast population on global basis. It finds ready acceptance in confectionery as well. For instance, it has been shown that 25% of milk solids (Khoa) used in making Gulab Jamun (Indian sweet preparation) can be replaced with this soy flour without affecting the physical or sensory properties of the finished product. On the nutritional side, the flour contains approximately 48% protein, 6% fat, 14% dietary fiber and 4,000 micrograms/gram of total isoflavones.

Product quality

Protein: Under controlled conditions of operation, the ExPress® system yields protein meal that can be milled into soy flour having good functionality for various food applications. When processed from de-hulled soybean, the flour contains approximately 50% protein. The "beany and grassy" flavor associated with solvent extracted soy flour has been a constraint against its use in high concentrations in food systems. The dry extrusion process deactivates the enzyme systems responsible for off flavor development before they act. Hence, soy flour produced by the ExPress® system is free of off flavors. It has been demonstrated that this soy flour can be used as raw material for textured soy protein as well as an ingredient for co-processing with cereal grains into snacks. Finely milled soy flour has been used at levels of 10-30% in raised breads, flat breads, specialty breads, cookies and crackers, pasta, and confectionery products. Because the ExPress® process does not involve solvents or aqueous extraction, the naturally occurring soybean isoflavones are retained in the meal almost quantitatively. Hence, ExPress® soy flour is an ideal ingredient to supply soy protein, isoflavones and other beneficial phytochemicals to food systems.

Oil Quality: Hill (1990) reported that soybean oil extracted by the ExPress® process has lower contents of free fatty acids, trace metals, sterols,

phosphatides and unsaponifiable matter as compared to crude solvent extracted oil processed from the same lot of beans. Because of the low phosphatide content (less than 0.5%), the oil is being consumed without further processing in markets where the natural color of the oil is not objectionable. The small amount of residual phosphatide, however, tends to result in foaming during deep frying applications. It was shown that simple water de-gumming could eliminate this problem. In any case, it has been demonstrated that this oil could be physically refined even when light color and blandness is important for marketing the oil. ExPress® soybean oil contained 1,566 PPM of total tocopherols. The high content of tocopherols imparts oxidative stability to the oil. According to Hill's study, considerable amount of the tocopherol was retained even after full refining of the oil under the conditions of his experiments.

Commercialization

Insta-Pro International is currently supplying complete ExPress® systems on a global basis. More than 170 operations have been established in the United States and overseas. The production capacities of existing plants vary from 8 - 200 Metric Tons of soybean input per day. In recent times, the company has begun supplying small-scale oil refining systems to further add value to the oil extracted by the ExPress® system.

The success of the ExPress® system is primarily due to its relatively low capital cost and high quality of processed products. It allows value added processing of oilseeds and vegetable protein at the rural level, benefiting producers, farmer's cooperatives, and small agribusiness enterprises. Above all, the technology is helping to better utilize vegetable protein for nutrition and health.

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Soy ingredients in bakery and other cereal products

W. F. Limpert[1]

Abstract

Commercially available soy protein ingredients (soy flour and grits, soy concentrate, and soy isolate) have been used for many years in the bakery and cereal industries. These products are utilized in these applications for a variety of functional and nutritional reasons, commonly at levels of two to five percent. The common functionalities of soy protein products include solubility, water absorption and binding, viscosity control, gelation, cohesion/adhesion, elasticity, emulsification/stabilization, fat absorption/repulsion, flavor binding, foaming/whipping, and color control. Various bakery and cereal applications can utilize all of these functionalities. This high level of functionality is attributed to the soy protein's polymer chain, which contains lipophilic, polar, non-polar, as well as negatively and positively charged groups, which enable soy protein to associate with many different types of compounds. Bakers have long understood soy flour to provide moisture retention, whiten crumb color, darken crusts, extend shelf life, shorten baking time, and decrease fat absorption. Soy flour can also be an excellent low-fat source of isoflavones as well as protein, by providing all of the amino acids essential for human health. The PDCAAS (Protein Digestibility Corrected Amino Acid Score) of soy protein products ranges from 0.93 to 1.0 (maximum obtainable). Soy flour, concentrates and isolates are utilized in blends with inexpensive dairy components, or by themselves, to replace Non Fat Dry Milk or other costly dairy components in many formulations. Many egg components can be replaced by soy proteins, such as the use of lecithinated soy flour to substitute for a substantial percentage of egg yolk solids. High protein pasta products, such as spaghetti, can be fortified with soy flour to increase their nutritional value. Breakfast cereals and bars now use soy proteins (powered or texturized) to boost protein quality and quantity. Many of these uses are further enhanced in several nations, by the ability to utilize a soy health claim. It allows for the addition of specific levels of soy protein to foods for the claim of potential reduction of coronary heart disease. A number of common bakery and cereal products, including breads, can now be formulated to contain substantial levels (~35% by weight) of various soy protein products.

[1] Cargill Incorporated Minneapolis, Minnesota USA bill_limpert@cargill.com

Soy Products with Bakery Potential

- Defatted (<1.0% fat) Soy Flour
  - 20, 70, 90 PDI (industry standards, any value possible)
  - Defatted Grits
- Relecithinated or Refatted Soy Flour (3-20%)
- Full-Fat (20%) or Low Fat (6-8%) Soy Flour: enzyme active or toasted and toasted Full-Fat Grits
- Soy Concentrate
- Soy Isolate

Why Add Soy Protein?

- **Water absorption**
  - Flour & concentrate 2 – 5 parts water to 1 part protein
  - Isolate 5 – 7 parts water to 1 part protein
- **Fat absorption or repulsion**
- **Emulsification**
  - Heat stable protein:water:fat matrix
  - Ratio of protein:water:fat
    - Varies for type of soy and how it is manufactured
- **Gelling**
  - Heat stable protein:water matrix

Why Add Soy Protein?

- **Viscosity Control**
  - Water and fat absorption, emulsification
- **Texture Enhancement**
  - Viscosity
  - Gelling
  - Mimic rolled oats, crisped rice or other particulates in bakery products
    - Textured soy flour and textured concentrate
- **Protein fortification**
  - Cost per pound of protein
    - Cost / pound divided by % protein (x 100)
  - **$0.20 / 52 x 100 = $0.39 / lb for soy flour**
  - Health claim
    - 6.25 grams soy protein / RACC
  - **(Soy protein / RACC / .% protein in soy ingredient) x 100 = % soy protein ingredient in product**

- Bread at 50 grams RACC
  - » (6.25 / 50 / 0.86) x 100 = 14.5% soy isolate
  - » (6.25 / 50 / 0.65) x 100 = 19.2% soy concentrate
  - » (6.25 / 50 / 0.52) x 100 = 24.0% soy flour

Summary

- Soy Protein ingredients are used as highly functional components in many bakery products
  - Inclusion of soy can reduce costs and improve quality

- Due in large part to their composition, soy flour, concentrate, and isolate can be utilized as ingredients in "new" high-soy bakery items to deliver health benefits to consumers

Soymilk processing from soybeans – DOs and DONTs

I. Debruyne[1]

Abstract

Various roads exist to produce good quality soymilk. This article will focus on modern variations on the traditional Asian-type soymilk process. A modern process consists of consecutive processing steps: cleaning, (dehulling), soaking and/or blanching, grinding, separation, formulation and stabilization. For Western markets it is essential to obtain a bland neutral taste with no traces of beany taste or astringency. This can be realized by destroying the lipoxygenase and a few other enzymes early in the process. The review goes into more detail on important aspects to keep in mind and how to optimize soymilk yield.

Paper

Soymilk has been produced since thousands of years according to the traditional Asiatic process. This process can easily be applied on small-scale household level, or upgraded for large-scale production of soymilk and tofu product for Chinese cuisine. After overnight soaking, the slurry is grinded into slush and cooked. The process ends with a separation step yielding the soymilk. The perishable okara byproduct can be used in other food products. Typically, the traditional process yields a product with preferably a strong beany taste – not adapted to the Western taste pattern. For Western consumers, a more bland and neutral taste is preferred.

Various routes have been explored to come to such a bland-tasting product. Soybean ultramilling and extraction is a route in full development with many new powders and flakes coming to the market in recent years. One major advantage is claimed to be the excellent dispersibility of the product, thus reducing the need for extensive homogenization and limited fiber separation, if any. Fine powders have a disadvantage though, in that the risk for off flavor formation increases due to increasing surface and air contact risk (full fat flours are highly sensitive to oxidation).

This has been partially solved in the development of instant soymilk powders, based on isolated soy protein, which are then combined with a vegetable oil or fat, an emulsifier, sugar, salt, and other ingredients. Also spray-dried (enzyme treated) soymilk products are being developed (after fiber hydrolysis,

[1] Ignace Debruyne & Associates, Consultant, Izegem, Belgium, ignace.debruyne@advalvas.be

no okara is needed), again to be combined with other ingredients. Such products tend to be rather expensive due to the need to evaporate lots of water. Again oxidation stability is often at the origin of off-taste formation.

Reconstituted soymilks can be a good answer in such case. Basically isolated soy protein (ISP; >90% protein) is combined with vegetable oil, emulsifier, sugar, salt, flavors; minerals, and water. The product is easily formulated, and when a proper emulsifier and protein combination are used, a stable product will be obtained. Such tailor-made formulas can be produced under license of the ISP suppliers, who developed optimized formulas and processing. Reconstitution eliminates the need for soybean extraction, and reduces byproduct formation. It tends to be a good solution for medium-scale production, and is especially interesting for dairy milk plants that have most if not all of the equipment available.

However, tonyu or soymilk by definition is based on direct extraction of soybeans, yielding a so-called *Soy Base* to be used for further formulation or production of derived products such as desserts, yogurt, ice cream, or more traditionally tofu.

What are the main objectives of such soybean extraction technology yielding bland-tasting Soy Base?; Attaining the desired flavor, taste and texture is the first objective; this must be done while maximizing the soluble protein extraction yield; the need for stabilization of the product for long shelf life depends on the product marketing concept. It is important to realize that Taste is the Number One consideration. However, also nutrition is increasingly important. Even the pricing position can have significant consequences on processing level.

Sensory Quality basically will look at some major characteristics such as *absence of bitterness*, *absence of beany taste*, *smoothness* of structure, and intensity of the *color*. As will be explained below, beany taste is highly dependent on the activity of hydrolyzing and oxidizing enzymes such as lipoxygenase and of the naturally present polyunsaturated fatty acids (PUFA). Bitterness seems to be related to the amount and status of isoflavones and saponins in the final product (esterified isoflavones are more or less neutral in taste; after hydrolysis though, the bitterness of the aglycone form is easily detectable). Smoothness is a function of efficiency of homogenization and separation of the fiber fraction. Color is related to soybean color and can depend on dehulling conditions and emulsion stability.

Many descriptors have been used to describe the taste profile of soybean milk: color, viscosity, balance, fullness, flavoring and other aromatics,

sweetness, sourness, mouthfeel and aftertaste to name just a few (Soyatech and A.D. Little e-Sensory Perception™ – Sensory benchmarking: The U.S. Soymilk market 2001). A first priority is the quality of the soybean to be used: grade; characteristics; composition (protein; oil); color; size; taste; yield. Many varieties are specifically developed for soyfoods, mostly with a light yellow color and even with a clear hylum, equal size and shape, and specific biochemical composition. Important factors to look at are the Lipoxygenase activity (affects the flavor), the Trypsin inhibitor activity (re: protein digestibility), presence of oligosaccharides (which could induce flatulence), and last but not least the mouth catching factor(s) (astringency).

When looking at the soybean supply, also storage and transportation are important. A high protein level is essential, with limited moisture, freshness and properly maturated. Presence of excessive foreign material can be detrimental for the process. Extra specifications can focus on more transcendental virtues such as being non-GM or organic. Soybeans are stored all along the transport chain: at the farm, the cleaning and grading facility; during shipping; at the soymilk processor's site. Appropriate temperature, humidity and air circulation must be guaranteed.

Understanding how to avoid beany taste, includes knowledge of where the beany off-flavor is created in the process (fishy smell in oil). The origin depends on two factors: the presence of PUFA combined with soybean lipoxygenase. The lipoxygenase will oxidize PUFA, giving fatty acid hydroperoxides, and further transformed into short-chain aldehydes, which is the beany taste.

Enzyme reactions can be stopped in two different ways: removing or denaturation of the enzyme; inhibiting interaction with the substrates (PUFA and oxygen in this case). Heating, or thermal denaturation of the lipoxygenase is easy to obtain and can further be optimized by proper pH control. Substrate removal is only possible by reducing access to oxygen. Other options, such as removal of the reaction products are only curative. It is always better to prevent off-taste formation.

Controlling other flavor issues such as the bitter taste and astringency again depend a lot on proper processing conditions: reduction of isoflavone hydrolysis to the aglycone form; reduction of oil and protein hydrolysis. Of course a de-bittering step is possible, or by-products can be masked with bitter blockers. However, again this last option is curative and not a fundamental solution.

Heating operations and specific chemical treatment will influence these soy flavor issues. Processing could also create a conflict with other objectives such as improving nutritional value (digestibility; PDCAAS, reduction of trypsin inhibitor). Too much cooking can result in a sterile cooked flavor and mouthfeel. Only a proper process control can prevent a cooked taste formation.

In every Soy Base production process the same basic processing steps are found: soaking, rinsing, grinding and filtering, with somewhere in the process the necessary heating step. The first objective of the heating step remains the inactivation of lipoxygenase (and other degrading enzymes), with minimal reduction of protein solubility, and maximizing the protein extraction yield. Denaturation is a physico-chemical process. It depends on temperature, time, and humidity or free water content. Heating can be done on the dry soybeans, or somewhere down in the production process.

The first heating opportunity is during the dehulling step, if this is chosen as an important part of the process. But, is dehulling of soybeans necessary? No dehulling saves additional equipment investment, cost and time, and damaging soybeans could even lead to activation of the lipoxygenase and beany taste formation. Soy Base made from dehulled soybeans might have chalky taste. Hulls can even aid in the filtration step. Hot dehulling could also insolubilize the protein, and a moist bean surface is sensitive to mould infection. Dehulling though could be developed in such a way that lipoxygenase activity is strongly reduced, splits and damaged beans are better eliminated, and the overall process yield might significantly improve. Wet hulls also increase okara mass and moisture. In a dehulling step, most of the soy hypocotyl (soy germ, rich in isoflavones) will be removed, giving a less astringent product. When properly done, dehulling might even improve emulsion stability of the Soy Base. Anyway, with or without dehulling, soybeans always need to be properly cleaned by the supplier or at the entry of the production process: destoning; removal of foreign material and if possible also removal of splits; sizing.

The first step using water is the presoaking step. Do we need to presoak? And how long and at what temperature? This step has as main objective to tenderize the soybeans, which will considerably affect the life of the grinding and homogenization equipment down in the process line. Soybeans are notoriously abrasive stuff! The presoaking step is also the ideal point of heat treatment and lipoxygenase denaturation: it's at the beginning of the wet processing; lipoxygenase denaturation goes extremely fast at high moisture conditions (even below 60°C). This will result in less risk for the development of beany taste, and improve the protein extraction yield. It

will also make the soybeans soft and easy to grind. Soaking at low temperature is to be avoided because of the very high risk for lipoxygenase and other enzyme activity, except when this done in absence of oxygen. A variation on the presoaking step is blanching at elevated temperature. Blanching is typically done with vegetables to avoid unwanted (lipoxygenase) enzyme reactions during long-term storage. No blanching will result in a reduced loss of insoluble protein, as heat treatment is after separation; cooking could reduce protein solubility and extraction yield. Blanching eliminates lipoxygenase and other enzymes (lipases, phospholipases, proteases, TI, urease), and improves the taste by reducing the flavor deterioration in long shelf-life products. Loss of soluble components may affect mouthfeel and taste.

Some processes recommend adding sodium bicarbonate at this stage of the process. This will slightly increase the pH of the solution and soften the beans, while solubilizing more soy protein. Is this important? Probably not, since all protein will be denatured and insolubilized anyway as soon as the slurry is cooked. It will also increase the need for more chemicals addition and another pH adjustment afterwards to obtain good taste. Why add chemicals if it can be done without?

Next is the grinding step. Again this can be done hot or cold. At low temperature, the protein solubility argument may play a role: no protein denaturation early in the process could improve the extraction yield. But at low temperature the risk for higher abrasiveness of the soybeans increases. Hot grinding (>80°C) will eliminate lipoxygenase and other enzymes if not done before, and stabilize the taste profile. It will also make size reduction and grinding much more efficient, giving a better overall yield. Various types of grinding equipment can be used: from simple airless screen grinding to colloid mills, and even high-pressure homogenization.

One final step remains: the fiber separation process. Depending on the scale of the production unit this can be done with various types of equipment: with a decanter, centrifuge or by course filtration. Filtration is typically a small-scale solution; centrifugation is larger scale with semi-continuous okara discharge. Decanters with continuous okara discharge are used in large-scale processing.

Some companies also recommend using an extra deodorization step, removing traces of off-flavor. This will remove volatile off-flavors, remove the dissolved air from the Soy Base, and with the applied steam injection give an additional pasteurization effect. However, it will require an extra processing step, and if the final product is pasteurized or sterilized, maybe even using UHT, another deodorization might follow anyway.

The final product's physico-chemical stability is judged on absence of particle sedimentation and oil droplet creaming. These are governed by factors such as pH, emulsion stability (emulsifier; salt), homogenization and clarification efficiency, the use of a soaking step, the intensity of heat treatment and particle size (grinding efficiency), and the presence or absence of salt and stabilizers. The shelf life of the Soy Base depends on the quality of the raw materials, the stabilization process (pasteurization, sterilization or UHT), the storage and distribution temperature, the quality of the packaging, and the formulation of the soy drink (effect of salt and stabilizers).

European-type soymilk is generally formulated on 3.6% protein level, which corresponds with 2.5% oil and 3.4% sugars for the plain unsweetened type. A typical American soymilk will rather be on about 2.5% protein, and 1.8% oil. Most American products are heavily sweetened with sugar-levels up to 10%. South American products are often blends with fruit juice. Asiatic types of soymilk tend to fluctuate between 2 and 3.5% protein. Anyway, Soy Base is not only used for soymilk formulation, but can also be used for blending with other products such as fruit juice, or used as the starting material for tofu, yogurt, custard and ice cream.

Evidently, yield calculation will not look at the volume produced, since dilution to any protein level is theoretically possible. Yield will rather be expressed on dry matter or on protein level. Starting from whole soybeans less moisture (12 ± 2%), a first loss is found in the dehulling (8 to 15 to 25%). More losses follow in the okara separation. The final product yield can be expressed as a soy solids yield (*Ys*), or as a soy protein yield (*Yp*):

$$Ys = \frac{S*N*(1-L/100)}{B*(1-M/100)} \quad \text{and} \quad YP = Ys*Q/P$$

With B = soybeans (kg); M = moisture (%); L = dehulling loss (%); S = soymilk produced (kg); N = solids in soymilk (%; measured with moisture balance); P = protein in soybeans (% on moisture free basis); Q = protein in soymilk (% on moisture free basis).

Optimizing this yield is of utmost importance for a successful long-term profitable plant operation. Obviously, in small-scale plants, limitations are mainly due to grinding and separation efficiency. On average Soy Base at 4% protein should come from such type of processing. One kg of soybeans will then yield six to seven liter of Soy Base.

This can however, easily be optimized in larger scale operations by the introduction of more efficient grinders and homogenizers, and by using

more efficient centrifuges or decanters. A final optimization will come from countercurrent okara washing using fresh washing water. This will require at least one extra centrifuge or decanter (the pay-off for a second washing is mostly not interesting) and extra automation and process procedures. On average, one extra washing will result in a ten percent yield increase. Using an okara washing step also enables Soy Base production at 5% protein level, giving much more flexibility in downstream formulation. One kg of soybeans will then yield six to seven liter of a more concentrated Soy Base.

Companies interested in investing in soymilk production can build such a plant all by themselves and integrate the equipment of choice for every process step envisaged. However, for many operators, this will be way too complex and tedious to manage. In such case, a turnkey plant will give a complete solution.

The impact of soybean quality on the production of soybean meal, full fat soybean meal and soybean oil

P. G. Díaz[1]

Abstract

The degree of quality of the soybean used for crushing has an impact on the quality of the meals and oil that is produced in the mills. It is very important to define the specifications that the soybeans should meet to maintain a high level of quality in the meals and the refined oil

There are several characteristics and values in the soybeans that are intended for crushing in mills to extract the oil and to produce soybean meals and full fat soybean meals. All the characteristics of the soybeans have and impact on the quality of finished products as well as on the yields of the mills.

The yield of the mills and the economic figures of the oil refineries are affected by the variations on the specifications of the soybeans.

The soybeans can be classified and graded using different measurements. The U.S. soybeans are commonly traded using standards established by the U.S. Department of Agriculture (USDA). The USDA grades and grade requirements include the following parameters: Weight per bushel (lbs), Total damaged kernels %, heat damaged kernels %, foreign material %, splits % and soybean of other colors. Depending on the values of the samples, the soybeans are classified on Grade US Number 1, Number 2, Number 3 and Number 4

All the shipments of US soybeans exports are officially inspected by the Federal Grain Inspection Services. This inspection is mandatory on export shipments. Voluntary grading on domestic shipments us usually done by plants, elevators and laboratories for controlling storage and processing practices at elevators and crushing plants. The US Grades Numbers are also used to established the prices for the soybeans

The US soybeans that the crushing industries usually crush is grade US No 2 that requires Minimum test per bushel of 54 Lbs, and maximum level as follows: heat damage kernels 0.5 %, Total damaged kernels 3.0 %, foreign material 2.0 % splits 20 % and soybean of other colors 2.0 %

[1] American Soybean Association, Mexico – pgonzalez@soyamex.com.mx; pgonzalezdiaz@aol.com

Moisture was eliminated as a grading factor, but it is required to be shown on the certificate.

Other specifications such as oil content and protein content can be established on the contracts.

The trading of soybeans is of international scope and the signature of purchasing / buying contract is recommendable. The National Oilseed Processors Association (NOPA) edited Yearbook and Trading Rules 2003-2004 that include trading rules for soybeans, soybean meals and soybean oil, methods of analyses, sampling procedures, certification of installation, automatic sampler & mechanical divider, official referee laboratories and chemists, sales contracts, inspection, export trading rules and foreign trade definitions.

The specifications of the soybeans are affected by storage and transportation and it is a common practice to take the samples of the soybeans and conduct the evaluation at the point of shipment.

When the soybeans are received in the mill the shipment is sampled, analyzed and evaluated against the specifications and the contract of purchase.

The impact that each quality factor has on the meals and soybean oil is analyzed separately

Moisture: There is an optimal value of moisture of soybean for proper storage that is related to the temperature of the surrounding air and to the relative humidity of the place. The temperature of the grains and the percentage of moisture determine the allowable storage time (Spencer, M.R. J. Amer. Oil Chem. Soc. 53: 238. (1976)

High moisture of soybean will reduce the allowable storage due to the proliferation of microorganisms

High moisture will also reduce the yield of the mills and the nutritional value of soybean meal

High moisture content of soybeans affect the dehulling operation and difficult the formation of correct flakes for oil extraction

Low moisture content on the contrary, will produce an excessive amount of dust in the breaking of soybeans.

Too much variation in the moisture content affects the preparation of the soybeans, the breaking, dehulling and flaking operations

The utilization of Silos for equilibrium of moisture is recommendable

Density lbs/Bu.- Variation in the density of soybeans is due to the different sizes of the kernels. Too much variation in the size affects the preparation of the soybeans. Small grains will produce small particles and small flakes will produce too much fines with negative effect in the oil extraction

Heat damage kernels .- This damage usually presented when the grains were not properly dried or, when were transported at high level of moisture, the damage will reduce the protein content of meals, the palatability of the meals and will affect the color of the oil.

Total damaged kernels.- An excessive amount of damaged kernels will reduce the yield of the mills, affect the preparation and all the operations necessary for oil extraction.

Foreign material.- An excessive amount of FM reduce the yield of soybean meals and oils, affect negatively the costs of the mill and reduce the protein content of the meals.

Splits.- Broken grains and splits have active enzymes that initiate degradation of the oil and will produce negative effects on the shelf – life of the oil with objectionable flavors and oxidative reactions

Soybean of other colors .- When the grains are green will produce oil with green tones that are very difficult to remove. The shelf life of the oil may be affected due to the presence of pro oxidant materials

Protein content.- This value is of key importance for the production of meals of high protein content.

Oil content.- The oil content of the soybeans will determine the yield of the extraction plant and in combination with the protein content determine the value of the soybeans for the mills and the refineries

The level of quality of the 2002 US crop of soybean is analyzed

Do findings from the women's health initiative have implications for soyfoods and soybean isoflavones?

P. Albertazzi and S. Lecturer[1]

Hormone replacement therapy (HRT) is used mostly for the relief of menopausal symptoms such as hot flushes, and for the prevention of osteoporosis. Its effectiveness and safety continue to be tested and debated. In 2002 the Women's Health Initiative reported the results of a study on the effects of combined oestrogen plus progestogen on healthy postmenopausal women. They found increased rates of breast cancer, coronary heart disease, stroke, and venous thromboembolism and decreased rates of hip fracture and colorectal disease but no "global" benefit.[1] It was concluded that combined oestrogen and progestogen is not suitable for the prevention of chronic diseases and since then there has been a dramatic decrease of HRT use in many part of the world.[2,3]

This has sharpened the interest both in women and in health cares providers in non estrogenic alternatives to treats symptom and condition related to the menopause.[4] In the last decade, the dietary supplements industry has grown rapidly and the popularity of alternative therapies, mostly dietary supplements, for the management of the symptoms of menopause is unprecedented.[5] Food supplements are perceived to be safer than HRT. Furthermore, since are generally not prescribed by doctors, some women report that with their use, they are taking more personal control over their health care.[6] Since self care has become so attractive, manufacturers have responded by developing a wide array of dietary supplements mostly containing soy derived isoflavones.

Epidemiological data has suggest that Asian women have a much lower incidence of hot flushes, coronary artery disease, osteoporosis and estrogen dependent cancers such as that of the breast compared to Western women. In vitro studies show marked biological effects of soy isoflavones, but prospective studies performed so far in Western Women using different types of isoflavones have failed to meet this degree of efficacy. In particular the effects of different preparation of soy isoflavones on hot flushes have been mild at best, and no effect has been found in women treated for breast cancer. Trial design, difference in metabolism, suboptimal quality of product used, insufficient information about doses are all been blamed for these sub optimal results.

[1] Centre For Metabolic Bone Disease, HS Brocklehurst Building, Hull Royal Infirmary, Hull, HU3 2RW, UK.

But while research is still needed to find the most effective way to administer soy for climacteric complains, postmenopausal women all over the Western World have caught onto the idea that phytoestrogens may be beneficial to their health. In 2000 the value of the European market for phytoestrogens was estimated to be € 106. Dietary supplements containing isoflavones are an expanding market and presently represent 9% of the phytonutrient market value. Research from market analyst predicts that the combined phytonutrient market will increase to an estimated €163 million by 2008. [7] [8] Unfortunately, the fast pace at which these products enter the marketplace leads the general public and health professional alike to question their quality and safety. The several research reported by the media have reinforced the doubt that many dietary supplements do not actually contain the ingredients or the ingredient quantities listed on their labels. These reports create confusion among both consumers and health professionals alike about which manufacturers are credible and which products are suitable for use.

This presentation will critically review the data available in order to extrapolate indications for research on products with improved effectiveness to be used in menopause.

Key Word: Phytoestrogen, Soy, Menopause, Hot flushes, Isoflavones

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Coronary benefits of soya: cholesterol reduction and beyond

C. W. C. Kendall and D. J. A. Jenkins[1]

Abstract

There is much evidence indicating that soy protein is effective in reducing serum cholesterol concentrations and this was supported when the Food and Drug Administration of the United States permitted a health claim for soy protein and heart health. While the mechanism(s) responsible for the lipid-lowering effects of soy are not agreed on and there is need for further study, several have been suggested. Most recently the lipid lowering effect of soy has been attributed to the isoflavonoids associated with the protein fraction. A number of lines of evidence indicate that soy isoflavones due to their polyphenolic structure may also reduce oxidation of LDL cholesterol, thereby further reducing risk of cardiovascular disease. Soy protein and isoflavones may have additional benefits in terms of cardiovascular health beyond that of cholesterol reduction and the prevention of LDL-oxidation. Recent studies indicate that soy may decrease homocysteine levels, reduce blood pressure, inhibit platelet aggregation and enhance vascular reactivity. These lines of evidence point to the inclusion of soy as part of a heart healthy diet. Indeed, our own recent studies indicate that a Portfolio Diet containing soy protein and other cholesterol-lowering dietary components may be as effective as the first generation statins in reducing LDL-cholesterol.

Introduction

Cardiovascular disease (CVD) continues to be the leading cause of mortality in Western civilizations. For cardiovascular disease, preventive strategies have focused in the past on serum lipids and blood pressure reduction where considerable gains have been made in terms of diet and pharmacological approaches. However other major risk factors for cardiovascular disease, including oxidative stress, inflammatory biomarkers, serum homocysteine levels, clotting factors and vascular reactivity, have recently been identified. The impact of dietary factors, including soya, are now being investigated to determine if these markers of cardiovascular risk may be modified through dietary interventions.

[1] PhD. Clinical Nutrition & Risk Factor Modification Center. St. Michael's Hospital; Department of Nutritional Sciences, Faculty of Medicine, University of Toronto; Toronto, Ontario, Canada
Address correspondence to Dr. Cyril W.C. Kendall at the Department of Nutritional Sciences, Faculty of Medicine, University of Toronto, Toronto, ON, M5C 3E2, Canada.
Phone: (416) 978-6527; Fax: (416) 978-5310; EM: cyril.kendall@utoronto.ca

Elevated low-density-lipoprotein (LDL) cholesterol increases risk for CVD. Amongst dietary components, soy occupies a central position in lipid lowering related to its effect, possibly associated with isoflavones, on serum cholesterol reduction (1). However soy also contains a number of components including an oil low in saturated fat and relatively high in polyunsaturated fats together with plant sterols, all of which, together with its viscous soy polysaccharide fiber, will tend to lower serum cholesterol (Table 1). Add to the lipid-lowering effect of soy its antioxidant activity, its possible effects on homocysteine and clotting factors and its effects on vascular compliance and vascular reactivity, and soy appears to be ideally suited to CVD risk reduction.

The purpose of the present paper is to discuss the potential benefits of soy in CVD risk reduction including but not exclusive to its effects on cholesterol and to outline our recent experience with soy in combination with other dietary components to maximize cholesterol reduction.

Soy and cholesterol reduction

It has been suggested that soy food consumption may be part of the reason for the low rates of CVD in the industrialized nations of the Orient, where deaths from CHD are half those of North America. Interest in the hypocholesterolemic effect of soy was greatly increased when Dr. James Anderson published his meta-analysis of data from 38 controlled studies of soy protein and blood lipids (2). In these studies total cholesterol was reduced by 8.3%, LDL cholesterol was reduced by 13%, importantly the HDL was increased by 2.4%, and triglycerides, another risk factor for heart disease, was reduced by 10%. While the mechanism(s) responsible for the lipid-lowering effects of soy are not agreed on and there is need for further study, several have been suggested (Table 2) (3-10). Some researchers believe that the amino acid profile of soy may be responsible for the lipid-lowering effect, as arginine levels are higher in soy protein than in animal proteins and an elevated ratio of lysine to arginine has been found to be more atherogenic in some studies. Other possible mechanisms include particular peptides (7s and 11s globulins), higher glucagon:insulin ratio, higher free thyroxine levels or alterations in bile acid balance (Tables 1 & 2). Most recently the lipid lowering effect of soy has been attributed to the isoflavonoids associated with the protein fraction. These compounds, often referred to as phyto-estrogens due to their structural similarity to estradiol, may act on sex hormone receptors as agonists and antagonists.

Table 1. Active Ingredients in Soya Beans

- Soy Polysaccharides (fiber)
- ω-3 Fatty Acids
- Plant Sterolds
- Amino Acid Profile (*arginine rich*)
- Peptides (*7S and 11S globulins*)
- Protein Hydrolysate
- Saponins, Trypsin Inhibitors, Phytic Acid
- Isoflavonoids (*tamoxifen-like phenolics*)

Table 2. Soya: Mechanism for Cholesterol Reduction.

Author	Journal	Date	Mechanism
Carroll	J Am D Assoc	1991	↑ Arginine or ↓ Lysine: Arginine ratio
Carroll	J Am D Assoc	1991	↓ Insulin: Glucagon ratio
Fumagalli	Atherosclerosis	1982	↑ Bile acid losses
Hori	Biosci Biotechnol Biochem	2001	↑ Steroid losses
Huff	Atherosclerosis	1977	↑ VLDL apo B turnover
Lovati	JCI	1987	↑ LDL-receptor activity
Setchell	J App Bacteriol	1985	Tamoxifen-like effect
Anthony	Arterio Thromb Vasc Biol	1995	Isoflavonoids responsible for 70-80% of effect (cynomolgus monkeys)

Recently, several studies have specifically investigated the role of isoflavones in cholesterol reduction. Isoflavones naturally present in soy have been implicated in influencing the serum lipid changes associated with soy consumption in some but not all of these studies (Table 3) (11-18). Interestingly, isolated isoflavones given in pill form do not appear to reduce serum cholesterol concentrations, indicating that other components present in soy protein are either acting independently or synergistically with the isoflavones to improve serum lipids. Regardless of the role of the isoflavones, the majority of studies continue to indicate that soy protein, even at relatively low intakes, is an effective dietary component for decreasing serum cholesterol concentrations.

Antioxidant activity

Studies on the effect of soy on reducing measures of oxidative stress have generally yielded positive data. Soy isoflavones have been shown to reduce lipid and lipoprotein oxidation in vitro (19-24) and soy feeding was associated with reduced circulating leukocyte DNA oxidative damage assessed in 11 men by the comet assay (25) despite no change in serum cholesterol.

Table 3. Effect of Soy Foods Varying in Isoflavone Content on LDL-Cholesterol Reduction

Authors	Isoflavones	LDL Effect	
Hodgson et al, 1998	55 mg/d	no effect	
Baum et al, 1998	56, 90 mg/d	no effect	
Crouse et al, 1999	3, 27, 37, 62 mg/d	↓ LDL	Dose-response
Merz-Demlow et al, 2000	10, 65, 129 mg/d	↓ LDL	Dose-response
Wangen et al, 2001	7, 65, 132 mg/d	↓ LDL	Dose-response
Gardner et al, 2001	3, 80 mg/d	↓ LDL	Dose-response
Dent et al, 2001	4, 80 mg/d	no effect	
Hermansen et al, 2001	165 mg/d	↓ LDL	

At present the antioxidant activity of soy isoflavones appears a likely reason for the antioxidant activity of soy foods (28). However as has been pointed out the isoflavone concentration in LDL is low after soy feeding despite increased resistance to ex vivo oxidation (26) and recent studies using 55 mg isoflavonoid daily in 5-9 subjects in an 8-week parallel study failed to detect changes in F_2 isoprostanes as markers of in vivo lipid peroxidation (29). Other components of soy may also play a role. Nevertheless the combination of lipid-lowering with antioxidant activity would appear to be a good combination to reduce atheroma formation and hence CHD risk (28,30).

Our own studies have consistently shown reduction in native conjugated dienes in the LDL fraction of serum for hyperlipidemic subjects in the absence of ex vivo copper-ion oxidation. We have observed these effects at soy protein intake levels as low as 13 g/d in ad libitum diets (31). In metabolic diets similar effects have been seen both in subjects taking vitamin E (400-800 mg/d) and those not taking supplements (32). We have observed the effect even when serum lipid levels were not reduced in subjects who took soy in breakfast cereal form and the comparison was therefore between soy protein and starch (i.e. a high soy protein cereal versus a high starch cereal) (33). We have also seen the effect at high (69 mg/d) and low (9

mg/d) levels of isoflavone intake with no significant difference in effect between treatments (34).

Soya and cardiovascular disease risk reduction: beyond cholesterol

Soy may have additional cardiovascular disease benefits beyond its effects on cholesterol, including reductions in homocysteine and blood pressure, improved arterial function and inhibition of platelet aggregation (Table 4). Soybeans are rich sources of folate and vitamin B_6 and may therefore have favorable effects in terms of reducing homocysteine, an independent risk factor for cardiovascular disease. This was recently investigated in a cross-sectional study of premenopausal women living in Japan. Soy intake was found to be positively associated with serum folate concentrations ($r=0.15$, $P=0.04$) and inversely correlated with serum homocysteine ($r=-0.15$; $P=0.04$) (35). The ability of soy to reduce serum homocysteine has recently been demonstrated in clinical studies both in hyperlipidemic subjects (34,36) and in individuals with type 2 diabetes (18).

In a recent cross-sectional study conducted on Japanese women, the correlation between soy intake and systolic pressure was found to be borderline significant ($r=-0.10$, $P=0.09$) with no correlation observed for diastolic blood pressure (37). The results of clinical studies are also equivocal with soy protein being associated with reduced blood pressure in perimenopausal women (38) in hyperlipidemic men but not women (34) and no effect being observed in subjects with type 2 diabetes (18,39). Part of the discrepancy may relate to the difficulty of accurately assessing blood pressure, due to day to day variation and the rapidity of blood pressure changes to external stimuli. The ability of soy to reduce blood pressure remains unclear and quite obviously more research is required in this area.

Normal arterial function is critical in the prevention of ischemic heart disease. It is measured by endothelial mediated vasodilatation (i.e. the ability of an artery to dilate) and by vascular compliance (i.e. a measure of arterial stiffness) (40). Soy protein has been found to improve endothelial function in hypercholesterolemic men (41). However all studies are not in agreement as one recently found that soy improved arterial dilatation in postmenopausal women yet worsened it in men (40). In animal studies, fermented soybean extract was found to suppress intimal thickening and inhibit thrombos formation in mice (42) and intravenous injection of the isoflavone genestein was found to prevent thrombogenesis and inhibit ex vivo platelet aggregation induced by collagen (43).

Portfolio diet

Diet continues to be stress by the major health agencies as the cornerstone for managing raised blood lipids for primary prevention of coronary heart disease (CHD). Recently, in addition to dietary guidelines that emphasize reductions in saturated fat and dietary cholesterol, the National Cholesterol Education Program (NCEP) Panel III has recommended plant sterols (2g/d) and viscous fibers (10-25g/d) as additional dietary options to maximize the effectiveness of diet and the American Heart Association (AHA) has drawn attention to the benefits of soy proteins. In the United States, the Food and Drug Administration (FDA) now permits health claims for CHD risk reduction for foods delivering adequate amounts of plant sterols, viscous fibers and soy proteins. However, the effectiveness of these dietary factors in combination has not been investigated. In view of the differences in possible mechanisms of action, the cholesterol-lowering effects of these various dietary components may be additive. A Portfolio Diet combining vegetable protein (emphasizing soy and almonds), viscous fiber and plant sterols was therefore investigated. Preliminary studies indicate that a Portfolio Diet containing soy protein may be as effective as the first generation statins in reducing LDL-cholesterol (44-46).

Table 4. Soya and Cardiovascular Disease Risk Reduction.

Cholesterol Effects:
• decrease LDL
• inhibit LDL oxidation
Non-cholesterol effects:
• decrease homocysteine
• decrease blood pressure
• enhance vascular reactivity
• inhibit platelet aggregation

Conclusion

The data for the cardioprotective effects of soy are strongest for the lipid lowering and antioxidant activities, which together make an attractive pairing for CHD risk reduction. Although isoflavones may play a role in both these changes it is likely that other aspects of soy have major roles as yet to be clearly defined. Beyond the cholesterol effects of soy the data are less clear and more research is required to determine possible benefits on

homocysteine reduction, blood pressure, arterial function and platelet aggregation. However, dietary approaches that include soy to lower serum cholesterol maximally have shown dramatic reductions in LDL-cholesterol, similar in magnitude to those achieved with starting doses of current cholesterol lowering medications.

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Soy intake and cognitive function: results from clinical trials

S. E. File; S. Elsabagh and D. E. Hartley[1]

Introduction

Cognitive function is an umbrella term used to encompass a range of mental functions, such as attention, learning, memory, mental flexibility and planning. Many drugs, dietary insufficiency and physiological factors, such as sleep deprivation, can impair cognitive function.

With increasing interest in potential health benefits of soy, a crucial question is whether there might be cognitive benefits following soy administration. Three recent studies of soy administration from 3-6 months provide some evidence that this might be the case (File et al, 2001; Duffy et al, 2003; Kritz-Silverstein et al, 2003) and the results of our latest study (File et al., 2004) suggest that the onset of benefit may be as soon as 6 weeks. Two important questions arise with respect to the effects of soy. Firstly, and most importantly, is whether the reported benefits are reliable, or whether they could have occurred by chance. Secondly, are the benefits specific to particular cognitive processes, or are they rather non-specific in nature. One important confounding factor to exclude is any possible mood changes, since, for example, if one group was more depressed than the other, this could result in different performance levels in the cognitive tests. None of the studies on the effects of soy found any mood changes and hence this indirect influence on cognition can be ruled out. All the studies on soy also took care to match the two groups for IQ and educational level, again factors that can have an important influence on cognitive performance. It is also possible to see a non-specific improvement in performance of cognitive tasks through an action to reduce reaction times. However, there is no evidence from any of the studies that administration of soy has a general effect of speeding up reaction times.

Attention

File et al (2001) randomly allocated student volunteers to receive 10 weeks of a low (0.5 mg total soy isoflavone/day) or high (100 mg total soy isoflavones/day) soy diet. They were tested at baseline, before the dietary

[1] Psychopharmacology Research Unit, Centre for Neuroscience, Hodgkin Building, King's College London, Guy's Campus, London SE1 1UL, UK.

regime started, and after 10 weeks. Three tests of attention were given. The digit-symbol substitution test, which measures attention and psychomotor speed, the digit-cancellation test, which measures focussed attention, and the paced auditory serial addition task (PASAT), which measures sustained attention. In none of these tasks was any effect of the soy diet apparent.

Duffy et al (2003) randomly allocated post-menopausal women to receive placebo or a soy supplement (60 mg total isoflavone equivalents/day) for 12 weeks. They were tested at baseline and at the end of the treatment period. The only test of attention included in this study was PASAT. The group that had received the soy supplement showed significantly better performance, but only at one speed of presentation. In our most recent study (File et al, 2004) 50 post-menopausal women were randomly allocated to placebo or soy supplements (60 mg total isoflavone equivalents/day) and tested at baseline and after 6 weeks of treatment. No effects in the PASAT were found.

