

Soybean tempeh with maximum bioconversion of β -glucoside isoflavones into aglycones

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Abstract

The aim of this work was to investigate the effect of process variables on the production of soybean tempeh with maximum bioconversion of β -glucoside isoflavones into aglycones using a Central Composite Design (CCD) 2³. The soybean cultivar BRS 267 was used to prepare the tempehs and the evaluated variables were: soaking time (6, 12 and 18 h); cooking time (15, 30 and 45 min) and fermentation time (18, 24 and 30 h) with *Rhizopus oligosporus* at 37°C. The function response aglycones content was evaluated and quantified by Ultra Performance Liquid Chromatographic (UPLC). To prepare the soybean tempeh with maximum aglycones content is recommended 18 h of fermentation with *Rhizopus oligosporus* and minimum conditions of 6 h soaking and 15 min of cooking time.

Keywords: aglycones, central composite design, fermentation, *Rhizopus oligosporus*

Introduction

Tempeh is a traditional food, marketed in different forms and obtained by fermentation of soybean grains (Nout and Kiers, 2005).

The conditions of preparation of soybean tempeh vary and include soaking and cooking process of the grains, inoculation with *Rhizopus oligosporus* and finally the fermentation (Farnsworth, 2008; Karyadi, 2001). In the preparing of tempeh, generally the soybean grains are soaked in water for 10 to 12 h at room temperature to facilitate hulls removal. Then, the cotyledons are cooked in boiling water, drained, cooled at 35-40°C inoculated with microorganism and fermented at 37°C for 24 to 48 h (Farnsworth, 2008). During soaking and cooking process of soybean grains losses of important chemical constituents can occur. Villares et al. (2011) observed in tempeh that 12 % of isoflavones were leached to soaking water and 49 % were lost during the cooking. Fermentation is the major stage to obtain the tempeh due to the changes that occur in the sensory characteristics and bioconversion of isoflavones (Nout and Kiers, 2004). In the fermentation, β -glucosidase enzyme hydrolyse the β -glucosidic isoflavone forms to their corresponding aglycone forms which are readily available to human organism and has high biological activity (Liggins et al., 2000). Isoflavones, mainly aglycones, have been studied due to their ability to reduce cardiovascular disease risk (Zhuo et al., 2004), inhibit some types of

cancer cell growth (Lund et al., 2004), reduce the risks of diseases, including osteoporosis (Liggins et al., 2000) and relieve symptoms of menopause (Messina and Hughes, 2003).

The aim of this study was to investigate the effect of soaking, cooking and fermentation time on tempeh production with maximum bioconversion of β -glucoside isoflavones into their corresponding aglycones using the Central Composite Design (CCD) 2^3 .

Material and Methods

Soybean cultivar BRS 267, harvest in 2011/2012, obtained from Embrapa-Soybean, Londrina, Parana, Brazil, were used to prepare the tempehs. *Rhizopus microsporus var. oligosporus* was obtained from INTSOY (International Soybean Program, University of Illinois. EUA). All reagents were of analytical grade from different sources.

The tempehs were prepared according to traditional method describe by Wei (1991), with some modifications. Central Composite Design (CCD) 2^3 , with 3 replicates of central point with a total 17 random assays was used to evaluate the effects of process variables during tempeh production, and on the bioconversion of β -glucoside isoflavones into their aglycones. The variables investigated were: X_1 (soaking time, h), X_2 (cooking time, min) and X_3 (fermentation time, h). To each assay, 100 g of soybean grains, proportion 1:10 (w/v) were soaked (X_1) and then, the grains were debulbed by hand. The cotyledons were cooked into boiling water (X_2), drained and cooled at 25°C. inoculation was done using 2 g of *Rhizopus oligosporus* for 100 g of cooked cotyledons. The cotyledons were homogenised, package in perforated polypropylene bags, maintained at 37°C and fermented (X_3) according to experimental design. Coded and real levels of independent variables and response function ($Y_1 = \text{mg aglycones } 100 \text{ g}^{-1}$ of tempeh) obtained experimentally were shown in Table 1.

The lyophilized tempeh were defatted with hexane (1:10 w/v) for 30 min at room temperature in continuous rotary agitation and then vacuum filtered. The response function Y_1 was determined. Mathematical model was as follow: $Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_{11}X_1^2 + \beta_{22}X_2^2 + \beta_{33}X_3^2 + \beta_{12}X_1X_2 + \beta_{13}X_1X_3 + \beta_{23}X_2X_3 + e$, where Y = response function, x_1 , x_2 and x_3 = coded variables, β = estimated coefficients of the response surface model and e = pure error.