Kritz-Silverstein et al (2003) randomly allocated post-menopausal women to receive either 6 months of soy supplement (110 mg total isoflavones/day) or placebo. Subjects were tested at baseline and after the 6 months of treatment, and there was no effect in the Trails A test.

It would therefore seem that there is little evidence that soy supplementation alters attention. Therefore, this factor can be excluded as an explanation for any improvement found in other cognitive tasks.

Short-term memory

File et al (2001) included two tests of short-term memory, immediate story recall and delayed matching to sample, a non-verbal task. After the 10 weeks of high soy diet the students were significantly better in both of these tasks than those that received the low soy diet. Duffy et al (2003) also found improved short-term memory in the post-menopausal women receiving soy supplements for 12 weeks. This nearly reached significance ($p < 0.06$) for the story recall, but not in the DMTS task. In our study on the effects of 6 weeks of soy supplement to post-menopausal women (File et al, 2004) we found significantly better performance in the DMTS task in the soy group and improved immediate story recall that just missed significance ($p < 0.08$). Kritz-Silverstein et al (2003) tested short-term verbal memory using immediate story recall. After 6 months the soy treatment group showed a greater improvement than the placebo group on this test, but this was not significant.

Thus, overall these studies support the conclusion that soy supplementation

can improve short-term memory, both verbal and non-verbal. The onset of improvement seems to be fairly rapid, around 6 weeks, but the effects are relatively modest. There is no evidence that the effects are more marked in the older group than in the young students, so the actual baseline level of performance is not an important factor. All of the studies used relatively small samples ($n = 27$-53) and it is quite likely that the marginal levels of significance are due to this factor.

Long-term memory

In the study of the effects of a high soy diet in student volunteers, File et al (2001) found that the group consuming the high soy diet showed significantly better long-term recall of pictures than did the low soy diet group. Significantly better performance was also found in this task in the post-menopausal women receiving 12 weeks of soy supplementation (Duffy et al, 2003). However, when post-menopausal women were tested after 6 weeks of soy supplementation, there were no differences in picture recall, or in long-term story recall (File et al., 2004). It is therefore possible that treatment for at least 10 weeks is necessary in order to obtain benefits in long-term memory. However, although Kritz-Silverstein et al (2003) found improved long-term memory in the delayed paragraph recall task in post-menopausal women receiving soy supplements for 6 months, this did not reach significance. It is thus possible that some tolerance might develop to the benefits of soy with longer term treatment. It is quite possible that different cognitive functions will display a different time-course in response to soy supplementation, but only further experimentation will be able to resolve the pattern with respect to long-term memory.

Another aspect of long-term memory has been assessed using a category generation task. This task has elements of verbal fluency and semantic memory. File et al (2001), Duffy et al (2003), and File et al (2004) all found no effects in this task, suggesting that at least up to 3 months of treatment with soy is ineffective. However, after 6 months of soy supplements, Kritz-Silverstein et al (2003) found significantly better performance in post-menopausal women. Thus, it is possible that the mechanism underlying the cognitive functions engaged by this task need a relatively long period of treatment before it is subject to change by soy.

Frontal lobe functions

a) Mental Flexibility

In our three studies, mental flexibility was assessed in a task of rule learning, reversal and rule shifting. We used the IDED task from the CANTAB computerised battery (CeNeS, Cambridge), which is an automated version

of the Wisconsin card-sorting task. File et al (2001) found that the students on the high soy diet showed significantly greater improvement in the trials to complete this task than did those on the low soy diet. Our post-menopausal women found this task difficult and several were unable to complete all the stages. However, it was possible to analyse their performance as far as the stages of rule reversal. Whilst the soy supplementation had no effect on the ability to learn the rules, the women receiving soy were significantly better (i.e. completed the task more rapidly) at learning rule reversals than those receiving placebo. This was found after both 6 and 12 weeks of supplementation, see Figure 1. It would therefore seem that an improvement in mental flexibility is a consistent finding following soy supplementation, and that it can be seen in both young students who show a high ability in this task, and in an older group in which the performance level is much lower.

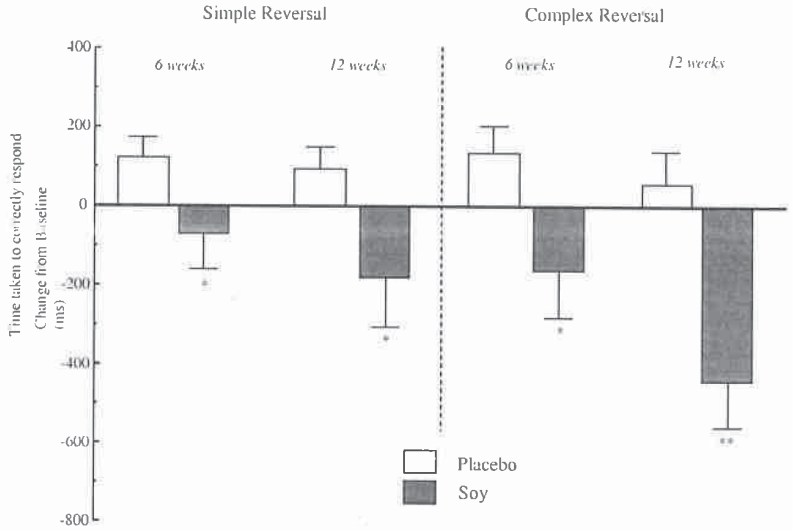

Figure 1. Mean ± SEM change in performance from baseline day in the time (ms) taken to learn simple and complex rule reversals in a test of mental flexibility (IDED) by subjects after 6 weeks and 12 weeks of treatment with either placebo or soy. *Pd"0.05, **Pd"0.01

b) Planning

Ability to plan was measured in our studies in the Stockings of Cambridge test from the CANTAB. Once again our student volunteers showed a higher level of performance in this test than did our post-menopausal groups. The

students were able to complete all stages of the task and all the male students showed a significant improvement in the number of moves to complete the hardest part of the test (5 moves task) from the first test at baseline to the second one after 10 weeks. Of the female students only those on the high soy diet showed a significant improvement from baseline to week 10 (File et al, 2001). In the studies on post-menopausal women, significant improvements were seen in the time to complete the 4 moves part of this task in the group receiving 12 weeks of soy supplements (Duffy et al, 2003), and in a reduction in the number of moves to complete this task after 6 weeks of soy (File et al., 2004). Kritz-Silverstein et al (2003) found no effect of 6 months of soy supplements in the Trails B test, which measures attention and ability to plan. However, it is possible that this a less sensitive measure of planning ability than the stockings of Cambridge test.

Discussion

The results of the four studies discussed in this paper allow us to draw some preliminary conclusions about the possible cognitive benefits of soy. Firstly, it seems that overall there is some consistency in the pattern of results and that benefits can be seen in tasks measuring memory and frontal lobe function.

After 6 weeks of soy supplements to post-menopausal women and 10 weeks of a high soy diet in young volunteers there were significant effects on short-term memory. Short-term memory was also improved in a group of post-menopausal women receiving soy supplements for 12 weeks, but this just missed significance. In the group tested after 6 months of soy, there was again a trend for improved short-term memory, but this was not significant. It is therefore possible that there is a relatively rapid onset of improvement in short-term memory, but that this benefit decreases with prolonged treatment. In contrast, there was no evidence for any effect of 6 weeks of soy on long-term memory, although after 10 weeks of high soy diet or 12 weeks of soy supplements there were improvements. Thus, there might be a slower onset of effect of soy on this memory process. Although the post-menopausal women who received 6 months of soy supplements showed better long-term memory, this did not reach significance and it is possible that prolonged treatment is less effective. However, after 6 months of supplements the group receiving soy were significantly better in the category generation task, although none of the shorter periods of treatment showed any benefit in this task. Thus, although broadly speaking soy administration can be said to improve memory, there might be differences in the time-courses of onset for the different memory functions.

In the tests of frontal function, the pattern of benefits from 6 weeks of soy supplements to post-menopausal women was exactly the same as that found after 12 weeks. It was also the same as that found after 10 weeks of a high soy diet. This raises the interesting possibility that soy may particularly benefit frontal lobe functioning. However, although the post-menopausal women tested after 6 months of soy supplements were better at planning, this was not significant. This could be because of the use of a less sensitive measure of planning.

Overall, whilst the findings of the Kritz-Silverstein et al (2003) study show the same general pattern as our three studies, the findings did not reach significance except for the category generation task. This suggests that with longer treatment with soy isoflavones there is some loss of benefit, or that for some other reason the Kritz-Silverstein study had reduced sensitivity to ours. It is unlikely to be due to a difference in dosing regimens as the dose used in their study (110 mg/day) was similar to the dose used in the 10 week soy diet study (100 mg/day; File et al 2001), where effects were found. In addition the group size used by Kritz-Silverstein was similar to that used by File et al (2003, 2004), and the two groups were matched for demographic characteristics. Possible factors that may have masked the effects of soy in the Kritz-Silverstein study include the failure to exclude subjects who had recently used hormonal replacement therapy in the months preceding the trial, and the failure to enquire about subjects' use of supplements other than soy products. Estrogen replacement therapy (Duka et al, 2000) and certain herbal supplements, such as ginkgo biloba (Hartley et al, 2003) and ginseng (Kennedy et al, 2001) are known to possess cognitive enhancing effects, which could have masked the effects of soy if.

Conclusion

The evidence indicates that soy specifically and reproducibly improves short-term memory and frontal lobe function. These effects can be extrapolated to situations of everyday life, where planning and short-term memory are essential processes needed to perform simple tasks. If there were a slight improvement on each task performed throughout the course of the day, the overall effects would be accumulative. Improvement in cognitive function, however slight, is something most people would consider an asset, particularly groups such as post-menopausal women who are subject to declining cognitive function.

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Does soya alleviate hot flushes?

E. Laudanna[1]

Comparative clinical study between a Soybean Protein Isolate versus Classical HRT for the Treatment of Menopause Symptoms. Esther Laudanna, Angela Maggio da Fonseca; Claudia Pinto M. de Oliveira; Vicente Renato Bagnoli; Hans Wolfgan Halbe; Paulo Augusto de Almeida Junqueira; José Aristodemo Pinotti. Clinical Nutrology Group (Department of Gastroenterology) and Climacteric Group (Department of Gynecology and Obstetrics) – Clinical Hospital of the University of São Paulo – Brazil.

Objective

Evaluating the action of a food based on soybean protein isolated rich in isoflavone, in a known dose, in comparison to the hormone replacement therapy (HRT).

Methods

98 symptomatic and menopause women were clinically evaluated in an open randomized study, being divided into two groups. Group A 50 women aged 54.65 ± 7.24 years; menopause period of 5.76 ± 5.06 years; Quetelet Body Mass Index (BMI) of 28.64 ± 4.5kg.m^{-2} and Kupperman Menopausal Index of 22.83 ± 9.62. Group B 48 women aged 52.02 ± 3.93 years; menopause period of 4.07 ± 3.41 years; BMI of 27.55 ± 4.03kg.m^{-2} and KMI of 19.94 ± 8.97. To the women in group A, during 4 months, were given 30g of a food (Previna®) in two doses of 30g/day, containing 45mg of isoflavones, 17g of soy protein and 360mg of calcium per dose. GroupB received an hormone replacement containing 2mg of estradiol + 1mg of norethisterone acetate, once a day. All the patients were clinically evaluated in the beginning and at the end of the 4 months in relation to Arterial Blood Pressure (ABP), Quetelet Body Mass Index (BMI), Kuppermann Menopause Index (K.I.) and adverse Symptoms related by the patient.

Results

Group A, in which the initial average KMI was 22.83 ± 9.62, had it reduced to 6.73 ± 5.58. This did not differ from the final KMI in group B, which was 5.41 ± 5.28 (p = 0.001). There were no differences between the two groups between the beginning and the end. Group A 8 patients in

[1] Collaborator Doctor from Nutrology and Gynecology Groups from FMUSP HC. Rua Martiniano de Carvalho, 934 – Apto 42 – Paraíso - São Paulo/ SP – Brasil - CEP 01321.000 – E-mail: pesquisa@estudosojamenopausa.com.br

discontinued the treatment (16%), group B 13 patients in discontinued the treatment (26.53%). In GroupA, 3 women felt flatulence, 1 woman showed allergy to soy and 1 showed an increase in blood uric acid. In group B, 6 women had return of menstruation and 4 had breast pain.

Discussion

The treatment of the symptoms and signs of climacteric used in the last 50 years is hormone replacement (TRH). The combinations and doses of the hormones used have varied, as well as the forms of administration. However, there are some women who cannot or do not want to use this treatment, because of fear. At the same time, some formal contraindications limit the use of TRH in some cases. Previous studies conducted in Brazil (8, 22) compared the effects of the ingestion of isoflavone pills with those of placebo on menopause symptoms and bone metabolism. Most studies conducted with isoflavones to treat menopause symptoms have used isoflavone pills or pure protein isolate versus placebo.

The subject remains controversial, nevertheless, and it requires further clinical investigation. It is enough to mention St. Germain et al. 2001 (14), which concluded that soybean isoflavones are inefficacious in the control of climacteric symptoms. This gap was the objective of the present work. In order to objectively characterize climacteric symptoms, we used the scored Kupermann Index. We did not use placebo with the control group, but the classical hormone replacement therapy (HTR), who's favorable and adverse results are well-known. The results were comparable in both groups, with evident improvement of the climacteric symptoms in both groups. In fact, in group A the Kupperman index dropped from 22.83 ± 9.62 to 6.73 ± 5.58; in group B it dropped from 19.94 ± 8,97 to 5.41 ± 6.28. These data are statistically equivalent when analyzed by the Wilcoxon score and by the t Student test for $p < 0.05$. These results show the efficaciousness of the food studied (Previna®) in the treatment of menopause symptoms. This observation legitimates the therapeutical prescription of this food as an alternative to HTR in the treatment of climacteric symptoms. It should observed, however, that, in the present work, we have had no significant complications in the group treated with the food. In group B, submitted to HRT, we observed mastodynia in 4 patients and metrorrhagia in 6 patients.

Having met the scope of this publication, we now turn our attention to the metabolic and hormonal aspects such as a possible action on the lipid profile, on osteoporosis, among other alterations resulting from climacteric hypoestrogenism, something we intend to cover in another paper.

Conclusions

This paper demonstrates that the tested food is as efficient in the treatment of menopause symptoms. Adverse reactions observed during the treatment with HRT, such as, breast pain, tromboflebite, among others, weren't observed in the group that received the food. In this group, no adverse effect was observed.

Key words: hormone replacement, menopause, isoflavones, Kupperman Menopausal Index, soybean, Previna®.

AGLICONES:	daidzein, genistein and glycitein
GLYCOSIDES:	daidzin, genistin and glicitin
ACETYL GLYCOSIDES:	6"-0-acetyldaidzin 6"-0-acetylgenistin 6"-0-acetylglicitin
MALONYLGLYCOSIDES:	6"-0-malonyldaidzin 6"-0-malonylgenistin 6"-0-malonylglicitin

Chart 1. Kinds of isoflavones and isomeric forms found in soybeans

Table 1. Arterial blood pressure, Body Mass Index and Kupperman Index of women consuming a food based on soybean protein isolated rich in isoflavone (Previna®) versus conventional HRT.

	GROUP A (Previna®)		GROUP B (HRT)	
	Initial	Final	Initial	Final
Kupperman Index	22.83 ± 9.62	6.73 ± 5.58	19.94 ± 8.97	5.41 ± 6.28
BMI (kg/m2)	28.64 ± 4.51	28.62 ± 4.42	27.55 ± 4.04	27.35 ± 3.90
Systolic pres. (mmHg)	128.76 ± 23.7	127.81 ± 16.59	134.6 ± 21.02	130.83 ± 5.88
Diastolic pres. (mmHg)	81.21 ± 14.6	81.07 ± 13.45	81.42 ± 11.72	80.30 ± 9.73

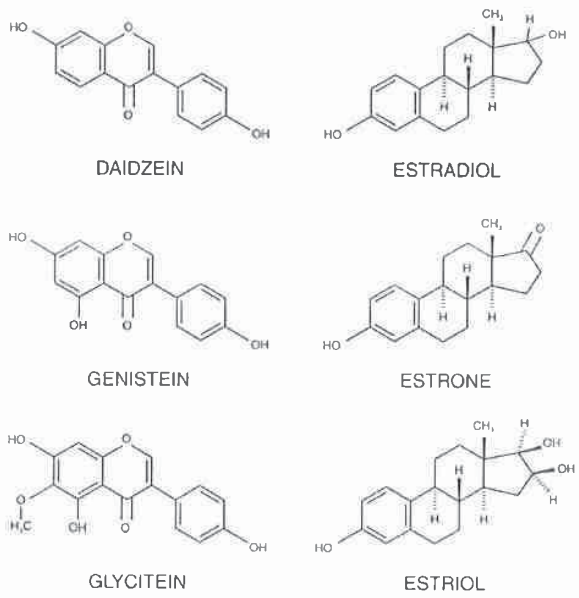

Figure 1. Comparison of the structures of estrogens and soybean isoflavone aglycones.

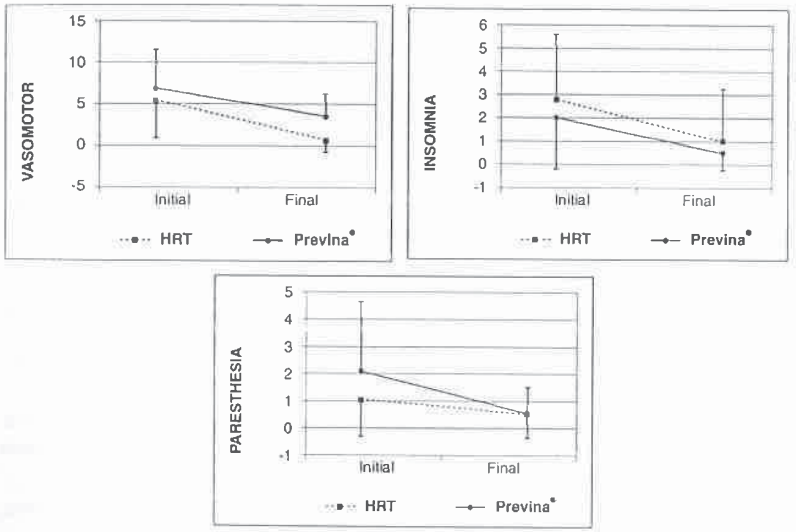

Figure 2. Vasomotor symptom, Paresthesia and insomnia reported for the women consuming a food based on soybean protein isolated rich in isoflavone (Previna®) versus conventional HRT.

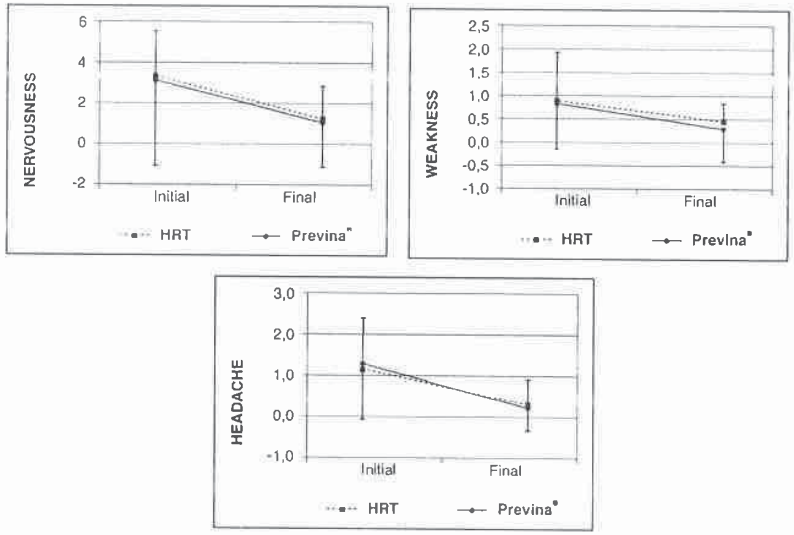

Figure 3. Nervousness, Weakness and Headache reported for the women consuming a food based on soybean protein isolated rich in isoflavone (Previna®) versus conventional HRT.

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Sensory properties of soy foods

S.-Y. Lee[1]

Abstract

Consumption of soy and soy-related food products have been related to many health benefits, such as lowering serum concentration of total cholesterol, LDL cholesterol and triglycerides (Bakhit et al., 1993; Widhalm and Brazda, 1993; Anderson et al., 1995), reducing the risk of breast cancer (Messina et al., 1994), reducing menopausal symptoms (Messina et al., 1994) and preventing osteoporosis by maintaining calcium levels in the bone (Breslau et al., 1988; Anderson et al., 1995). Moreover, in 1999, Food and Drug Administration's health claim on cardiovascular benefits of soy protein consumption has opened the door for a vast market in the soy foods area in the U.S.

Although the many health benefits of soy promise a great demand in the soy foods area, soy foods are still a niche market, mainly due to their off-flavors. Some aspects of soy flavor that have been reported are lack of desirable flavors, presence of unfamiliar flavor notes, presence of off-flavor developed from lipid oxidation, from various processing steps, and removal and/or masking of flavor (MacLeod and Ames, 1988). However, there still remains systematic understanding of soy flavor, due to the lack of appropriate sensory testing of soy foods. In order to appropriately conduct sensory testing of soy foods, specific objectives of the research need to be matched with sensory test objectives.

Recently, in an attempt to standardize the language used to describe soy foods, a lexicon for soymilks has been developed (N'Kouka et al., 2003), which provides a frame of reference for sensory profiling of other soy-related products. Moreover, thresholds for various isoflavones which were previously identified as the major compounds responsible for bitterness and astringency in soy food have been determined (Grady 2001, Robinson et al., 2003). The threshold studies demonstrated that the individual isoflavones do not elicit bitter/astringent off-flavors at the levels they are present in conventional soy foods.

The main goals of this presentation are to introduce various sensory evaluation methods and to discuss appropriate use of the sensory methods to fulfill specific research objectives.

[1] Dept. of Food Science & Human Nutrition – University of Ilinois, Urbana-Champaign, USA

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Improvement of food texture with soluble polysaccharides derived from soybean

H. Maeda[1]

Abstract

Soluble Soybean Polysaccharide, "SSPS", is a water-soluble polysaccharide from soybean cotyledon. It can be extracted with high purity from 'okara', the insoluble residue formed during the manufacture of soy protein isolate, and is marketed by Fuji Oil as "SOYAFIBE-S". It is mainly composed of galactose, arabinose and galacturonic acid and has an estimated molecular weight of several hundred thousand. It is spherical in shape with low viscosity and high stability in aqueous solution. SSPS can be used as a raw material for fiber-fortified foods, but also has many other food applications. It has useful properties related to dispersion, stabilization, emulsification, and adhesion, and one of its most popular applications is in beverages. It stabilizes acidified milk beverages and this, combined with its low viscosity, makes it especially suitable for milk beverages with lower pH and less non-fat milk solids. In cooked rice products and ready-to-eat noodles, SSPS helps to avoid stickiness by coating the particle surfaces. It also holds moisture and helps to conserve food quality. The polysaccharide binds to the surface of objects such as milk protein particles, cooked rice and noodles, and the driving force for its surface absorption appears to be its backbone galacturonan, while its side chains form a thick coating on particle surfaces.

Manufacture and structure

SSPS is made from the cotyledons of the seed of *Glicine max* MERRILL. It is produced from okara, the insoluble residue formed in the manufacture of soy protein isolate, by heating in weak acidic conditions. After extraction by an efficient patented technique, it is refined, pasteurized, and spray-dried, and comes out in powder form as shown in Fig. 1. A report on the extraction of SSPS from okara has already been published (Furuta et al., 1998).

Table 1 shows typical analytical values of a common type of SSPS, SOYAFIBE-S-DA100. The component sugars are mainly galactose, arabinose, and galacturonic acid but there are also many others such as rhamnose, fucose, xylose and glucose. Different types of SOYAFIBE-S are produced, each adapted to a specific function.

[1] Fuji Oil Co., Ltd., Food Science Research Institute Yawara, Tsukuba-gun, Ibaraki Pref. 300-2497, Japan.

Chromatographic analysis of SSPS by HPLC reveals three main components of approximate molecular weights 550,000, 25,000, and 5,000, with a mean value of several hundred thousand (Fig. 2). Recently, Nakamura et al. (2001 and 2002) have almost clarified the chemical structure of the polysaccharide of molecular weight 550,000, as shown in Fig. 2. The major fraction of the polysaccharide has a main backbone consisting of long rhamnogalacturonan and short homogalacturonan chains, with many long homogeneous galactosyl and arabinosyl neutral sugar side-chains bound to the rhamnogalacturonan region via rhamnose(Fig. 3). In total, each polysaccharide molecule seems to have a spherical structure, which results in relatively low viscosity and high stability in aqueous solution.

Basic material properties and characteristics

As shown in Table 1, the content of dietary fibers measured by the official AOAC method is greater than 70%; hence SSPS has the same physiological function as other dietary fibers.

It dissolves in both cold and hot water without gelation, and has a relatively low viscosity compared to that of other gums/stabilizers such as guar gum, thus allowing highly concentrated (more than 30%) solutions to be produced. Furthermore, its viscosity is not substantially affected by acid, heat, or salts (Maeda, 2000).

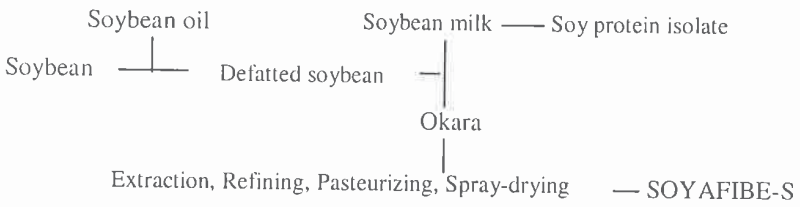

Figure 1. The manufacturing process of SOYAFIBE-S

Table 1. Chemical composition of SOYAFIBE-S-DA100

Moisture (%)	Crude Protein (%)	Crude Ash (%)	Dietary fiber content[1] (%)	Sugar composition[2] (%)						
				Rha	Fuc	Ara	Xyl	Gal	Glc	GalA
6.0	5.6	7.9	73.3	5.8	2.8	23.4	3.6	44.2	1.1	19.1

[1] The analysis was conducted by the official AOAC method (Prosky method: 1973).

[2] Neutral saccharides were analyzed by GLC after being converted to alditol acetate, and galacturonic acid was analyzed by the Blumenklanz method.

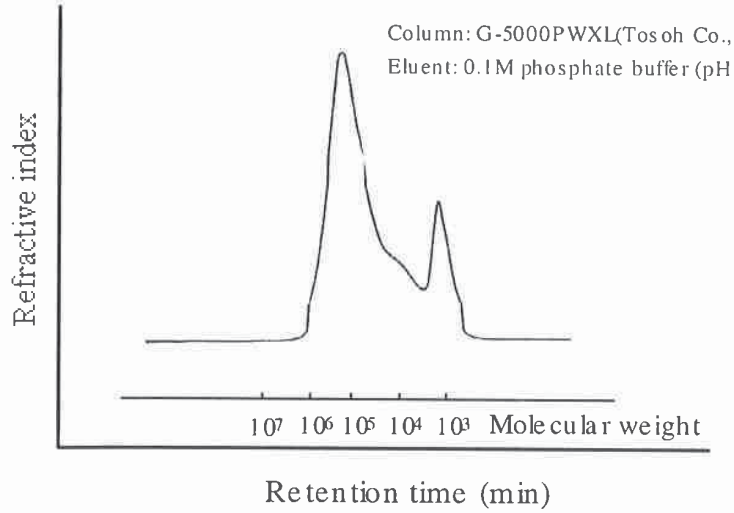

Figure 2. Molecular weight distribution of SOYAFIBE-S

SSPS has superior film- forming properties. Film processed from an aqueous solution without using any additives showed resistance to tension approximately as great as that of Pullulan (Nakamura, 1986). This film-forming property makes it easy to produce colorless, transparent, water soluble, and edible films. It can also be exploited to coat the surface of foods such as tablets.

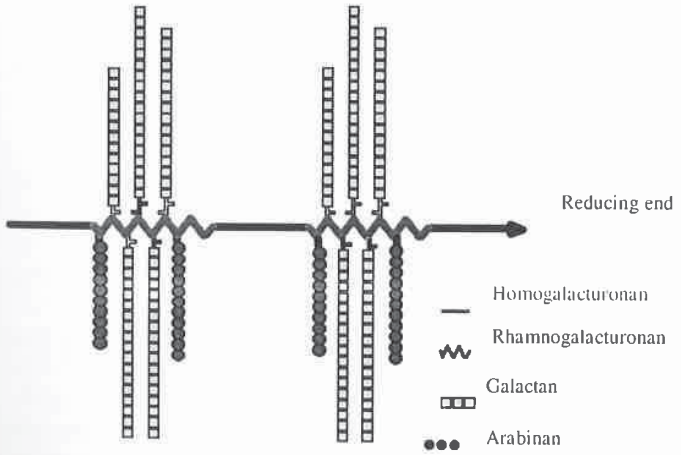

Figure 3. Main Structure of Soluble Soybean Polysaccharide.

Functions and applications of SSPS

Small amounts of SSPS can greatly improve the physical properties of a food. Table 2 gives the properties and applications of SOYAFIBE-S. Its dispersion stabilizing and anti-sticking effects are outstanding among its properties.

Table 2. Properties and applications of SOYAFIBE-S

Properties	Applications	Type of SOYAFIBE-S
Soluble Dietary Fiber	General dietary fiber, fortified foods	All types
Stabilizing effects under acidic conditions	Drinkable yogurt, Ice cream, Acidic dessert, Sour cream	DN, DA100, DA300
Emulsifying	Flavor emulsion, Powdered flavor, Coffee	EN100 LN
Emulsion stabilizing	cream, Dressing, Cleaner	LA200
Adhesion	Edible film, Coating agent, Particle	DN
Film forming property	forming agent, Snack, Bakery	FA100
Dispersing	Various types of paint, Agricultural chemicals, Ceramics, Cement	DN LA200
Foam stability	Meringue, Surfactant	DN DA100 LA200
Loosing effect	Various types of cocked rice and noodles	DN DA100 LA200
Softening effect	Bread, Cake, Ham, Sausage, Kamaboko (boiled fish paste), Sauce	All types

Stabilization of protein dispersions under acidic conditions

Under acidic conditions, SOYAFIBE-S prevents protein particles from coagulating and precipitating. Unlike HM-pectin (high methoxyl pectin), the most commonly used stabilizer under acidic conditions, for example in drinkable yogurt (Pedersen and Jorgensen, 1991), the point of interest with SOYAFIBE-S is its ability to stabilize protein particles at low pH without raising viscosity. This means that one can produce acidic milk drinks of low viscosity and light taste that have no sticky feeling in the mouth (Asai et al., 1993).

Figure 4 compares the viscosity, rate of precipitation and protein particle size of drinkable yogurts made with SOYAFIBE-S and HM-pectin as stabilizers. The acidic milk drink with SOYAFIBE-S as stabilizer has a lower viscosity than the drink stabilized with HM-pectin. In the lower pH range (pH < 4.0), SOYAFIBE-S has excellent stabilizing properties whereas at higher pH (pH = 4.4), HM-pectin is more effective. In other words, SOYAFIBE-S is suitable for acidic milk drinks with lower pH and less non-fat milk solids. This function of SOYAFIBE-S can be exploited in many other products, such as beverages, ice creams, desserts, etc., and provides excellent stability and refreshing taste under acidic conditions. From the data on particle size in Fig. 4, it appears that the mechanism of dispersion with SSPS is rather different from that with HM-pectin. SSPS contains about 20% galacturonic

acid, located in the backbone of the major molecule. These anionic groups probably bind to the surface of the cationic protein particle, and electric repulsion prevents them from coagulating. Moreover, the layer of SSPS formed is thick, because of the many side chains of galactose and arabinose. Therefore we assume that the main force of repulsion in the case of SSPS derives from the steric effect of the thick coating layer around the surface.

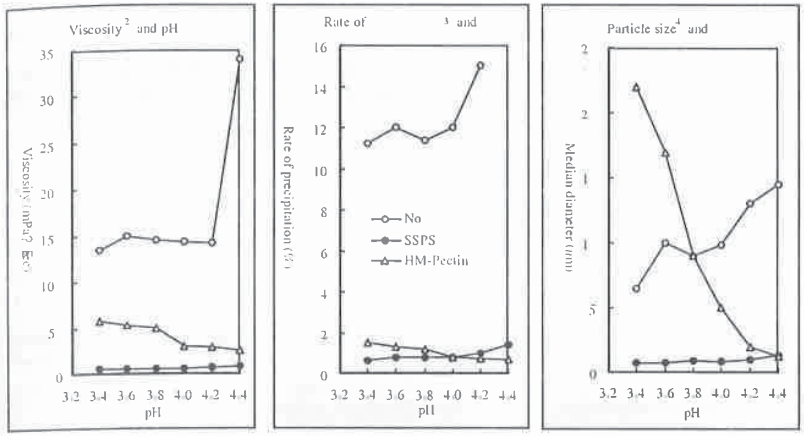

Figure 4. Effects of SSPS and HM-pectin on dispersion of acidified milk

1) Formulation of acidic milk drink: non-fat milk 8.0%, stabilizer 0.4%, sugar: 7.0%.
2) Measured with a B type Viscometer Model BM at 10! (Rotor No.1, 60rpm).
3) After each 50 gram of solution was centrifuged at 3,000g for 20 min., the supernatant was decanted and the residue allowed to stand for 20 min. in order to remove the remaining supernatant before measuring the extent of precipitation. Rate of precipitation = {precipitate (g) / 50 (g)} X 100 (%)
4) Measured with a Laser Diffraction Particle-size Distribution Analyzer (SALD-2000A, Shimadzu Corp.)

Anti-sticking effect of cooked rice and noodle

SOYAFIBE-S keeps cooked rice such as plain rice or rice with other ingredients like pilaf, non-sticky for many hours after being boiled. Hence SOYAFIBE-S is a great help when other ingredients are mixed with cooked rice. The same effect is obtained when SOYAFIBE-S is used in retort or frozen rice. Furthermore, as rice is boiled head by the addition of SOYAFIBE-S, more water can be added before it is boiled so that the yield of boiled rice increases. The rice obtained in this manner has the advantage that it is does not easily harden during cold storage, as shown in Fig 5.

SOYAFIBE-S also keeps cooked noodles from sticking for many hours. Just dipping the boiled noodles in SSPS water solution or spraying the

solution onto the noodles prevents them from sticking. When SOYAFIBE-S is added to sauce for spaghetti or chow mein, the noodles remain non-sticky for many hours.

Other applications

Many other applications are possible, for example as an emulsifying and suspending agent, as a film-coating agent for tablets and a softener for baked foods. It may therefore be useful not only in foods, but also in a number of industrial applications. As SSPS has a dispersing effect on inorganic particle similar to its stabilizing effect on protein particles it could be useful as a dispersion agent for water colors, ceramics and cement.

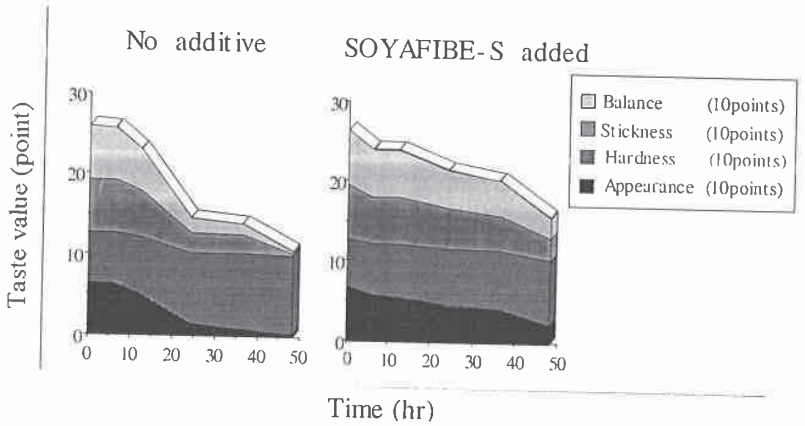

Figure 5. Change in the taste value of boiled rice (stored at 10 degrees C)

Rice was boiled with 1.2 times its weight of water when no additive was present. When SOYAFIBE-S was added the rice was boiled with 1.44 its weight of water. The rice was stored at 10 degrees C and the change in its taste value analyzed at intervals with a Rice Taste Analyzer STA-1A (Satake Corp; Mikami 1997).

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III CONGRESSO BRASILEIRO DE SOJA

MARCH 1

Perspectivas futuras da cultura da soja no Brasil: produção, produtividade, expansão de área

J. de B. França Neto[1]

Resumo

A soja, espécie originária da China, foi introduzida no Brasil e nele se distribuiu de Norte a Sul. Tal expansão deveu-se, em grande parte, aos trabalhos de melhoramento para a inclusão dos genes do período juvenil, realizados na Embrapa Soja. A soja é hoje a principal cultura agrícola do País, representando mais que 10% das exportações brasileiras. O Brasil é o segundo maior produtor mundial de soja e as taxas de expansão da produção têm atingido índices anuais de até 24,6%, conforme ocorrido na safra 2002/03, em relação à safra anterior. Nesse ritmo, em cerca de três a quatro anos, o Brasil será o líder mundial em produção de soja. Na safra 2003/04, espera-se colher uma produção variando entre 58 a 60 milhões de toneladas. O Brasil é o País com o maior potencial agrícola no mundo. Possui clima excelente para a cultura e solos diversificados, que, após correção de suas características químicas, poderão ser utilizados para a sua produção. O potencial do cultivo com a soja, conforme demonstra o presente trabalho, pode variar de 110 a 145 milhões de hectares, que, com uma produtividade média de 3.000 kg/ha, poderão produzir de 330 a 435 milhões de toneladas de grãos. Esses valores representam de 1,7 a 2,3 vezes a produção mundial de grão da espécie, obtida em 2003, que foi de 190 milhões de toneladas.

Abstract – Future perspectives of soybeans in Brazil: production, yield and area expansion

Soybean is a species originated from China. It was introduced into Brazil, and its cultivation expanded from Northern to Southern areas of the Country, mainly due to the breeding studies with the juvenile trait carried out by Embrapa Soybean. It is the major agricultural crop in Brazil, representing over 10% of the total Brazilian exports. Brazil ranks second in the production of the crop in the world. Rates of expanding the production from the 2001/02 to 2002/03 crops reached the expressive value of 24,6%. It is estimated that the 2003/04 crop will yield from 58 to 60 million tons. If this expansion rate is kept, in three to four year period, Brazil will be the world leader in the

[1] Embrapa Soja, Caixa postal 231, CEP 86001-970, Londrina, PR; Brasil. Consultor Técnico junto à Sementes Adriana, Rondonópolis, MT; franca@cnpso.embrapa.br.

production of this crop. Brazil is the country with the greatest agricultural potential. Its climate is excellent for the crop, and its soils can be easily corrected for the cultivation of soybeans. Soybean has a potential of being cultivated in Brazil in an area from 110 to 145 million hectares. Considering an average productivity of 3,000 kg/ha, this area will yield from 330 to 435 million tons of the grain. These values represent from 1.7 to 2.3 times the world production of the crop obtained in 2003, which reached over 190 million tons.

Introdução

A soja tem sua origem na China, onde é utilizada na alimentação humana há mais de cinco mil anos. Lá ela é considerada um "grão mágico" devido às suas características nutricionais (Embrapa Soja, 2003).

Ela foi introduzida no Brasil em 1882 como uma espécie forrageira, entrando em nosso país pela Bahia. O seu cultivo comercial para produção de grão foi implementado a partir dos anos 40 do século passado em algumas regiões dos Estados do Rio Grande do Sul e São Paulo. Entretanto, foi na década de 70 que a cultura teve uma expansão marcante, devido principalmente aos preços atrativos que naquela época eram pagos pelos seus grãos, de até US$400,00 por tonelada.
Nos anos 80, com a introdução dos genes do período juvenil longo e com o lançamento da cultivar Doko, o cultivo da soja foi expandido para as diversas regiões do Cerrado brasileiro e posteriormente, com o lançamento da cultivar Tropical, tal cultivo atingiu áreas do Norte e Nordeste brasileiros (Embrapa Soja, 2003). Deve-se destacar que a introdução dessa característica, responsável pela utilização e expansão da soja para as regiões brasileiras de baixa latitude, foi fruto de trabalhos coordenados na época pela Embrapa Soja. Com isso, e também em função dos trabalhos da Embrapa em conjunto com outras instituições de pesquisa oficiais e privadas, o Brasil é considerado ponto de referência em relação à tecnologia de produção agrícola em regiões tropicais. Novas cultivares mais produtivas e novas tecnologias específicas são disponibilizadas a cada ano para as diversas regiões agroecológicas brasileiras.

O complexo soja inserido no agronegócio brasileiro

Nos últimos 100 anos, o desenvolvimento econômico do Brasil tem se pautado, em grande parte, no incremento do setor agrícola, o que tem sido a maneira mais rápida e menos custosa de agregar valor às exportações, atrair novos investimentos ao País e de criar novos empregos. Hoje, o agronegócio representa mais de 40% do PIB brasileiro.

O complexo soja brasileiro é hoje o maior setor exportador do País, representando mais de 10% do total das exportações. Além disso, tem a maior participação do PIB agrícola e apresenta perspectivas de crescimento contínuo.

Dentre as principais razões desse sucesso, destacam-se os esforços incansáveis dos sojicultores, que, com o seu espírito empreendedor e com a coragem de assumir riscos, tornaram a sojicultura em um meganegócio a nível nacional. O desenvolvimento pela pesquisa e a adoção de tecnologias de ponta e o uso de novas cultivares mais produtivas e adaptadas às condições brasileiras tiveram também papel fundamental para esse sucesso. O Brasil é hoje o segundo maior produtor de soja, com uma produção de 52,1 milhões de toneladas obtida na safra 2002/03. Esse número é 24,6% maior que a produção do ano anterior, incremento esse jamais relatado em nenhum outro país, na história evolutiva da produção de soja. Tal acréscimo foi fruto de um incremento de 9,7% na produtividade (média recorde de 2.818 kg/ha) e de 13,5% de expansão da área cultivada, que foi de 18,5 milhões de ha, segundo dados da CONAB (2003). As produtividades médias obtidas no Brasil nos últimos 10 anos têm sido compatíveis ou superiores às dos EUA, o que demonstra o alto nível tecnológico utilizado na produção dessa espécie no País. No presente ano, a produtividade média brasileira foi 24,1% superior ao valor de 2.270 kg/ha obtido nos EUA, segundo o USDA (2003). Deve-se mencionar que tal valor foi devido à ocorrência de intensas secas nas regiões produtoras daquele país.

Hoje, a produção de soja se concentra principalmente nas regiões Centro-Oeste e Sul, que produziram 45,4% e 40,8% respectivamente do total da produção nacional. A região Sudeste vem a seguir com 7,7%, a Nordeste com 5,0% e a Norte com 1,1%. Dentre os principais Estados produtores, destaca-se o Mato Grosso, com 13,1 milhões de toneladas, seguido pelo Paraná, com 10,9 milhões, e o Rio Grande do Sul com 9,6 milhões de toneladas.

Para semear os cerca de 18,48 milhões de ha com soja, foram necessárias aproximadamente 1,3 milhões de toneladas de sementes. Dentre as principais culturas produzidas no Brasil, a soja consome cerca de 58% do volume de sementes total produzido no País, segundo a ABRASEM (2002).

Em todo o País, cerca de 240 mil produtores cultivam soja, dos quais cerca de 140 mil no Rio Grande do Sul, onde se concentram pequenos e médios produtores(IBGE, 1996). Já, no Mato Grosso, o principal Estado produtor, cerca de apenas 3.000 produtores foram responsáveis pela produção dos mais de 13 milhões de toneladas. Estima-se que cerca de 1,3 milhões de empregos são gerados diretamente pelo setor produtivo da soja.

Perspectivas da expansão da sojicultura em curto prazo

Em 2003, a sojicultura propiciou um outro recorde histórico ao Brasil, que assumiu a liderança mundial nas exportações de soja, superando pela primeira vez os Estados Unidos, com um faturamento aproximado de US$ 8 bilhões. O crescimento da soja tem sido fantástico no País; para a safra 2003/04 estima-se uma produção aproximada de 58 a 60 milhões de toneladas, o que será um outro recorde nacional. Mantendo esse ritmo, em três anos, ou seja, na safra 2006/07, o País assumirá a liderança mundial na produção de soja, com cerca de 80 milhões de toneladas de grãos produzidos.

Razões para o sucesso da sojicultura no Brasil

Diversas são as razões que têm contribuído para a expansão da cultura no Brasil. Dentre elas, destacam-se: utilização de cultivares mais produtivas e adoção de novas e modernas tecnologias de produção, incluindo práticas de semeadura direta; melhor gerenciamento das propriedades rurais, que hoje são encaradas como empresas rurais; expansão em áreas previamente cultivadas com outras culturas e pastagens degradadas; expansão de novas fronteiras nos Estados do Mato Grosso, Tocantins, Bahia, Maranhão e Piauí, mesmo em áreas arenosas, com o uso de novas tecnologias de produção; condições climáticas favoráveis; boas perspectivas de comercialização da produção, apesar da recente valorização do Real frente ao Dólar; disponibilização de novos instrumentos e operações para a comercialização; baixo custo de produção; franca expansão dos consumos interno e internacional, com forte tendências de produção e exportações de produtos de soja e de carnes suína e de aves à Ásia e Europa; estabilidade da economia brasileira; tradição brasileira em produzir soja e seus derivados de alta qualidade; e melhorias na estrutura de transporte, com melhores ferrovias e hidrovias, o que tem representado em substancial redução dos custos.

O intermodal de transporte

Entretanto, apesar de algumas mudanças estarem ocorrendo no sistema de transporte interno dos grãos, muito ainda tem de ser melhorado. Hoje, grande porção da safra, ou seja, 60%, é transportada por rodovias, as quais, em sua maioria, encontram-se em estado lastimável de conservação. As rodovias pedagiadas estão em boas condições, porém o seu uso onera o custo do transporte. Apenas 30% da produção são transportados por ferrovias e 7% por hidrovias, segundo dados da ABIOVE (2003). Nos EUA esses números são respectivamente 23% e 61%. Apesar dessa imensa discrepância entre os sistemas de transporte dos dois países, deve-se destacar que investimentos têm sido realizados na diversificação dos meios

de transporte brasileiros, o que reduzirá os custos, tornando o agronegócio ainda mais competitivo. Nesse sentido, segundo o Anuário Brasileiro de Soja (2003), a Brasil Ferrovias, que controla as operadoras Ferroban, Novoeste e Ferronorte, vem registrando crescimento anual no movimento de soja do Centro-Oeste para o porto de Santos: em 2003 foram transportadas 6,8 milhões de toneladas, ou seja, 50% a mais que no ano anterior. Em relação às hidrovias, significante progressos têm sido constatados, principalmente nas hidrovias dos rios Madeira, de Porto Velho (RO) a Itacoatiara (AM) e a do Rio Tietê, em São Paulo.

Perspectivas em médio e longo prazos

O Brasil é hoje o País com a maior potencialidade agrícola do mundo. Segundo a Organização das Nações Unidas (Em Dia, Embrapa Soja, 2003a), em 12 anos o Brasil será o líder mundial em diversos segmentos agrícolas. Nele, existem amplas regiões com clima propício à produção de soja e de outras culturas.
Sua diversidade de solos é imensa, abrangendo solos de excelente qualidade físico-químicas, a solos com qualidade marginal, mas com possibilidade de serem corrigidos e utilizados na produção. É o caso dos solos arenosos, que até poucos anos não eram passíveis de cultivo, devido à sua baixa acidez, baixa fertilidade e baixos índices de matéria orgânica. Hoje existem técnicas para a correção desses solos e para a produção de soja, com resultados finais compatíveis com os solos mais argilosos.

Além disso, no Brasil existem cultivares de soja e tecnologias de produção adequadas para todas as suas latitudes, desde o ponto mais ao Norte, com 7° latitude N, ao extremo mais meridional, com 33° latitude S. É com orgulho que reconhecemos que tais cultivares e técnicas de produção foram desenvolvidas por instituições de pesquisa brasileiras, o que tornou o País como centro de referência para a produção de soja nos trópicos.

Os Cerrados brasileiros são constituídos por uma área aproximada de 204 milhões de ha. Neles, se concentram mais de 50% da produção de soja nacional. Seus solos apresentam uma ampla diversidade em relação à composição física, indo de argilosos a extremamente arenosos, com até 2% a 3% de argila. Há cerca de 25 anos atrás, tais solos eram considerados improdutivos e sem perspectivas de utilização agrícola, devido à inexistência, na época, de tecnologias apropriadas para o seu cultivo.

Desses 204 milhões de ha, 62%, ou seja, 127 milhões de ha, são solos aráveis; 49 milhões são cobertos por pastagens, dos quais cerca de 30 milhões estão degradados; 10 milhões de ha são cultivados com culturas anuais; e dois milhões por culturas perenes (Ribeiro & Barros, 2002).

Considerando uma possível ocupação desses 127 milhões de ha com o cultivo da soja, mais cerca de 12 milhões na região Sul, e os cerca de seis milhões de ha de pastagens degradadas existentes na região Amazônica, que são passíveis de serem cultivados com soja, ter-se-á um total de 145 milhões de ha. Considerando uma produtividade média de 3.000 kg/ha, essa área apresenta um potencial para a produção de 435 milhões de toneladas de soja. Cabe ressaltar que em 2003, a produção mundial de soja ficou ao redor de 190 milhões de toneladas e esses 435 milhões representam 2,3 vezes essa produção.

Porém, trabalhando-se com perspectivas mais conservadoras, utilizando os dados apresentados em relatório da ONU, durante a UNCTAD (Conferência sobre o Comércio e o Desenvolvimento), ocorrida em Genebra em 2003, o Brasil conta com nada menos que 90 milhões de hectares de terras potencialmente aráveis e que poderiam ser utilizadas, sem que se tenha de derrubar uma única árvore (Em Dia, Embrapa Soja, 2003a). Adicionando-se os cerca de 20 milhões de hectares atualmente cultivados com a cultura a esses números apresentados pela ONU, ter-se-á um total de 110 milhões de hectares para o cultivo da soja. Considerando uma produtividade média de 3.000 kg/ha, o potencial com soja brasileiro será de 330 milhões de toneladas, o que significa 1,73 vezes a produção mundial atual, e o principal, sem derrubar árvore alguma.

Em suma, o potencial de produção de soja no País estará entre 435 e 330 milhões de toneladas, que são números impressionantes. Logicamente, tais valores são meramente hipotéticos e com grande margem de especulação. A produção desse montante de soja só será viável, mediante real demanda do mercado mundial pelo produto.

Restrições e limitações para a expansão do cultivo da soja

Até o momento, no presente relato foram apresentadas as vantagens e razões para a expansão da cultura no Brasil. Obviamente existem restrições e limitações que poderão dificultar essa expansão no futuro, sendo que algumas delas serão citadas a seguir: alto custo do transporte; elevadas despesas portuárias, consideradas as mais caras do mundo; falta de boas rodovias e pobre infraestrutura de ferrovias e hidrovias; estrutura insuficiente de armazenagem; excessiva taxação interna; elevada taxação internacional em alguns países para o óleo e o farelo da soja; dificuldades na comercialização; fundos insuficientes para o financiamento da produção; fundos insuficientes para a pesquisa; risco de ocorrência de novas doenças, como tem ocorrido com a ferrugem asiática; e o baixo nível tecnológico adotado por alguns produtores.

É de suma importância que as autoridades competentes, quer sejam do setor público ou do privado, tomem atitudes visando superar tais limitações, que poderão restringir a contínua expansão da sojicultura nacional.

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Competitividade da cultura da soja no Brasil e no mundo

A. C. Roessing[1]

Resumo

Apesar de o setor sojícola nacional ainda se deparar com importantes problemas, como o elevado Custo Brasil e o protecionismo internacional, o País, no contexto mundial, apresenta as maiores vantagens competitivas para a exploração da soja. Diante disso, o objetivo deste trabalho é discutir importantes aspectos relacionados com essa competitividade, que se deve, entre outras coisas, ao fato de existirem excelentes condições técnicas, novas fronteiras agrícolas e grandes excedentes exportáveis do produto. Baseado nessas vantagens, e caso não haja decréscimo significativo na demanda mundial, a produção de soja nacional, até o ano de 2010, deverá ultrapassar a produção dos Estados Unidos, que atualmente é o maior produtor mundial. O trabalho também mostra que, em termos comerciais, grande parte da produção sojícola brasileira é destinada às exportações, especialmente, para a União Européia e a Ásia, que são responsáveis pela importação de 80% da soja produzida no mundo. Por fim, são efetuadas discussões sobre oferta e demanda de soja, que podem afetar os preços e a rentabilidade futura.

Abstract – Competitiveness of soybean crop in Brasil and in the world

The Brazilian soybean sector is the world most competitive in spite of important limitations, such as high off-farm costs and international protectionism. This paper discuss several aspects related to the country's competitive advantages, especially those concerning availability of technology, of farming land and of large amount of soy products and sub-products for export. Based on these advantages and assuming no demand decrease in the near future, this paper concludes that the Brazilian soybean production should exceed the USA's, the present largest world producer, by 2010. This paper also shows that a large amount of the Brazilian production is exported, mainly to the European Union and Asia, which together import 80% of the world soybean production. A discussion on soybean production and demand relationship affecting prices and future returns is also included.

[1] Embrapa Soja, Caixa Postal 231, CEP 86001-970, Londrina-PR, Brasil e-mail: acr@cnpso.embrapa.br

Competição

Dentre os países que produzem soja, que poderiam competir com o Brasil, praticamente nenhum deles possui potencial para significativos aumentos de área e produtividade, gerando portanto grande volume de produção para atender o aumento da demanda, como possui o Brasil.

A China, produzindo atualmente cerca de 16 milhões de toneladas de soja, não aumenta sua área de plantio há 40 anos, pois em 1964 já cultivava 10 milhões de ha, no entanto, com uma produtividade de 786 kg/ha. Atualmente essa produtividade mais do que dobrou. Mesmo assim, a produção total não é suficiente para o consumo interno do país. A China está se tornando o mercado mais promisor para os países que tem potencial para aumentar sua produção de soja, chegando a importar 20 milhões de t de soja grão em 2003.

A Índia, outro produtor de soja da Ásia, começou a aumentar sua área somente a partir dos anos 90, porém ainda com produtividades extremamente baixas. Em 5,6 milhões de ha produz 5,0 milhões de t. Inúmeros problemas existem que retiram esse país da lista dos concorrentes internacionais na comercialização de soja, inclusive a sua própria demanda que ultrapassa a capacidade produtiva, tornando-o um importador do produto.

Outro país que se caracteriza como principal competidor do Brasil na produção de soja é a Argentina. Esse país passou de 6,2 milhões de ha e 11,2 milhões de t em 1996 a 12,3 milhões de ha e 32,5 milhões de t em 2002, mais do que dobrando sua produção. No entanto esse aumento obrigou o país a substituir culturas tais como girassol e milho por soja. Não existem fronteiras agrícolas que sustentem aumentos significativos de produção.

Semelhante à Argentina, o Paraguai se desponta como país produtor de soja e competidor no mercado internacional devido ao seu baixo consumo interno dos produtos do complexo soja. No entanto, a curto prazo, esse país não apresenta ameaça à produção da soja brasileira, além do que, em sua maioria, produz soja cujas cultivares são brasileiras.

A Bolívia aumentou bastante a sua produção de soja na década de 90, porém não possui grandes excedentes exportáveis. Em adição a isso, sua possibilidade de expansão de área para cultivo é muito limitada.

Sem dúvida, o maior competidor brasileiro na produção mundial de soja é os Estados Unidos. Mesmo assim, esse país já chegou ao seu teto de produção e a produtividade não vem aumentando como no Brasil. A fronteira agrícola americana está esgotada e qualquer aumento de produção só pode ser alcançado por meio de substituição de culturas ou aumento de produtiviade. A continuar a demanda mundial em torno de 5,3% ao ano, sem dúvida o único país que poderá ofertar a soja necessária ao atendimento dessa demanda será o Brasil.

O potencial brasileiro de produção soja até o ano de 2010 é ultrapassar a produção americana, chegando a 85 milhões de t, caso não haja decréscimo significativo na demanda mundial.

Oferta e demanda

Apesar do crecimento de 5,3% ao ano na demanda mundial de soja, a tendência dos estoques finais tem sido de alta. Estima-se um aumento de 9,8% nos estoques para a temporada comercial 03/04 quando comparada com 02/03. Essa tendência, não sendo acompanhada pelo aumento da demanda, pode deprimir os preços no futuro. O aumento de produção de soja da América do Sul, liderado pelo Brasil e Argentina, é o responsável pelo aumento dos estoques mundiais.

Evolução dos preços

Quando se analisa o histórico dos preços da soja percebe-se que dificilmente se repetirá o ocorrido no início da década de 70, quando a tonelada de soja chegou, a preços corrigidos de 2003, ao valor de US$ 804,00/t. No entando, em 1995 e 2000/2001 atingiu os menores preços históricos até então registrados. A partir de 2002 começou a recuperar os preços e vem atingindo a média histórica, que gira em torno de US$ 230,00/t a US$ 240,00/t. No entanto, caso a demanda não aumente e a continuar o aumeto da oferta, os preços poderão cair novamente abaixo da média histórica.

Mercado

Os maiores mercados mundiais de soja são a União Européia e a Ásia. Essas duas regiões são responsáveis pela importação de 80% da soja produzida no mundo. A Ásia possui potencial para se tornar o maior mercado mundial de soja e derivados, devido a sua população e baixa renda *per capita* relativamente aos países ocidentais. A elasticidade-renda da demanda de alimentos nos países da Ásia é normalmente alta. Qualquer aumento da renda provoca grandes aumentos de demanda de alimentos.

Projeções para a safra 2003/2004

Algumas considerações podem ser feitas a respeito do desempenho da safra 2003/2004, desde que as condições consideradas não sofram fortes variações. Entre essas considerações destacam-se:

- manutenção ou mesmo aumento da demanda mundial, que nos últimos seis anos esteve em torno de 5,3% ao ano;
- preços internacionais entre US$220,00/t a US$250,00/t;
- existência de cultivares altamente produtivas, aumentando a competitividade brasileira frente a concorrentes internacionais;
- manutenção da lucratividade na produção de soja em torno de 30% a 40%, como na safra 2002/2003;
- elevada liquidez na negociação do produto;
- melhor opção econômica para ocupação de terras, tanto desbravadas como pastagens degradadas ou substituição de culturas de menor valor econômico ou de liquidez menos imediata;
- monitoramento eficiente de pragas e doenças, principalmente da ferrugem asiática, que pode comprometer seriamente a produtividade;
- garantia do repasse das variações do câmbio na comercialização da soja e, acima de tudo, manutenção da taxa de câmbio acima de R$2,8 por dólar;
- oferta abundante de crédito privado para o custeio.

Não havendo mudanças drásticas nessas considerações, estima-se uma área no Brasil, para a safra 2003/2004, entre 20 milhões de ha a 21 milhões de ha e uma produção de 58 milhões de t a 60 milhões de t.

No entanto, existem algumas considerações que podem atuar do lado negativo em relação ao desempenho da safra 2003/2004, tais como:

- política agrícola nacional direcionada preferencialmente ao milho;
- boa expectativa de mercado para o crescimento de outras culturas como o algodão e o arroz;
- o mercado de soja, embora firme, não indica maior agressividade que a temporada anterior;
- aumento significativo nos custos de produção;
- custo de transporte aliado às condições das rodovias;
- protecionismo internacional;
- custos portuários, tributação e outros elementos de infra-estrutura (Custo Brasil)

III CONGRESSO BRASILEIRO DE SOJA

MARCH 2

A agricultura e as plantas geneticamente modificados

A. L. Nepomuceno[1]

Abstract – The agriculture and the genectically modified plants

Use of Genetically Modified Plants (GMP) has been the focus of debate around the world where strong positions against and in favor have emerged. We are just looking at the dawn of a new era. In the first phase of commercial use of GMP agronomical characteristics like herbicide, insect and disease resistance have been introduced. In a second phase more complex agronomic traits will be introduced, as for example drought and flooding tolerance. However, the exchange of genes among species by means of genetic engineering will revolutionize agriculture. Many organic compounds produced before only in the donor organisms, will now be produced in large scale using plants as vectors. The commercial importance of this technology is evident, however, its potential to solve many humanity problems is what gives more reflection. Like any other new technology it must be developed following rigorous biosafety and bioethical principles. However, excessive requirements to prove a risk free technology might block agriculture progress in countries that do not approve GMP technology.

A agricultura e as plantas geneticamente modificados

O uso comercial de organismos geneticamente modificados (OGM) na medicina e na industria já vem de décadas desde que pela primeira vez foi possível produzir insulina humana através de bactérias recombinantes. Atualmente, e principalmente em países desenvolvidos vários medicamentos, alimentos industrializados e substâncias de uso na industria são produzidos com o uso de organismos transgênicos. Entretanto, o uso de plantas geneticamente modificadas (PGM) têm sido alvo de um grande debate nacional e internacional onde posições acirradas têm sido apresentadas tanto a favor quanto contra plantas modificadas geneticamente por técnicas de biologia molecular. Não há dúvida da importância do debate que discute possíveis efeitos destas plantas no ambiente e na saúde humana e animal, não só do ponto de vista de como aquele gene (ou genes) introduzido modificou o metabolismo daquela planta, mas também como aquela planta modificará o ambiente ao qual será introduzida. Entretanto, parte da polêmica talvez tenha como base o fato de que a primeira onda de plantas modificadas geneticamente tenha sido promovida pelo interesse de grandes empresas privadas e a maneira extremamente competitiva utilizada por estas empresas

[1] Embrapa Soja, Caixa Postal 231, CEP 86001-970 Londrina, PR, Brasil, anepo@cnpso.embrapa.br

para conquistar seus mercados. Nesta primeira onda das PGM, estão sendo introduzidas, principalmente, plantas capazes de tolerar a ação de herbicidas (exemplos: Glifosato, gene *CP4 EPSPS*; Glufosinato de amônia, genes *bar* e *par*; Imidazolinonas, gene *ahas*; etc) e ao ataque de insetos (exemplo: plantas *Bt* com os genes Cry1(b)(c), CryF e Cry9, e responsáveis pela produção da toxina inseticida, retirados da bactéria *Bacillus thuringiensis*). Estas características são importantes agronomicamente, podendo favorecer o manejo das lavouras e, em algumas situações, reduzir os custos de produção. Em breve ainda veremos, nesta primeira onda, outras características de tolerância a diferentes moléculas de herbicidas e insetos (exemplo: baculoviroses, gene *ACMNPV* expressando a toxina TXP-1 específica para controle de Lepidópteros) mas, também, começarão a aparecer, em várias culturas, genes de resistência a fungos, bactérias (exemplo: genes de quitinase), vírus (exemplo: genes da capa proteíca de vírus; batata resistente ao Vírus Y; mamão resistente ao Vírus da Mancha Anelar) e estresses abióticos como à seca (exemplo: genes envolvidos no metabolismo do açúcar Trehalose, DREB genes – "Dehydration Responsive Elements Binding proteins" – sendo desenvolvido na Embrapa Soja em Parceria com o Jircas – "Japan International Research Center for Agricultural Sciences") e alagamentos (exemplo: gene da desidrogenase alcoólica).

É importante chamar atenção, entretanto, que esta é somente a primeira onda de plantas modificadas geneticamente. A engenharia genética tem propiciado ir muito além da introdução de simples características agronômicas como resistência a herbicidas e insetos. Uma segunda onda de PGM já está a caminho. Nesta onda, genes que conferem características de interesse mais direto aos consumidores finais estão sendo incorporadas. Por exemplo, o aumento da qualidade nutricional em soja, canola, milho e girassol são algumas das características sendo introduzidas. Em soja, plantas que produzem óleo mais saudável para o consumo humano com menos gorduras saturadas exemplo: *high oleic Soybean*, já sendo comercializada nos EUA), grãos com maiores teores de sacarose que melhoram o sabor para consumo direto, assim como, com maiores teores dos aminoácidos lisina e metionina, já estão em fase final de liberação no ambiente. Características como altos teores de ácido laúrico e esteárico, compostos básicos que aumentam a qualidade dos produtos produzidos a partir do óleo de canola, também estão nas etapas finais de teste. Na maioria das culturas comercialmente exploradas já existe algum tipo de característica nutricional sendo introduzida através de técnicas de biotecnologia. Alguns exemplo são: o milho com baixos teores de ácido fítico que reduz a quantidade de fósforo nos dejetos animais diminuindo a poluição ambiental; o girassol com baixos teores de ácidos graxos saturados; o trigo com alteração nos teores de glútem e amido; o arroz com teores elevados de Ferro e Vitamina A, entre outros.

Outras ondas de PGM virão nas próximas décadas, introduzindo características talvez inimagináveis até então. A gastroenterite suína, por exemplo, é causada por uma espécie de vírus (Transmissible Gastroenteritis Virus – TGEV) e tem causado grandes perdas anuais na suinocultura mundial. Pesquisadores estão conduzindo trabalhos visando o desenvolvimento de plantas forrageiras geneticamente modificadas cujos genes introduzidos expressam proteínas que imunizam os suínos que se alimentam destas plantas. A viabilização desta tecnologia (que vem sendo chamada de Vacinas Comestíveis), provavelmente, facilitará e ampliará a imunização do rebanho suíno. Entretanto, o potencial dessa tecnologia vai muito além do uso somente em pecuária. A produção em plantas, não só de vacinas comestíveis contra diarréias infantis e outras doenças, mas também de outras substâncias como por exemplo, fatores de crescimento para humanos, insulina, anti-corpos contra câncer, é alvo de pesquisa de vários instituições públicas e privadas no mundo inteiro. A medida que estas tecnologias tornam-se mais aceitas pela sociedade, existe um grande otimismo no sentido de que o baixo custo de produção de substâncias terapêuticas livres de patógenos e toxinas através de plantas geneticamente modificadas será uma importante ferramenta na manutenção da saúde pública no futuro.

Um outro exemplo do potencial do uso de plantas para produção de diversos compostos é o trabalho da equipe do Dr. Kenneth Gruys, publicado na revista "Nature Biotechnology" que mostra que será possível, num futuro próximo, a produção comercial de bioplástico através das plantas. O bioplástico é um excelente concorrente do plástico produzido a partir do petróleo, pois além das características básicas de maleabilidade e resistência do plástico comum, o bioplástico é biodegradável. Neste trabalho foram introduzidos quatro genes retirados originalmente da bactéria Ralstonia eutropha, que vem sendo utilizada deste os anos 80 para produção de bioplástico. O custo de produção de bioplástico pela bactéria em biofermentadores ainda é elevado. Entretanto, apesar das plantas transgênicas originadas nesse trabalho terem sido capazes de produzir bioplástico somente em pequenas quantidades (sinalizando que ajustes da tecnologia ainda são necessários), os cálculos do custo de produção sugerem um grande potencial para competir com os custos de produção de plásticos derivados de petróleo. O sucesso deste tipo de tecnologia permitirá que várias substâncias orgânicas de interesse para a industria possam ser produzidas em larga escala e a baixos custos.

A adoção das próximas fases das plantas geneticamente modificadas provavelmente ocorrerá de forma lenta por uma série de razões. Primeiro, é imperativo que a sociedade esteja ciente e bem informada do que esta tecnologia realmente é, e dos seus riscos potenciais que devem ser

analizados caso a caso. É também essencial que a sociedade esteja segura de que todas medidas de proteção ambiental, de segurança à saúde e de anti-monopólio das tecnologias estejam sendo aplicadas e constantemente reavaliadas. Entretanto, também é chave que a sociedade perceba o potencial destas tecnologias no futuro bem estar da humanidade. Segundo, a adoção das PGM passará por um processo de conhecimento, onde os diversos setores envolvidos se confrontarão com questões de determinação de preços, rotulagem, certificação, competição com produtos convencionais, valor real da característica agregada, etc. O que se visualiza para o futuro é a criação de nichos especializados, onde alguns produtores produzirão somente alguns hectares ou metros quadrados daquela espécie geneticamente modificada que produzirá um produto final com um alto valor agregado. Em outros nichos de produção, haverá a produção em grande escala, como é hoje, de espécies como a soja e o milho tendo talvez algumas características transgênicas relacionadas a fatores agronômicos que possam favorecer a redução dos custos de produção. E, caso persista a não aceitação de PGM por alguns setores da sociedade, existirão ainda nichos de produção direcionados a estes setores.

O que se vê até agora, em termos de plantas modificadas geneticamente, é somente o amanhecer de uma nova era. Muitas características estarão sendo disponibilizadas aos produtores e aos consumidores em geral. O importante é que como sociedade, estejamos atentos às mudanças, exigindo das instituições estabelecidas total seriedade e comprometimento no sentido de que, com bom senso, identifiquem e quantifiquem riscos evidentes e potenciais destas tecnologias. O princípio da precaução e o cuidado devem estar sempre em destaque. Entretanto, o exagero e a supervalorização de riscos pouco prováveis de ocorrer poderão prejudicar o desenvolvimento tecnológico de países reticentes em adotar a tecnologia das PGM. Como sociedade também temos que estar cientes de que a biotecnologia, em especial a engenharia genética e as plantas geneticamente modificadas, fazem parte da evolução tecnológica da humanidade. Se o homem não enfrentasse riscos calculados visando a melhoria de suas condições de vida, talvez anda estivesse vivendo em cavernas.

Dinâmica da população e manejo de plantas daninhas na cultura da soja RR: vantagens e riscos

D. L. P. Gazziero[1] and C. E. C. Prete[2]

Resumo

As plantas daninhas no Brasil tem composição e dinâmica própria de um país tropical. Com o plantio da soja transgênica resistente ao glyphosate, ocorrerão profundas mudanças nos sistemas de controle das plantas daninhas. Os quase 40 produtos ou combinações de produtos utilizados atualmente na soja convencional serão substituídos por um único ingrediente ativo, o glyphosate. Inicialmente serão observadas apenas os benefícios, mas após três a cinco anos certamente começarão a surgir os problemas como a seleção das espécies consideradas tolerantes e até mesmo resistência de plantas daninhas.

Manejar plantas daninhas não significa apenas aplicar produtos. Trata-se de uma filosofia de trabalho, cujos resultados e benefícios se manifestarão ao longo dos anos. Por isto, para evitar as conseqüências negativas do uso continuado de um mesmo herbicida, é necessário a adoção de técnicas de manejo de plantas daninhas, mesmo se tratando da soja transgênica resistente ao glyphosate.

Abstract – Population dynamics and management weeds in the RR soybean crop: advances and risks

The diversity and dynamic of weeds in Brazil is typical of a tropical Country. The utilization of transgenic soybean resistant to glyphosate will cause important changes in the weed management systems. There are currently, almost 40 chemical herbicides and mixtures of herbicides indicated for non transgenic soybean, which will be substituted for only one active ingredient , the glyphosate.

Initially, only the advantages of the systems will be observed. After three to five years however, problems are expected with selection of weed species considered tolerant or even resistant to glyphosate. Management of weeds has a broader meaning other than just herbicide application. It is a procedure whose results will only be observed only after some years of adoption. Thus, in order to avoid the negative consequences of the utilization of the same herbicide continuously, it is recommended the application of weeds management techniques, even on transgenic soybean resistant to glyphosate.

[1] Engº Agrº. Pesquisador da Embrapa Soja. Caixa Postal 231, CEP 86001-970, Londrina-PR. E-mail: gazziero@cnpso.embrapa.br
[2] Engº Agrº. Professor da UEL. E-mail: cassio@uel.br

Introdução

Soja infestada com plantas daninhas está sujeita a diferentes formas de interferências. Os danos podem se manifestar diretamente com conseqüências sobre o rendimento e a qualidade do produto ou indiretamente influenciando negativamente o manejo da cultura, a eficiência técnica da colheita e o beneficiamento de grãos.

As plantas daninhas podem também servir de hospedeiras de pragas, doenças e nematóides, representando riscos não só para a soja como também para outras culturas em sucessão ou rotação, a exemplo do milho e trigo.

Mesmo sendo poucos os métodos de controle disponíveis, recomenda-se que a eliminação das invasoras seja feita dentro de um programa de manejo integrado o qual deve estar inserido no manejo da área e da propriedade, e contemplar o uso contínuo e tecnificado do solo. É preciso que o problema planta daninha seja tratado o ano inteiro.

Dada as condições tropicais, tem sido observado no Brasil que espécies com características de verão como o picão-preto e amendoim-bravo estão se estabelecendo e se multiplicando em outras estações do ano, aumentando o banco de sementes. Situação inversa ocorre com a nabiça, planta característica de inverno que se adaptou ao verão.

Desta forma, as culturas chamadas de "safrinhas" como o milho e o sorgo, desde que não tratadas com herbicidas, bem como os períodos de pousio no outono nas regiões mais frias e inverno/primavera nas mais quentes, tornaram-se uma fonte incontestável de multiplicação de plantas daninhas, e como consequência, nestas condições, não tem sido possível obter controle satisfatório na safra de verão.

A soja é uma cultura que se caracteriza pelo alto consumo de herbicidas. O controle químico é o meio mais usual, dada as características de praticidade, eficiência e rapidez na execução. O uso de boas práticas agrícolas propicia um ambiente favorável para o desenvolvimento vigoroso da soja fazendo com que ela possa competir com vantagem com as plantas invasoras, e com isso criar condições para que os herbicidas funcionem adequadamente, possibilitando ao longo dos anos a redução de doses, e em certos casos até mesmo a eliminação de produtos.

Portanto é preciso ter a consciência de que manejar não significa simplesmente aplicar produtos. Trata-se de uma filosofia de trabalho que transcende a uma dada cultura e cujos resultados e benefícios se manifestarão ao longo dos anos.

A soja RR

Glyphosate é um herbicida sistêmico, não seletivo com espectro de ação sobre cerca de 154 espécies ocorrentes no Brasil, utilizado em doses que variam de 1 a 6 L ha^{-1} e disponível no mercado a quase 30 anos (Rodrigues & Almeida, 1998). Atualmente, é aplicado em pós-emergência das plantas daninhas, antes da emergência das culturas no sistema de semeadura direta, em áreas não cultivadas, pomares e reflorestamentos. Não possui efeito residual, pois é fortemente adsorvido ao solo, onde é degradado principalmente pela atividade microbiana. Atua na síntese de aminoácidos de cadeia aromática inibindo a enzima EPSPs (enol-piruvil-shiquimato-fosfato sintase) cuja rota sintetiza proteínas, vitaminas (K e E), hormônios, alcalóides e outros produtos essenciais ao crescimento e desenvolvimento das plantas. Estima-se que 35% ou mais da massa seca deriva da via do ácido shiquímico, e que 20% do carbono fixado na fotossíntese segue essa rota metabólica. Os sintomas aparecem de forma relativamente lenta, podendo variar de 10 a 30 dias conforme a espécie, tamanho e condições de umidade e temperatura. As plantas afetadas paralisam o crescimento, murcham, ficam cloróticas, necrosadas e morrem (Trezzi et al., 2001).

As culturas modificadas geneticamente para resistência ao glyphosate apresentam uma sequência alterada para a enzima EPSPs o que é feito pela introdução de um gene denominado CP4 proveniente de uma bactéria do gênero *Agrobacterium*, encontrada no solo e que confere insensibilidade à enzima EPSPs (Madsen & Jensen, 1998; Trezzi et al., 2001). Além da soja, o algodão, o milho, o trigo, o arroz, o fumo e o tomate também foram alterados para resistência a esse produto.

A possibilidade de uso de glyphosate aplicado após a emergência representa uma nova alternativa de controle em função da eficiência e viabilidade econômica, características essenciais no conceito de praticabilidade.

Para Shaner (1996), o número de variedades resistentes a vários herbicidas tende a aumentar podendo representar uma importante alternativa de controle, desde que a tecnologia seja utilizada como parte de um programa de manejo integrado. Ateh & Harvey (1999) analisaram o controle de plantas anuais em soja resistente ao glyphosate, e concluíram que o manejo das invasoras, da cultura e do herbicida são fatores fundamentais e que interferem diretamente nos resultados. Barnes & Oliver (2003) observaram ser possível obter bons resultados de controle de *Senna obtusifolia* e prevenir o acúmulo de sementes no solo associando a aplicação de glyphosate com épocas de semeadura, também destacando a importância do manejo da cultura no controle de plantas daninhas.

Uma outra vantagem, ainda que temporária, da cultura geneticamente modificada para resistência ao glyphosate, é a oportunidade de usar um herbicida com diferente mecanismo de ação para controlar biotipos resistentes (Divine, 2000). No entanto, desvantagens também poderão ocorrer quando se relaciona o manejo de plantas daninhas resistentes com o manejo de culturas exigindo análise individual de cada tipo de problema (Darmency, 1996). Já se fala sobre a perda da eficiência de glyphosate nos Estados Unidos. Culpepper et al. (2003) considera a *Commelina benghalensis* a principal planta daninha em algodão resistente ao glyphosate indicando alternativas para associação com outros produtos visando melhorar o nível de controle, já que a aplicação de glyphosate sozinho tem permitido a seleção desta espécie. Na Argentina, foram registradas mudanças na comunidade das plantas daninhas sendo citado que oito espécies estão sendo selecionadas, estando entre elas uma da família *Commelinaceae* e outra *Convolvulaceae* (Papa et al., 2002).

Trabalhos objetivando o uso de glyphosate combinado com herbicidas utilizados na soja convencional sobre diversos alvos foram conduzidos por Gonzini et al. (1999), Payne & Oliver (2000), Culpepper et al. (2000), Vangessel et al. (2000), Kranz et al. (2001), Shaw & Arnold (2002) e Krausz & Bryan (2003) que observaram aumentos significativos na percentagem de controle, no número de espécies controladas, redução na dose de glyphosate e benefícios em relação a época de aplicação, sem que houvesse interferência das plantas daninhas ou fitointoxicação. Mas nem sempre estas combinações foram vantajosas (Gower et al., 2002), havendo em certos casos antagonismo, segundo Oliver (1996) citado por Shaw & Arnold (2002). Para Ellis & Griffin (2003) uma vez obtido 85% de controle de gramíneas e folhas largas com a aplicação de glyphosate sozinho, a adição de latifoliadicidas não aumenta a produção, não evidenciando vantagens nestas condições. Porém não se pode esquecer que a presença de plantas não controladas pode aumentar o banco de sementes e reduzir a eficiência da colheita e a qualidade dos grãos.

A soja RR no Brasil

O Brasil é um país de clima tropical, portanto, a composição e a dinâmica da comunidade infestante possui características próprias, dificultando a transferência de informações de outros países. As infestantes reagem as ações do homem adaptando-se as novas condições.
Com o advento da soja geneticamente modificada para a resistência ao herbicida glyphosate, os quase 40 produtos ou combinações de produtos utilizados atualmente na soja convencional serão substituídos por um único ingrediente ativo, o glyphosate.

Algumas espécies são altamente sensíveis a este produto, enquanto outras são consideradas tolerantes e preocupam pela possibilidade de disseminação e aumento de infestação nas áreas cultivadas com a soja transgênica. Vários pesquisadores tem se posicionado quanto aos riscos e benefícios do controle de plantas daninhas nestas condições.

Para Gazziero (2003) pelo grau de importância que ocupa atualmente na soja convencional, a trapoeraba (*Commelina benghalensis*) é uma das espécies com grande potencial para se tornar rapidamente um sério problema de controle desde que, medidas de manejo não sejam adotadas. Trata-se de uma espécie que se adapta com facilidade em diferentes ambientes, apresenta intensa resposta a calagem e adubação do solo, hospedeira de pragas e moléstias. É uma planta considerada perene que se reproduz por sementes aéreas, subterrâneas e multiplica-se também à partir do enraizamento de porções do caule (Rocha 1999; Rocha et al., 2000).

A corda-de-viola (*Ipomoea* spp) também compõe a relação das espécies consideradas como de difícil controle por glyphosate. A julgar pelo que se observa na relação plantas daninhas – glyphosate, é possível incluir outras espécies com condições de expandir sua população na soja transgênica. Entre elas estão a poaia-branca (*Richardia brasiliensis*), a erva-de-santa-luzia (*Euphorbia pilulifera*), a erva-de-touro (*Tridax procumbens*), o capim-branco (*Chloris dandyana*) e a erva-quente (*Borreria latifolia*).

Segundo Nonino (2003) a curto prazo a percepção do usuário da soja RR é de que se trata de uma ferramenta versátil com controle de quase todas as espécies. Mas, a longo prazo, irá predominar espécies tolerantes como a trapoeraba, corda-de-viola, erva-de-touro, erva-quente (*Borreria latifolia*) e erva-tostão (*Boechavia diffusa*). O pesquisador entende ainda que em muitas lavouras serão necessárias de 2 a 3 aplicações, abrindo assim a possibilidade de mistura de herbicidas de ação residual com o glyphosate.

Vidal (2003) concorda em relação a futura necessidade da mistura em tanque com outros produtos e prevê a manifestação de plantas caruru (*Amaranthus* spp) e capim pé-de-galinha (*Eleusine indica*) resistentes ao glyphosate, assim como a seleção de corda-de-viola.

A experiência sobre o uso de glyphosate em pomares de maçã no Rio Grande do Sul é relatada por Vargas (2003), que observou a seleção de poaia-branca, leiteiro, trapoeraba e trevo-branco, além da resistência de azevém (*Lolium* sp) sobre os quais tem sido aplicado doses de 16 L ha^{-1} do produto comercial sem sucesso. Nas áreas com mais de 15 anos de uso tem sido feitas 3 aplicações/ano em doses que variam de 2 a 8 L ha^{-1}.

Segundo Theisen (2003) muitos produtores de soja transgênica (RR) no Rio Grande do Sul deixam de fazer o controle das plantas daninhas que nascem antes da semeadura da soja aproveitando para controlar com a aplicação de pós-emergência feita na cultura. Em muitos casos nestas condições, por ocasião da aplicação, já iniciou o processo de competição entre a cultura e as plantas indesejadas trazendo prejuízos à cultura. O pesquisador tem observado aumento na população de corda-de-viola, erva-de-touro, amendoim-bravo, poaia-branca e queixas de agricultores sobre a ocorrência de trapoeraba.

As opiniões quanto aos benefícios são unânimes em relação a facilidade de controle e a vantagem de se dispor de um herbicida com mecanismo de ação diferenciado para manejar plantas daninhas resistentes aos inibidores das enzimas ALS e ACCase. Exemplo disto tem ocorrido com picão-preto (*Bidens* spp), amendoim-bravo e capim-marmelada (*Brachiaria plantaginea*) no Rio Grande do Sul. Todos concordam também que os problemas iniciarão à partir do 3° ao 5° ano da adoção da tecnologia.

Apesar da legislação vigente no Brasil ter limitado a instalação de experimentos com soja geneticamente modificada, os resultados obtidos em nosso país permitem antever a possibilidade de se conseguir eficiência aceitável de controle, mesmo em espécies tidas como tolerantes através do manejo da dose, da época de aplicação e da combinação com outros compostos (Marochi & Zagonel, 2002; Schon & Marochi, 2002; Ferreira Neto & Marochi, 2002a; Ferreira Neto & Marochi, 2002b; Rodrigues et al., 2002; Kuva et al., 2002; Gazziero, 2003).

Segundo Duke (1998) os próximos dez anos deverão definir o impacto das culturas transgênicas como nova e poderosa ferramenta no controle e manejo das plantas daninhas. Para Gazziero et al (2001) os erros e as consequências do uso continuado de um mesmo herbicida já são conhecidos no Brasil e devem ser considerados para que os riscos inerentes aos programas como o da soja transgênica sejam evitados ou minimizados.

Ainda para Gazziero (2001, 2003) a adoção de técnicas de manejo da área, das plantas daninhas e do produto, na entressafra, poderá facilitar o controle na cultura da soja transgênica, pois na medida em que o produtor organiza a ocupação do espaço, dificulta o estabelecimento das plantas daninhas. O autor obteve bons resultados com a associação de glyphosate com produtos de pré e pós emergência atualmente utilizados na soja convencional assim como com aplicação sequencial de glyphosate, mesmo para espécies consideradas tolerantes como a trapoeraba.