The response function (Y_1) was used to perform regression analyses and analyses of variance (ANOVA) for the regression. The equation model was fitted to experimental data to yield the proposed model. Response surface graphs and desirability parameters were generated to obtain a tempeh with maximum aglycones content. All executed analysis, desirability and response surfaces were performed with Statistica 8.0 software (StatSoft Inc, 2007).

The isoflavones extraction was performed using a mixture solvent extractor containing ultra pure water, acetone and ethanol (1:1:1, v/v/v) described by Yoshiara et al. (2012). Extraction was performed in triplicate with 0.3 g of sample with 6 mL of organic solvent mixture, then vortexing every 15 min for 1 h at room temperature. The mixture was placed in an ultrasonic bath for 15 min at room temperature, centrifuged (2500 xg at 4 °C and 15 min) (Centrifuge 5804R - Eppendorf, Hamburg, GE) and filtered (Millex filter - H (0.22 μm)). Triplicate aliquots of 1.4 μL of filtrate were automatically injected into the Waters liquid

chromatography UPLC (Acquity UPLC® System, Waters, United States). The column was a reversed-phase type (model ACQUITY - UPLC BEH C18, Waters, United States) with dimensions of 2.1 mm x 50 mm and particle size of 1.7 μm . Elution was performed with non-linear gradient using two solvent systems: A containing acidified water at pH 3.0 adjusted with glacial acetic acid, and B contained acetonitrile. The flow rate was 0.7 mL min^{-1} at 35 °C. The gradient began with 90% eluent A and 10% eluent B; at 8 min gradient elution ratio reached 0% A and 100% B. Returning to the initial conditions at 9 min with a total run time of 10 min. The detector was a Photo diode array (Waters) adjusted in a wavelength of 260 nm. All isoflavones (β -glucosides, malonil-glucosides and aglycones) peaks were identified by their spectra and retention time, and the standard curves were prepared with pure standards from Sigma Co. (St. Louis, EUA). In this study, only the aglycones daidzein and genistein concentration were determined and quantified. The aglycone glycitein was not detected. The results were expressed in mg of aglycones 100 g^{-1} of defatted tempeh.

Results and Discussion

Soybean cultivar BRS 267 showed only 8.80 mg of aglycones 100 g^{-1} . According to Carrão-Panizzi et al. (2003) soybean grains presents lower contents of aglycone forms.

According to regression and variance analysis (ANOVA), only the variable X_3 (fermentation time, h) showed a significant linear negative effect ($\beta_3 x_3 = -4.55x_3$) at a 5 % probability on the response function Y_1 (mg of aglycones 100 g^{-1}). The mathematical model, considering only the significant variable, was developed: $\hat{Y}_1 = 30.87 - 4.55x_3$. The lack-of-fit of model was not significant (at 95 %) and 85 % (R^2) of the experimental data was properly adjusted to the model. The response surface and desirability parameters (Figure 1) suggest that the maximum aglycones content ($\hat{Y}_1 = 30.21$ mg aglycones 100 g^{-1} of tempeh) was obtained when $x_1=+1$ or $X_1=18$ h soaking time, $x_2=0.5$ or $X_2=37.5$ min cooking time and $x_3=-1$ or $X_3= 18$ h de fermentation time. This estimated result of the maximum aglycones content is according with the assay 7 ($Y_1 = 39.11$ mg aglycones 100 g^{-1} of tempeh) (Table 1). By the equation ($\hat{Y}_1 = 30.87 - 4.55x_3$), to obtain tempeh with higher aglycones content, recommended to use the lower level condition $x_3 = -1$ and 18 h of fermentation and independent of the variables X_1 or X_2 . Considering the confidence interval of the assay 7 (CI = 29.98 to 43.18), it was observed that the response function of the assays (1, 5 and 13) where $x_3 = -1$, all functions response are within interval of the assay 7, confirming that the variables X_1 and X_2 are not significant to obtain tempehs with maximum aglycones contents. However, the response function of assay 3 was not within the CI, probably due the maximum condition of variable X_2 (45 min of cooking).