Conclusões

É de se esperar que profundas mudanças venham a ocorrer com o cultivo em escala comercial da soja transgênica resistente ao glyphosate, trazendo inicialmente uma série de benefícios no controle das plantas daninhas. Por outro lado, problemas poderão ocorrer, como por exemplo a seleção de espécies. Para solucionar os problemas, é preciso que os conceitos básicos de manejo de plantas daninhas não sejam desconsiderados, sob pena de se perder as vantagens obtidas. Espécies tolerantes poderão ser melhor controladas com o uso de aplicações sequenciais de glyphosate e combinações deste produto com outros compostos químicos. A integração do controle químico com cultural poderá propiciar condições para que o herbicida atinja elevados níveis de controle, mesmo se o alvo for uma espécie tida como tolerante , como a trapoeraba.

Quanto a manifestação da resistência de plantas daninhas, sabe-se que é possível que possa ocorrer e isto tem sido visto em países como os Estados Unidos, Chile, Austrália, Malásia e África do Sul, com espécies de caruru, azevém, buva, capim pé-de-galinha e tanchagem ou língua-de-vaca. No Brasil, já foi oficialmente confirmada a presença de azevém (*Lolium multiflorum*) resistente ao glyphosate. Porém, devido ao mecanismo de ação do glyphosate espera se que este fato não assuma a proporção e a rapidez observada com outros mecanismos de ação. Casos de resistência podem ser previnidos. Uma vez ocorrido o problema, tanto na soja convencional como na transgênica (RR) são resolvidos com a adoção de técnicas de manejo.

Glyphosato é um herbicida de amplo espectro, praticamente atoxico à soja RR , quando utilizado em condições normais. No caso de lavouras mau conduzidas poderão ocorrer aplicações fora da época recomendada. Nestes casos, além dos efeitos negativos da convivência da soja com as plantas daninhas haverá também redução no nível de controle, devido ao estágio de desenvolvimento de certas espécies. Paralelamente questionamentos podem ser feitos sobre a relação ghyphosato-soja especialmente no que se refere a resíduo na cultura, visto que as informações sobre o período de carência ainda não foram estabelecidas publicamente. Portanto, aplicações tardias ou no final do final do ciclo não deverão ser feitas.

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Integração lavoura – pecuária no Sul do Brasil

A. de Moraes [1]; S. J. Alves[2]; A. Pelissari[1] and P. C. de F. Carvalho[3]

Abstract – Agriculture-livestock integration in Southern of Brazil

Grain and livestock farmers in southern Brazil are seeking alternative production systems to improve efficiency and profitability. University and state research institutions are leading development of sustainable no-tillage systems that integrate livestock grazing with cash grain production. These systems diversify farm income and increase efficiency on the same land base. The integration occurs primarily in two forms: rotation of annual grain crops with perennial pastures, and livestock grazing of winter cover crops or annual pastures in rotation with annual grain crops. A systems approach is being employed to study soil-plant-animal interactions, providing a basis for best management practices. Studies include evaluations of grazing intensity and animal performance on winter annual pastures, nutrient cycling, and animal grazing impact on soil physical and chemical properties and subsequent grain crop production. Research and farmer adoption are demonstrating these no-till integrated systems improve profitability and environmental sustainability, and will likely increase competitive ability of southern Brazilian farmers.

Introdução

A agricultura no Brasil foi praticada durante décadas na forma convencional. Como a maior área agrícola no País concentrava-se no Rio Grande do Sul, foi neste Estado que se verificaram os primeiros sintomas de um processo de degradação cada vez mais acelerado dos solos do então celeiro do Brasil. Muitos agricultores desta região migraram em busca de novas fronteiras agrícolas que surgiam no País, atraídos pelos baixos preços da terra e buscando solos mais produtivos. Foi na década de 70, inicialmente no Estado do Paraná, que surgiu o plantio direto, um novo sistema de cultivo representando um alento aos agricultores que buscavam alternativas de sustentabilidade do solo agrícola. Avançou-se muito em controle de invasoras e em tecnologia de semeadura, porém, em muitas regiões, a rotação de cultivos tem sido relevada. A falta de diversificação de cultivos tem levado a um aumento de problemas no manejo de plantas daninhas, pragas e doenças nas principais culturas de lavoura que participam do sistema de

[1] Professor Dr. Departamento de Fitotecnia e Fitossanitarismo – UFPR – anibalm@ufpr.br
[2] Pesquisador, Dr. Instituto Agronômico so Paraná – sja@iapar.br
[3] Professor Dr. Departamento de Plantas Forrageiras e Agrometeorologia – UFRGS – paulocfc@vortex.ufrgs.br

rotação. A diversificação representa a chave essencial para superar estes e outros problemas, podendo assegurar uma eficiente, produtiva e estável agricultura no futuro. Entre as possibilidades de diversificação, encontra-se a integração lavoura-pecuária

Neste sentido o ingresso da pastagem no sistema representa um degrau a mais na escalada de uma agricultura agroecológicamente sustentável pois permite uma diversificação do sistema de forma muito mais flexível e contrastante. Também o proprietário fica distanciado dos riscos inerentes de uma propriedade baseada somente nos cultivos agrícolas, independente do tamanho desta propriedade e das diferentes condições edafoclimáticas em que se encontra. Além disto ainda permite um fluxo de caixa mais condizente com o orçamento familiar principalmente para o pequeno proprietário.

A utilização da pecuária na propriedade aparentemente representa uma atividade de pouca remuneração para o produtor, não permitindo atingir uma renda mínima para sua subsistência, quando não houver uma disponibilidade de área muito grande. De fato, como atividade isolada isto pode ser verdadeiro, mas tem outra conotação quando integrada num sistema agrícola e praticada em rotação de lavouras, em plantio direto, com uso de genótipos melhorados (cultivares e animais) e insumos para gerar produtos facilmente comercializáveis e de valor no mercado como o leite e a carne. Não se pode comparar a atividade pecuária com a agrícola, enquanto na primeira utilizam-se resultados obtidos de uma pecuária incipiente, praticada em áreas marginais dentro da propriedade, e os resultados da lavoura são registrados nas áreas mais nobres e mais cuidadas. São situações distintas e não comparáveis. No momento em que a produção animal passa a ser praticada nas mesmas áreas e com tecnologia similar a utilizada em lavouras, pode-se avaliar o verdadeiro potencial da pecuária em gerar renda na propriedade.

O plantio de coberturas de solo ou de culturas de alto risco econômico como cereais de inverno, leva o agricultor a buscar alternativas econômicas durante este período. A formação de pastagens hibernais torna viável a terminação de bovinos, durante a entressafra e surge como alternativa para o aumento de rentabilidade das propriedades. Porém, o sucesso dos sistemas de integração lavoura-pecuária, depende de diversos fatores, que por sua vez, são dinâmicos e interagem entre si.

A pesquisa no Brasil vem ganhando espaço neste tema para uma melhor compreensão do efeito da entrada do animal no sistema agrícola em plantio direto. A seguir estará contemplado uma abordagem sobre a ação da pesquisa em diferentes regiões do Sul do País e seus principais resultados e impactos regionais.

No Sul do Brasil a pesquisa na integração lavoura-pecuária desenvolve-se dentro de duas realidades distintas: a) Em regiões tipicamente agrícolas: Neste caso a pecuária entra como opção de diversificação das propriedades e possibilita a utilização na alimentação animal de plantas de cobertura e/ou pastagens anuais e/ou perenes em rotação com cultivos anuais de grãos e b) Em regiões tipicamente pecuárias: Neste caso a agricultura entra como uma opção na reforma de pastagens.

Região tipicamente agrícola no Sul do Brasil

No Sul do Brasil os Estados do Paraná e Rio Grande do Sul destacam-se como grandes produtores de grãos e pela avançada tecnologia utilizada em suas áreas agrícolas, contudo, nos últimos anos, devido à baixa rentabilidade da exploração de cereais de inverno e à ausência de outras alternativas, a maior parte das áreas agricultáveis tem sido destinada, nesta época, para o plantio de espécies exclusivamente protetoras de solo ou, então, deixadas em pousio. Teoricamente, os sistemas de produção agrícola poderiam ser beneficiados por alternativas econômicas de rotação de culturas, que fossem capazes de contribuir com uma melhoria na utilização dos investimentos, e que buscassem intensificar o uso da terra e desenvolver sistemas de produção mais estáveis.

As regiões tipicamente agrícolas na região dos Planaltos do RS ao PR contam com boa infra-estrutura e as propriedades têm normalmente mão de obra mais qualificada e são melhores gerenciadas. A pecuária entra como uma opção de diversificação destas propriedades, possibilitando uma renda adicional pela utilização na alimentação animal de plantas de cobertura e/ou pastagens anuais em rotação com cultivos anuais de grãos. As forrageiras anuais utilizadas variam de acordo com a região considerada. Na região Sul do País tem-se utilizado principalmente o azevém e aveia preta, já em regiões mais tropicais normalmente se utiliza o milheto e o sorgo, em combinação ou não, na mesma propriedade, com áreas menores de pastagens perenes de verão (visando manter os animais na propriedade o ano todo e também aproveitar áreas com menor aptidão agrícola).

Impacto do animal sobre as características físicas e químicas do solo

Com a utilização do plantio direto nas propriedades agrícolas, cria-se a necessidade de promover uma boa cobertura do solo no período de outono/inverno e parte da primavera. As gramíneas utilizadas com esta finalidade (aveias e/ou azevém) são excelentes forrageiras capazes de suportar a atividade pecuária durante estas estações do ano, as quais representam o período mais crítico para a pecuária praticada no Sul do Brasil.

Esta região possui solos e clima propícios para o desenvolvimento deste sistema integrado, além de agricultores com suficiente infra-estrutura para superar os desafios da nova técnica. Entretanto, sempre surgem dúvidas a respeito de como se comporta o sistema quando utilizado por longo tempo (Eltz et al. 1989). Estas dúvidas ficam ainda mais fortes quando se associa a atividade animal em áreas agrícolas cultivadas dentro do sistema plantio direto. Acredita-se que a entrada de animais em áreas de lavoura possa causar compactação ou outra alteração que comprometa o rendimento das culturas em sucessão à pastagem. Além disso, são relativamente raros no Brasil, experimentos de longa duração que permitam avaliar os efeitos de diferentes formas de preparo sobre as propriedades físicas e químicas do solo (Eltz et al., 1989). O conhecimento destas alterações em condições específicas de solo e clima é importante no entendimento da potencialidade dos sistemas de manejo em relação à produtividade de culturas e adoção de práticas no sentido de contornar possíveis limitações advindas da sua utilização (Bayer e Mielniczuk, 1997).

Do ponto de vista das propriedades químicas do solo, assim como ocorre no sistema de plantio direto, há uma melhoria na fertilidade do solo devido ao acúmulo de matéria orgânica, alteração na reciclagem de nutrientes, melhoria na eficiência do uso de fertilizantes e capacidade diferenciada de absorção de nutrientes (Lustosa,1998; Assmann 2001; Bona Filho 2002).

É inquestionável o fato dos animais causarem prejuízos nas características físicas do solo pelo efeito do pisoteio. Também é inquestionável a ação regeneradora que a própria pastagem exerce no sentido de reverter este processo. O resultado destas ações antagônicas estará mais direcionado para um sentido ou outro, em função das práticas de manejo adotadas no ecossistema solo-planta-animal. Todas as ações adotadas visando incrementar a produtividade primária da pastagem representam benefícios ao solo no âmbito físico, químico e biológico. Dentre estas sobressai práticas de calagem e adubação que visam garantir uma boa condição nutricional para as plantas, que associadas ao correto ajuste da lotação e do sistema de pastejo, representam a questão chave na manutenção da produtividade do sistema.

O efeito da descompactação também pode ser obtido biológicamente pela ação do sistema radicial da própria pastagem e pela atividade da mesofauna do solo. Isto é possível de se obter quando a pastagem é submetida a adequada intensidade de pastejo, suficiente para promover um bom acúmulo de fitomassa aérea, que será um suporte a um melhor desenvolvimento radicial.

Estes efeitos foram observados por Coimbra (1998) e Consalter (1999) avaliando o efeito do impacto do animal no solo de áreas de plantio direto

envolvidos na integração lavoura-pecuária. Os parâmetros físicos do solo, avaliados nos meses seguintes ao pastejo mostraram que os efeitos negativos do pisoteio são rapidamente revertidos após o cultivo da lavoura de verão, representada no caso, pela cultura do milho. O mesmo foi observado por CASSOL (2003), no Rio Grande do Sul utilizando como tratamentos diferentes alturas de manejo da pastagem de inverno, tendo a soja como cultura subseqüente.

Resposta das lavouras cultivadas em plantio direto sobre áreas utilizadas com pastagem no inverno

Nos municípios de Guarapuava (Lustosa,1998; Consalter 1998) e Campo Mourão no Paraná, foi avaliada a resposta da lavoura de soja em sucessão à pastagem de inverno. Não se observou diferença significativa quando a soja foi cultivada sobre áreas pastejadas em condições de diferentes ofertas de forragem, que geraram resíduos diferenciados, e em distintos locais amostrados, que sofreram o efeito de graus de intensidade do pastejo mais intenso (áreas próximas dos cochos) e menos intenso (áreas de circulação normal dos animais). O uso de semeadeira de plantio direto com facão rompeu bem esta camada e as sementes conseguiram germinar e as plântulas desenvolveram-se bem. Já Cassol (2003) no Rio Grande do Sul encontrou diferença na produtividade da soja nas parcelas que sofreram uma maior intensidade de pastejo no período de inverno apenas no ano em que houve um período de estiagem associado a época do plantio da soja. Neste caso não foi utilizada a semeadeira equipada com o facão. Em todos os trabalhos houve algum comprometimento das características físicas do solo nos locais de concentração, entretanto, nestas áreas encontrou-se maior disponibilidade de nutrientes, devido a maior deposição de urina e fezes. É interessante observar que os locais de concentração representam menos de 1% da área total do piquete. Estes resultados contradizem com o conceito de que animais em áreas de lavoura compactam e reduzem a produção das culturas. Um fato comum a todos os trabalhos citados é a utilização de adubações na pastagem de inverno o que assegurou uma elevada produção de biomassa que foi incorporada no sistema tendo passado ou não pelo trato digestivo dos animais.

Manejo da cobertura de inverno e resposta dos animais em pastejo.

Normalmente como cobertura de inverno, no Sul do Brasil, são utilizadas as gramíneas como aveia-preta (Avena strigosa) e branca (Avena sativa) e o azevém (Lolium multiflorum). Estas espécies proporcionam diferentes períodos de utilização em função da velocidade de estabelecimento e ciclo de vida.

Na integração lavoura-pecuária a pastagem é cultivada em condições de solos corrigidos, aproveitando o residual das adubações realizadas na

lavoura. Neste caso existe uma ampla oportunidade de se trabalhar com leguminosas de inverno, que são espécies pouco presentes nas propriedades, por serem mais exigentes quanto à fertilidade do solo (Pelissari *et al.*1997; Castro Junior, 1998 e Lustosa,1998).

Buscando-se conhecer melhor estas relações, numa pastagem formada por aveia, azevém, trevo branco e trevo vermelho em sistema de plantio direto, foi conduzido um trabalho na região agrícola de Entre Rios, município de Guarapuava -PR, com o objetivo de definir o nível crítico de biomassa que deve permanecer sobre o solo após a retirada dos animais do pasto, de forma a permitir um bom estabelecimento da cultura de verão semeada em plantio direto. Esse nível não deve ser o mesmo para a lavoura e para a pecuária, pois para o estabelecimento do sistema de semeadura direta exige-se uma alta quantidade de biomassa, ao passo que para a produção pecuária, quanto maior a biomassa menor a eficiência de pastejo. O desafio, portanto, em sistemas integrados, é encontrar um nível intermediário de biomassa que beneficie, tanto a cultura de verão instalada no sistema de semeadura direta, quanto a produção animal no período de pastejo, de forma a garantir uma alta produtividade do sistema.

Na região da Depressão Central do Rio Grande do Sul, Cassol (2003) avaliou um experimento, nos invernos de 2001 e 2002, tendo como um dos objetivos determinar o nível crítico de biomassa que deve permanecer sobre o solo após a retirada dos animais do pasto. A variação na biomassa foi criada em função de diferentes alturas em que a pastagem foi manejada durante o período de inverno (10 cm, 20cm, 30cm e 40cm)

Fica evidente que, em condições de solo com boa fertilidade e com pastagens de boa qualidade como aveia, azevém e trevo branco, bem manejados, é possível obter-se elevado rendimento animal por área. Todavia considerando a necessidade de se manter uma boa cobertura de palhada para o posterior cultivo das lavouras em rotação permanece ainda um questionamento sobre qual seria a quantidade de palhada mínima que seria necessário para o bom funcionamento do sistema em plantio direto. A pesquisa tem demonstrado que quando o pastejo é de intensidade não limitante a manutenção de uma adequada cobertura (acima de 3 t de MS/ha) então pode-se deixar os animais até o momento do plantio (Cassol, 2003).

Dentre as limitações ainda existentes, quanto a difusão do sistema, a principal dúvida era sobre o que fazer com os animais no período de verão, caso os mesmos não fossem terminados durante o período de inverno. Optou-se pelo estudo de espécies forrageiras perenes e anuais de verão, semeadas em parte da propriedade e com uso intensivo de tecnologia.

Nesta proposta, a pastagem de verão deveria obrigatoriamente competir em rentabilidade com as lavouras de verão e permitir a manutenção dos animais na propriedade o restante do ano. Em diversas unidades de validação foram obtidas produções superiores a 1000 kg de PV/ha utilizando-se diferentes espécies e cultivares (Sorgo forrageiro, Tifton 85, Tanzânia + Arachis pintoi, Mombaça, Brizantão, etc.).

Região tipicamente pecuária

As regiões tipicamente pecuárias normalmente contam com menor infra-estrutura e/ou limitações edáficas importantes para uma atividade agrícola intensiva. Neste caso a agricultura entra como uma opção na reforma de pastagens. A utilização da agricultura no processo de recuperação da capacidade produtiva das áreas destinadas às pastagens possibilita um controle de invasoras, uma diminuição de pragas e doenças e uma fertilização de forma econômica, além da diversificação da renda das propriedades.

Adicionalmente podem-se implantar forrageiras anuais nas áreas agrícolas em épocas em que não inviabilizem o plantio das lavouras principais (inverno, início ou final do período chuvoso) e/ou produzir silagem nestas áreas. A utilização destas na alimentação animal no período de inverno possibilita um incremento da produção pecuária.

Esta é uma linha de pesquisa que foi desenvolvida na região do Arenito Caiuá em um projeto envolvendo a UFPR, IAPAR e a UEM.

Este projeto trabalhou com um sistema de rotação de cultivos, onde as lavouras de milho, soja, feijão e sorgo representavam alternativas que complementavam às condições particulares de cada propriedade. Outro diferencial do projeto é não estabelecer um "pacote tecnológico" para a região, devido ao fato de que o contexto sócio-econômico, cultural e estrutural de cada propriedade deveria levar o técnico a encontrar as soluções mais adequadas à realidade do produtor. A complexidade de um sistema integrado Lavoura - Pecuária, em solos com alta suscetibilidade a erosão exige uma ação prioritária de pesquisa visando gerar informações confiáveis para que os técnicos da região possam tomar decisões ajustadas à sua realidade. "O lucro deve andar de mãos dadas com a sustentabilidade".

Desde o início deste projeto em novembro de 1996, observaram-se ótimas respostas de produção para a cultura da soja, semelhantes às obtidas nas melhores regiões produtoras do Estado.

Estas áreas de lavoura, posteriormente utilizadas em pastejo com aveia preta no inverno, permitiam uma opção de forragem de elevada qualidade

no período mais crítico do ano, reduzindo o déficit alimentar dos animais e possibilitando que os animais pudessem atingir ganhos próximos a 1,0 kg/animal/dia, desde que o pastejo fosse adequadamente manejado (Grise, 2000), em oposição ao normalmente observado na região, em que os animais perdem até 500 g/animal /dia.

Após dois anos de lavouras no período de verão e do pastejo da aveia no período de inverno foi implantada uma pastagem perene de verão de alto potencial de produtividade e exigente em fertilidade de solos (Tanzânia) e com a qual se buscou a consorciação com uma leguminosa (Arachis).

Os resultados obtidos nos dois anos consecutivos de avaliação da consorciação de Tanzânia com Arachis, implantada em sucessão aos cultivos agrícolas mostram o elevado potencial de resposta animal desta região (Perin,2003).

Alguns paradigmas têm sido quebrados com o desenvolvimento deste projeto, e talvez isto tenha representado um dos maiores êxitos da proposta:

Um paradigma fortemente estabelecido no passado era de que a produção animal em pastejo era menos rentável que a agricultura e que a adubação de pastagens era pouco viável economicamente. Com o desenvolvimento do projeto e a instalação de unidades demonstrativas, utilizando-se alta tecnologia, mostrou-se que a adubação de pastagens é viável e que a produção animal em pastejo pode resultar em alto retorno financeiro, inclusive muito superior ao observado em áreas agrícolas.

Outro paradigma era em relação à impossibilidade de utilização de forrageiras de inverno na região, pois se considerava que as precipitações no período de inverno seriam limitantes ao cultivo da aveia. Isto decorreu de trabalhos realizados no passado em condições de plantio convencional onde o estoque de água no solo é rapidamente perdido.

O trabalho mostrou que o Noroeste do Paraná tem condições edafoclimáticas excepcionais para a produção animal em pastejo e que altos desempenhos animais podem ser obtidos a pasto, tanto para leite quanto para corte.

Uma das mais importantes virtudes do projeto foi o de comprovar o grande potencial de uso de culturas agrícolas nos solos do Arenito Caiuá, antes considerado impróprio e até proibitivo para esta atividade. Este conceito também tem uma razão histórica por considerar resultados obtidos com uma agricultura praticada no modelo convencional que á altamente degradante em solos suscetíveis a erosão como no caso do Arenito Caiuá.

O projeto, que foi pioneiro na implantação de soja no arenito, teve uma grande repercussão inicial. Centenas de visitas de produtores, inúmeras notas em jornais e na televisão, cálculos sobre a possível rentabilidade do sistema causaram impacto. A região passava por um processo de forte empobrecimento, o que levou algumas prefeituras e em particular a de Umuarama, a desenvolveram ações no sentido de viabilizar a proposta. A prefeitura de Umuarama iniciou um programa visando o arrendamento de áreas de pastagens degradadas para agricultores de outras regiões. Muitos agricultores se deslocaram para o arenito do Paraná, levando máquinas, equipamentos e pessoal e iniciou-se assim, o que seria posteriormente conhecida como a nova fronteira agrícola do Paraná. Motivando inclusive a abertura de linhas de crédito e que o IAPAR se envolvesse mais decisivamente na pesquisa nesta região e dentro de um novo enfoque. Até então, este órgão não trabalhava com soja no arenito e nem com plantio direto. Suas ações estavam mais voltadas para a reforma de pastagens em preparo convencional e utilizando-se principalmente dos cultivos de algodão, milho e mandioca ou para o estabelecimento de espécies perenes, como café, frutíferas e reflorestamento.

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Resultados da integração agricultura x pecuária no arenito Caiuá

A. Sacoman[1]

Resumo

O objetivo deste trabalho é avaliar os resultados da integração agricultura x pecuária na região do arenito caiuá. A análise econômica do sistema de integração agricultura e pecuária é composta por duas alternativas: Sistema de Integração Agricultura e Pecuária e Sistema de Reforma de Pastagem Após Agricultura com Arrendamento de Terras. Analisaram-se os efeitos combinados das duas alternativas, e o método de avaliação econômica usado foi o Valor Presente Líquido. Verificou-se que, com os resultados de produtividade e custos de produção, as duas alternativas são viáveis economicamente. Os resultados da integração se mostram consistentes, tanto do ponto de vista econômico quanto técnico, o que pode ser comprovado pela rápida ocupação das áreas de arenito sob a forma de integração, com a melhoria dos solos, elevação dos níveis de produtividade e de renda.

Palavras-chave: Resultados, integração, agricultura e pecuária, arenito

Abstract – Agriculture-livestock integration results in the sandy e Caiuá soil

The objective of this work is to evaluate results related to the integration agriculture and cattle raising in the "cauiá" sandstone soil region. The economic analisis of this integration system is composed of two alternatives: i) integration agriculture and cattle raising system, ii) new pasture rotation system after agriculture with land leasing. The combined effects of the two alternatives were analised using the Net Present Value method. The two alternatives were economically viable when yield and production costs were taken into account. The results were consistent economically as well as tecnically, backing up a rapid occupation of sandstone soil areas with integration systems, resulting in soil improvement and higher yields and profits.

Key words: Results, Integration, agriculture, cattle raising, sandstone ("arenito") soils

[1] Engenheiro Agrônomo pela Universidade Estadual de Maringá, Maringá, 198, com MBA em Agribusiness pela FGV. Coordenador Técnico de Desenvolvimento do Arenito e Grãos da Cocamar Cooperativa Agroindustrial. Estrada Oswaldo de Moraes Corrêa, 1000 – Maringá – PR – Brasil. Telefone (44) 221-3007. Fax (44) 221-3040. cocamar@cocamar.com.br

Introdução

O objetivo geral do Projeto é implantar um sistema de produção planejado na região do arenito caiuá, através da utilização racional de recursos naturais e econômicos a médio e longo prazo, otimizando a produção agropecuária, com sustentabilidade e estabilidade econômico-financeira.
As razões que levaram a Cocamar a desenvolver o Projeto são a baixa produtividade da pecuária, a existência de grandes áreas de terras ociosas e/ou improdutivas, o êxodo rural, o empobrecimento da região e, ainda, a demanda por novas fronteiras agrícolas.

Localização

De acordo com a SEAB-PR, dos 20,0 milhões de hectares que compõem o Paraná, cerca de 3,2 milhões são caracterizados como solo arenito caiuá, correspondendo a 16% do Estado. São 107 municípios localizados em sua maioria na Região Noroeste do Estado, ocupados, em sua maior parte por pastagens (Figura 1).

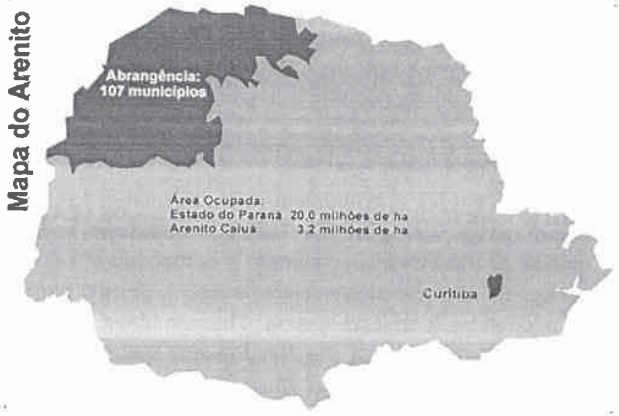


Figura 1. Mapa do Arenito

Antecedentes

Características da região

Na seqüência são apresentadas as principais características da região do arenito, em relação à chuva e solo, possibilitando subsídios para análise das condições regionais (Tabelas 1 e 2).

Tabela 1. Chuvas Registradas – Média de 20 Anos

Municípios	Quantidade (mm)
Palotina	1.660
Cianorte	1.668
Umuarama	1.645
Paranavaí	1.511

Tabela 2. Arenito - Características do Solo

Itens	Características
Textura	Média a arenosa 85-90% de areia
Fósforo/Potássio/Clacio/Magnésio	Níveis Críticos
Alumínio	Baixo
Erosão	Vulnerável

Utilização das áreas

De acordo com os dados do IAPAR/IBGE/SEAB, em 2002 a área total de arenito no Paraná era de 3,2 milhões de hectares, dos quais 69% ocupados com pastagens, 16% com agricultura, 5% com matas e florestas e 10% com outras utilizações.

A área média das propriedades existentes na região era de 56,5 hectares, sendo que 678.400 hectares estão compostos por propriedades com menos de 50 hectares e 2.521.600 hectares com mais de 50 hectares, caracterizando assim uma estrutura fundiária formada por grandes áreas.
A área total do arenito ocupada com a atividade agrícola em 2002 totalizava 527.000 hectares, 35% destinados a soja, 24% a cana-de-açúcar, 12% a mandioca, 11% ao milho, 8% ao algodão e os demais 6%, sendo amora, citrus e outras culturas (Tabela 3).

Tabela 3. Arenito – Produtividade Agrícola

Culturas	Unidade por Ha	Produtividade	Culturas	Unidade por Ha	Produtividade
Café em Coco	Sc 40 kg	38	Milho	Sc 60 kg	32
Cana-de-Açúcar	Toneladas	70	Soja	Sc 60 kg	32
Mandioca	Toneladas	12	Algodão	Arroba	80

A área ocupada com pastagens na região era de 2,24 milhões de hectares, com um rebanho de 3,5 milhões de cabeças com média de 1,56 cabeça/hectare. Na seqüência destacamos as características gerais das pastagens existentes:

- Pastos degradados
- Solos suscetíveis à erosão
- Solos pobres em fertilidade
- Solos degradados física e quimicamente
- Pastagem de baixa qualidade (Grama Matogrosso, Capim rabo de burro e favorito)

Considerando ainda a lotação média das pastagens da região, verifica-se que 42,1% dos municípios apresentavam lotação abaixo de 1,20 unidade animal por hectare e 57,9% acima de 1,20.

Integração agricultura X pecuária

Obstáculos

- Conscientização do pecuarista quanto às necessidades de mudança
- Conscientização de que a atividade pecuária não pode continuar degradando o solo
- Conscientização de que se deve tratar a pastagem como as demais culturas, necessitando também de bom manejo e adubação
- Conscientização de que a integração com a agricultura em plantio direto, e rotação de cultura, confere pasto de boa qualidade permitindo alta lotação de gado
- Conscientização de que diversificação da atividade proporciona maior produtividade e lucratividade, com redução de custos
- Qualificação de assistência técnica
- Cobrança pelos pecuaristas de elevados percentuais pelo arrendamento de terras
- Envolvimento superficial das lideranças políticas municipais

Princípios tecnológicos

- Sistematização do terreno quando necessário
- Formação de palhada com aveia/milheto
- Plantio direto na palha
- Alimentação do gado no inverno com aveia/milheto/silagem
- Rotação de espécies plantadas na área de agricultura
- Sistema de rotação agricultura com pecuária
- Controle de erosão
- Proteção das áreas de nascente

Planejamento Estratégico do Sistema (Figuras 2 e 3)

INTEGRAÇÃO AGRICULTURA X PECUÁRIA
PLANEJAMENTO ESTRATÉGICO DO SISTEMA - SUGESTÃO

ESTÁGIO	TALHÃO 01	TALHÃO 02	TALHÃO 03	TALHÃO 04
SITUAÇÃO ATUAL	Pasto Velho	Pasto Velho	Pasto Velho	Pasto Velho
1º ANO	Agricultura	Pasto Velho	Pasto Velho	Pasto Velho
2º ANO	Agricultura	Agricultura	Pasto Velho	Pasto Velho
3º ANO	Agricultura	Agricultura	Agricultura	Pasto Velho
4º ANO	Pasto Novo	Agricultura	Agricultura	Agricultura
5º ANO	Pasto Novo	Pasto Novo	Agricultura	Agricultura
6º ANO	Pasto Novo	Pasto Novo	Pasto Novo	Agricultura
7º ANO	Agricultura	Pasto Novo	Pasto Novo	Pasto Novo

FONTE: IAPAR/UEM COCAMAR

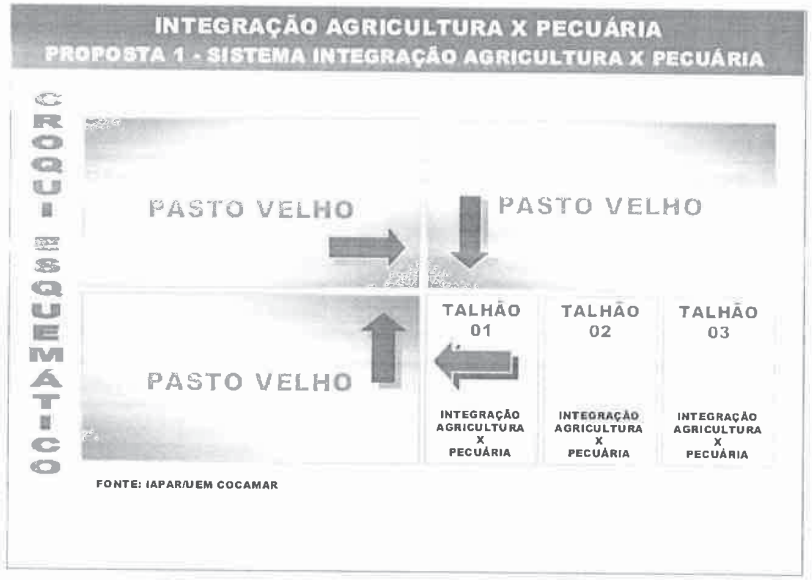
INTEGRAÇÃO AGRICULTURA X PECUÁRIA
PLANEJAMENTO ESTRATÉGICO DO SISTEMA - SUGESTÃO

DESCRIÇÃO	TALHÃO 01	TALHÃO 02	TALHÃO 03
VERÃO	SOJA (Sem Pagamento de Renda)		
OUTONO/ INVERNO	Milho Safrinha	Aveia forrageira para pastoreio	Sorgo para silagem
SISTEMA	Agricultor semeia a aveia a lanço após 30 dias da semeadura do milho	Pecuarista recebe área semeada com aveia para pastoreio	
VANTAGENS PARA O PECUARISTA	O pastoreio da aveia de julho/setembro permite GMD¹ de 0,6 kg/PV² /animal, ou seja, cerca de 3,6 a 4,8 arrobas/ha de carne no período. Para a vacada, o pastoreio permite melhoria do estado corporal para o período de monta, dando maior índice de fertilidade.	O pastoreio da aveia de maio/setembro permite até 1,0 kg/PV/animal, ou seja, 10,7 arrobas/ha de carne no período. Para a vacada, o pastoreio permite melhoria do estado corporal para o período de monta, dando maior índice de fertilidade. Permite, ainda, sistema de novilho precoce.	Agricultor semeia o sorgo. Pecuarista faz silagem e usa rebrote para pastoreio. O sorgo pode produzir 17 toneladas/ha de matéria seca, equivalente a 30 @ de carne, alimentando 32 animais adultos por 120 dias. O rebrote do sorgo pode ainda se pastoreado no período crítico de agosto/setembro.

NOTA EXPLICATIVA:
GMD¹ = ganho médio diário; PV² = peso vivo;
Para o plantio da forragens para o gado, o agricultor fornece apenas o serviço.

FONTE: IAPAR/UEM COCAMAR

Figura 2. Planejamento Estratégico do Sistema



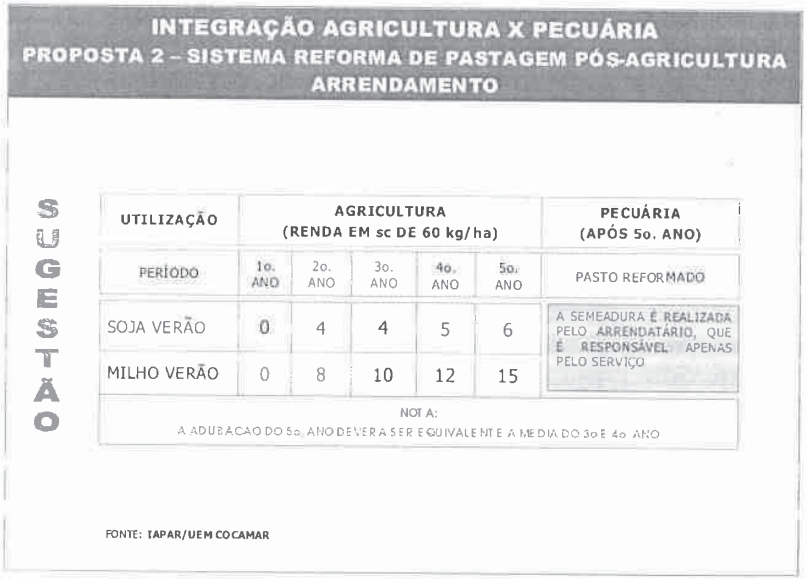


Figura 3. Propostas: Integração A x P e Arrendamento

Vantagens

- Retorno mais rápido do capital investido
- Produção de forragem em períodos críticos (inverno/seca): Aveia, milheto, sorgo, milho
- Recuperação da produtividade da pastagem
- Fornecimento de nutrientes: Adubação residual, nitrogênio fixado pela soja
- Economia na implantação de pastagem
- Recuperação do solo e Elevação da matéria orgânica
- Maior armazenamento de água e Melhor cobertura do solo
- Elevação da produção de grãos e Redução do custo de produção
- Sustentabilidade/estabilidade econômica-financeira e Equilíbrio do fluxo de caixa
- Elevação da produção de carne
- Geração de empregos diretos e indiretos

A atividade de integração da agricultura com a pecuária comparativamente ao sistema atual, possibilita ainda, a geração de empregos diretos em escala, ou seja, registra-se uma elevação de 1 a 2 empregos para de 6 a 8 empregos a cada 1.000 hectares.

Quanto aos empregos indiretos, o efeito multiplicador da atividade proporcionará inúmeros empregos na atividade agroindustrial, no comércio e no setor de serviços (estimativa de 28 empregos adicionais a cada 1.000 hectares de integração AxP).

Análise econômica e financeira

A análise econômica do sistema de integração agricultura e pecuária é composta por duas alternativas: Sistema de Integração Agricultura e Pecuária e Sistema de Reforma de Pastagem Após Agricultura com Arrendamento de Terras.

O Sistema de Integração Agricultura e Pecuária considera para as análises, três anos de pasto de inverno mais três anos de pasto reformado, sendo, portanto, pecuária e agricultura, conforme demonstrado na seqüência (Tabela 4).

Tabela 4. Alternativa – Agricultura e Pecuária (R$/ha)

Descrição	Verão - Soja (5 Anos)		Verão - Milho (5 Anos)	
	Total	VPL 12%	Total	VPL 12%
Custo	4.591,01	3.298,82	6.150,86	4.395,08
Receita	7.175,00	6.487,19	10.260,00	7.201,12
Resultado	2.583,99	3.188,37	4.109,14	2.806,04
Rentabilidade	98,2%		63,9%	

Fonte: Iapar/UEM/Cocamar

No Sistema de Reforma de Pastagem Após a Agricultura com Arrendamento de terra, o pecuarista arrenda sua área por um período de 5 anos, onde com o plantio de grãos (soja e milho) obtêm a reforma da área de pastagem. Após os 5 anos é implantada pastagem de alta produtividade (Tabelas 5 e 6).

Tabela 5. Alternativa – Pecuária (R$/ha)

Descrição	Inverno Pecuária (6 Anos)		Verão – Soja (3 Anos)		Verão – Milho (3 Anos)	
	Total	VPL 12%	Total	VPL 12%	Total	VPL 12%
Custo	5.833,02	3.829,28	2.495,12	2.012,44	3.297,93	2.648,81
Receita	17.472,50	10.853,77	5.250,00	4.171,52	5.580,00	4.386,23
Margem Total	11.639,48	7.024,49	2.754,88	2.159,08	2.282,07	1.737,42
Rentabilidade	183,4%		107,3%		65,6%	

Fonte: Iapar/UEM/Cocamar

Tabela 6. Alternativa – Pecuária Pasto Novo (R$/ha)

Descrição	Soja/Pasto Novo (8 Anos)		Milho/Pasto Novo (8 Anos)		Gado/Pasto Velho (8 Anos)	
	Total	VPL 12%	Total	VPL 12%	Total	VPL 12%
Receita	4.232,00	2.062,09	4.377,00	2.152,84	858,40	533,03
Relação de Renda	386,1%		404,1%		100,0%	

Fonte: Iapar/UEM/Cocamar

Resultados da integração

Na seqüência apresentamos alguns resultados da integração, com ênfase na utilização de áreas, potencial de rendimento e resultados de lavouras comerciais, com base nos dados da Secretaria da Agricultura e da região da Cocamar (Tabelas 7 a 9 – Figuras 4 e 5).

Tabela 7. Evolução – Região do Arenito Caiuá – Paraná (mil hectares)

Descrição	2000	2004	Variação 2000-2004
Pastagem	2.300	2.009	-12,65%
Agricultura	432	723	67,36%
Matas/Florestas	161	161	-
Outros Usos	307	341	0,11
Total	3.200	3.234	0,01
Soja	87	344	295,40%
Outras Culturas	345	379	9,86%
Agricultura	432	723	67,36%
Crescimento Anual Soja	-	-	41%
Crescimento Anual Outras	-	-	2%

Tabela 8. Utilização de Áreas no Arenito (hectares)

Descrição	2002	2003	Var 2002-2003	2004	Var 2003-2004
Arenito Paraná - Total	3.200.000	3.200.000	0,0%	3.200.000	0,0%
Matas e Florestas	161.000	161.000	0,0%	161.000	0,0%
Outras Áreas	307.000	307.000	0,0%	307.000	0,0%
Agricultura de Verão	527.000	613.000	16,3%	723.117	18,0%
Agricultura de Inverno	145.000	197.000	36%	220.100	12%
Pastagem	2.205.000	2.119.000	-3,9%	2.008.883	-5,2%

Tabela 9. Utilização de Áreas no Arenito - Agricultura (hectares)

Descrição	2002	2003	Var 2002-2003	2004	Var 2002-2003
Agricultura de Verão	527.000	613.000	16,3%	723.117	18,0%
Soja	186.000	253.000	36,0%	344.080	36,0%
Milho	57.000	55.000	-3,5%	43.600	-20,7%
Outras	284.000	305.000	7,4%	335.437	10,0%
Agricultura de Inverno	145.000	197.000	35,9%	220.100	11,7%
Trigo	40.000	43.000	7,5%	45.000	4,7%
Milho	84.300	132.000	56,6%	153.000	15,9%
Canola	1.400	2.800	100,0%	2.800	0,0%
Outras	19.300	19.200	-0,5%	19.300	0,5%

POTENCIAL DE RENDIMENTO

PRODUTO	RENDIMENTO (hectare)	RENDIMENTO (alqueire)
Soja – 1o. Ano	3.200 kg	129 sc
Soja – 2o. Ano	4.000 kg	161 sc
Milho	9.000 kg	363 sc
Sorgo Granífero	7.400 kg	298 sc
Sorgo Silagem	18 t de MS	44 sc de MS
Aveia Granífera	4.200 kg	169 sc
Feijão de Outono	3.000 kg	121 sc
Canola	2.200 kg	89 sc
Girassol	2.300 kg	93 sc
Trigo	4.000 kg	161 sc
Triticale	4.000 kg	161 sc
Aveia Forrageira	12 t de MS	29 sc de MS

RENDIMENTO DA SOJA

PALOTINA E UMUARAMA – SAFRA 2001/2002

SEMEADURA: 14 e 15/10/1999 – 10 e 27/03/2000

ADUBAÇÃO: 200 E 250 kg/ha de 00-20-20 + 20 kg de K2O

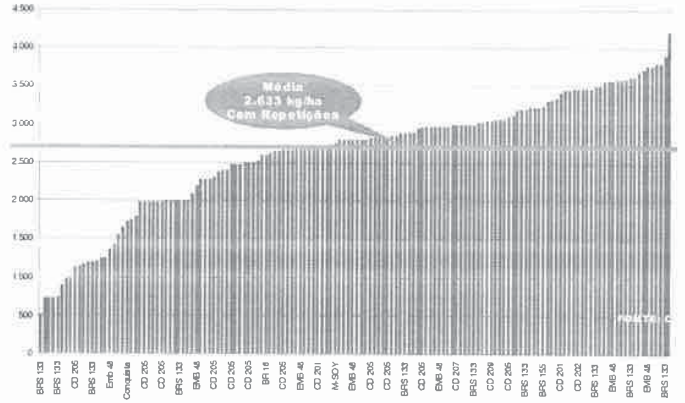
PALOTINA		
Cultivar	Kg/ha	sc
BRS 136	4.329	175
CD 209	3.592	145
BRS 133	3.520	142
CD 208	3.357	135
CD 204	3.322	134
EMB 58	3.197	129
CD 202	2.895	117
CD 207	2.813	113

UMUARAMA		
Cultivar	Kg/ha	sc
BRS 136	4.337	175
BRS 133	3.917	158
CD 209	3.851	155
CD 204	3.149	127
EMB 58	3.121	126
CD 202	2.607	105
CD 208	2.354	95

Figura 4. Potencial de Rendimento

RESULTADOS DE LAVOURAS COMERCIAIS

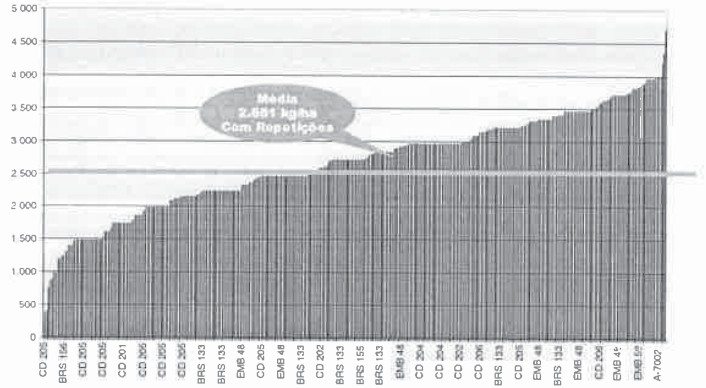
ARENITO – PRODUTIVIDADE DE SOJA POR VARIEDADE SAFRA 2001/2002 (kg/ha)



Amostragem ao acaso

RESULTADOS DE LAVOURAS COMERCIAIS

ARENITO – PRODUTIVIDADE DE SOJA POR VARIEDADE SAFRA 2002/2003 (kg/ha)



Amostragem ao acaso

Figura 5. Resultado de Lavouras Comerciais

Ressalte-se quanto a recuperação de pastagens, quantificando os valores químicos em soja após três anos de plantio, podemos estimar a melhoria dos solos em 9,00 mgP.dm³ do fósforo e 0,20 cmolc.dm³ de potássio. Isto equivale a 6,74 sacas de soja por hectare/ano, que denominamos de renda anual de fertilidade.

III CONGRESSO BRASILEIRO DE SOJA

MARCH 3

Produtividade potencial da soja: mapeamento de plantas

J. A.Costa[1]

Resumo

O mapeamento é um método para a análise morfológica da distribuição da frutificação das plantas de soja, em resposta ao ambiente. Comumente é realizado na maturação. Com a seqüência de mapeamentos de uma mesma área origina-se o monitoramento. Este levantamento permite a avaliação metódica e a descrição morfológica de uma comunidade de plantas quanto ao vigor, crescimento, desenvolvimento e frutificação ao longo do ciclo. Permite detectar situações limitantes do potencial de rendimento e, consequentemente , do rendimento real. O mapeamento é uma poderosa ferramenta de manejo, sendo de grande utilidade na documentação e entendimento das respostas morfológicas da soja ao ambiente. O mapeamento seqüencial ou monitoramento, permite registrar em tempo real o diálogo entre as plantas e as situações potencialmente limitantes do ambiente de cultivo. Este trabalho objetiva mostrar como o mapeamento de plantas pode ser útil no desenvolvimento do manejo para a obtenção de altos rendimentos de soja.

Abtract – Soybean yield potential: plant mapping

Plant mapping is a method for the morphological analysis of the didtribution of the fruiting of the soybean plant in response to the environment. Originally was performed at the end of the growing season. When mapping is done in sequence, in the same area, it is possible to monitor the field. This data collection allows for a permanent evaluation, and morphological description, of a soybean community in relation to vigor, growth, development and fruiting, during the growing season. It allows for the detection of situations that could be limiting to the potential yield and final seed yield. Plant mapping is a powerful management tool and is also helpful in documenting and understanding soybean morphological responses to the environment. The sequential mapping or monitoring permit the recording in real time of the dialog between plant and potential limitation conditions of the environment of cultivation. The objective of this paper is to show how plant mapping can be used in the improvement of the management of the soybean in order to reach high yields.

[1]Prof. Titular da Universidade Federal do Rio Grande do Sul. Bolsista do CNPq. jamc@ufrgs.br

Mapeamento

O mapeamento de plantas é um método empregado para a análise morfológica da distribuição da frutificação das plantas de soja. Comumente é realizado na maturação (R8), pela coleta de número adequado de amostras para a representação de uma comunidade de plantas. Pode ser utilizado para representar uma parcela experimental ou uma lavoura.

Estes mapas de plantas tem sido usados para (1) determinação de quais locais de frutificação são mais importantes para o rendimento. Este levantamento pode ser feito para mostrar diferenças ou semelhanças em relação ao hábito de crescimento (Gai et al., 1984), distribuição vertical dos frutos, no perfil das plantas, como topo, região mediana e parte inferior do dossel (Weibold et al., 1981; Marchezan e Costa, 1983), ou em caule e ramos (Board et al., 1990, 1992; Settimi e Board, 1988), ou, ainda, os dados podem ser coletados e separados por caule, ramos primários e ramos secundários (Gai et al., 1984; Munier-Jolain et al., 1994); e (2) determinar o efeito de práticas de manejo no rendimento (Costa et al., 2002; Maehler et al., 2003; Pires et al., 2000).

Quando realizado na maturação, o mapeamento pode se tornar uma ferramenta poderosa de manejo, pela documentação e entendimento da resposta morfológica da soja ao ambiente. No entanto, sua aplicação é limitada, pois o conhecimento gerado pelos resultados só poderão ser incorporados na safra seguinte, objetivando a modificação do manejo para alcançar o potencial de rendimento.

Monitoramento

De muito maior utilidade, a curto e longo prazo, é utilizar o mapeamento seqüencial da área cultivada, originando o monitoramento durante o ciclo de desenvolvimento da cultura (Costa, 1993). É um método não destrutivo e bastante eclético, podendo ser repetido, nas mesmas plantas, quantas vezes forem necessárias ou desejáveis, durante o ciclo. Possibilita a avaliação metódica e a descrição morfológica de uma comunidade de plantas de soja, quanto ao vigor, crescimento, desenvolvimento e frutificação ao longo do ciclo. Permite detectar problemas como infestação de pragas, fertilidade do solo, deficiência hídrica e outras situações limitantes do potencial de rendimento.

O monitoramento é uma ferramenta poderosa para a avaliação de áreas cultivadas com soja, de forma criteriosa, sistemática e tecnicamente correta, utilizando os dados levantados como indicativo para a tomada de decisões de manejo, bem como na mensuração de efeitos de tratamentos

experimentais. Para os produtores esta metodologia pode ajudar no entendimento de como a soja responde às opções de manejo, objetivando a obtenção de rendimentos elevados, bem como, pode ajudar na quantificação da variabilidade espacial e temporal nas áreas cultivadas, proporcionado benefícios ao sistema, pelo melhor entendimento do efeito favorável da rotação de culturas e da integração lavoura-pecuária.

Graças a determinação do potencial de rendimento através do monitoramento, é possível estabelecer metas realistas de rendimento em cada região, propriedades e mesmo para glebas distintas dentro de áreas maiores.

Com base nos dados obtidos, pode-se tomar decisões que irão influenciar o rendimento atual da lavoura, como cobertura de solo, irrigação, controle de pragas, aplicação de inoculante em cobertura, tratamento químico da parte aérea, para preservação da área foliar fotossinteticamente ativa, controle de doenças de final de ciclo, bem como àquelas que vão determinar o desempenho futuro da área, como a inoculação da(s) cultura(s) antecessora(s), análise de solo – em mais de uma profundidade – incluindo micronutrientes, correção de características químicas do solo em profundidade, uso de sementes de qualidade, de cultivares adaptadas com alto potencial de rendimento, tratamento de sementes, inoculação anual, adoção de espaçamentos menores entre fileiras, exame das raízes, verificação da existência de camadas compactadas, avaliação da quantidade e qualidade da nodulação, análise foliar, estimativa do rendimento e preservação do potencial.

O monitoramento identifica e registra a estatura da planta, o número de nós no caule, número de ramos, comprimento dos ramos e número de nós nos ramos, nós férteis no caule e nos ramos, acúmulo de matéria seca no caule e nos ramos, produção e retenção de flores, legumes e grãos no caule e nos ramos.

Efetuado de maneira sistemática na lavoura, através da amostragem seqüencial de plantas, ajuda a identificar problemas a tempo de corrigi-los. Para isso, deve começar nos estádios vegetativos. Para que o monitoramento tenha qualidade e atinja suas finalidades, é necessário o domínio do conhecimento de como a planta de soja cresce e se desenvolve e, através de amostragens representativas, generalizar para a comunidade de plantas de áreas experimentais ou de lavoura.

Monitoramento nos estádios vegetativos

O mapeamento de plantas, se usado desde o início do crescimento, no período vegetativo ou pré-floração, oportuniza o conhecimento de como a cultura esta se desenvolvendo, dando ao sojicultor a flexibilidade de aplicar opções de manejo disponíveis, capazes de dar a soja as melhores oportunidades de alcançar o potencial de rendimento.

No período vegetativo, sugere-se de uma a quatro amostragens, aos 10, 20, 30 e 40 dias após a emergência, objetivando determinar a população de plantas, a nodulação, o vigor das plantas e o potencial de rendimento da lavoura, o grau de estresse ocorrido e a necessidade de aplicação de, por exemplo, irrigação, e, se não tiver sido feita, o tratamento químico e inoculação das sementes no ano seguinte. Nesta etapa é determinada a estatura das plantas, o número de nós no caule, o número e a matéria seca dos nódulos, a área foliar, a matéria seca das folhas, a matéria seca total das plantas.

No caso de se decidir por apenas uma amostragem, sugere-se que a mesma seja feita aos 30 dias após a emergência. Deve-se, nessa ocasião, dar especial atenção a quantidade e qualidade da nodulação.

Monitoramento nos estádios reprodutivos

O período reprodutivo, abrange a floração, o desenvolvimento dos legumes, o desenvolvimento dos grãos e a maturação. Cada uma dessas etapas é constituída por dois estádios.

O mapeamento pode ser feito em cada um dos estádios reprodutivos ou em apenas alguns, dependendo dos objetivos. Para demonstração do método, vamos mostrar as determinações em R2 (floração plena), R5 (início do enchimento de grãos) e R8 (maturação). Na floração plena (R2) o objetivo é quantificar o desenvolvimento e vigor das plantas, o surgimento de ramos e flores, determinar a matéria seca das folhas, caule e ramos, acumulada pelas plantas até este estádio. Determinar parâmetros fisiológicos e o potencial de rendimento com base no número de flores.

Em R5, início de enchimento de grãos, se procura determinar o vigor das plantas pelo crescimento do caule e ramos e do acúmulo de matéria seca, bem como pelos parâmetros fisiológicos. As plantas devem estar em condições de, através das folhas fazendo fotossíntese, fornecer as reservas necessárias para o enchimento dos grãos. Verifica-se quantitativa e qualitativamente a nodulação das raízes.

Com o número de flores e legumes, calcular o potencial de rendimento neste estádio de desenvolvimento.

O mapeamento final, na maturação (R8), concluindo o monitoramento, tem como objetivo fazer estimativa do rendimento, utilizando a população de plantas e pelos demais componentes do rendimento. Pelo percentual de fertilidade de nós, legumes e grãos, pode-se determinar a diferença entre o rendimento obtido e o potencial de rendimento calculado.

O mapeamento é uma poderosa ferramenta de manejo. É de grande utilidade, também, na documentação e entendimento das respostas morfológicas da soja ao ambiente.

O mapeamento seqüencial, que é o monitoramento, permite registrar em tempo real o diálogo entre as plantas e as situações potencialmente limitantes do ambiente de cultivo.

Ações de manejo para maximizar o potencial de rendimento

A seguir são arroladas algumas sugestões de iniciativas de manejo que objetivam aproximar o rendimento real do potencial de rendimento. Obviamente não existe a veleidade de esgotar as ações possíveis de serem adotadas, nem se espera que as mesmas sejam adotadas conjuntamente, mesmo porque, algumas delas, para mostrar seus efeitos positivos, requerem algum tempo de adoção.
Ações antecedentes à instalação da lavoura.

- Adoção de plantio direto de qualidade;
- Inoculação da cultura antecessora;
- Uso de semente de qualidade;
- Uso de insumos de qualidade;
- Escolha de cultivares adaptadas e de potencial de rendimento comprovado;

Ações na instalação da lavoura.

- Utilizar equipamentos com precisão na profundidade de colocação das sementes e na distância adequada entre elas na fileira. Isto resultará em uniformidade das plantas na lavoura o que aumenta as chances de obtenção de rendimento elevado;
- Inoculação com produto de qualidade e em maior quantidade;
- Usar a quantidade de adubo necessária para a obtenção do rendimento almejado;
- Antecipação da época de semeadura, seguindo a recomendação, para melhor aproveitamento da radiação incidente, nos períodos críticos;
- Usar espaçamento entre fileiras e população de plantas que tem apresentado acréscimo de rendimento;

Ações durante o período de pré-floração.

- Manutenção da integralidade e funcionalidade da área foliar, através do controle eficiente de pragas e doenças;
- Controle eficiente de plantas daninhas;
- Avaliação quantitativa e qualitativa da nodulação, para que as plantas disponham de nitrogênio suficiente para o vigor e manutenção do potencial de rendimento;

Ações durante o período reprodutivo.

- Produção e fixação do maior número possível de flores;
- Produção e fixação do maior número possível de legumes;
- Produção e viabilização do maior número possível de grãos;
- Alcançar o potencial de cada estrutura;
- Maximizar a duração da fixação simbiótica de nitrogênio para potencializar a manutenção das estruturas reprodutivas;
- Usar irrigação, se disponível e econômica;
- Controle de doenças foliares, para manutenção da atividade fotossintética nos períodos críticos, pela preservação e prolongamento da duração da área foliar;

Ações para a próxima safra.

- O mapeamento sistemático para o monitoramento das áreas permitirá:
- o registro do histórico das áreas, com a identificação de fatores de estresse que causam redução do potencial de rendimento;
- a identificação de cultivares com melhor adaptação e maior potencial de rendimento nestas áreas;
- planejar alternativas de manejo para que o rendimento real se aproxime do potencial de rendimento;

Considerações finais

A disponibilidade hídrica no solo é o fator ambiental primário limitante para alcançar rendimentos elevados.

A deficiência de umidade durante o período vegetativo resulta em redução da atividade fotossintética e, como conseqüência, da fixação de nitrogênio e do metabolismo da planta, se refletindo em menor crescimento e portanto, plantas com menor vigor. Plantas vigorosas, com metabolismo intenso, exsudam maior quantidade de substâncias de melhor qualidade, que serão liberadas para a solução do solo e que vão influir favoravelmente na micro, meso e macro fauna e flora que habitam o ambiente rizosférico. Este ambiente qualificado influenciará o metabolismo das plantas, formando um

ciclo de enriquecimento do ambiente de produção. Estes ciclos se repetirão ao longo da estação de crescimento, qualificando cada vez mais o ambiente produtivo, dando lugar ao surgimento de condições favoráveis para a expressão do potencial de rendimento das cultivares.

Durante o período reprodutivo, a falta de quantidade adequada de água no solo provoca a diminuição da atividade simbiótica e, como conseqüência, queda de flores, de legumes, abortamento de grãos, grãos de menor tamanho, de menor densidade e com teores de óleo e proteína diminuídos. O resultado final será a redução do rendimento e da qualidade da matéria prima produzida.

Duas são as maneiras possíveis de aumentar a disponibilidade de água para a soja. Uma é por meio da irrigação, que se constitui em uma prática onerosa e nem sempre possível de ser utilizada, principalmente pela competição do uso da água na agricultura com destinações mais nobres desta riqueza natural. A outra maneira é pela conservação, no solo, da maior parte possível da precipitação, o que pode ser feito de forma racional e benéfica com o plantio direto.

Para finalizar, gostaria de deixar a idéia que o manejo racional da lavoura de soja se constitui de um conjunto interminável de interações favoráveis, o que se torna um facilitador para a expressão do potencial, aproximando o rendimento real do potencial de rendimento.

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Potencial genético da soja: progressos e limitações para alta produtividade

C. A. A. Arias[1]

Resumo

Constantes recordes de produção e produtividade vêm ratificando a posição de destaque do Brasil como um dos principais produtores mundiais de soja. Tecnologias genuinamente brasileiras garantiram melhorias no potencial genético das cultivares e nos processos que permitem a expressão desse potencial no campo. O avanço para produtividade de grãos no Brasil, considerando fatores genéticos e ambientais, foi da ordem de 0,9% ao ano no período 1940-2003 (cerca de 24,5 kg $ha^{-1}ano^{-1}$). O maior e o menor progresso na produtividade de grãos foram obtidos, respectivamente, pelos Estados do Mato Grosso (61 kg $ha^{-1}ano^{-1}$) e Rio Grande do Sul (22,9 kg $ha^{-1}ano^{-1}$). Fatores climáticos, principalmente relacionados à disponibilidade de água, determinam o nível e a estabilidade das produtividades ao longo dos anos. O progresso genético anual tem se mantido acima de 0,5% no Brasil, ultrapassando 1% para cultivares com ciclos mais longos. O potencial de ganhos genéticos disponível nas novas cultivares só se concretiza, comercialmente, à medida em que estas são cultivadas pelo produtor. A expansão de lavouras implantadas com grãos de materiais tolerantes ao glifosate, sem os devidos testes de adaptação regional, representa uma séria ameaça para a realização desses ganhos. Os incrementos no índice de colheita, obtidos nas novas cultivares, confirmam a necessidade de avaliações constantes dos níveis econômicos estabelecidos para auxiliar na tomada de decisão envolvendo a adubação e o controle fitossanitário em soja.

Abstract – Soybean genetic potential: progresses and limitations for high productivity

Records on yield and productivity ratify the excellence Brazilian position as one of the major soy producer in the world. Genuine Brazilian technologies supported the increments both on genetic potential of varieties and on processes allowing the expression of this potential in field conditions. The progress on grain productivity, taking into account genetic and environmental factors, reached 0,9% annually in Brazil during the period 1940-2003 (about 24.5 kg $ha^{-1}year^{-1}$). The highest and lowest progress were obtained, respectively, by the states of Mato Grosso (61 kg $ha^{-1}year^{-1}$) and Rio Grande do Sul (22,9 kg $ha^{-1}year^{-1}$). Environmental factors, essentially related to

[1] Embrapa Soja, Caixa Postal 231, CEP 86001-970, Londrina, Paraná, Brasil. arias@cnpso.embrapa.br

water supply, determine the magnitude and stability for the productivity along the years. In Brazil, the annual genetic progress have keep up to 0.5%, exceeding 1% for late maturity varieties. The potential for genetic gains accumulated in the new varieties are only commercially reliable when they are cultivated by producers. The expansion of glifosate tolerant crops established with non-registered materials lacking regional assessment, represents a serious threat for the achievement of those gains. The increase in the harvest index observed for the new varieties ratify the need of constant assessments about the established economic levels to help the decisions concerning fertilization and phytossanitary control in soybean.

Potencial genético da soja para alta produtividade

Produzindo, na safra 2002/03, cerca de 52 milhões de toneladas de soja em uma área cultivada de 18,5 milhões de hectares e produtividade média de 2818 kg/ha (Kist et al., 2003), o Brasil ratifica sua posição de destaque na produção mundial, seguindo a trilha traçada por sua aptidão agrícola. O investimento brasileiro em pesquisas direcionadas para a soja, principalmente nos últimos 30 anos, trouxe estabilidade para esse agronegócio e assegurou o desenvolvimento de tecnologias genuinamente brasileiras, que permitiram o avanço da soja para as baixas latitudes e o cultivo em, praticamente, todo o território brasileiro. Além da melhoria do ambiente de cultivo em conseqüência de melhores tecnologias de manejo das características físicas, químicas e biológicas envolvendo o solo e a planta, houve também um forte progresso no potencial genético das cultivares de soja. São materiais genéticos capazes de produzir mais, com ampla adaptação e com menores riscos de perdas por causas bióticas ou abióticas.

O modelo matemático que melhor pode representar os avanços de rendimento ao longo dos anos é o logístico, pois prevê a existência de um patamar máximo de rendimento possível de ser alcançado pelos materiais genéticos. Já foi utilizado, em anos anteriores, um patamar de 8000 kg/ha para a soja (Kiihl, R.A.S, informação pessoal), um valor possível, visto que já temos exemplos de médias de ensaios finais acima de 6000 kg/ha, a exemplo de Brasilândia, PR, onde observou-se genótipos com média acima de 7.000 kg/ha (Embrapa Soja, Ensaios Finais da safra 2002/03, dados não publicados). Apesar disso, o modelo utilizado nesse trabalho sobre os rendimentos do Brasil e de alguns estados brasileiros, ao longo do tempo, foi o linear, por representar adequadamente a tendência para períodos curtos de tempo e por ser de mais fácil compreensão. A evolução do potencial genético da soja pode ser observada nas médias mínimas e máximas obtidas ao longo das décadas, desde a safra 1939/40 (Figura 1). Os patamares médios de rendimento no Brasil nunca foram tão altos como nos últimos

anos. Tomando-se os dez últimos anos, observa-se que os rendimentos médios mais baixos foram maiores que os rendimentos mais elevados obtidos até a década de 80, demonstrando que tanto o potencial produtivo quanto a estabilidade de produção aumentaram.

O resultado provocado, conjuntamente, pelas melhorias genéticas e ambientais pode ser verificado no avanço do rendimento de grãos da soja brasileira (Figura 1) e que foi da ordem de 0,9% ao ano (cerca de 24,5 kg $ha^{-1}ano^{-1}$). Dos cinco estados brasileiros com as maiores áreas plantadas, o Mato Grosso é o que apresentou o avanço mais intenso (61 kg $ha^{-1}ano^{-1}$), seguido por Goiás (47,9 kg $ha^{-1}ano^{-1}$), Mato Grosso do Sul (38,9 kg $ha^{-1}ano^{-1}$), Paraná (37,4 kg $ha^{-1}ano^{-1}$) e Rio Grande do Sul (22,9 kg $ha^{-1}ano^{-1}$). Os coeficientes de determinação (R^2) medem a proximidade dos pontos observados em relação à reta estimada . O maior R^2 foi observado para o MT e o menor para o RS. Esse coeficiente também pode ser interpretado como um índice de estabilidade ambiental e permite inferir que as restrições ambientais à expressão do potencial genético da soja são maiores no RS que no MT. A água é o fator ambiental determinante dessa diferença, sendo que, historicamente, a quantidade e a distribuição das chuvas ao longo do ciclo da soja no RS não são as ideais para altas produtividades. Maior instabilidade da produção ao longo dos anos significa conviver com maior risco de perdas e com tendência de redução de investimentos, incluindo insumos básicos como a semente, que precisa ter qualidade genética e fisiológica para alcançar os níveis de produtividade desejados. No MT a situação se inverte: administra-se com menor risco, redundando em maior investimento em insumos e em terra, aumentando-se a escala de produção e reduzindo custos. Além disso, o MT em geral não tem clima favorável para que o agricultor produza sua própria semente, constituindo-se em um interessante mercado para o setor sementeiro, o qual, por sua vez, pode investir mais em pesquisas que garantam a sustentabilidade do negócio. Cooper (2003) também aponta a água como principal fator restritivo, principalmente quando o objetivo é alcançar altas produtividades. Além da água, o acamamento e a ocorrência de pragas e doenças são descritos pelo mesmo autor como fortes barreiras para a alta produtividade. Enquanto um acamamento leve é comumente observado em lavouras de alta produtividade, níveis elevados de acamamento reduzem drasticamente o potencial produtivo da soja, além de dificultar o processo de colheita mecânica. Já no caso das pragas e doenças, a importância relativa dos danos causados pelos agentes varia de acordo com os níveis de produtividade desejados. Como exemplo, podemos citar o caso das doenças de final de ciclo, normalmente observadas em lavouras de soja, cuja ocorrência sob média severidade pode não representar uma barreira importante para níveis médios de produtividade, mas constituem forte fator restritivo para altas produtividades.

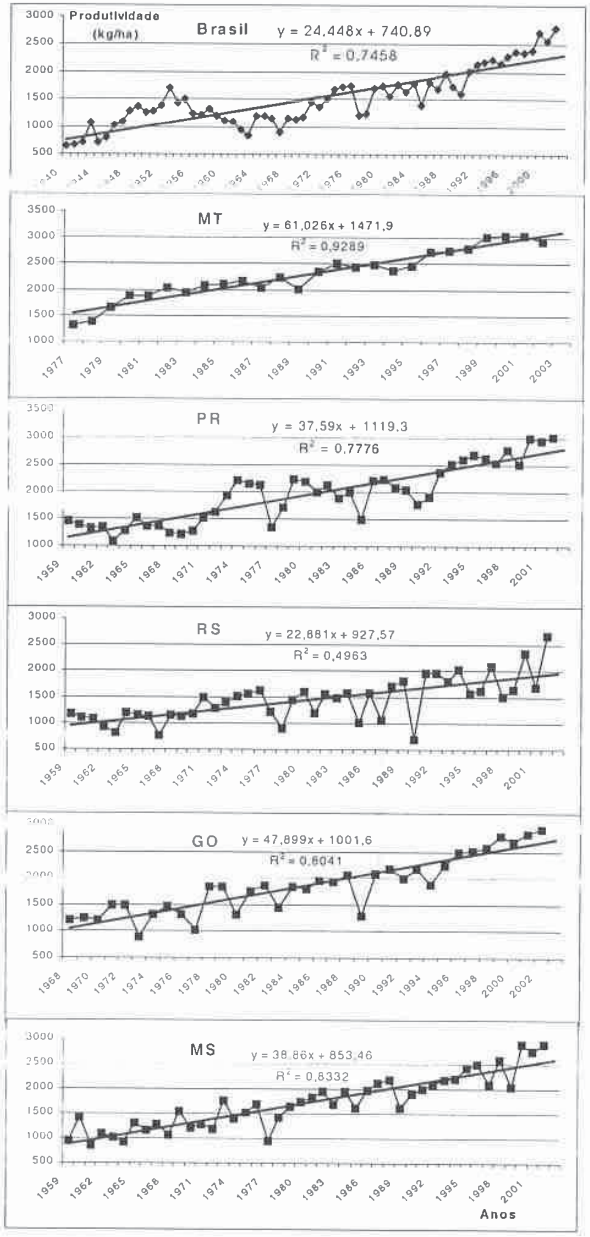

Figura 1. Evolução da produtividade da soja no Brasil e nos principais estados produtores ao longo dos anos: dados originais e regressão linear estimada.

Mas quanto dos avanços da produtividade de soja observados no Brasil se deve a causas genéticas? No Brasil, ganhos genéticos da ordem de 0,38% a 0,89% ao ano no período 1985/86 a 1989/90 (Alliprandini et al., 1993) foram descritos, considerando-se materiais de diferentes ciclos. Avanços em torno de 0,5% ao ano também foram observados em outros países por diversos autores (Voldeng et al., 1997; Morrison et al, 1999). Tomando-se dados experimentais das safras 2000/01 a 2002/03, incluindo 67 ambientes conduzidos pela parceria entre Embrapa Soja e a Fundação Meridional para os estados do PR, SP e SC, observa-se que as cultivares com maior potencial produtivo de cada ciclo, a BRS 230 no ciclo precoce, a BRS 232 no semi-precoce e a BRS 215 no médio, foram 4,5%, 7,6% e 5,1% superiores em relação aos melhores padrões de cada ciclo (CD 202, Embrapa 48 e BRS 134, respectivamente). Levando-se em conta os anos de lançamento das cultivares e dos padrões, e considerando que elas foram submetidas aos mesmos ambientes, pode-se calcular um ganho genético realizado anual de 0,76%, 1,08% e 1,03%, respectivamente, para os ciclos precoce, semi-precoce e médio. O potencial genético dessas cultivares ainda não se reverteu em benefícios para a sociedade, pois são lançamentos recentes que ainda não ocupam grandes percentuais da área cultivada nesses estados. Todo esse potencial genético pode ser perdido sem um processo de transferência de tecnologia adequado. Além disso, a utilização de grãos transgênicos tolerantes ao glifosate, sem os testes regionais e, portanto, sem adaptação comprovada para garantir a estabilidade de produção, representa uma séria ameaça para a realização desses avanços. A opção mais apropriada para o agricultor seria utilizar essa tecnologia apenas após a disponibilização das sementes desenvolvidas pela pesquisa pública e privada brasileira.

O que mudou nas cultivares atuais em relação às antigas para permitir maiores rendimentos? Vários autores demonstram que as cultivares atuais são mais eficientes, não apenas na produção, como também na distribuição dos fotoassimilados, otimizando seu armazenamento nas vagens. Para tanto, as plantas de hoje tendem a ser menos viçosas, tem folhas menos espessas, menor índice de área foliar (área foliar/área de solo ocupada), maior concentração de clorofila e maior índice de colheita (produção de grãos / peso total da parte aérea). Morrison et al. (1999) estudando detalhadamente as mudanças fisiológicas provocadas pelo melhoramento de soja no Canadá entre 1934 e 1992, observaram que não houve mudança significativa no ciclo e no peso seco das cultivares, ao longo desse período. Entretanto, observaram um incremento de 0,5% ao ano na produção de grãos, no índice de colheita e na taxa fotossintética, enquanto o índice de área foliar diminuiu em 0,4% ao ano. Concluíram que as cultivares de soja atuais são mais eficientes em produzir e alocar as fontes de carbono nas sementes, em relação às cultivares mais antigas.

Ao que tudo indica, essa tendência ainda deve se perpetuar ao longo dos anos, ou seja, aumento do índice de colheita. Assim, pode-se pensar que, com o aumento do índice de colheita, necessário para atingir os 8.000 kg/ha, cada centímetro quadrado de folha passa a ter uma importância relativa maior e que o percentual da parte aérea sendo exportado nas sementes aumenta a cada ano. Com isso, várias questões relacionadas às mais diversas áreas de pesquisa precisariam ser reavaliadas, como por exemplo, aquelas relativas à fertilidade do solo e nutrição das plantas e ao controle de plantas daninhas e, principalmente, de pragas e doenças, onde os níveis críticos para perdas econômicas podem mudar.

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Manejo da fertilidade do solo e da nutrição da soja para a máxima produtividade

A. F. Lantmann[1] and C. de Castro[2]

Resumo

A produtividade da soja no Brasil teve nos últimos 10 anos um aumento médio de 1.200 kg ha^{-1}. Assim, para sustentar essa maior produtividade são necessários melhores condições de fertilidade do solo, para possibilitar o maior rendimento das novas variedades disponíveis no mercado. A máxima produtividade da soja é normalmente obtida quando os nutrientes estão disponíveis em quantidades adequadas e, já estabelecidas para as principais regiões produtoras de soja no Brasil. Porém, as respostas da soja aos fertilizantes, não estão só condicionadas as quantidades de nutrientes. Interferem também nas respostas, condições climáticas e de solo, tais como sistema de plantio e de rotação de culturas, calagem, quantidades de fertilizantes aplicadas e produtividades nas culturas anteriores, forma de aplicação de fertilizantes, equilíbrio de nutrientes nos solos, distribuição de chuvas e comportamento varietal da soja. O trabalho apresenta, um conjunto de relatos em que os rendimentos da soja foram afetados pela fertilidade dos solos e pela aplicação ou não de adubos, com o objetivo de esclarecer quais as condições de fertilidade do solo a soja poderia alcançar o máximo rendimento.

Abstract – Soil fertility and soybean nutrition management for maximum yield

The soybean productivity in Brazil has increased about 1.200 kg ha^{-1} in the last ten years. In order to support this higher productivity are necessary better conditions of soil fertility to make possible a better yield of new cultivars available in market. The maximum soybean yield is normally obtained when the nutrients are available in adequate amounts and theirs levels already established for the main regions in Brazil. However, the soybean answer for fertilizers aren't only conditioned to the nutrient amounts. The weather and soil conditions also interfere such as planting system and crop rotation, liming, fertilizer amounts used and productivity in the last crop, the way of using fertilizer, soil nutrient balance, rain distribution and cultivars behavior. The work presents a group of reports showing how soybean yield has been affected by soil fertility and by using

[1] Eng. agrônomo Consultor
[2] Embrapa Soja, Caixa Postal, 231, CEP 86001-970, Londrina-PR, Brasil.

or not fertilizers, with the objective of making clear which soil fertility conditions the soybean could reach the maximum yield.

Introdução

A produtividade da soja no Brasil teve nos últimos 10 anos um aumento médio de mais de 1200 kg ha^{-1}; algumas regiões com rendimentos médios de 3000 kg ha^{-1}, um grande conjunto de agricultores com médias acima de 4400 kg ha^{-1} e em ensaios de pesquisa são freqüentemente observados rendimentos acima de 5000 kg ha^{-1}, atingindo até 6500 kg ha^{-1}. Assim, essa maior produtividade estaria requerendo melhores condições de fertilidade, do que as atuais existentes, para assegurar o máximo rendimento determinado pelas novas variedades existentes no mercado. Por outro lado, a maior parte dos solos do Brasil, tem deficiências quanto aos principais nutrientes necessários para o pleno desenvolvimento da soja. Grandes quantidades de fertilizantes e de calcário são necessárias para promover esses solos de uma situação conceituada de baixa fertilidade para situações de boa fertilidade. As máximas produtividades da soja são normalmente obtidas quando os nutrientes estão em níveis adequados, conforme, o que, para a maior parte das regiões onde se cultiva a soja no Brasil, já foi estabelecido. Porém as respostas da soja ao uso de fertilizantes, não estão só condicionadas a quantidade de nutrientes. Interferem nas respostas, condições climáticas e de solo, tais como, tempo de uso com o sistema de plantio direto, sistemas de rotação de culturas, tempo posterior há ultima calagem, quantidades anteriores de fertilizantes aplicadas, índices anteriores de produtividade, forma de aplicação de fertilizantes, equilíbrio de nutrientes nos solos e comportamento varietal da soja a diferentes condições de fertilidade. As quantidades de nutrientes para o cultivo da soja são definidas com base nos resultados das análises de solos, bem como, em função de todos os itens de condições do solo, acima citados. O presente trabalho apresenta, um conjunto de relatos onde os rendimentos da soja foram afetados pelas condições de fertilidade dos solos e de adição ou não de fertilizantes, com o objetivo de tentar esclarecer quais as situações de solo onde a soja teria máximo rendimento.

Considerações sobre algumas situações de fertilidade do solo e nutrição da soja e respectivos efeitos sobre o rendimento da soja

Nas Tabelas 1 e 2 são apresentadas as quantidades de macro e micronutrientes necessárias para produção de uma tonelada de soja bem como para as culturas de milho, trigo e girassol. Comparando as necessidades nutricionais da soja com as das outras culturas, se percebe que, a soja demanda mais nutrientes que o trigo e o milho e um pouco menos do que seria necessário para a cultura do girassol. Essa observação

faz pressupor que a soja também demandaria uma maior quantidade de fertilizantes e da mesma forma maiores concentrações desses nutrientes nos solos. Porém, observando os resultados apresentados na Tabela 3, (Rendimentos de grãos de milho, soja e trigo, cultivados em sucessão, após oito anos de fertilizantes aplicados para a sucessão soja-trigo), os melhores rendimentos da soja (3.147 kg ha^{-1} e 3.539 kg ha^{-1}) são encontrados onde as concentrações no solo de fósforo (9,3 mg dm^{-3}) e de potássio (0,20 $cmol_c$ dm^{-3}) não são as mais altas. Contudo, os melhores rendimentos de milho e de trigo, foram necessários com maiores concentrações de fósforo (17,8 mg dm^{-3}) e de potássio (0,44 $cmol_c$ dm^{-3}). Verifica-se também na Tabela 3, que na safra 98/99, a soja com produção de 3.539 kg ha^{-1} foi obtida no tratamento que não recebeu adubação fosfatada ou potássica, diferentemente do observado para as culturas de milho e trigo, em que, os melhores rendimentos ocorreram quando se adubou para ambas os cultivos.

Tabela 1. Quantidades médias de macronutrientes para a produção de 1t de grãos.

Nutrientes	Soja	Milho	Trigo	Girassol
	kg/1000kg			
N	83,5	24,9	28,3	79
P	8,4	4,3	6,9	9,8
K	32,1	18,2	20,6	49,5
Ca	15	3,9	3,7	30,3
Mg	8,0	4,4	2,1	9,9
S	8,2	2,6	4,3	8,4

Tabela 2. Quantidades médias de micronutrientes para a produção de 1 t de grãos.

Nutrientes	Soja	Milho	Trigo	Girassol
	g/1000kg			
B	55	18	20	64
Fe	513	236	37	281
Mn	165	43	106	180
Cu	30	10	6	66
Zn	64	49	20	98

Tabela 3. Rendimentos de grãos de milho, soja, trigo e soja, cultivados em sucessão após oito anos de fertilizantes aplicados para a sucessão soja/trigo, e respectiva concentração de P e K no solo, em Latossolo Roxo distrófico. Embrapa Soja.

Tratamentos				Produtividade			
Soja		Trigo		Milho	Soja	Trigo	Soja
P_2O_5	K_2O	P_2O_5	K_2O	96/97	97/98	98	98/99
				kg ha^{-1}			
0[1]	0[1]	0	0	4079	2654	2578	2883
P = 5,3		K = 0,17					
0	0	50	30	5250	3147	3100	3539
P = 9,3		K = 0,20					
30	50	50	30	5878	3238	3228	3621
P = 15,1		K = 0,32					
60	100	50	30	6210	3225	3180	3368
P = 17,8		K = 0,44					

[1] cultivo sem adubação. P em mg dm^{-3} e K em cmol$_c$ dm^{-3}.

PRODUTIVIDADE DA SOJA. Caso A

Localidade: Balsas, MA 1993
Análise de solo

pH	Al	Ca	Mg	P	K	M.O.	V
	cmolc dm^{-3}			mg dm^{-3}		%	
5,2	0,0	2,8	0,9	13	84	2,4	48

- Adubação com macronutrientes: 430 kg/ha 02 - 20 - 18
- Adubação com micronutrientes: 2,5 kg de Zn, 0,2 kg de B, 0,12 kg de Mo, 0,4 kg de Cu - 0,30 kg de Mn e 0,03 kg de Co.
- Cultivar: Cristalina Produtividade: 5.684 kg ha^{-1}

Fonte: POTAFOS 1996.

PRODUTIVIDADE DA SOJA. Caso B

Localidade: Dourados MS 1997
Análise de solo

pH	Al	Ca	Mg	P	K	M.O.	V
	cmolc dm^{-3}			mg dm^{-3}		%	
5,2	0,0	10,6	1,4	24	318	2,8	72

Cultivar: BR - 16.

1) Adubação com macronutrientes: Sem adubação
Produtividade: 4.860 kg ha^{-1}
2) Adubação com macronutrierntes: 450 kg/ha 00 - 20 - 20
Produtividade: 4.860 kg/ha
FONTE: Embrapa CPAO

Outras duas situações são mostradas em "Produtividade da soja caso A e caso B". No caso A, análise de solo revela uma situação de fertilidade muito pobre, com baixas concentrações de Ca, de Mg, de P e de K e também uma baixa concentração de bases (V = 48%) e nesse caso, a soja foi adubada com 430 kg ha^{-1} da fórmula 00-20-18 mais mistura de micronutrientes, resultando um rendimento de 5.684 kg ha^{-1}. No caso B, a análise de solo revela uma condição de alta fertilidade, com concentrações elevadas de Ca, de Mg, de P e de K e uma alta saturação de bases (V = 72%) em que, a soja foi adubada com 450 kg ha^{-1} da formula 00-20-20 e não se observou diferença de rendimento quando se comparou com o obtido pela soja sem qualquer adubação (4.860 kg ha^{-1}). A análise destes dois casos evidencia que, a soja pode apresentar uma alta resposta em função da adubação praticada, caso a situação de fertilidade seja baixa, e pode também apresentar ausência de resposta a adubação, caso a condição de fertilidade seja elevada.

Excessos de adubação ou de calagem podem em muitos casos promover menores rendimentos de soja. Na Tabela 4, são apresentados os rendimentos médios de soja, de vários locais da região dos cerrados do Brasil, em função de diferentes quantidades de calcário que determinaram diferentes condições de saturação de bases. Como se observa, os melhores rendimentos de soja (3.500 kg ha^{-1}), foram obtidos com saturação de bases perto do valor de 50% e menores rendimentos com saturação de bases menores ou maiores que 50%. Esses resultados mostram que também para a calagem a soja pode apresentar decréscimos de rendimento caso a mesma seja praticada com excessos ou dose menores que a mais adequada.

Tabela 4. Relação entre produção de grãos de soja e saturação por bases da camada arável do solo na região dos cerrados. Embrapa cerrados.