In the present study, soybean cultivar BRS 267 presented only 8.80 mg aglycones 100 g^{-1} and tempeh obtained in assay 7 presented 39.11 mg aglycones 100 g^{-1} , showing an increase of 4.4 times due to fermentation process. Therefore, models to obtain tempeh with maximum aglycones level were investigated, and the following conditions are observed: $x_1 (-1)$; $x_2 (-1)$ and $x_3 (-1)$.

To achieve the maximum bioconversion of the β -glucosides into their aglycones, it is recommended to use smaller conditions of variables in the process, that is according with the 1 assay 6 h soaking, 15 min cooking and 18 h fermentation.

Table 1. Central Composite Design (CCD) 2^3 and response function (Y_1) to aglycones content of the tempehs produced with BRS 267 soybean cultivar.

Assays	X_1 (x_1)	X_2 (x_2)	X_3 (x_3)	Y_1
1	6 (-1)	15 (-1)	18 (-1)	32.77
2	6 (-1)	15 (-1)	30 (+1)	27.5
3	6 (-1)	45 (+1)	18 (-1)	24.97
4	6 (-1)	45 (+1)	30 (+1)	21.35
5	18 (+1)	15 (-1)	18 (-1)	31.57
6	18 (+1)	15 (-1)	30 (+1)	23.52
7	18 (+1)	45 (+1)	18 (-1)	39.11
8	18 (+1)	45 (+1)	30 (+1)	21.31
9	6 (-1)	30 (0)	24 (0)	32.42
10	18 (+1)	30 (0)	24 (0)	32.47
11	12 (0)	15 (-1)	24 (0)	34.79
12	12 (0)	45 (+1)	24 (0)	22.26
13	12 (0)	30 (0)	18 (-1)	33.11
14	12 (0)	30 (0)	30 (+1)	22.31
15	12 (0)	30 (0)	24 (0)	32.15
16	12 (0)	30 (0)	24 (0)	29.41
17	12 (0)	30 (0)	24 (0)	32.05

x_1 , x_2 and x_3 are coded variables; X_1 (soaking time, h), X_2 (cooking time, min) and X_3 (fermentation time, h) are real variables; Y_1 = mg aglycones 100 g^{-1} of tempeh.

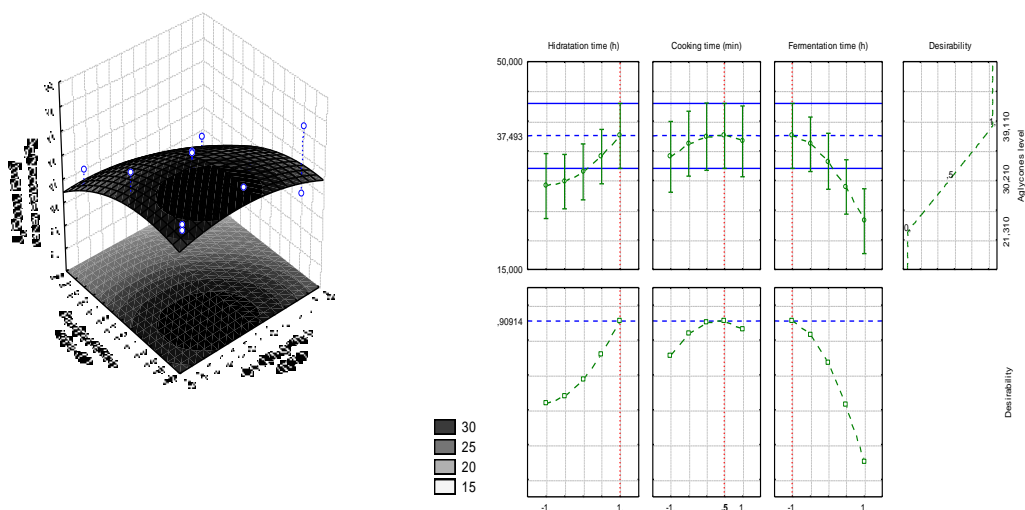


Figure 1. Response surfaces and desirability parameter to response function Y_1 (mg aglycones 100 g^{-1} of tempeh)

Conclusion

To a tempeh with maximum aglycones contents is recommended 18 h of fermentation with *Rhizopus oligosporus* and a minimum conditions of 6 h of soaking and 15 min of cooking time.

Acknowledgements

This work was partially funded by project PRONEX/Fundação Araucária/PR., CNPq/MCT and EII thanks CNPq for the research fellow. We thank Embrapa-Soybean by donation of the soybean.

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