Saturação por bases	Rendimentos
%	kg ha^{-1}
20	2.700
28	2.840
35	3.250
45	3.300
50	3.500
55	3.400
60	3.100
70	2.800
80	2.500

Fonte: Sousa, et al.1993.

A análise foliar apresenta-se como uma possibilidade complementar, as interpretações das análises de solo e capaz de identificar os nutrientes que estariam comprometendo a maior produtividade da soja. A utilização da análise foliar como critério de diagnóstico é baseada na premissa de que existe uma relação significativa entre o suprimento de nutrientes e seus respectivos níveis, e que aumentos ou decréscimos nas concentrações estão relacionados a produções mais altas ou mais baixas, respectivamente. A Tabela 5 apresenta, o rendimento da soja produzida em função de quantidades aplicadas de P e ou K para a sucessão de culturas soja - trigo e concentração de macro micro nutrientes nas folhas de soja. Os maiores rendimentos de soja foram obtidos, com ausência de fertilizantes (3.539 kg ha^{-1}) e com a aplicação de P e K (3.542 kg ha^{-1}) e para esses rendimentos as concentrações de P e K nas folhas de soja estiveram sempre acima de 3,0 g kg^{-1} 20,0 g kg^{-1}.

Tabela 5. Produtividade da soja, safra 98/99, e concentração de nutrientes nas folhas em função de fertilizantes aplicados para a sucessão soja/trigo, em Latossolo Roxo distrófico. Embrapa Soja. Londrina, PR. 2001.

Tratamentos				Produtividade	Concentração de nutrientes em folhas										
Soja		Trigo													
P_2O_5	K_2O	P_2O_5	K_2O		N	P	K	Ca	Mg	S	Zn	Mn	Fe	Cu	B
kg ha^{-1}					g kg^{-1}						mg kg^{-1}				
0	0	0	0	2884	49,3	2,75	20,1	9,13	4,10	2,63	49,6	138	127	10,3	75,9
0	0	50	30	3539	55,6	3,62	22,0	8,35	3,98	2,75	43,7	134	122	11,3	69,1
0	0	50	0	3344	54,1	3,70	20,1	10,0	4,67	2,69	49,3	149	149	10,6	69,2
0	0	0	30	2510	45,5	2,54	20,1	14,5	5,44	2,47	84,6	205	239	7,44	61,3
30	50	50	30	3542	57,6	3,82	22,2	9,51	4,09	2,13	48,2	160	172	8,26	50,7
60	100	50	30	3193	55,7	4,31	22,2	8,73	3,71	1,97	43,2	117	169	7,53	44,6

Conclusões

Os máximos rendimentos da soja estão condicionados as diferentes condições de fertilidade dos solos, determinada por oferta dos nutrientes, condições de manejo dos solos e de quantidades de fertilizantes.

Nutrição nitrogenada da soja para obtenção de altos rendimentos

R.J. Campo and M. Hungria[1]

Abstract – Sources of nitrogen to reach high soybean yields

Soybean grain yield largely depends on N supply. In order to produce one ton of soybean, 83 kg of N are necessary. As a comparison, to produce the same amount of soybean, 38 kg of potassium and 15,4 kg of phosphorus, must be absorbed by the soybean plants. The main source of N to soybeans come from fertilizers and from biological N_2 fixation (BNF). In Brazil, hundreds of experiments have been performed testing sources, levels, methods and timing of N fertilizing, in order to obtain the maximum economic results. As the experiments demonstrated, no economic benefit is achieved using N fertilizers (mineral N), and the key to obtain higher yields is to maximize the BNF. Additionally, it was observed that low yields correlate to low N and low protein content in the grain. Recent studies proved that the contribution of BNF ranged from 72 to 94% of N accumulated by soybean plants. The BNF efficiency depends on: (i) the adequate of environmental factors (temperature, water supply and photoperiod); (ii) the interaction among environmental factors and the soybean plant, like the efficiency of soybean cultivar in fixing N_2, soil fertility conditions, macro and micronutriente supply; and (iii) the bacteria strain competitiveness, the amount and the quality of the inoculant, the care in the inoculation process and the absence of antagonic agrochemicals in the seed. On experimental conditions, when more efficient *Bradyrhizobium* strains plus other recommended technologies (highly productive cultivars, no-tillage cultivation, etc.) were used, yields in excess of 5.000 kg of soybean/ha were easily obtained. However, top yields have rarely been observed in commercial fields, probably due to the difficult to control the set of factors limiting higher soybean yields. These factors will be further discussed.

Introdução

A cultura da soja é extremamente dependente do fornecimento de nitrogênio (N) para obtenção de altos rendimentos. Para cada 1.000 kg de grãos de soja são necessários, aproximadamente, 83 kg de N. A titulo de exemplo, o potássio e fósforo, segundo e terceiro nutrientes mais exigidos pela cultura

[1] Embrapa Soja, Caixa Postal 231, 86001-970, Londrina, PR, Brasil, rjcampo@cnpso.embrapa.br.

da soja, participam, respectivamente, com 38 kg e 15,4 kg para cada 1.000 kg de grãos. Basicamente as fontes de N disponíveis para a cultura da soja são os fertilizantes nitrogenados e a fixação biológica do nitrogênio (FBN).

Fertilizantes nitrogenados

São a forma assimilada com maior rapidez pelas plantas, mas a um custo elevado, porque possuem baixa eficiência de utilização pelas plantas (raramente ultrapassam a 50%). O N não absorvido pelas plantas é perdido principalmente por lixiviação e desnitrificação, podendo causar sérios problemas de poluição, resultando em acúmulo de nitrogênio em águas dos rios e lagos. Além disso, os adubos nitrogenados aumentam a velocidade de decomposição da matéria orgânica do solo e são altamente inibidores do processo de FBN, que é a principal fonte de N para a soja.

Desde a introdução da cultura da soja no Brasil, centenas de trabalhos envolvendo fontes, doses, métodos e épocas de aplicação de N foram executados. Nos últimos anos, em função dos aumentos sucessivos dos rendimentos da soja aliados à maior demanda de N, novamente, diversos experimentos foram conduzidos para avaliar a importância desse nutriente visando a obtenção de altos rendimentos em soja. Gan et al. (2003), em trabalhos com adubação nitrogenada com a soja na China, encontrou que a melhor dose e época para aplicar N por cobertura foi de 50 kg/ha na floração. No Brasil, diversos trabalhos foram efetuados em diversas regiões (Tabela 1). Os resultados mostram que a soja brasileira não responde à adubação nitrogenada, seja ela aplicada na semeadura da soja ou em cobertura. Dos 20 experimentos incluídos na Tabela 1, somente em cinco deles haveria pequena vantagem econômica de se aplicar N na forma de fertilizante mineral. Muito provavelmente isso se deveu à baixa eficiência da FBN desses experimentos, devido a ocorrência de algum fator que limitou a eficiência do processo de FBN.

Fixação biológica do nitrogênio (FBN)

fonte de N indispensável para a cultura da soja nas condições brasileiras. Bactérias do gênero *Bradyrhizobium*, quando em contato com as raízes da soja, infectam as raízes via pêlos radiculares, formando nódulos. Dentro dos nódulos, as bactérias, através de um complexo enzimatico chamado nitrogenase, conseguem quebrar a tripla ligação do N_2 atmosférico e provocar a sua redução para NH_3. A essa amônia são incorporados íons H+ abundantes nas células das bactérias, ocorrendo a transformação em íons amônio (NH4+). Os íons amônio são, então, metabolizados e distribuídos para a planta hospedeira sendo, posteriormente, incorporados na forma de N orgânico (Hungria et al, 1997).

Tabela 1. Resumo dos principais resultados de pesquisas realizados nos últimos ano envolvendo doses e épocas de aplicação de N na cultura da soja e lucros auferidos pela adubação nitrogenada. Londrina, 2003.

Local (Citação)	Tratamento doses de N	Época aplicação	Rend. Kg/ha	Custo da Técnica	Lucro R$/ha
Dourados, MS	SI[1]	-	3141	0	2199,00
Mercante et. al. 2003.	200 kg N	50 % + 50%	3798	400,00	2259,00
	I[2]. c/ 5019	-	4052	10,00	**2826,00**
Ponta Porã, MS.	SI	-	2006	0	1404,20
Mercante, et al. 2003.	200 kg N	50% + 50%	2453	400,00	1317,00
	120 kg N	4(pl,r1,r2, r5)	2054	240,00	1198,00
	30 kg N	Plantio	2022	60,00	1355,40
	I c/ 5019	-	2133	10,00	**1483,10**
Jaciara, MT	SI	-	2498	0	1748,60
Loureiro et.al. 2001	200 kg	50% + 50%	2668	400,00	1467,60
	30 kg	Plantio	2535	60,00	1714,50
	50	Pré r1	2580	100,00	1706,00
	50	R5	2527	100,00	1668,90
	I c/ (vários)	-	2639	10,00	**1837,30**
Londrina, PR	SI	-	3503	0	**2452,10**
Crispino et. al. 2001.	200 kg	50% + 50%	3407	400,00	1984,90
Embrapa 48	30	Pl.	3333	60,00	2273,10
Convencional	50	Pré R1	3253	100,00	2177,10
	50	R5	3573	100,00	2401,10
	I	-	3497	10,00	2437,90
Londrina, PR	SI	-	3635	0	2544,30
Crispino et. al. 2001.	200 kg	50% + 50%	3556	400,00	2089,20
Embrapa 48	30	Pl.	3634	60,00	2483,80
Direto	50	Pré R1	3681	100,00	2476,70
	50	R5	3917	100,00	**2641,90**
	I	-	3517	10,00	2451,90
Londrina, PR	SI	-	3139	0	2197,30
Crispino et. al. 2001.	200 kg	50% + 50%	3179	400,00	1825,30
BRS 134	30	Pl.	3280	60,00	2236,00
Convencional	50	Pré R1	3230	100,00	2161,00
	50	R5	3455	100,00	**2318,50**
	I	-	3214	10,00	2239,80
Londrina, PR	SI	-	3039	0	**2127,30**
Crispino et. al. 2001.	200 kg	50% + 50%	3229	400,00	1860,30
BRS 134	30	Pl.	3060	60,00	2082,00
Direto	50	Pré R1	3039	100,00	2027,30
	50	R5	3076	100,00	2053,20
	I	-	2747	10,00	1912,90
PontaGrossa, PR			3005	0	2103,50
Crispino et al. 2001.	200 kg	50% + 50%	3285	400,00	1899,50
Embrapa 48	30	Pl.	3414	60,00	**2329,80**
Convencional	50	Pré R1	3052	100,00	2036,40
	50	R5	2985	100,00	1989,50
	I	-	3178	10,00	2214,60

[1]. sem inoculação, [2] inoculação. Preço kg soja = R$ 0,70, Preço kg N = 2,00

A soja é uma das leguminosas mais eficientes no processo de fixação do N_2. No Brasil, as taxas de fixação biológica têm sido estimadas entre 70% a 94% do N acumulado pela planta (Hungria et al., no prelo). Estudos desenvolvidos por Peoples & Craswell (1992), verificaram que a soja pode fixar até 450 kg/ha, que seriam suficientes para produzir 5.420 kg de soja, considerando a necessidade de 83 kg N/1.000 kg grãos. Esses resultados demonstram que a FBN pode, dependendo de sua eficiência, fornecer todo o N que a soja necessita para obter altos rendimentos. Para isso, algumas

técnicas de cultivo da soja, bem como alguns cuidados especiais na inoculação da soja devem ser observados e adotados pela assistência técnica e os produtores de soja.

A eficiência de FBN depende de uma série de fatores inerentes ao ambiente onde a simbiose ocorre, à planta e à bactéria. Ao ambiente incluem-se os fatores abióticos, como altas temperaturas e estresse hídrico. Á interação ambiente/planta incluem-se a capacidade de FBN das cultivares de soja e os fatores nutricionais, como excesso de acidez do solo com presença de Al e Mn; deficiência de P, K, Ca, Mg e deficiência de micronutrientes, especialmente, Mo e Co, que são importantes no processo de FBN. Com relação à bactéria, independente da eficiência da fixação simbiótica e da competitividade de cada estirpe, sabe-se que, aumentando a população de células viáveis da bactéria na semente, através da inoculação, independente da população existente no solo, aumenta-se a ocorrência de nódulos no sistema radicular da soja, que são os nódulos que possuem maior eficiência de FBN (Weaver & Frederick, 1974). Com uma maior população de células na semente aumenta-se, ainda, o número de nódulos na coroa do sistema radicular da planta, aumentando-se a eficiência de FBN e a quantidade de N fixado. Assim, todos ao fatores que influenciam a população de células nas sementes, tais como: quantidade e qualidade dos inoculantes; cuidados na inoculação; distribuição uniforme do inoculante em todas as sementes, aderência dos inoculantes nas sementes e aplicações de fungicidas e micronutrientes nas sementes, são fundamentais para o sucesso da FBN.

Recentes trabalhos desenvolvidos por Cooper (2003) em Wooster, Ohio Estados Unidos, demonstraram que a principal barreira para obtenção de altos rendimentos de soja são o binômio fotoperíodo e temperatura. Soja plantada na primavera com altas temperaturas floresceu duas semanas antes do que soja plantada na mesma época porém, com temperaturas normais, o que resultou em um aumento do potencial produtivo da soja. Testes com soja cultivada em condições controladas, dias longos e altas temperaturas após o plantio, mostraram rendimentos que variaram de 5.383 a 5.963 kg/ha, enquanto que, sob condições normais de temperatura, os máximos rendimentos anuais variaram de 3.575 a 4.862 kg/ha.

Os trabalhos de pesquisa de soja, no Brasil têm desenvolvido novas tecnologia de cultivo de soja, com aumentos sucessivos de rendimento, implicando em necessidades crescentes de N. Níveis de rendimento de soja acima de 5.000 kg/ha (83 sacos/ha) têm sido obtidos, com certa freqüência, em trabalhos de pesquisa, comprovando a capacidade produtiva das cultivares ora disponíveis e da capacidade de FBN das estirpes atualmente recomendadas. Entretanto, em lavouras comerciais, raramente a soja produz mais que 4 000 kg (66 sacos/ha), comprovando a existência de fatores limitantes ao rendimento.

Tem-se observado, com freqüência, que baixos rendimentos de soja estão relacionadas com baixos teores de N nos grãos, agravando o problema do baixo teor de proteína dos grãos destinados à industria de farelo. Assim, torna-se indispensável a busca de tecnologias mais eficientes para aumentar a nodulação, a FBN, o rendimento e os teores de proteína nos grãos.

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Manejo da cultura da soja para máxima produtividade

R. G. de Araújo

Resumo

A Fazenda Couro do Boi II, localizada na região de Londrina, PR, sob clima do tipo Cfa, segundo Köeppen, já teve produtividades de soja inferiores a 3200 kg/ha e, após uma readequação em seu sistema de manejo, já alcançou picos superiores a 4000 kg/ha. Entre as principais técnicas de manejo adotadas estão: otimização do uso de herbicidas e fungicidas, sistema de Plantio Direto na Palha com rotação de culturas, qualidade no plantio e colheita, e introdução à Agricultura de Precisão, que unidas à dedicação e ao meu comprometimento em seguir as recomendações técnicas traçadas contribuem para a maximização de produtividade na cultura da soja.

Abstract – Soybean crop management for maximum yield

The Couro do Boi II farm, located in the region of Londrina, PR, under Cfa type of clime, according to Köeppen, sometime ago had soybean yields below 3,200 Kg/ha. After some changes in the production management system, attained yields are already up to 4,000 Kg/ha. Among the main management methods used are: optimization of herbicide and funcicide use; adoption of no till system with crop rotation; quality of sowing and harvesting; and, introduction of precision agriculture practices. The compromise of the farm in following the technical recommendations contributed for the maximization of soybean yield.

Introdução

A Fazenda Couro do Boi II:

A propriedade rural localiza-se no município de Bela Vista do Paraíso, região de Londrina, estado do Paraná, dispõe de uma área agrícola de 614,30 ha, e é de propriedade autor.

• Caracterização:
Solo: latossolo vermelho eutroférrico (LVef).
Clima: o clima da região é do tipo Cfa segundo a classificação de Köeppen: subtropical; temperatura média no mês mais frio inferior a 18°C (mesotérmico) e temperatura média no mês mais quente acima de 22°C, com verões quentes, geadas pouco freqüentes e tendência de concentração das chuvas nos meses de verão, contudo sem estação seca definida.

• Histórico de Produtividades de Soja:
Safra 1999/2000: produtividade média 3120 kg/ha
Safra 2000/2001: produtividade média 3520 kg/ha
Safra 2001/2002: produtividade média 3410 kg/ha
Safra 2002/2003: produtividade média 3617 kg/ha, máxima 4181 kg/ha (talhão CB2-P5 / 35,85 ha)

• O passado:
Introduzi em minha propriedade o sistema de rotação de culturas porém retornei à monocultura.
• O Presente:
A partir da safra 2002/2003 (quando se integrou ao grupo Insolo) houve uma total readequação do manejo das áreas cultivadas da propriedade, com vistas à otimização dos fatores de produção.

Dentre os procedimentos realizados na readequação do manejo podemos citar programação de rotação de culturas, levantamento da fertilidade do solo e programação de corretivos e fertilizantes, recomendação de variedades adequadas a cada área e época de plantio, programação de herbicidas, inseticidas e fungicidas, de acordo com as necessidades da cultura e de cada talhão.

Aliado a essas técnicas de manejo tem-se também o acompanhamento semanal do engenheiro agrônomo na área, junto ao administrador da fazenda, de modo a atender todas as necessidades de cultivo e efetivar constatações prévias e recomendações eficazes, evitando-se desperdícios e perdas desnecessárias. As informações obtidas no campo, desde o plantio até a colheita são armazenadas num banco de dados padronizado, para posterior avaliação de resultados de safra.

A tecnologia mais recentemente incorporada ao manejo é a Agricultura de Precisão, a qual fornece subsídios para a determinação precisa da quantidade de fertilizantes e corretivos a serem aplicados na área, de acordo com as necessidades de cada parte dos talhões.

Estratégias para obtenção de altas produtividades

Manejo do Sistema de Produção

O sistema de cultivo aplicado na Fazenda Couro do Boi II, é o Sistema de Plantio Direto na Palha com rotação de culturas em 100% da área, a rigor sem qualquer tipo de operação de preparo do solo. Porém considerando-se o fato de ter-se um solo de textura muito argilosa, a compactação do terreno é esperada, e para evitar os inconvenientes advindos desse processo

procede-se a um monitoramento da compactação do solo e, ao se constatar a real necessidade é realizada uma escarificação sobre a área compactada.

Fertilidade do solo: os fertilizantes recomendados para o cultivo são escolhidos através das necessidades apresentadas no balanço de nutrientes do sistema, e os micronutrientes determinados através de análise de tecido para posterior avaliação e recomendação .

Escolha de Variedades: as variedades a serem utilizadas são criteriosamente escolhidas após avaliação de desempenho em diversas áreas da propriedade e de outras propriedades assistidas no grupo de que faço parte. Materiais de empresas como Embrapa, Coodetec, Monsoy, entre outras, são designadas de acordo com sua adaptabilidade a cada talhão da fazenda e também de acordo com o seu ciclo e época de plantio dentro da rotação de culturas.

Escolha da época de plantio: nas áreas programadas sem safrinha, o plantio é realizado a partir do segundo decêndio de outubro até o segundo decêndio de novembro. Áreas programadas com safrinha têm o plantio iniciando no final do primeiro decêndio de outubro estendendo-se até o primeiro decêndio de novembro, e fazendo uso variedades precoces. O plantio dessas áreas é antecipado justamente para que a época de colheita da soja não se sobreponha ao plantio do milho safrinha em fevereiro, garantindo maiores produtividades do milho na safrinha.

Manejo fitossanitário: é realizado com vistas à otimização do uso de herbicidas, fungicidas e inseticidas. A lavoura é monitorada constantemente para que não sejam feitos aplicações desnecessárias, nem atraso na aplicação.

Plantas Invasoras: para o controle procede-se a dessecações seqüenciais; uma inicial, para atingir plantas que estejam sementeando antes do plantio da soja, e outra em pré-plantio, utilizando herbicida 2,4-D e Glifosato. Os herbicidas são programados em rotação com objetivo de se controlar e até mesmo evitar a existência de plantas resistentes a herbicidas.

Fitopatógenos: o controle de patógenos era feito apenas para as doenças de final de ciclo, porém com o advento da Ferrugem da Soja foram inseridos no programa produtos triazóis e estrubirulinas, aplicados dentro de um programa de monitoramento, para evitar a entrada e estabelecimento do patógeno na área.

Insetos: o Manejo Integrado de Pragas é realizado através da avaliação semanal dos talhões pelo funcionário da fazenda responsável pelo

levantamento de pragas, através da contagem de insetos por batida de pano; e quando necessário for, faz-se uso de inseticidas fisiológicos para controle de lagartas e organofosforados para o controle de percevejos.

Qualidade de plantio: uma área produtiva é reflexa de um plantio bem feito. Regulagem precisa de máquinas, sementes tratadas e inoculadas, condições de tempo ideais são elementos determinantes de uma lavoura uniforme.

Equipamentos Agrícolas: a fazenda dispõe de equipamentos modernos, como semeadoras capazes de plantar trigo após milho, soja em altas palhadas de trigo, específicas para plantio direto. O engenheiro agrônomo responsável oferece treinamento aos funcionários da fazenda, sobre regulagem de semeadoras e pulverizadores, antes do início da safra.

Tratamento de sementes: para o plantio é realizado o tratamento com fungicida e a inoculação das sementes com rizóbio, garantindo sanidade e facilitando o aproveitamento de nitrogênio pela planta. Esse assunto também é abordado durante o treinamento pelo qual os funcionários passam antes da safra.

Condições de tempo: o acompanhamento das previsões do tempo também é um importante aliado na realização de um plantio de qualidade. Chuvas em quantidades moderadas contribuem pra germinação e emergência uniformes.

Rotação de culturas

A rotação de culturas dentro do Sistema de Plantio Direto na Palha é um fator importante na maximização da produtividade, seja na quebra de ciclo de pragas e doenças pela troca anual de cultura sobre a área, seja no fornecimento de palha para proteção do solo contra o processo de erosão tão freqüente em locais de monocultura, ou no fornecimento de elementos como Nitrogênio e Fósforo para as culturas posteriores.

O sistema de rotação de culturas aplicado na Fazenda Couro do Boi II, idealizado pela assistência técnica, está descrito na tabela abaixo e é chamado "Rotação ¼ de Milho de Verão com ¼ Milho Safrinha". Consiste em plantio de verão com 25% de milho e 75 % de soja, e Inverno com 50% de trigo, 25 % de milho safrinha e 25 % de aveia.

Área	153,57 ha	153,57 ha	153,57 ha	153,57 ha
	1/4	1/4	1/4	1/4
Inverno/01	TRIGO	TRIGO	MILHO SAFR.	AVEIA
Verão/01	SOJA PREC.	SOJA	SOJA	MILHO
Inverno/02	MILHO SAFR.	AVEIA	TRIGO	TRIGO
Verão/02	SOJA	MILHO	SOJA	SOJA PREC.
Inverno/03	TRIGO	TRIGO	AVEIA	MILHO SAFR.
Verão/03	SOJA	SOJA PREC.	MILHO	SOJA
Inverno/04	AVEIA	MILHO SAFR.	TRIGO	TRIGO
Verão/04	MILHO	SOJA	SOJA PREC.	SOJA

Agricultura de precisão

É a mais nova tecnologia aplicada ao sistema produtivo na Fazenda Couro do Boi II, com objetivos de otimizar condições de solo para que a planta explore todo seu potencial produtivo. Consiste em mapear a área da propriedade, determinando sua necessidade de fertilizantes e corretivos a partir de análise de solo amostrado com auxílio de equipamento de posicionamento global com sinal diferencial (DGPS).

As figuras abaixo são de mapeamento de fertilidade do solo realizada na fazenda no segundo semestre de 2003, e demonstram a variabilidade de elementos considerados na avaliação da fertilidade do solo em toda a propriedade, bem como sua necessidade produtos corretivos e fertilizantes.

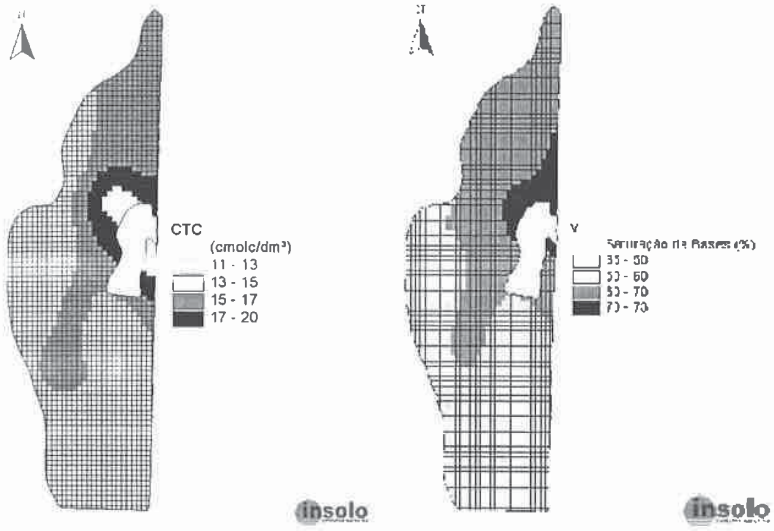

Figura 1. mapeamento da Capacidade de Troca de Cátions (CTC) e Saturação de Bases (V%) do solo através de agricultura de precisão. Fazenda Couro do Boi II-2003

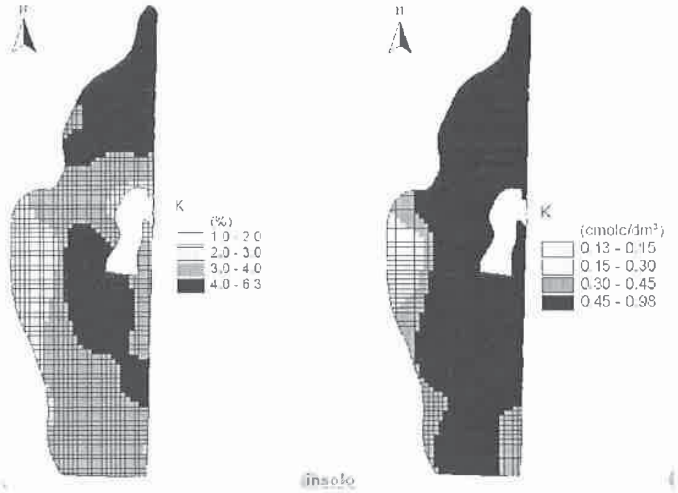

Figura 2. mapeamento do elemento Potássio (K) em porcentagem da CTC e em cmol/dm³ do solo através de agricultura de precisão. Fazenda Couro do Boi II-2003

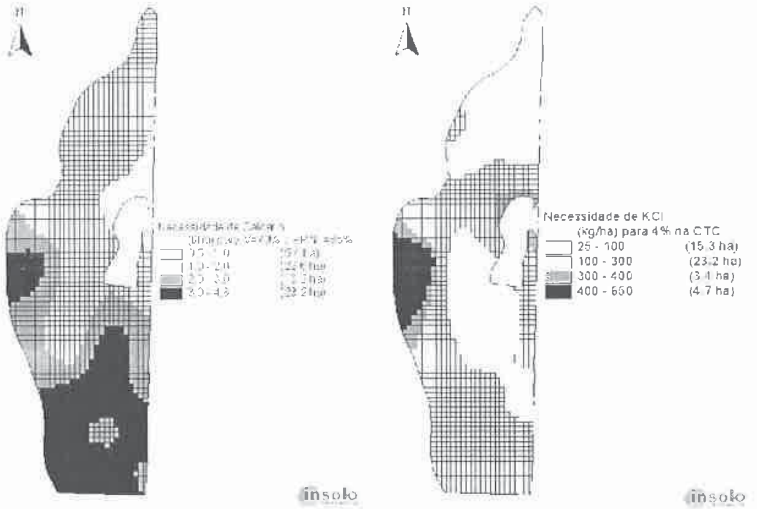

Figura 3. Mapeamento da Necessidade de Calcário (NC) e Necessidade de Cloreto de Potássio (NK) do solo através de agricultura de precisão. Fazenda Couro do Boi II-2003

Limitações do ambiente à obtenção de máximos rendimentos de soja

J. R. B. Farias[1]

Resumo

A cultura da soja ocupa uma posição de destaque na economia brasileira, o que justifica a busca de novas informações no sentido de otimizar seu cultivo e de reduzir os riscos de prejuízos aos quais a cultura está sujeita. A expressão do rendimento máximo de grãos de uma cultura é determinada, principalmente, por suas características genéticas e pelo grau de adaptação ao ambiente predominante, podendo ser afetada pelo manejo humano. Dentre os fatores inerentes à produção agrícola, o clima aparece como aquele de mais difícil controle e de maior impacto sobre à obtenção de máximas produtividades. Os principais fatores climáticos que determinam o rendimento máximo são temperatura, radiação solar e água, embora outros como ventos, fotoperíodo e umidade do ar possam também estar relacionados. Dos elementos do clima, o que se apresenta como mais limitante é a água. A variabilidade na disponibilidade hídrica durante a estação de crescimento da cultura é a principal limitação à expressão do potencial de rendimento da soja. A adoção de algumas práticas de manejo ajuda a minimizar os efeitos negativos sobre o rendimento de grãos. Porém, qualquer intervenção no sistema de produção tem por objetivo obter uma situação econômica ótima, fundamentada numa situação técnica ideal. Provavelmente, no futuro, muito do potencial para obtenção de altos rendimentos de soja estará relacionado à tolerância das cultivares a curtos períodos de seca. Práticas de manejo, que visam maior armazenamento ou incremento da camada de extração da água do solo, em muito, também, poderão contribuir para o alcance de elevadas produtividades.

Abstract – Environmental limitations to maximum soybean yield

Soybean occupies a prominent position in the Brazilian economy, which justifies the search for new information to optimize its cultivation and reduce the risks of damage. The expression of the maximum yield of a plant is determined mainly for its genetic characteristics and the adaptation level to the predominant environmental conditions, factors that can also be affected by human management. Among the factors involved in crop production, climate appears to be the most difficult to control and the factor with the greatest impact on maximum yields. The principal climatic factors determining

[1]Embrapa Soja, Caixa Postal 231, CEP 86001-970, Londrina, PR, Brasil. jrenato@cnpso.embrapa.br

maximum yield are temperature, solar radiation and water, although others such as wind, photoperiod and atmospheric humidity can also play a role. Of the climatic elements, the most restrictive is water. The variability of water availability during the crop growth season is the main limitation to the expression of maximum soybean yield potential. The adoption of appropriate crop management practices can help minimize the negative effects of water shortage grain yields and farm revenue. However, any intervention in the production system must strive to obtain a optimum economical returns, based on sound technical knowledge. In the future, much of the potential increases in soybean yields will probably be related to the tolerance of new varieties to short drought periods. The adoption of Management practices, that increases water storage and/or plat available water in the soil, will also contribute greatly to improve soybean productivity.

O setor agrícola tem se destacado por contribuir com um superávit considerável na balança comercial do Brasil. Embora não facilmente mensurável, a contribuição indireta das principais culturas de grãos na movimentação da economia brasileira tem sido de vital importância para o desenvolvimento do país, seja pela geração de empregos ou pela adição de valor ao produto industrializado, Nesse cenário, destaca-se a participação do agronegócio da soja. A soja representa, hoje, uma importante fonte de divisas, contribuindo com uma parcela significativa das exportações brasileiras, além do seu crescente uso na alimentação humana. Afora isto, há um contingente de famílias cuja renda está diretamente relacionada aos empregos gerados pelos complexos de produção, transporte e industrialização da soja.

Com a globalização dos mercados agrícolas, incrementos nos rendimentos e redução dos custos e dos riscos de insucesso passaram a ser exigências básicas à competitividade. O aumento de eficiência no uso de recursos e de insumos, a melhora qualitativa dos produtos agrícolas e a preservação dos recursos naturais, são desafios da moderna agricultura. Para manter e/ou melhorar a competitividade da sojicultura nacional, face à forte concorrência externa, há uma demanda crescente por conhecimentos e informações de natureza tecnológica que atendam essas necessidades. O grande desafio da agricultura brasileira é a necessidade de produzir com qualidade para abastecer o mercado interno e gerar excedentes para exportação, a um custo competitivo com o praticado pelos principais mercados internacionais, sem perder de vista o compromisso de preservar os recursos naturais. Diante desse contexto, a busca por rendimentos máximos aparece como uma das principais demandas do setor produtivo.

Rendimento máximo de uma cultura nada mais é do que aquele obtido por uma variedade altamente produtiva e bem adaptada ao ambiente de

crescimento, sem a ocorrência de fatores limitantes durante todo o seu ciclo. A expressão do rendimento máximo de grãos de uma cultura é determinado, principalmente, por suas características genéticas e pelas respostas ao ambiente predominante, podendo ser afetada pelo manejo do cultivo. A bagagem genética varia de uma cultivar para outra, devendo-se, portanto, dar preferência aos materiais mais adaptados às características edafoclimáticas da região de cultivo. Os fatores de ordem ambiental que afetam o rendimento podem ser divididos em bióticos e abióticos. Os bióticos consistem do conjunto de seres vivos (fungos, bactérias, insetos, etc.) que podem atuar sobre a cultura, tanto de forma benéfica como maléfica. Todos os fatores ambientais não atribuíveis à atividade biológica podem ser incluídos como de ordem abiótica. Dentre os principais destacam-se a disponibilidade de nutrientes, as características físicas e químicas do solo e o clima. Para vários desses fatores, já foram desenvolvidas alternativas tecnológicas ou se está trabalhando para amenizar seus efeitos.

Apesar da boa disponibilidade de tecnologias de ponta recomendadas para a cultura da soja e adotadas por muitos produtores, como cultivares adaptadas, manejo integrado de pragas e doenças, manejo do solo e da cultura, entre outras, ainda hoje são bastante desuniformes e relativamente baixos os rendimentos médios de soja no País.

Dentre os fatores inerentes à produção agrícola, o clima aparece como aquele de mais difícil controle e de maior impacto sobre a obtenção de máximas produtividades. Aliado a isso, a imprevisibilidade das variações climáticas confere à ocorrência de adversidades climáticas a posição de principal fator de risco e de insucesso na exploração da cultura da soja. Consta no relatório sobre seguridade agrícola, elaborado pelo Ministério do Planejamento (Göpfert et al., 1993), a ocorrência de secas como principal evento sinistrante (71% dos casos) nas culturas de verão, seguido por chuva excessiva (22% dos casos), granizo e geada. Além destes, são mencionadas, ainda, perdas devido à tromba d'água, vento frio, vento forte, variação excessiva de temperatura e enchentes. Entre os fatores bióticos, são informadas, também, perdas por ocorrência de pragas e de doenças (0,20% das perdas nas safras de verão e 0,05% nas de inverno). Além disso, a ocorrência de muitas doenças e de pragas pode estar associada às condições climáticas favoráveis ao patógeno ou inseto, ou adversas ao desenvolvimento da planta, deixando-a mais fraca e suscetível ao ataque externo.

Estresses abióticos como seca, excesso de chuvas, temperaturas muito altas ou baixas, baixa luminosidade, etc., podem reduzir significativamente os rendimentos das lavouras e restringir os locais e os solos onde espécies comercialmente importantes podem ser cultivadas. Os principais fatores

climáticos que determinam o rendimento máximo são temperatura, radiação solar e água, embora outros como ventos, fotoperíodo e umidade do ar possam também estar relacionados.

Em geral, a temperatura determina a taxa de desenvolvimento da cultura, afetando a duração do período de crescimento necessário à formação da colheita. Existem relatos informando que altas produtividades foram obtidas em anos de ocorrência precoce de altas temperaturas que, juntamente com o fotoperíodo, induziram precocemente a floração quando os dias são mais longos e a intensidade luminosa é maior. Como a duração do ciclo total da planta é similar a dos anos normais, ocorreu, neste caso, um período reprodutivo mais longo, permitindo maior produtividade (Cooper, 2003). Isso indica que cultivares que florescem mais cedo podem apresentar incremento da produtividade.

A radiação solar é o fator desencadeador da fotossíntese, na qual o CO_2 atmosférico, com a participação da água, é transformado em carbohidratos. Esses carbohidratos serão usados para o crescimento (aumento de tamanho) e para a manutenção (recomposição dos tecidos devido à respiração). A diferença entre o ganho e a perda de carbono é chamada de Taxa Fotossintética Líquida. À medida que aumenta a incidência de radiação solar sobre a cultura, aumenta a taxa fotossintética (absorção de CO_2), até um certo limite. A temperatura afeta a respiração (consumo de CO_2). A fotossíntese é afetada, também, pela temperatura, e o vento e a umidade relativa do ar estão envolvidos no transporte biofísico do CO_2 até os cloroplastos. O rendimento máximo de soja é determinado pela capacidade da planta em interceptar a maior quantidade possível de radiação solar durante os estádios vegetativos e nos estádios reprodutivos iniciais. Maior interceptação de luz, associada a condições hídricas e nutricionais adequadas, aumenta o potencial produtivo da soja. Resumidamente, a radiação solar é responsável pela produção de matéria seca.

A sensibilidade da soja ao fotoperíodo é uma das principais restrições à adaptação de uma cultivar a um determinado local. O desenvolvimento das plantas de algumas cultivares de soja é muito dependente do fotoperíodo, pois o desenvolvimento reprodutivo é normalmente iniciado quando as plantas estão submetidas a dias curtos. Dessa forma, o cultivo de tais cultivares de soja em áreas de baixa latitude, onde o fotoperíodo é sempre relativamente curto, resultaria em florescimento precoce e, conseqüentemente, plantas pequenas e com baixos rendimentos de grãos. A introdução do período juvenil longo possibilitou ampliar a adaptação de uma cultivar, permitindo-lhe maior estabilidade de produção no tempo (épocas de semeadura) e no espaço (regiões). Segundo Kiihl & Garcia (1989),

período juvenil é o nome dado à fase inicial de crescimento durante a qual a planta não é induzida a florescer, mesmo sob condições indutivas. O período juvenil longo parece impedir o florescimento, independentemente do fotoperíodo, até que transcorra um período de tempo mínimo, fortemente determinado pelo genótipo e pela temperatura. Uma vez transcorrido este período, a soja torna-se "apta a florescer" e o florescimento destes materiais tende a ser menos sensível ao fotoperíodo. Uma cultivar de soja com período juvenil longo permanece vegetativa por mais tempo do que cultivares convencionais, quando expostas a dias curtos, mas poderá florescer mais cedo do que algumas cultivares convencionais sob dias longos. Assim, as cultivares com período juvenil longo parecem ser menos sensíveis ao fotoperíodo do que a maioria das cultivares tradicionais. A identificação desta característica e a sua incorporação em novos genótipos pelo melhoramento genético estão entre os principais fatores responsáveis pela adaptação da soja às regiões de baixa latitude, permitindo a exploração da cultura em latitudes de até zero grau.

Dos elementos do clima, o que se apresenta como mais limitante é a água. A variabilidade na disponibilidade hídrica durante o período de desenvolvimento da cultura é a principal limitação à expressão do potencial de rendimento da soja, independentemente do ciclo da cultivar, da época de semeadura e do local. Todavia, há variabilidade entre as regiões na magnitude das perdas de potencial de rendimento por falta de água à cultura. Clima e solo são as variáveis que explicam as diferenças regionais dos impactos da deficiência hídrica na cultura da soja, principalmente, em função da capacidade de armazenamento de água disponível no solo e do regime pluviométrico (Cunha et al, 1998).

A deficiência hídrica provoca reações fisiológicas e morfológicas na planta, como fechamento dos estômatos, murchamento de folhas, redução da área foliar, menor estatura da planta, queda de flores e frutos, redução na fixação do nitrogênio, ajustamento osmótico etc., que reduzem a fotossíntese, afetando negativamente o crescimento e o rendimento de grãos. A ausência de chuvas, isoladamente, não significa ocorrência de déficit hídrico. Ocorre déficit hídrico quando a evapotranspiração real for menor do que a evapotranspiração máxima da cultura. A soja possui duas fases mais críticas à disponibilidade hídrica: a primeira vai da semeadura à emergência, quando a falta de água afeta o estabelecimento das lavouras e a população de plantas; e a segunda, da floração ao enchimento de grãos, mais relacionada à formação do rendimento de grãos. A ocorrência de seca severa durante a fase vegetativa também pode afetar o rendimento de grãos, devido ao menor desenvolvimento das plantas e menor população. A floração é a fase de maior consumo de água, porém a falta de água é

mais crítica durante o enchimento de grãos. O efeito da deficiência hídrica sobre o rendimento de grãos depende da sua intensidade, da duração, da época de ocorrência, da cultivar, do estádio de desenvolvimento da planta e da interação com outros fatores determinantes do rendimento.

A água passa a ser limitante aos altos rendimentos em várias regiões produtoras brasileiras, dependendo da produtividade esperada. Se a produtividade almejada for ao redor de 3000 kg/ha, pode-se afirmar que a água não será limitante, numa determinada região. No entanto, se a produtividade esperada for de 6000 kg/ha, a água poderá ser o fator limitante da produtividade nessa mesma região (Cooper, 2003).

A partir do momento que a água for removida como fator limitante da produtividade, a resposta aos outros fatores de produção, incluindo cultivares, aumenta significativamente. Porém, a remoção exclusivamente da água como um fator limitante da produtividade, não necessariamente resultará em produtividade recorde da soja.

Em algumas regiões de excelente disponibilidade hídrica durante toda a estação de crescimento e na ausência de outro fator limitante ao desenvolvimento das plantas, a ocorrência de acamamento tem sido apontado, também, como um dos fatores limitantes à obtenção de máximos rendimentos. Algumas pesquisas têm mostrado que o acamamento precoce pode reduzir a produtividade potencial em até 25% (Cooper, 2003). Para solucionar este problema, deve-se, a longo prazo, direcionar programas de melhoramento específicos para ambientes de alta produtividade e, a curto prazo, amenizar o problema de acamamento manejando o espaçamento entre linhas e a população de plantas (Cooper, 2003).

As interações existentes no meio biológico são inúmeras e muito complexas. São vários os efeitos dos diferentes níveis dos diversos fatores envolvidos na produção de grãos. Quando se busca compreender os efeitos das interações entre esses níveis, a complexidade acentua-se drasticamente. Muitas vezes, os efeitos das interações dificilmente conseguem ser visualizados/demonstrados no campo. Devido a esta grande complexidade dos fatores envolvidos na tomada de decisão num sistema de produção, feqüentemente são usadas ferramentas como a modelagem, para melhor entender e compreender as diferentes interações existentes no meio biológico. A associação da pesquisa convencional com técnicas de modelagem e simulação tem se mostrado de grande utilidade para atender as crescentes demandas do setor agrícola. O enfoque sistêmico viabilizado pelo uso de modelos de simulação de desenvolvimento permite integrar os efeitos de diferentes condições edafoclimáticas sobre o comportamento da

cultura, criando ferramentas eficazes para apoiar a tomada de decisões operacionais e estratégicas, contribuindo para que o setor agrícola brasileiro se torne mais eficiente, competitivo e ambientalmente sustentável. A idéia básica em modelagem é expressar um conhecimento de forma quantitativa, através de equações preditivas, e combiná-las de forma integrada, permitindo uma perfeita descrição e entendimento do conjunto.

Apesar dos grandes prejuízos advindos da ocorrência de adversidades climáticas, pouco ou quase nada se tem para oferecer como solução ao produtor, sem que haja um aumento do custo de produção. Isto justifica a busca de novas informações no sentido de otimizar o sistema produtivo, possibilitando a obtenção de maiores rendimentos com menores riscos.

A adoção de algumas práticas de manejo da cultura e de fatores envolvidos na produção ajudam a minimizar os efeitos negativos sobre o rendimento de grãos. Essas práticas devem permitir que a planta expresse o máximo possível do seu rendimento potencial. Porém, qualquer intervenção no sistema de produção tem por objetivo obter uma situação econômica ótima, fundamentada numa situação técnica ideal. A rigor, na prática, é o critério econômico que, em última instância, norteia a ação de manejo. O manejo integrado das informações e das tecnologias disponíveis, fundamentado no conhecimento das variabilidades espacial e temporal, é fundamental para a obtenção de maiores rendimentos. O manejo integrado de pragas e doenças permite maior conservação e permanência da área foliar fotossinteticamente ativa, favorecendo o processo da fotossíntese e, desta forma, contribuindo para a obtenção de maiores rendimentos. O perfeito controle de ervas daninhas reduz a competição por água, deixando maior quantidade disponível à cultura, o que pode ser de grande valia em épocas e períodos de menor disponibilidade hídrica. Embora possa ser facilmente resolvido com as recomendações de adubação indicadas pela pesquisa, deve-se ter em mente que, na correção do solo ou no suprimento das necessidades da cultura, devem-se considerar os níveis desejados de rendimento de grãos.

Conhecendo-se o rendimento máximo de uma cultura, pode-se, através do uso de tecnologias e/ou práticas de manejo, procurar alcançá-lo. O manejo adequado da cultura consiste num conjunto de práticas, considerando-se todas as interações possíveis do meio biológico, que propiciem a expressão do máximo rendimento de grãos. Não existe uma receita pronta para a obtenção de altos rendimentos. É necessário bom conhecimento e muita sensibilidade para tomar decisões certas, no sentido de ajustar os diversos níveis dos fatores envolvidos na produção de grãos. Alguns princípios básicos devem ser observados na condução de atividades agrícolas, como por

Exemplo:

- manejo adequado do solo, através de práticas como a semeadura direta e a rotação de culturas, favorece a drenagem e o armazenamento de água, propiciando melhor desenvolvimento do sistema radicular;
- aumento da profundidade do sistema radicular amplia a camada de extração de água do solo, propiciando maior reservatório e, conseqüentemente, maior disponibilidade de água para sustentar o crescimento da planta durante curtos episódios de seca das camadas superiores do solo;
- elevada condição de nutrição às plantas, com restabelecimento dos níveis de fertilidade do solo e reposição das necessidades da cultura, condizente com o nível de rendimento almejado;
- utilização de cultivares adaptadas à região, com alto potencial produtivo, resistentes ao acamamento e às principais doenças da cultura. Quando se dispõe de excelentes condições de lavoura e alta tecnologia de produção, deve-se optar pela utilização de cultivares com elevado potencial de rendimento, expresso pela maior produção de estruturas reprodutivas.
- Planejamento da semeadura para a época de melhor aproveitamento da radiação solar e melhor disponibilidade hídrica ao desenvolvimento da cultura, considerando, principalmente, as fases de desenvolvimento das plantas mais críticas à falta de água;
- Manutenção da planta sadia ao longo do ciclo, com as folhas plenamente desenvolvidas, sem manchas ou danos e com controle eficiente de plantas daninhas.

Grande progresso tem sido conseguido na adaptação da cultura da soja para a obtenção de altos rendimentos. Em áreas de baixa latitude, a limitação do fotoperíodo foi superada através do desenvolvimento de germoplasma menos sensível ao fotoperíodo. O baixo pH e a alta disponibilidade de alumínio nesses solos foram eliminados com calagem. A pesquisa em desenvolvimento para tolerância ao alumínio em soja continua. Atualmente, é provável que o principal fator que limita os rendimentos em áreas de baixa latitude seja a disponibilidade de água nos solos. Os rendimentos de grãos são extremamente dependentes da água disponível e, desta forma, provavelmente, será necessário aumentar a disponibilidade de água para atender a transpiração da cultura e, conseqüentemente, obter-se incrementos na produtividade de grãos. Maior profundidade do sistema radicular parece ser crítico à obtenção de rendimentos crescentes sob condições limitantes de água. Por conseguinte, aumento na profundidade das raízes para camadas do solo com baixo pH e alto alumínio disponível, é um grande desafio. Além disto, a fixação de N_2 pela soja é muito sensível à disponibilidade de água no solo. Pesquisas recentes têm procurado identificar germoplasma

que expresse tolerância considerável da fixação de N_2 à menor disponibilidade de água no solo (Sinclair e Purcell, 2002). No sul do Brasil, o maior entrave à expressão de altos rendimentos da soja tem sido a variabilidade na distribuição de chuvas durante o período de primavera-verão (Cunha et al, 1998).

Apesar de toda tecnologia hoje existente e disponível, a obtenção de máximos rendimentos continua extremamente dependente do clima. Provavelmente, no futuro, muito do potencial para obtenção de altos rendimentos de soja estará relacionado à tolerância dos materiais a curtos períodos de baixa disponibilidade hídrica. Práticas de manejo, que visam maior armazenamento ou incremento da camada de extração da água do solo, em muito, também, poderão contribuir para o alcance de elevadas produtividades.

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III CONGRESSO BRASILEIRO DE SOJA

MARCH 4

Ferrugem da soja: panorama geral

J. T. Yorinori[1]

Resumo

Desde a sua primeira detecção no Paraguai e no Paraná, em 2001, a ferrugem "asiática" se espalhou por todo o Paraguai, Bolívia, quase todo o Brasil e parte da Argentina. Nos dois anos seguintes (2002 e 2003), causou perdas de soja estimadas em 4,011 milhões de toneladas ou o equivalente a US$884,425 milhões. Os Estados mais afetados foram: MT, BA, RS, GO e MG. O fungo *P. pachyrhizi* é inibido por temperaturas acima de 30° C. Sob condições favoráveis, infecta a soja e desenvolve-se com rapidez, exigindo imediata ação de controle. Os principais fatores que dificultam o controle da ferrugem são: a. a falta de cvs. resistentes; b. a variabilidade patogênica do fungo; c. a dificuldade em diferenciar a ferrugem inicial de outras doenças foliares; d. a continua produção do fungo em sojas "safrinha" e guaxas e o longo período de semeadura; e. a dificuldade de pulverização em períodos chuvosos, e f. a falta de fungicidas. Onze fungicidas dos grupos dos triazóis e strobilurinas são recomendados. Na safra 2003/04, a incidência antecipada (18 de dezembro) e a falta de fungicidas, poderão favorecer a incidência generalizada da ferrugem nas regiões onde a distribuição das chuvas for normal.

Abstract – Soybean rust: general overview

Since its first detection in Paraguay and in the state of Paraná, in 2001, the "Asian" rust has spread to all Paraguay, Bolivia, most of Brazil and parts of Argentina. In the two following years (2002 and 2003) it caused and estimated 4.011 million tons in grain losses or the equivalent to US$884.425 millions. The States most affected were: MT, BA, RS, GO and MG. The fungus *P. pachyrhizi* is adversely affected by temperatures above 30° C. Under favorable weather it develops extremely fast demanding quick action for control. The main difficulties in controlling rust are: a. lack of resistant cultivars; b. the variability of the fungus; c. the difficulty in early distinction from other foliar diseases; d. the continuous production of inoculum on second soybeans and volunteer plants and the long sowing period; e. the excess rain that makes timely spraying difficult, and f. the shortage of fungicides. Eleven compounds of triazol and strobilurin fungicides are recommended for rust control. In 2003/04 crop season, the continuous production of inoculum on fall-winter soybeans and the early rust outbreak

[1] Embrapa Soja, Caixa postal 231 CEP: 86001970; Londrina, PR. Fone: (43)-3371-6250/6251e-mail: tadashi@cnpso.embrapa.br

(Dec. 18) may result in general incidence if normal rainfall prevails throughout the growing season.

Introdução

A soja [*Glycine max* (L.) Merrill] é infectada por duas espécies de *Phakopsora* que causa a ferrugem: a *P. meibomiae* (Arthur) Arthur, nativa no Continente Americano, e a temida *P. pachyrhizi* Sydow & Syd, presente na maioria dos países asiáticos e na Austrália e ausente nas Américas até a safra 1999/00. A ferrugem "americana" (*P. meibomiae*), raramente causa perdas, ocorre em condições de temperaturas amenas (média abaixo de 25° C) e umidade relativa elevada, estando localizada nas regiões dos Cerrados com altitudes superiores a 800m e na Região Sul. A ferrugem "asiática" (*P. pachyrhizi*), está adaptada a temperaturas mais elevadas (até 30° C) e pode causar severas perdas de soja em todas as regiões onde ocorra períodos de molhamento de folha por mais de 10 horas. A diferenciação das duas espécies só é feita através do teste de DNA.

Evolução da ferrugem "asiática"

A seguir é apresentada uma síntese da expansão da doença, das perdas causadas e a situação atual (janeiro de 2004) no Brasil. Após a primeira constatação no Paraguai e no estado do Paraná, em 2001, a ferrugem "asiática" espalhou-se rapidamente pelo Paraguai, Bolívia, quase todo o Brasil e partes da Argentina.

Safra 2000/01

A ferrugem "asiática", causada pela *P. pachyrhizi*, foi identificada pela primeira vez no Continente Americano, no dia 5 de março de 2001, na localidade de Pirapó (Itapúa), Paraguai, pelo pesquisador Engo. Agro. Wilfrido Morel Paiva (CRIA, Capitan Miranda, Itapúa). Posteriormente, levantamentos realizados no período de 23 a 28 de maio de 2001, mostraram que a doença estava amplamente disseminada no Paraguai e no oeste e norte do Paraná.

Safra 2001/02

Nessa safra, a ferrugem foi constatada nos estados do RS, PR, SP, MS, GO, MG e MS, atingindo cerca de 60% da área brasileira de soja. Os municípios mais atingidos foram: Passo Fundo, Cruz Alta, Ciríaco (distrito de Cruzaltinha) (RS); Ortigueira, Ponta Grossa, Guarapuava (PR); Chapadão do Sul, Costa Rica, São Gabriel D´Oeste (MS); Chapadão do Céu, Rio Verde, Jataí, Mineiros, Portelândia, Santa Rita do Araguaia (GO) e Alto Taquari, Alto Araguaia, Alto Garças (MT). Perdas de rendimento de 30% a 75% foram registradas em Chapadão do Sul. Somente nos municípios de

Chapadão do Sul e Costa Rica (MS) e Chapadão do Céu (GO), com cerca de 220.000 ha de soja, houve perdas de 30% da produção (cerca de 59.281,4 t) ou o equivalente a US$13,00 milhões (US$220,50/t). Em Cruzaltinha (Distrito de Ciriaco)(RS), foi determinada redução de rendimento de 46% (1.383 kg/ha), entre áreas com (3.015 kg/ha) e sem fungicida (1.632 kg/ha). Baseados em relatos e levantamentos de perdas e comparações com dados de área e de produção da CONAB (junho de 2003) a ferrugem causou perdas de grãos estimadas em 569.200t ou o equivalente a US$125,513 milhões (US$220,50/t). Nessa safra os produtores estavam totalmente despreparados contra a ferrugem e a maioria das aplicações de fungicidas foi feita tardiamente.

Safra 2002/03

Nessa safra, o quadro de ocorrência da ferrugem foi diferente da anterior. Nas localidades onde a ferrugem foi severa em 2001/02, apesar da chuva abundante, as altas temperaturas impediram o desenvolvimento da doença, exceto no Rio Grande do Sul e Santa Catarina (Papanduva), onde plantios tardios foram afetados. Em São Paulo, um foco da doença foi identificado em janeiro de 2003 (Itapeva), porém, não houve evolução durante a safra e só ocorreu com severidade em "safrinhas" semeadas de fevereiro a março em Taquarituba (próximo a Itapeva).

Em Mato Grosso, Goiás e Mato Grosso do Sul, onde a ferrugem tinha sido severa na safra anterior, a baixa incidência da doença deve ter sido influenciada pelo uso intensivo de fungicidas e pela falta de inoculo oriunda da região Sul, onde as altas temperaturas inibiram o fungo.

Por outro lado, onde a ferrugem não havia sido registrada anteriormente, as condições climáticas favoráveis e uma nova raça de *P. pachyrhizi* causaram grandes perdas. Os estados da Bahia, Goiás, Minas Gerais e Mato Grosso, foram severamente atingidos. A primeira constatação da ferrugem foi feita no início de fevereiro mas, pelos níveis de severidade, a doença já devia estar nas lavouras da Bahia ("Roda Velha" e Bela Vista, no chamado "Anel da Soja") e Mato Grosso (Sorriso e Tapurah), pelo menos, desde início de janeiro de 2003. A partir dessas localidades, o fungo disseminou-se rapidamente, atingindo a totalidade das lavouras de soja da Bahia, partes de Goiás e Minas Gerais e a maior parte de Mato Grosso.

Apesar da intensa campanha de alerta e orientações sobre métodos de identificação e controle, através de dias de campo, palestras, publicações e outros meios de divulgação, realizada em 2002 e janeiro/fevereiro de 2003, a assistência técnica e a maioria dos produtores não estavam

preparados para o controle da ferrugem. Em muitas lavouras, a aplicação de fungicidas foi atrasada devido à falta do produto e/ou por excesso de chuva que impossibilitou a pulverização.

Com base em dados de levantamentos de danos e perdas, estimativas do custo do controle químico, obtidos através de consultas a produtores, agrônomos da assistência técnica, experimentos de fungicidas, de comparações de severidade da doença com a idade das plantas afetadas e com base nos dados de área e produção nacional de soja da CONAB (jun/03), o volume de perdas de grãos foi estimado em 3,442 milhões de toneladas ou o equivalente a US$758,868 milhões. O custo do controle químico (média de duas aplicações, incluindo fungicida e aplicação em 80% da área de soja nacional) foi avaliado em US$40,00/ha, atingindo um total de US$592,00 milhões. Portanto, o custo ferrugem estimado para a safra 2002/03 atingiu a cifra de US$1,351 bilhão.

Safra 2003/04

Essa safra que, no momento (jan/2004), está na metade de seu curso, teve como antecedentes uma safra com ocorrência severa de ferrugem, causada por uma nova raça de *P. pachyrhizi* e presença contínua de inoculo na entressafra, em lavouras irrigadas ou não, no Cerrado (BA, GO, MA, MG, MT, SP e TO). Apesar da presença contínua de inoculo no Cerrado, o início da safra caracterizou-se por chuvas irregulares e temperaturas elevadas o que, provavelmente, não favoreceu a explosão da ferrugem como seria de se esperar. Além disso, a experiência das perdas na safra anterior deixaram os produtores de prontidão e "armados" para o controle químico. Lavouras de cultivares precoces, semeadas de meados de setembro a início de outubro, irrigadas por pivô central ou sem irrigação (Lucas do Rio Verde, Sorriso, Primavera do Leste, MT) colhidas do início a meados de janeiro, em geral, foram pulverizadas até duas vezes. Em outras regiões, lavouras não tratadas (Planura, MG; Guairá, Miguelópolis, Paranapanema, Itaí, Taquarituba e Itaberá, SP) ou tratadas com deficiência (Bela Vista e Luis Eduardo Magalhães, BA), foram afetadas pela ferrugem, em diferentes graus de severidade e representam fontes de inoculo para áreas vizinhas, mais tardias.

Na região Sul (PR e Paraguai), o início da safra foi caracterizado por temperaturas mais amenas e chuvas freqüentes que favoreceram o surgimento precoce da ferrugem. No Paraguai, a primeira constatação da ferrugem a campo, foi feita em kudzu (*Pueraria lobata*), em 29 de outubro, em níveis de traços, porém, um mês mais tarde (28 de novembro) a doença havia progredido atingindo níveis severos, passando para lavouras vizinhas de soja, infectando plantas com 25 – 30 dias da semeadura. O mês de

dezembro seguiu chuvoso e estimulou o desenvolvimento da ferrugem, exigindo a aplicação de fungicidas na fase vegetativa, porém, em janeiro houve pouca ou nenhuma chuva e altas temperaturas, o que paralisou a doença. No Paraná as primeiras ocorrências de ferrugem foram registradas em 18 (Londrina) e 23-26 de dezembro (Rolandia, Mauá da Serra, Bela Vista do Paraíso), porém, a progressão da doença no norte e oeste do estado foi impedida pela forte estiagem e altas temperaturas no mês de janeiro. Os primeiros relatos em outros Estados tiveram o seguinte cronograma: em São Paulo, foram registradas em 18 de dezembro, em Guaira e Miguelópolis; em Minas Gerais, foram registradas em 9 (Conceição das Alagoas) e 18 de dezembro (Planura); em Goiás, em início de novembro, em Goiânia (E.E. do CTPA), em 9 de dezembro (Rio Verde e Santa Helena), em 11 de dezembro (Campo Alegre, Montividiu Santo Antonio da Barra), em 17 de dezembro (Mineiros) e em 20 de dezembro (Porteirão). Desde então, novos focos tem sido detectados quase que diariamente. Até final de janeiro de 2004, a ferrugem foi constatada nos estados da Rio Grande do Sul, Paraná, São Paulo, Mato Grosso do Sul, Goiás, Minas Gerais, Bahia, Tocantins, Piauí e Maranhão. Poucos são os relatos de ocorrências severas de ferrugem e estão restritos a plantios antecipados, irrigados com pivô central e onde a tratamento químico não foi realizado ou foi atrasado (Planura, Uberaba, MG; Guaira e Miguelópolis, SP). Seguindo-se períodos normais de chuva e temperaturas favoráveis, o potencial de danos por ferrugem e/ou o custo do controle químico da doença poderão ser superiores ao ocorrido na safra 2002/03. Corre-se, ainda, o risco de haver falta de produtos no momento mais necessário, principalmente, em áreas tratadas antecipadamente que necessitem mais de uma aplicação. Essa situação está ocorrendo em Joviânia (GO), Uberlândia e Uberaba (MG). O efeito da ferrugem sobre a safra 2003/04 e, principalmente, sobre as lavouras semeadas a partir de meados de novembro, irá depender das condições climáticas dos meses de fevereiro e março de 2004 e da eficiência do controle químico.

Viabilidade dos uredosporos

Estudos realizados por pesquisadores em diversos países do Oriente tem mostrado grande variabilidade na longevidade dos uredosporos. Estudos preliminares realizados na Embrapa Soja, com diferentes métodos de coleta dos esporos, mostraram que os uredosporos foram viáveis por um período de 28 a 50 dias, quando mantidos em condição ambiente.

Germinação dos uredosporos

Sob condições de ambiente de laboratório (temperaturas de 25° C a 27° C) 80% dos uredosporos germinaram em uma hora, considerando como uredosporo germinado quando o tubo germinativo atingiu o dobro do maior diâmetro do esporo.

Rapidez do desenvolvimento de sintomas e esporulação

Sob condição de casa-de-vegetação, com molhamento contínuo de folha durante a noite, os primeiros sintomas foliares foram observados 48 horas após a exposição de plantas sadias em ambiente com abundante produção de inoculo. As primeiras urédias com esporulação foram observadas do sexto ao sétimo dias de exposição à infecção.

Reação das cultivares brasileiras

Dentre 452 cultivares testadas com isolados de *P. pachyrhizi* obtidos em 2002 (raça do Centro-Sul e Sul), as seguintes mostraram-se resistentes (R) a moderadamente resistentes (MR): BRS-134 (R), BRSMS-Bacuri (R), Campos Gerais (MR), CS 201 (Esplendor)(R), FT-2 (MR), FT-3 (MR), FT-17 (Bandeirantes)(R), FT-2001 (R), KI-S 601 (MR) e OCEPAR 7 (Brilhante)(MR). Todas essas cultivares mostraram-se suscetíveis à raça ocorrida nos Cerrados em 2003. No momento, o programa de melhoramento de soja para resistência à ferrugem está considerando as cultivares e germoplasmas que apresentaram reação do tipo castanho-avermelhado (RB), independentemente da severidade da doença. A seleção é feita sob elevada pressão de inoculo em casa-de-vegetação.

Variabilidade genética do fungo

A alta severidade da ferrugem nos Cerrados em 2003 e o fato das cultivares resistentes em 2002 serem suscetíveis aos isolados de *P. pachyrhizi* dos Cerrados, é uma clara indicação da variabilidade genética do fungo. Uma questão não esclarecida é a ocorrência dessa nova raça em regiões onde praticamente não havia registro da doença na safra anterior. Comparações das reações de testes feitos na Embrapa Soja e no Paraguai (Engo. Agro. Wilfrido Morel Paiva, da E.E. do CRIA, Capitan Miranda), com isolados da safra 2002, com inoculações em germoplasmas que possuem os quatro genes maiores resistentes à *P. pahcyrhizi*, Rpp1, Rpp2, Rpp3 e Rpp4, mostraram que são muito semelhantes, com vários germoplasmas comportando-se como resistentes. Todavia, quando estes foram inoculados com isolados dos Cerrados e comparados com os testes de 2002, tornaram-se suscetíveis. Por outro lado, quando os resultados dos testes com os isolados dos Cerrados foram comparados com os testes feitos nos Estados Unidos (Dr. Reid D. Frederick, FWSR/ USDA/ARS, em Fort Detrick, Maryland), com um isolado do Zimbábue, o isolado dos Cerrados foi praticamente idêntico ao do Zimbábue. Esta semelhança do isolado dos Cerrados com o do Zimbábue torna ainda mais provável que a nova raça tenha vindo da África, pelo vento, cruzando o Oceano Atlântico.

Medidas de controle

O prolongado período de semeadura da soja nas diferentes regiões, de meados de setembro de 2003 (Lucas do Rio Verde, Sorriso, Primavera do Leste, Campo Verde, MT) a início de janeiro (BA), torna difícil a recomendação de uma medida padrão de controle da ferrugem. Além disso, na fase inicial da ferrugem, tem havido muita dificuldade em distinguir seu sintoma de outras doenças foliares como a mancha parda (*Septoria glycines*), crestamento bacteriano (*Pseudomonas savastanoi* pv. *glycinea*) e danos por herbicida pós-emergentes, o que está resultando em uso indevido e antecipado de fungicidas.

Como medidas gerais de controle, são sugeridas as seguintes estratégias de controle:

1. semear, preferencialmente, cultivares mais precoces e no início da época recomendada para cada região (semeaduras tardias sofrerão mais danos devido à multiplicação do fungo nos primeiros plantios); o objetivo é escapar ao período de maior potencial de inoculo do fungo;
2. evitar plantios adensados que dificultam a penetração do fungicida no interior das plantas;
3. vistoriar a lavoura desde o início do crescimento da soja; ao primeiro sinal da doença e, havendo condições climáticas favoráveis, poderá haver necessidade de aplicação de fungicida; neste caso, buscar orientação específica para a escolha correta do fungicida diante da situação; os fungicidas e dosagens (g p.a./ha e L p.c./ha) recomendados são (Subcomissão de Fitopatologia, XXV RPSRCB, Uberaba, 11-13 de agosto de 2003): preventivos/protetores: fluquinconazole (62,5g i.a./ha) (Palisade 250 CE- 0,25L p.c./ha); Difenoconazole (50g)(Score 250 CE – 0,20L); azoxyxtrobin (50g)(Priori 250 – 0,20L) e trifloxystrobin + propiconazole (50g + 50g)(Stratego 125 + 125 – 0,4L); preventivos e curativos: tebuconazole (100g)(Folicur 200 CE – 0,5L, Orius 250 CE – 0,4L); epoxyconazole + pyraclostrobin (25g + 66,5g)(Opera – 0,5L); flutriafol (50g – 75g)(Impact 125 SC – 0,4L a 0,6L); tetraconazole (50g)(Domark 100 CE – 0,5L); trifloxystrobin + ciproconazole (56,25g + 15g)(Sphere 187,5 + 50 – 0,3L) e azoxystrobin + ciproconazole (60g +24g)(Priori Xtra 200 + 80 – 0,3L);
4. o volume da calda para aplicação terrestre deve ser de 150-250 L/ha e de 40 L/ha, para aérea;
5. utilizar bicos de pulverização dos tipos cone ou duplo leque e seguir as orientações técnicas de aplicação correta: umidade relativa, temperatura, velocidade do vento, etc.; e
6. o momento da aplicação, em relação ao estádio de desenvolvimento da soja, terá que ser determinado caso a caso e dependerá da presença e

severidade da ferrugem na lavoura ou na região, das condições climáticas e da data de início da semeadura na região.

Perspectiva futura

A história da ferrugem "asiática" no Continente Americano é ainda muito recente (três anos) e muitos detalhes sobre a epidemiologia e variabilidade do fungo necessitam ser pesquisados. A expectativa é de que a ferrugem da soja venha a ser uma doença severa e permanente nas regiões mais chuvosas e altas dos Cerrados (acima de 800m) e na Região Sul, onde há abundante formação de orvalho e temperaturas mais amenas no verão. Ao longo dos anos, em função das variações climáticas, deverão ocorrer flutuações na severidade ou até ausência da doença. O cultivo ininterrupto da soja em áreas irrigadas ou não, representa ameaça contínua, tendo a própria soja como hospedeira alternativa na entressafra. No Paraguai e no Paraná (Palmeira), a presença da leguminosa kudzu (*Pueraria lobata*), altamente eficiente como hospedeira alternativa, aumenta o potencial de risco e torna o controle da ferrugem mais difícil.

A limitada fonte de resistência genética diante da variabilidade do fungo e a presença contínua de inoculo e sojas "safrinha" ou guaxas na entressafra, tornam difícil a solução por esse caminho, a curto e médio prazos. A forma mais eficiente de controle no momento é o controle químico, porém, seu uso eficaz e econômico depende: a. da capacidade de identificar a doença na fase inicial; b. do levantamento, mapeamento, acompanhamento das primeiras ocorrências e da vistoria contínua das lavouras; c. da redução do período (janela) de plantio; d. da adequação da densidade de semeadura para maior penetração do fungicida no dossel foliar; e. da escolha correta do(s) fungicida(s), em relação ao estádio de desenvolvimento da soja e da severidade de infecção; f. da observação das condições climáticas no momento da aplicação; g. da adoção de equipamento e tecnologias corretas de aplicação, tanto para tratamentos aéreo como terrestre; h. da capacidade operacional para aplicação do fungicida no momento correto, principalmente, em períodos chuvosos, e i. do equilíbrio nutricional e/ou do nível de fertilidade do solo.

A convivência com a ferrugem "asiática", sem que haja danos significativos a cada safra e custos desnecessários com controle químico, irá exigir a combinação de várias estratégias, entre as quais, a rotação de culturas, evitar o plantio de "safrinha" e a eliminação de plantas guaxas. Diversos estudos estão em andamento, buscando informações sobre o grau de tolerância das cultivares e novos germoplasmas e sobre a eficiência relativa dos fungicidas, o número e freqüência de aplicações, em função da época

de semeadura e do clima. A dificuldade na identificação inicial da ferrugem exige treinamento e capacitação contínua dos técnicos de campo na identificar da doença, de suma importância para que o controle químico seja feito no momento correto.

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Ferrugem da soja: safra 2002/2003 na Bahia

A. C. B. de Oliveira[1]

Resumo

O controle da Ferrugem da Soja (*Phakopsora pachyrhizi* Sydow & Sid) por meio da aplicação de fungicidas tem sido o método mais viável na ausência de cultivares resistentes. Os produtos que obtiveram registro para o controle da ferrugem na safra 2002/2003 foram os mesmos já utilizados para controle das doenças de final de ciclo na cultura da soja, existindo uma ausência de informações sobre o comportamento dos diferentes fungicidas em condições de alta pressão de ferrugem. Na safra 2002/2003 do Oeste da Bahia, focos iniciais foram observados em São Desidério em 07 de fevereiro e no decorrer da safra foi observado um progresso rápido da doença em função das condições climáticas favoráveis e do plantio de variedades suscetíveis.

Com o intuito de avaliar princípios ativos no controle da ferrugem foi instalado um ensaio para controle curativo da doença em condições de campo sob alta pressão de inóculo.

Todos produtos testados apresentaram resultados (produtividade e desfolha) estatisticamente superiores a parcela não tratada ao nível de 5% de significância pelo teste de Tukey. A eficiência dos produtos recomendados, em caráter emergencial, foi diferente daquela obtida em regiões onde a doença ocorreu com um menor nível de intensidade.

Abstract – Soybean rust in Bahia State: 2002/2003 crop season

Control of soybean rust (*Phakopsora pachyrhizi* Sydow & Sid) through application of fungicides has been the most viable method, since no resistant soybean varieties are available. The chemicals registered for controlling soybean rust in the 2002/03 season were the same chemicals already used for controlling the other diseases affecting soybean at the end of the season. No information however, is available on the efficiency of the fungicides when applied under high pathogen pressure. In Westhern Bahia State, season 2002/03, initial pustules were found in São Desirério County, on February 7. It was observed a quick process of infection of the disease through out the season due to the favorable climatic conditions and utilization of susceptible soybean varieties.

[1] Embrapa Soja, Caixa Postal 231, 86001-970 – Londrina, PR, Brasil.

An experiment was planted to evaluate the active ingredients of chemicals to control soybean rust under high pathogen pressure and at field conditions. All the chemicals tested presented results (yield and defoliation) better than the check treatment (with no chemical), at 5% of significance of Tukey test. The efficiency of the chemicals recommended under emergency conditions was different compared with the regions where the disease occurred in lower intensity.

Introduzida no Oeste da Bahia no fim dos anos setenta, a soja desempenha o papel de "carro chefe" gerando renda para investimentos em outras áreas, possibilitando uma verdadeira revolução, trocando a pecuária extensiva pela exploração profissional de produção de grãos, fibras, café, frutas e pecuária intensiva. A área cultivada com soja cresceu 66% nos últimos oito anos e a produção no mesmo período cresceu 188% (área de 800.000 ha e produção de 1.464.000 toneladas). Nesse período, a produtividade média dobrou, aumentando de 1200 kg/ha (20 sacos/ha) para 2400 kg/ha (40 sacos/ha). A cultura da soja ainda não é um sistema estável em função da crescente expansão para novas fronteiras agrícolas com diferentes condições edafoclimáticas e devido à introdução de novas variáveis a cada ano ameaçando sua produtividade e competitividade.

Na safra 2002/2003 a Uma mostra clara disto foi o que aconteceu durante a safra 2002/03 de soja do Oeste da Bahia onde o fator mais limitante é o regime pluviométrico, o qual, na safra 2002/2003, foi ideal em termos absolutos mas inadequado com relação à distribuição.

Com uma área plantada foide de 850.000 hectares sendo a produção esperada era de 2.040.000 t, mas foram colhidas apenas 1.428.000 t . Esta redução foi devida a diferentes fatores: 1) atraso no início do período chuvoso; 2) distribuição irregular das chuvas nos primeiros trinta dias, ocasionando veranico durante o período de plantio (mês de novembro) com duração de 15 a 35 dias dependendo da região, levando a um percentual elevado de replantio (30%). Este período seco levou a uma alta incidência da lagarta Elasmo (*Elasmopalpus lignosellus*), devido às dificuldades encontradas para o controle desta lagarta o que causou inadequado estande da cultura, abaixo do ideal.

Este período seco proporcionou alta incidência da lagarta Elasmo (*Elasmopalpus lignosellus*), dificultando o seu controle, como conseqüência o estande ficou abaixo do ideal.

Além disso, o surgimento de uma nova doença no final do ciclo da soja, a Ferrugem Asiática, cujo agente causal é o fungo *Phakopsora pachyrhizi*

Sydow & Sid, causou sérios danos às lavouras. As condições de alta umidade e a dificuldade de identificação dos sintomas iniciais favoreceram a doença, levando a uma alta pressão de inóculo e uma rápida disseminação do mesmo, dificultando o controle da ferrugem.. A eficiência dos produtos recomendados, em caráter emergencial, foi diferente daquela obtida em regiões onde a doença ocorreu com um menor nível de intensidade. Também foram detectados problemas com as tecnologias de aplicação dos fungicidas.

O controle da Ferrugem da Soja (*Phakopsora pachyrhizi* Sydow & Sid) por meio da aplicação de fungicidas tem sido o método mais viável na ausência de cultivares resistentes. Os produtos que obtiveram registro para o controle da ferrugem na safra 2002/2003 foram os mesmos já utilizados para controle das doenças de final de ciclo na cultura da soja, existindo uma ausência de informações sobre o comportamento dos diferentes fungicidas em condições de alta pressão de ferrugem. Na região Oeste da Bahia não havia sido constatada a presença da ferrugem na safra 2001/2002. Na safra 2002/2003 focos iniciais foram observados em São Desidério em 07 de fevereiro na propriedade Ceolin Agropecuária e no decorrer da safra foi observado um progresso rápido da doença em função das condições climáticas favoráveis e do plantio de variedades suscetíveis.

Com o intuito de avaliar princípios ativos no controle da ferrugem foi instalado um ensaio para controle curativo da doença em condições de campo sob alta pressão de inóculo. O ensaio foi conduzido no município de São Desidério, na Bahia, durante os meses de fevereiro e abril de 2003, a cultivar utilizada foi a Uirapuru, para avaliação do efeito de diferentes princípios ativos no controle da ferrugem da soja foram utilizados os tratamentos, dose do ingrediente ativo e do produto comercial, descritos na Tabela 01.

Os tratamentos com fungicidas foram realizados de forma curativa em 19/02/2003 quando as plantas se apresentavam no estádio R5.1, com aproximadamente 10% de severidade de ferrugem. Para a aplicação dos fungicidas foi utilizado pulverizador costal pressurizado com CO_2 utilizando-se volume aproximado de 300 L/ha e bicos RG000026 pretos cônicos. O delineamento experimental foi blocos ao acaso com seis repetições, cada repetição sendo constituída de uma parcela de 1,9 m por 6,0 m (área útil de 5,7 m^2). Os tratamentos com fungicidas foram realizados no final da tarde, sem vento.

A avaliação da desfolha foi realizada quando a testemunha sem controle apresentava-se com aproximadamente 100% de desfolha. As análises estatísticas foram realizadas por meio do teste de Tukey a 5% de significância para comparação de médias.

Tabela 1. Descrição dos tratamentos utilizados para controle de ferrugem em condições de campo, em São Desidério (BA).

Tratamentos		Nome comum	g i.a./ha[1]	p.c./ha[2]
1.	Testemunha		-	-
2.	Palisade[3]	Fluquinconazole	87,5	350
3.	Palisade[4] + Derosal	Fluquinconazole + Carbendazin	62,5 + 250	250 + 500
4.	Caramba	Metconazole	45	500
5.	Folicur	Tebuconazole	100	500
6.	Folicur + Derosal	Tebuconazole + Carbendazin	60 + 175	300 + 350
7.	Systhane	Miclobutanyl	125	250
8.	Score	Difenoconazole	50	200
9.	Priori[5]	Azoxystrobin	50	200
10.	Stratego	Trifloxistrobina + propiconazol	50 + 50	400
11.	Stratego	Trifloxistrobina + propiconazol	62,5 + 62,5	500
12.	Opera	Epoxiconazole + Pyraclostobin	25 + 66,5	500

[1] g i.a./ha = gramas de ingrediente ativo por hectare.
[2] g ou ml p.c./ha = gramas ou mililitros de produto comercial por hectare.
[3] Neste tratamento com Palisade foi acrescentado o Attach (0,350 L/ha)
[4] Neste tratamento com o Palisade foi acrescentado o Attach (0,250 l/ha)
[5] Em todos tratamentos com Priori foi acrescentado o adjuvante Nimbus 0,5% v/v

Entre os tratamentos testados para o controle da ferrugem (Tabela 02) pôde ser observado um acréscimo de produtividade variando de 11 a 29 sacas/ha, em função do produto utilizado. Considerando o melhor tratamento como o potencial produtivo da cultivar (3194 kg/ha) pode-se estimar um dano potencial de 54% redução de produtividade, quando comparado com as parcelas não tratadas (1471 kg/ha).

Todos produtos testados (Tabela 2) foram estatisticamente superiores a parcela não tratada ao nível de 5% de significância pelo teste de Tukey. Entre os ingredientes ativos testados, os que apresentaram uma maior eficiência de controle foram epoxiconazole 25 + pyraclostrobin 66,5 g i.a/ha, Metconazole 45 g ia/ha e tebuconazole 100 g ia/ha.

A eficiência dos produtos recomendados, em caráter emergencial, foi diferente daquela obtida em regiões onde a doença ocorreu com um menor nível de intensidade. Também foram detectados problemas com as tecnologias de aplicação dos fungicidas.

Dentro deste cenário a produtividade alcançada nas lavouras do oeste da Bahia oscilou muito entre as diferentes regiões e as propriedades. Foram alcançados patamares médios de 900 kg/ha (15 sacos/ha) a 3.000 kg/ha (50 sacos/ha), com média da região em torno de 1.800 kg/ha (30 sacos/ha).

Tabela 2. Dias para maturação, desfolha (%),Peso de cem sementes (g), produtividade (kg/ha) e acréscimo de produtividade (sacas/ha) nos diferentes tratamentos.

	TRATAMENTOS	g i.a./ha[1]	Dias para maturação	Desfolha (%)	Peso de cem sementes (g)	Produtividade (Kg/ha)	Acréscimo produtividade (sc/ha)
1	Testemunha		128,5 e	98 c	7,6 d	1471 a	-
2	Fluquinconazole	87,5	132,3 cd	92 c	8,7 bcd	2112 b	11
3	Fluquinconazole + Carbendazin	62,5 + 250	133,5 abcd	89 bc	9,6 abcd	2512 bc	17
4	Metconazole	45	135,5 ab	77 a	10,8 a	2910 cd	24
5	Tebuconazole	100	136,0 ab	68 a	10,7 ab	2918 cd	24
6	Tebuconazole + Carbendazin	60 + 175	134,8 abc	76 a	10,4 abc	2614 bc	19
7	Miclobutanyl	125	133,7 abcd	84 b	9,9 abc	2404 b	16
8	Difenoconazole	50	131,3 cde	94 c	8,9 abcd	2267 b	13
9	Azoxystrobin	50	131,5 cde	94 c	9,1 abcd	2267 b	13
10	Trifloxistrobina + propiconazol	50 + 50	131,3 cde	95 c	9,0 abcd	2113 b	11
11	Trifloxistrobina + propiconazol	62,5 + 62,5	130,8 ed	96 c	8,4 cd	2279 b	13
12	Epoxiconazole + Pyraclostobin	25 + 66,5	137,0 a	68 a	10,9 a	3194 d	29

Médias seguidas mesma letra não diferem entre si ao nível de 5% de probabilidade pelo teste de Tukey

[1] g i.a./ha = gramas de ingrediente ativo por hectare.

[2] Em todos tratamentos com Palisade foi acrescentado o Attach

[3] Em todos tratamentos com Priori foi acrescentado o adjuvante Nimbus 0,5% v/v

No período de final de ciclo da cultura, houve intenso ataque de percevejos, causando aumento do percentual de grãos ardidos e diminuição do peso dos grãos. Chuvas excessivas também, concorreram para maior incidência de doenças de final de ciclo.

Dentro deste cenário a produtividade alcançada nas lavouras do oeste da Bahia oscilou muito entre as diferentes regiões e as propriedades. Foram alcançados patamares médios de 900 kg/ha (15 sacos/ha) a 3.000 kg/ha (50 sacos/ha), com média da região em torno de 1.800 kg/ha (30 sacos/ha).

A ferrugem da soja em Mato Grosso do Sul

P. J. M. Andrade[1]

Resumo

Na região nordeste de Mato Grosso do Sul, a ferrugem da soja vem se comportando de forma diferente a cada ano, desde que foi detectada pela primeira vez, no final de fevereiro de 2002. Naquele ano, as perdas ocasionadas pela doença foram significativas, seja pelo fato de ter sido descoberta tardiamente, seja pela utilização, na ocasião, de fungicidas do grupo dos benzimidazóis no controle das doenças de final de ciclo (DFCs), pouco eficientes contra a ferrugem. Na safra 2002/2003, a doença surgiu tardiamente, no final de janeiro de 2003, quando a maioria das lavouras já estava protegida com fungicidas aplicados visando as DFC, mas também eficientes contra a ferrugem. Nessa safra não foram registradas perdas ocasionadas pela doença. Na safra atual (2003/2004), a ferrugem foi verificada em dezembro de 2003, em lavouras que já estavam na fase reprodutiva. A partir dessa primeira detecção, contrariando as expectativas, a doença passou a progredir muito lentamente, comportamento que só foi alterado em meados de janeiro. A Fundação Chapadão/Embrapa Agropecuária Oeste está fazendo o acompanhamento da doença desde seu aparecimento. Muito ainda há por elucidar em se tratando da ferrugem da soja e seu manejo.

Abstract – Soybean rust in the State of Mato Grosso do Sul

In the northeastern region of Mato Grosso do Sul, soybean rust is behaving differently every year, since it was first detected at the end of February 2002. In that year the yield losses caused by the disease were significant both by its late detection and due to the use of benzimidazole fungicides which were meant for the control of late season diseases (DFCs) and with little effect against rust. In 2002/2003 crop season the disease appeared late, by the end of January 2003, when most fields had been sprayed for DFCs but with fungicides that were efficient against rust. No yield losses were recorded for the 2002/03 crop season. For the current crop season (2003/2004), rust was found as early as December 2003 in fields at reproductive stage. From this first detection, contrary to the expectations, the disease progressed very slowly, which behavior was altered only by mid-January. The Fundação Chapadão/Embrapa Agropecuária Oeste is

[1] Eng. Agr°, M. Sc. Fitopatologista, Embrapa Agropecuária Oeste/Fundação Chapadão. Rod. MS 306, km 105- Chapadão do Sul, MS, CEP: 779560-000. E.mail: paulino@fundaçãochapadão.com.br

monitoring the disease since its first detection. Much still need to be learned about the soybean rust and its management.

Introdução

Na safra atual(2003/2004), a ferrugem da soja (*Phakopsora* sp.) foi detectada nos municípios de Chapadão do Sul e Costa Rica, no nordeste de Mato Grosso do Sul, em dezembro de 2003, em lavouras que já haviam atingido a fase reprodutiva de desenvolvimento. Da detecção até meados de janeiro, a doença progrediu com baixa intensidade, sendo necessário, comumente, analisar várias folhas das amostras coletadas no campo, para observar apenas algumas escassas lesões, em uma ou outra folha.

O desenvolvimento da ferrugem na região centro-sul de Mato Grosso do Sul, vinha sendo semelhante. A partir de 20 de janeiro, ela passou a se desenvolver mais rapidamente nas regiões nordeste e norte de MS. No centro sul do estado, com exceção da região de fronteira com o Paraguai, a doença ainda permaneceu com desenvolvimento lento.

Não ocorreram alterações significativas nas condições ambientais, comparativamente às safras anteriores, que pudessem justificar tal comportamento. O que poderia, então, explicar esse lento progresso da doença?

Em trabalhos conduzidos pela Fundação Chapadão/Embrapa Agropecuária Oeste, na safra 2001/2002, quando houve grande epidemia de ferrugem na região, verificou-se um comportamento diferenciado das cultivares em relação à incidência e severidade da doença, até uma determinada fase do desenvolvimento das plantas. A partir do período que ultrapassava a metade da granação, essa diferença já deixava de ser perceptível. Possivelmente, a partir desta fase, quando a demanda por fotoassimilados e nutrientes para o enchimento de grãos era muito grande, sucedesse alguma mudança na fisiologia da planta, mais precisamente, nos seus mecanismos de defesa, ocasionando o avanço rápido da doença. É provável que sob condições de alta pressão de inóculo, esse rápido progresso da doença acontecesse mesmo antes da meia granação.

Safra 2001/2002 x 2003/2004

Na safra 2001/2002, não se conhecia ainda a ferrugem da soja na região, que só foi detectada em final de fevereiro de 2002, quando a maioria das lavouras já se encontrava em adiantado estádio de desenvolvimento fenológico e a pressão da doença já era muito alta, tanto por causa das condições de ambiente altamente favoráveis ao seu desenvolvimento, quanto pelo fato

do hospedeiro encontrar-se desprotegido. A não utilização de fungicida para controle de doenças de final de ciclo (DFCs) e a utilização de fungicida sem eficiência no controle de ferrugem contribuíram sobremaneira para esse aumento de inóculo na ocasião. Levantando-se, nas principais revendas da região, os fungicidas que foram comercializados na safra 2001/2002 para o controle de DFCs, verificou-se que 75% dos mesmos pertenciam ao grupo dos benzimidazóis, que não são eficientes no controle da ferrugem, o que permitiu que a doença ocasionasse perdas médias em torno de 13% nos municípios de Chapadão do Sul, MS, e em Chapadão do Céu, GO.

Na safra atual está sendo feito, pela Fundação Chapadão e Embrapa Agropecuária Oeste, o acompanhamento sistemático da evolução da doença, desde o seu aparecimento.

Safra 2002/2003 x 2003/2004

Na safra 2002/2003, a ferrugem da soja só foi detectada na região NE de MS, em final de janeiro de 2003, época em que a maioria das lavouras já se encontrava protegida com fungicidas utilizados para controle de doenças de final de ciclo e eficientes também no controle da ferrugem (dados obtido de levantamento feito nas principais revendas da região mostravam que cerca de 80% dos fungicidas comercializados na safra 02/03 para utilização em aplicação foliar na cultura da soja eram eficientes no controle da ferrugem). Não ocorreram danos pela ferrugem nessa safra, o que pode ser comprovado pelos elevados rendimentos (os melhores verificados até então) alcançados na região.

Na entressafra de 2003, o cultivo de soja sob pivô na região do cerrado, no projeto Rio Formoso, em Tocantins, e até mesmo as lavouras de soja da Bolívia, funcionaram como "ponte verde", multiplicando a ferrugem nessa época do ano e favorecendo sua passagem para a safra atual (No norte de Mato Grosso, a primeira detecção se deu em outubro de 2003 e na região NE de MS, em dezembro do mesmo ano).

A expectativa é que nas lavouras estabelecidas mais tardiamente, a cultura esteja sujeita a uma maior pressão de doença, devido à multiplicação de inóculo nos plantios anteriores.

Por se tratar de doença nova em nosso país e pelo ainda pouco conhecimento que se tem a seu respeito, muitos estudos ainda são necessários para que se possa conviver de forma sustentável com a mesma.

O sistema Embrapa Soja de cooperação para o desenvolvimento de cultivares e a produção de sementes na atualidade

J. F. F. de Toledo[1]; L. A. de Almeida[1]; L. C. Miranda[2]

Resumo

Os programas de melhoramento genético desenvolvidos pela Embrapa Soja e os produtores de semente seguem um formato inovador, combinando as vantagens comparativas das instituições públicas e privadas. O bem treinado, experiente e eficiente grupo de pesquisadores da Embrapa se juntou à agilidade e à capacidade de atingir os mercados representadas pelos produtores de semente de soja. Esse arranjo, que funciona em todo o território nacional território nacional, possibilitou a obtenção de cultivares com grande eficiência – ganho genético de produtividade esperado entre 1,5% a 2,0% ao ano, estabilidade de produção, ganho de qualidade para o consumo humano e processamento industrial, além de redução dos custos para o produtor. Essas cultivares foram semeadas em mais de 60% da área cultivada com soja no Brasil em 2003 e representaram um importante componente do dinâmico complexo agroindustrial da soja que faturou 60 bilhões de dólares nesse ano. A produção total brasileira em 2003 foi de 52 milhões de toneladas de grãos, produzidas em 18,5 milhões de hectares, que representaram 26,5% da produção mundial e posicionaram o País como 2° maior produtor em volume e o 1° em produtividade (2.816 kg/ha).

Palavras chaves: parcerias, melhoramento genético, produção de sementes, *Glycine max*

Abstract – Current Embrapa Soybean cooperation system for cultivar development and seed production

The Embrapa Soybean breeding programs are carried out in partnership with seed producers in a creative format that allows the combination of the comparative advantages of an experienced and efficient public sector breeding team with the marketing expertise and dynamism of the private seed companies. The contribution of the genetic breeding programs to the development of Brazilian agribusiness has been very significant as the new cultivars are high yielding, high quality and well adapted to the various

[1] Engenheiro Agrônomo, Doutor, pesquisador da Embrapa-Soja, Rod. Carlos João Strass, Caixa Postal 231. CEP 860001-970 Londrina, PR. Correspondências para e-mail: toledo@cnpso.embrapa.br

[2] Engenheiro Agrônomo, Doutor, pesquisador da Embrapa Transferência de Tecnologia.

ecological regions and cultivation systems. The released cultivars led to an average genetic gain of 1.5% to 2.0% per year and represented an opportunity for reducing the environment impact of soybean cropping and production costs, while increasing crop yield, stability and quality. They were sowed in over 60% of the Brazilian soybean cropped area in 2003 and were an important component of the dynamic Brazilian soybean agro-industrial complex that was worth US$ 60 billion. The country's total soybean production in 18.5 million ha was 52.0 million tons that corresponded to 26.5 percent of the world production and placed Brazil as a second major world producer in volume and the first in yield (2,816 kg/ha).

Key words: partnership, genetic breeding, plant breeding, seed production, *Glycine max*

Introdução

Nos últimos anos aconteceram grandes transformações no cenário econômico mundial em função das facilidades de comunicação, de transportes e da abertura dos mercados. O Brasil cada vez mais se insere na economia mundial, se adaptando de forma rápida a essas transformações, principalmente no tocante à agropecuária, que tem sofrido modificações importantes em sua forma de produção e maneira de realizar negócios para ganhar novos mercados. As transformações na produção acontecem principalmente no sentido de trazer sustentabilidade para a atividade econômica com garantias de preservação do meio ambiente. O desenvolvimento científico e tecnológico tem sido fator preponderante de suporte a essas mudanças e, como não poderia deixar de ser na agropecuária, a Embrapa tem contribuído de forma importante.

No contexto de disponibilidade de soluções de P&D para a agropecuária, a cultura soja no Brasil é um dos melhores exemplos da contribuição da pesquisa. O complexo agroindustrial da soja faturou 60 bilhões de dólares em 2003 com uma safra de 52 milhões de toneladas de grãos, das quais 25,8 milhões foram processadas e resultaram em 5,16 milhões de toneladas de óleo e 20,64 milhões de toneladas de farelo. As exportações de grãos, farelo e óleo totalizaram 8,28 bilhões de dólares. As 52 milhões de toneladas de grãos produzidas em 18.480 hectares representaram 26,5% da produção mundial e posicionaram o País como 2° maior produtor em volume e o 1° em produtividade. O processo de busca de eficiência foi longo, mas os excelentes resultados dos últimos anos mostram que a estratégia adotada e as soluções encontradas foram adequadas. A produção de soja no Brasil aumentou aproximadamente 116,7% em relação à 1988/89, quando a produção foi de 24 milhões de toneladas. Esse aumento de produção se

deveu, essencialmente, a um significativo aumento da produtividade que passou de 1.940 kg/ha, em 1988/89, para 2.816 kg/ha, em 2002/03, enquanto a área cultivada evoluiu 51,6% no mesmo período.

Para esse cenário da cultura da soja, o desenvolvimento de cultivares representou contribuição importante. Cultivares que carregam em seu genoma os genes capazes de expressar alta produtividade, adaptação ampla e resistência e/ou tolerância a fatores bióticos ou abióticos adversos representam usualmente a contribuição mais significativa à eficiência de todo o setor produtivo. O programa de melhoramento genético da Embrapa Soja desenvolvido em parceria com produtores de sementes associados em fundações ou outra forma organizacional, atua em todo o território nacional, e possibilitou a obtenção de cultivares com grande eficiência – ganho genético de produtividade esperado entre 1,5% a 2,0% ao ano, estabilidade de produção, ganho de qualidade para o consumo humano e processamento industrial, além de redução dos custos para o produtor. Atualmente existem mais de 200 cultivares disponíveis que são semeadas em mais de 60% da área cultivada com soja no País.

O modelo de melhoramento genético em parcerias desenvolvido pela Embrapa Soja

A grande extensão territorial do Brasil sempre foi um grande desafio à atuação das Unidades de Pesquisa da Embrapa. Para a cultura da soja, que através do melhoramento genético havia sido incluída nos sistemas de plantio do Centro-Oeste, esse desafio exigiu a busca de soluções criativas. Apoiar a produção de soja com tecnologias, serviços e produtos que garantissem segurança e retorno econômico ao produtor, qualidade ao consumidor e sustentabilidade ambiental exigiu uma integração ampla entre vários agentes da cadeia produtiva e a pesquisa estatal.

A execução dos trabalhos propostos, as dificuldades enfrentadas e a experiência adquirida levaram a um aperfeiçoamento das formas de relacionamento e à seleção dos participantes. As parcerias mais óbvias, entre Unidades da Embrapa e com empresas do Sistema Nacional de Pesquisa Agropecuária – SNPA, foram capazes de suprir as demandas por pesquisa durante alguns anos. Logo ficou evidente que as restrições orçamentárias, as diferenças de visão dos dirigentes e os limites impostos à contratação de pesquisadores e de apoio nas empresas públicas eram fatores limitantes ao desenvolvimento dos trabalhos. Formas híbridas de cooperação, envolvendo unidades da Embrapa, empresas do SNPA e cooperativas ou outras formas de associações de empresas privadas, foram tentadas. Diversos problemas surgiram e diversas arestas tiveram que ser aparadas

durante o exercício dos trabalhos a que as parcerias se propuseram a fazer. A partilha das responsabilidades das tarefas, o dinamismo na busca das soluções, a conciliação do interesse público com o interesse privado, a garantia dos direitos das partes, tudo isso teve que ser equacionado. Vários parceiros desistiram no meio do caminho e nova formatação dos acordos foi necessária em vários casos. Por último, a aprovação da legislação brasileira de propriedade intelectual, que resultou na Lei de Patentes e na Lei de Proteção de Cultivares, e a conseqüente aprovação da política da Embrapa sobre proteção intelectual, forçaram novas revisões dos contratos das parcerias. As negociações para realizar as modificações contratuais e inserir o trabalho sob a égide dessa Lei e das novas normas da Embrapa foram consolidadas.

As parcerias que se ajustaram às novas diretrizes, principalmente pela sensibilidade dos produtores de semente que visualizaram as perspectivas de crescimento trazidas pelo trabalho conjunto com a Embrapa, prosseguiram. O produtor de semente é um cliente direto da pesquisa, interessado na existência de cultivares produtivas e bem adaptadas às várias regiões, capazes de garantir o seu negócio, que é a multiplicação e a venda de sementes. Esses produtores de semente associados em fundações privadas de apoio à pesquisa formaram as melhores parcerias para a criação e o desenvolvimento de novas cultivares de soja em troca da co-propriedade das mesmas ou da exclusividade na produção de semente. A Embrapa ganhou parceiros experientes e agressivos em comercialização, interpretadores das demandas por tecnologia, que apoiaram decididamente os trabalhos através do financiamento das despesas de custeio, pessoal e investimento.

Atualmente as parcerias estão evoluindo para outras áreas da pesquisa, porque logo ficou claro que as cultivares deveriam ser ofertadas junto com um conjunto de tecnologias como manejo da fertilidade do solo e das doenças e pragas, por exemplo, que garantissem o sucesso do produtor de grãos. Através da união dos esforços das instituições parceiras em cada estado, está sendo possível a implementação desses trabalhos nas dimensões necessárias, agregando a iniciativa privada envolvida com máquinas agrícolas, defensivos, fertilizantes, transporte e comercialização.

A experiência adquirida permitiu sintetizar os objetivos, as diretrizes e as vantagens mais aparentes dos trabalhos realizados. Os objetivos gerais das parcerias podem ser resumidos em: a) apoiar as atividades de P&D na busca de soluções tecnológicas demandadas pela cadeia produtiva; b) transferir e acompanhar o impacto causado pelas novas tecnologias; c) coletar e eleger prioridades para a pesquisa; d) captar recursos. As parcerias

foram regidas por diretrizes destinadas a criar um arcabouço legal para facilitar o trato da "coisa pública". Essas diretrizes podem ser resumidas: a) adesão à missão da Embrapa Soja; b) valorização da tecnologia; c) redução de custos; d) integração dos participantes da cadeia produtiva; e) proteção do conhecimento. As vantagens das parcerias ficaram logo aparentes: a) a iniciativa privada viabilizou recursos para financiar custeio, pessoal de apoio e o investimento nas pesquisas em melhoramento genético da soja; b) o quadro de pesquisadores, experiente e bem treinado, sentindo-se livre de restrições financeiras pôde expressar toda sua criatividade; c) o pesquisador foi colocado em contato direto com seu cliente e obteve, rapidamente, informações precisas que permitiram a criação de produtos que atendessem as expectativas e ou necessidades da cadeia produtiva; d) a capacidade de pesquisa da Embrapa Soja foi associada à agressividade do "marketing" do produtor de semente.

As parcerias

O estabelecimento de parcerias pela Embrapa Soja com o Setor Privado para o desenvolvimento de cultivares no Brasil foi o fator que mais contribuiu para agilizar a oferta de cultivares de alta qualidade em todo o território nacional. Graças a esse apoio, mais de 100 pontos de testes, estrategicamente distribuídos pelos principais ecossistemas produtores de soja do País, avaliam um volume superior a 250.000 progênies e 50.000 linhagens avançadas de soja, a cada ano.

a) Fundação Pró-Sementes de Apoio à Pesquisa
A parceria com a Fundação Pró-Sementes de Apoio à Pesquisa apóia os programas de melhoramento genético de soja e trigo da Embrapa para o desenvolvimento de cultivares. Tem sede no Rio Grande do Sul e atua nos estados do Rio Grande do Sul, Santa Catarina, Paraná, São paulo e sul do Mato Grosso do Sul.

b) Fundação Meridional de Pesquisa Agropecuária
A parceria com a Fundação Meridional de Pesquisa Agropecuária apóia os programas de desenvolvimento de cultivares da Embrapa nas culturas da soja e do trigo. Tem sede no Paraná e atua nos estados de Santa Catarina, Paraná e São Paulo.

c) Fundação de Pesquisa Vegetal Integrada
A parceria com a Fundação de Pesquisa Vegetal Integrada apóia o desenvolvimento de cultivares da Embrapa na região sul do Mato Grosso do Sul.

d) Fundação Triângulo de Pesquisa e Desenvolvimento
A parceria com a Fundação Triângulo de Pesquisa e Desenvolvimento e EPAMIG apóia o desenvolvimento de cultivares de soja para o Brasil Central.

e) Fundação Centro-Oeste de Pesquisa e Desenvolvimento
A parceria com a Fundação Centro-Oeste de Pesquisa e Desenvolvimento apóia o desenvolvimento de cultivares de soja para os estados de Mato Grosso e Rondônia.

f) Fundação de Apoio à Pesquisa e Desenvolvimento do Oeste Baiano
A parceria com a Fundação de Apoio à Pesquisa e Desenvolvimento do Oeste Baiano apóia a Embrapa no desenvolvimento de cultivares de soja para aquela região.

g) Centro Tecnológico para Pesquisa Agropecuária - CTPA
A parceria com o Centro Tecnológico Para Pesquisa Agropecuária – CTPA e AgenciaRural apóia o desenvolvimento de cultivares de soja para a região Central do Brasil.

h) Fundação de Apoio à Pesquisa do Corredor Norte de Exportação - FAPCEN
A parceria com a Fundação de Apoio à Pesquisa do Corredor Norte de Exportação – FAPCEN envolve trabalhos em melhoramento, produção de sementes, fitopatologia e fertilidade de solos. O trabalho de melhoramento genético é desenvolvido para criar cultivares para a região Norte do Brasil.

i) Fundação Estadual de Pesquisa Agropecuária - FEPAGRO
A parceria com a Fundação Estadual de Pesquisa Agropecuária – Fepagro visa desenvolver cultivares de soja para o Rio Grande do Sul.

Ex-parceiros

a) Fundação Mato Grosso
A parceria com a Fundação Mato Grosso não foi continuada pela falta de interesse dos seus colaboradores em atender as normas da Embrapa.

b) Fundação Cerrados
A parceria com a Fundação Cerrados não foi continuada dentro da nova política de parcerias da Embrapa pelo fato dos produtores de sementes ligados inicialmente a essa Fundação se constituírem em um grupo muito pequeno que preferiu aderir ao contrato do CTPA em Goiás.

c) Empaer – MS e Grupo GMS
A parceria foi desfeita por desinteresse do Estado em continuar apoiando a pesquisa e desenvolvimento de cultivares de soja.

Principais resultados obtidos

Atualmente cerca de 200 cultivares, bem adaptadas e altamente produtivas, disponíveis aos agricultores brasileiros são semeadas em mais de 60% da área cultivada com soja. Os benefícios para a sojicultura brasileira foram muito expressivos. A Tabela 1 mostra a evolução da produtividade da soja no Brasil em 28 anos, os primeiros 25 agrupados por qüinqüênios e o triênio mais recente. É notório o significativo aumento da produtividade nos dois últimos períodos comparativamente aos anteriores. Esse aumento coincide com o período de amadurecimento e forte ação das parcerias, que mesmo enfrentando problemas importantes como a doença cancro-da-haste e o nematóide de cisto foram capazes de criar cultivares que proporcionaram os ganhos observados. Essas vitórias fazem crer que os novos desafios, como a ferrugem da soja, também deverão ser enfrentados com sucesso.

Tabela 1. Área, produção e produtividade da soja brasileira em cinco qüinqüênios sucessivos entre 1975 a 2000 e o triênio 2001 a 2003.

Período	Área 10^6ha	Produção 10^6 tons	Produtividade kg/ha
1975 a 1980	7,35	11,43	1.555
1981 a 1985	8,88	15,25	1.717
1986 a 1990	10,56	18,38	1.740
1991 a 1995	10,59	21,69	2.048
1996 a 2000	12,34	28,76	2.330
2001 a 2003	15,98	42,92	2.684

Fonte: Conab

A área plantada com cultivares das parcerias que atinge hoje mais de 60% dos 18,5 milhões de hectares cultivados com soja, garante a sobrevivência de cerca de 550 empresas tipicamente nacionais no ramo de produção de sementes de soja.

Para os agricultores além das cultivares produtivas e adaptadas ainda há o benefício da semente a preços acessíveis, US$ 20-25 por saca de 50kg, cerca de metade da quantia paga por um produtor norte-americano. Para a Embrapa Soja houve o reconhecimento de que uma empresa estatal pode desempenhar eficientemente seu papel e benefícios extras como o aporte recursos diretos e indiretos em valores bastante significativos quando comparados com o total de recursos do tesouro nacional alocados para o custeio.

A agregação de setores tão distintos como pesquisa e produtores de sementes, permitiu que os produtores visualizassem nitidamente as vantagens de estarem organizados. Formas subseqüentes de associação e parcerias foram ou estão sendo buscadas. Empresas produtoras de insumos, de máquinas e de comercialização estão sendo chamadas para compor as parcerias. O desenvolvimento deste modelo de atuação coletiva está possibilitando ao agronegócio uma mudança estrutural, que irá se formalizar através de instrumentos contratuais que não sirvam apenas de controle às ações desenvolvidas, mas também incentivo e efetivação de novas alianças e parcerias.

Ganha o país que se mantém competitivo no estratégico setor agrícola e ganham as regiões que se desenvolvem através do maior dinamismo dos vários outros setores que se beneficiam do aumento da renda local. O agronegócio da soja talvez seja aquele onde o Brasil possua a maior capacidade de competir num mercado globalizado. Há disponibilidade de tecnologia, há capacidade instalada e há uma grande integração entre os vários setores da cadeia produtiva. A atuação da pesquisa, Embrapa e parceiros, foi fator determinante desse sucesso e da continuidade desse trabalho dependerá o desempenho futuro.

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Desafios tecnológicos para produção de semente de soja na região tropical brasileira

F. C. Krzyzanowski[1]

Resumo

A tropicalização da soja feita pela pesquisa agronômica brasileira e a sua expansão na região dos cerrados do Brasil gerou uma demanda científica e tecnológica que é a produção de sementes em condição climática distinta e adversa do ambiente originário da soja. A região tropical, devido ao clima quente e úmido predominante, propicia o desenvolvimento da deterioração por umidade, que é um dos fatores mais detrimentais que afetam a qualidade da semente de soja. A semente com deterioração por umidade é também altamente susceptível ao dano mecânico na operação de colheita. Tecnologias foram desenvolvidas pelos pesquisadores brasileiros de soja, visando criar um sistema de produção de sementes na região tropical. Decorrentes desses trabalhos, diversas práticas na produção passaram a ser utilizadas para minorar as conseqüências da deterioração no campo como: a colheita no momento adequado, a antecipação da colheita, a seleção de região mais propícia à produção de semente, a semeadura em época mais adequada para produção de semente, a utilização de fungicidas foliares, o manejo da fertilidade do solo e o manejo de percevejos. Além dos problemas inerentes aos fatores de campo, a produção de semente na região tropical também depara com desafios nas operações de colheita (em função do dano mecânico na semente), de secagem (devido os danos térmicos), de armazenamento (em função das condições climáticas prevalecentes na fase final) e de transporte até o consumidor final (devido a perdas significativas da qualidade nessa operação).

Abstract – Soybean seed production in the Brazilian tropical environment: technological challenges

The expansion of the soybean production area in Brazil, under tropical conditions, due to the improvement of cultivars with juvenile period, imposed a scientific and technological challenge concerning the soybean seed production under those environmental conditions. Weathering is the most detrimental factor for soybean seed physiological quality under humid and hot climate. Weathered seeds are very prone to mechanical damage at harvesting time. A system for soybean seed production was developed based on scientific and technological knowledge developed by the Brazilian soybean

[1] Embrapa Soja, Caixa Postal 231, 86001-970 – Londrina, PR, Brasil. E.mail: ickk@cnpso.embrapa.br

researchers such as: harvesting seed with high moisture content, selection of more suitable regions for seed production (750 meters above the sea level), best time for seeding, foliar fungicides to control late season diseases, soil fertility management (K supply), sting bug control, improvement of harvesting practices (mechanical damage monitoring), drying procedures (equipments and temperature), storage procedures (environment control and plastic bag) and transport management (losses monitoring and prevention during operation).

Revisão de literatura

A soja é oriunda da região temperada, pois segundo Hymowitz (1970), ela surgiu como cultura no nordeste da China (30° a 40° N), em torno do século XI A.C. A sua tropicalização, através do melhoramento genético com a descoberta do período juvenil e o desenvolvimento de cultivares adaptadas às diversidades edafo-climáticas da região tropical, abriu uma nova perspectiva para o seu cultivo, sendo o Brasil o principal país a cultivá-la nessas condições.

Concomitante à expansão do cultivo na região tropical criou-se a demanda por semente das novas cultivares adaptadas à região, o que, por conseguinte, estabeleceu grande desafio tecnológico e científico que é a produção de semente com altas qualidades fisiológica e sanitária nas condições ambientais predominantes.

A região tropical é caracterizada por ter clima quente e úmido no período de verão, que coincide com as fases de maturação e de colheita da semente de soja, o que como regra geral, a princípio, não atende às exigências da soja para produção de semente de alta qualidade, pois requer temperatura média, na fase de maturação das sementes ao redor de 22°C.

A semente de soja apresenta máximos vigor e viabilidade no ponto de maturação fisiológica. Entretanto, não pode ser colhida mecanicamente nesse estádio, pois apresenta elevado grau de umidade, cerca de 45%. A exposição da semente de soja durante a maturação, a ciclos alternados de umidades alta e baixa antes da colheita, devido à ocorrência de chuvas freqüentes ou às flutuações diárias da umidade relativa do ar associado com alta temperatura, comuns em regiões tropicais, resultará em deterioração por umidade ou deterioração de campo.

Sabe-se que deterioração ocorre após a maturação fisiológica, antes mesmo da semente ser colhida (Mondragon & Potts, 1974). Após esse estádio, a deterioração continua em taxas variáveis, porém é mais intensa

principalmente a partir do ponto em que a semente atinge pela primeira vez, 25% de umidade (Mondragon e Potts, 1974).

O intervalo entre a maturação fisiológica e a colheita, que pode variar de alguns dias a várias semanas, é caracterizado como um período de armazenagem e secagem natural e, raramente, as condições climáticas no campo são favoráveis para a preservação da qualidade da semente. Portanto, a deterioração por umidade é um dos fatores mais detrimentais que afetam a qualidade da semente de soja, principalmente nas regiões tropicais e subtropicais (França Neto et al., 1984c).

Quando expostas às condições de ambiente mais úmido, as sementes secas ganharão umidade e, conseqüentemente, terão os seus volumes expandidos. Uma redução de volume ocorrerá caso o oposto venha a ocorrer. Em algumas situações extremas, o grau de umidade das sementes pode alcançar níveis de até 27%, após chuvas intensas, conforme relatado por Queiroz et al. (1978). Com graus de umidade acima de 25%, o processo de respiração das sementes é ativado, resultando em consumo de suas reservas.

As sucessivas expansões e contrações do volume das sementes ocasionam a formação de rugas nos cotilédones, na região oposta ao hilo, cujas lesões bastante peculiares são nitidamente observadas através do teste de tetrazólio (França Neto et al., 1998a). Além disso, ocorre o cansaço físico dos tecidos, que pode resultar em ruptura do tegumento e dos tecidos embrionários, comprometendo o controle de permeabilidade das membranas aos níveis celular e sub-celular. Organelas, como as mitocôndrias, são particularmente afetadas por tal processo, havendo menor produção de energia (ATP) necessária para a germinação. Como conseqüência, os tecidos embrionários, nas regiões próximas ao tegumento, são danificados pela formação das rugas, o que resulta em redução na germinação e no vigor e em aumentos nos percentuais de plântulas anormais e infectadas e de sementes mortas (Moore, 1972; Pereira, 1974).

Além das conseqüências diretas na qualidade das sementes, a deterioração por umidade pode resultar em maior índice de danos mecânicos na colheita, uma vez que sementes deterioradas são extremamente susceptíveis aos danos mecânicos (França Neto et al., 1984b).

A deterioração no campo é intensificada pela interação com alguns fungos de campo, como *Phomopsis* spp. e *Colletotrichum truncatum*, que, ao infectar as sementes, podem reduzir o vigor e a germinação (Henning, 1984).

Diversas práticas de produção podem ser utilizadas para minorar as conseqüências da deterioração no campo como a colheita no momento

adequado, a antecipação da colheita, a seleção de região mais propícia à produção de semente, a semeadura em época mais adequada para produção de semente, a utilização de fungicidas foliares, o manejo da fertilidade do solo e o manejo de percevejos.

A semente deve ser colhida no momento adequado, evitando-se qualquer retardamento de colheita. A semente é normalmente colhida quando, pela primeira vez, o grau de umidade atinge a faixa de 15% a 13%, durante o processo natural de secagem a campo. O retardamento de colheita resultará em reduções de germinação e vigor da semente, especialmente quando associado com condições climáticas adversas (Costa et al., 1983a).

A antecipação da colheita poderá ser realizada com graus de umidade da semente ao redor de 18%. Tal operação poderá ser adotada, caso o produtor de semente tenha amplos conhecimentos das regulagens do sistema de trilha, visando a não ocorrência de elevados índices de danos mecânicos imediato e latentes. Além disso, uma estrutura adequada de secadores deverá estar disponível, para que o grau de umidade das sementes seja reduzido a níveis adequados, sem que ocorram reduções de germinação e de vigor.

A antecipação de colheita através do uso de dessecantes foliares não é recomendada, quando o objetivo é a produção de semente de soja de melhor qualidade. A aplicação de paraquat propiciou o aumento dos índices de semente infectada por fungos de campo, como o *Phomopsis* spp., agente da queima da haste e da vagem e do cancro da haste (Costa et al.,1983b; França Neto et al.,1998b). Tais resultados reforçam os relatados por Cerkauskas et al. (1983), que, além de aumentos na incidência de *Phomopsis* spp., reportou também maior ocorrência de *Colletotrichum truncatum*, agente da antracnose, associado à semente, após a aplicação de dessecantes foliares.

A produção de semente de alta qualidade requer que as fases de maturação e colheita ocorram sob condições climáticas secas, associadas com temperaturas amenas (22°C). Tais condições não são facilmente encontradas em regiões tropicais, porém podem ocorrer em platôs com altitude superior a 700 m. Para cada 160 m de elevação na altitude, ocorre, em média, uma redução de 1°C na temperatura (Costa et al.,1987).

Em regiões tropicais, a época de semeadura deve ser ajustada de tal forma que a maturação da semente ocorra sob condições de temperaturas amenas associadas a menores índices de precipitação. Através da comparação do ciclo das cultivares de soja utilizadas, observando especificamente a época da maturação e colheita com os padrões de chuva de uma determinada região, pode-se selecionar a data de semeadura que resultará nos menores

índices de deterioração por umidade na semente (Kueneman, 1982). De maneira geral, para os estados do Paraná, de São Paulo, do Mato Grosso do Sul, do Mato Grosso, de Goiás e de Minas Gerais, as melhores produtividades são obtidas quando a semeadura ocorre entre o final de outubro a meados de novembro. Entretanto, para a produção de semente de alta qualidade, os melhores períodos de semeadura ocorrem entre meados de novembro a meados de dezembro (França Neto et al., 1984e).

No Brasil, a aplicação de fungicidas foliares é recomendada para o controle da ferrugem asiática (*Phakospsora pachyrhizi*), de doenças de final de ciclo, como a mancha parda (*Septoria glycines)* e o crestamento foliar (*Cercospora kikuchii),* e para o controle do oídio (*Microsphaera diffusa)* (Embrapa Soja, 2003). Além de permitir o controle de tais doenças, a utilização dos fungicidas foliares, conforme recomendação, pode propiciar melhores rendimento e qualidade de semente, por permitir maturação uniforme da lavoura e por um período mais prolongado de retenção das folhas, propiciando a ocorrência de microclima no dossel da planta, mais favorável em termos de temperatura amena para a maturação da semente.

O uso contínuo do solo sem a reposição necessária do potássio extraído resultou no aparecimento de sérios sintomas de deficiência de potássio, maior ocorrência de plantas enfermas e menores produtividades (Borkert et al., 1988; Henning et al., 1985b). Semente de soja produzida em condições de baixa disponibilidade de potássio apresentou elevados índices de infecção por *Phomopsis* spp., altos índices de deterioração por umidade e de danos causados por percevejo, além de menores índices de vigor e de viabilidade (França Neto et al., 1985).

Outro tipo de dano que causa sérios prejuízos à indústria de semente na região tropical é o que resulta da incidência de percevejos (França Neto, 1984b). Quando o percevejo se alimenta da semente de soja, inocula a levedura *Nematospora coryli* Peglion (Sinclair & Backman, 1989). A colonização dos tecidos da semente por essa levedura causa sérias necroses, resultando em perdas de germinação e de vigor (Villas-Bôas et al., 1990). Os danos de percevejo podem resultar em redução de produtividades e, em algumas situações, podem causar problemas de retenção foliar e peciolar, o que dificultará a operação da colheita mecânica.

Além dos problemas inerentes aos fatores de campo, a produção de semente na região tropical também depara com desafios nas operações de colheita, de secagem, de armazenamento e de transporte até o consumidor final.

A colheita é a fase mais crítica de todo processo de produção de sementes de soja, pois os impactos causados pelos mecanismos de trilha, durante a

colheita, são a maior fonte de danos mecânicos à semente de soja. O tegumento da semente de soja atualmente sendo muito fino e contendo baixo percentual de lignina oferece pouca proteção ao eixo embrionário que está posicionado fragilmente abaixo (Agrawal & Menon, 1974; Alvarez et al.,1997). Proteção limitada é também oferecida pelo efeito amortecedor das células colunares (Pereira & Andrews, 1985) e por um tecido em formato de bolsa derivado do tegumento, que envolve a ponta da radícula (McDonald et al., 1987).

Os níveis de danos mecânicos são reduzidos se a semente de soja é colhida tão logo seja possível, após atingir graus de umidade entre 15% a 12% (Costa et al., 1979). Os menores índices de danos mecânicos e de perdas na colheita são obtidos quando a colheita ocorre com o grau de umidade da semente ao redor de 13%. Em regra geral, os índices de semente quebrada e "bandinhas" aumentam, quando o grau de umidade da semente está abaixo de 12%, e os de semente amassada e com dano mecânico latente aumentam com graus de umidade acima de 15% (Costa et al., 1979).

A semente de soja colhida com elevados graus de umidade, 15% a 18%, como prática da antecipação da colheita ou em condições de ano chuvoso, deve ser pronta e apropriadamente secada a 13% ou menos. O processo de secagem deve ser iniciado imediatamente após a colheita do material. O retardamento da secagem afeta as qualidades fisiológica e sanitária da semente (França Neto et al.,1983, 1984a). A umidade relativa do ar do ambiente interno da massa de sementes de soja colhida com 16% de umidade e armazenada a granel em moegas ou silo é acima de 80%, permanecendo nesse nível por um considerável período de tempo, independente da umidade relativa do ambiente externo, a não ser que a semente esteja sendo secada ou adequadamente aerada (Boyd, 1979). Segundo Pasin (1991), a aeração da semente em silo ventilável com alta taxa de renovação de ar (3 a 5 m^3/min/t) impede o aquecimento e a conseqüente deterioração da semente durante o período de espera de secagem.

Para as condições de armazenamento do Brasil, sugere-se que o grau de umidade da semente seja mantido nos seguintes níveis, de acordo com as regiões onde a semente será armazenada: 13,0% a 13,5%, para as regiões do Rio Grande do Sul, Santa Catarina e centro sul do Paraná; 11,5% a 12,0% para as regiões norte e oeste do Paraná, sul do Mato Grosso do Sul e São Paulo; 10,5% a 11,0% para as demais regiões dos cerrados (Pasin, 1991). É importante que, durante o período de armazenamento, tais níveis de umidade não sejam ultrapassados, caso contrário, a qualidade das sementes poderá ser prejudicada. Delouche (1975) relata que, em condições tropicais a semente de soja pode ser armazenada com 9,0% a 9,5% de umidade à temperatura de 25°C por períodos de até nove meses.

O baixo poder de armazenamento da semente de soja, conforme classificação realizada por Justice & Bass (1978), é amplamente reconhecido nos meios científicos e produtivos. Delouche (1982) complementa que semente dessa espécie deteriora mais rapidamente que semente de arroz, milho, sorgo, trigo e outras espécies, quando submetidas às mesmas condições de campo, de colheita, de secagem e de armazenamento.

Minor & Paschal (1982) demonstraram que mesmo semente com alta qualidade não suporta o período de armazenamento na região tropical. Esse fato foi comprovado no Brasil por Henning et al. (1985a), que compararam os efeitos de dois níveis iniciais de umidade de semente de soja 'Tropical' armazenada em câmara fria e ambiente natural, em Teresina, PI. Após 45 dias de armazenamento em ambiente natural o vigor e a germinação decresceram significativamente. Depois de três meses, a ocorrência de *Aspergillus* spp. era em média superior a 50% e a geminação e o vigor da semente foram drasticamente reduzidos. Por outro lado, quando o armazenamento foi efetuado em câmara fria (12°C-15°C e 65-70% UR) não ocorreram problemas por *Aspergillus* spp. e a qualidade da semente foi mantida por seis meses.

Segundo Harrington (1959) alta umidade é o fator isolado mais importante pelas perdas de viabilidade e vigor da semente durante o armazenamento.

Para manter a viabilidade e o vigor, a semente precisa ser secada a um nível adequado de umidade e armazenada em condições de temperatura e umidade que evitem a absorção de umidade. A semente é higroscópica, portanto seu grau de umidade está em equilíbrio com a umidade relativa do ar (Matthes et al., 1969).

Matthes & Butler (1968) recomendam graus entre 9%-10% de umidade para o armazenamento seguro da soja. Tal grau de umidade estaria em equilíbrio com uma umidade relativa de 55%.

O efeito do tipo de embalagem, do ambiente de armazenamento (convencional ou climatizado) e do grau de umidade da semente que adentra o armazém afetam a qualidade da semente. Amaral & Baudet (1983) observaram queda no vigor de semente armazenada em sacos de juta, polipropileno e papel multifoliado. O grau de umidade da semente variou de acordo com a UR do ambiente, independentemente do tipo de embalagem. Resultados semelhantes foram obtidos por França Neto et al. (1984d) os quais não observaram diferenças significativas no grau de umidade da semente de soja armazenada em sacos de juta, papel multifoliado e polipropileno trançado. O vigor da semente decresceu significativamente, após seis meses de armazenamento.

Os fungos de armazenamento (saprofíticos) estão normalmente associados com a semente. Todavia, permanecem inativos durante o período de armazenamento, caso o grau de umidade da semente seja mantido abaixo de 13%. Os fungos de armazenamento, principalmente *Aspergillus* spp. e *Penicillium* spp. (Henning, 1988), juntamente com a deterioração fisiológica, são as principais causas da perda da viabilidade da semente no armazém.

Muitas vezes, por falta de monitoramento das condições ambientais durante o período de armazenamento, produtores de semente não entendem a razão da perda da qualidade da semente, especialmente quando o seu grau de umidade no início e final do armazenamento estava abaixo de 13%. Porém, durante algum período, por poucas semanas talvez, esse grau de umidade elevou-se e manteve-se em níveis superiores a 14%, devido à ocorrência de períodos de alta umidade relativa do ar. Tal fato foi observado por Henning et al. (1985a) em Teresina, PI, o que resultou na perda total da viabilidade da semente, devido à proliferação de *Aspergillus* spp.

Nas regiões tropicais, a deterioração da semente durante o armazenamento, é uma das principais causas do baixo desempenho da semente no campo (Matthes et al. 1969). O armazenamento em ambiente condicionado (temperatura e umidade relativa baixas) é uma possível solução para o problema. Porém, além de economicamente onerosa, essa tecnologia muitas vezes não está disponível nas regiões onde ela é mais necessária (Camacho & Múnera, 1986).

O uso de embalagem plástica hermética tem apresentado grande potencial de aplicação. Sementes de soja com grau de umidade inicial variando de 8,5% a 9,0% foram mantidas em armazéns não climatizados, por períodos de até sete meses, em Cuiabá e Teresina, após serem acondicionadas em embalagens plásticas herméticas e em embalagens permeáveis, de pano ou de papel multifoliado. A sacaria plástica hermética preservou a qualidade da semente em níveis aceitáveis, muito superiores aos obtidos com as embalagens permeáveis (Henning et al., 1995a; Henning et al., 1997).

É comum, principalmente em regiões dos Cerrados, que lotes de sementes de soja sejam transportados por longas distâncias (1.500 km) das regiões de produção aos locais onde serão semeadas. França Neto et al (1993) demonstraram que o transporte rodoviário nessas condições resultou em reduções significativas de vigor e de viabilidade, devido à ocorrência de aumentos nos índices de deterioração por umidade e de danos mecânicos às sementes. Na grande maioria dos casos, a semente é transportadas em caminhões convencionais, sem compartimentos climatizados. Freqüentemente, a semente é exposta a altas temperaturas e chuvas durante

o transporte. Em temperatura ambiente de 30°C, semente de soja acondicionada em sacos de papel multifoliado expostos ao sol tiveram sua temperatura elevada a 43°C (J.B. França Neto, dados não publicados). Mudanças abruptas de temperatura podem reduzir a qualidade da semente, principalmente em semente com grau de umidade ligeiramente elevado (Justice & Bass, 1978).

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