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Effect of Soybean Cultivars on the Nutrients and Consumer Acceptance of Soymilk

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Abstract

Soymilk is one of the most available beverages, an alternative to dairy milk and is recognized for its nutritional value. The nutritional quality, the presence of anti-nutritional factors, isoflavones and sensory acceptability of soymilk depended on the soybean variety as well as the processing conditions. The soymilks from conventional and specialty Brazilian soybean cultivars were compared regarding the composition and consumer acceptance. There were significant differences on the protein, oil, sugars, isoflavones, presence of anti-nutritional factors (phytate and trypsin inhibitor activity) and NSI (nitrogen solubility index) among cultivars and soymilks and for sensory acceptance of soymilks. The preference mapping and cluster analysis identified three different segments of consumers. The soymilk from the conventional cultivar BRS284 achieved higher and similar acceptance score for the three consumer's segments while the lipoxygenase free cultivars (BRS213 and BRS257) and specialty cultivar BRS216 showed higher score for two segments of consumers. The sensory evaluation of soymilk from different soybean cultivars could improve consumer uptake.

Keywords

Preference Mapping, Cluster Analysis, Protein, Phytate

1. Introduction

There is a trend towards consumption of plant-based foods and the demand for soymilk has increased worldwide [1]. Soymilk is one of the most available beverages, an alternative for consumers that avoid or want to reduce the dairy milk products [2] [3]. It is recognized for its nutritional value, lactose and cholesterol free, presence of essential fatty acids, isoflavones and well-balance aminoacids [4].

The consumption of soy foods has been extensively studied and reviewed concerning the role of bioactive compounds (isoflavones, peptides, proteins, protease inhibitors, lectins, saponins, phytic acid, oligosaccharides, phytosterols, phenolic acids) related to health benefits such as cardiovascular diseases, some types of cancer, osteoporosis among others diseases [5]-[11].

Despite the nutritional value of soymilk, its consumption in the Western world is limited due to the off-flavor characterized as beany, green, grassy, painty, astringent, and bitter, which have been associated with oxidation of polyunsaturated lipids by lipoxygenases (LOX) present in soybeans [3] [11] [12].

Furthermore, there are anti-nutritional factors in soybean, such as anti-trypsin factor and phytates with negative effect on the protein digestion and mineral availability, respectively. Thermal treatment is commonly used for anti-trypsin factor inactivation while processes, such as blanching, roasting, hot grinding and pH adjustment of soaking water are used for LOX inactivation [3].

Technological alternatives for soymilk processing were developed by Nelson *et al.* [13] and Tanteeratarm *et al.* [14] called "Illinois process" and "INTSOY/Food science processes" resulting in bland and very smooth beverages. These processes for whole or dehulled soybeans may include soaking, and alkaline blanching (one or two steps) followed by cold or hot grinding. The alkaline soaking followed by alkaline blanch or alkaline blanching without soaking inactivated lipoxygenases and anti-trypsin factor. In addition to bland flavor, those steps also promote a reduction of the cooking time of soybeans. In Brazil, the processing of soymilk has been carried out with slight modifications (no soaking, reducing time of blanching and difference in bicarbonate concentration solution) by Felberg *et al.* [15]-[17] to meet the expectations of Brazilian consumers.

Tripathi *et al.* [18] compared several soybean processing for soymilk such as the traditional oriental process (soaking for 4 hours and grinding at room temperature) and different soaking (pH and temperature) and grinding conditions as well as the deodorization step of soymilk. The soymilk from the traditional process presented a strong "beany flavor" while higher sensory acceptance was obtained for soymilk from soaking and cooking with bicarbonate solution (Illinois Method) and for deodorized soymilk.

Nevertheless, the nutritional quality, the presence of anti-nutritional factors, isoflavones and sensory acceptability of soymilk depended on the soybean variety as well as the processing conditions [19] [20].

In Brazil, a breeding program to develop specialty soybean cultivars is carried out at the National Soybean Research Center (Embrapa). In the cross combinations, high-yielding cultivars adapted to the different soybean production regions, and genotypes with special traits such as better flavor (through absence of lipoxygenase), high protein content, reduced trypsin inhibitor, appropriate seed size (large or small), and hilum color (yellow), are being considered specialty ones in order to improve soybean for human consumption [21]. The Brazilian soybean cultivars BRS 213 and BRS 257 are lipoxygenase free and other specialty cultivars for human consumption are BRS 267 (large size, vegetable type for tofu and edamame) and BRS 216 (with high protein and isoflavones and small size for soybean sprouts) [22]-[25].

The lipoxygenase-free soybean BRS 213 was evaluated as soy beverage (prepared with 10% of spray dried soymilk) and the sensory acceptance was higher than two commercial brands of dried soymilk in two different cities in Brazil [26], which are very promising results. The specialty, conventional and LOX free cultivars from Embrapa were evaluated for tofu by Benassi *et al*. [27] and no difference was observed for acceptance but the internal preference mapping indicated consumer segmentation in three different groups. The internal preference mapping and cluster analysis showed the segmentation of consumer groups for soymilk which are useful tools to select among soybean cultivars [17].

There are few results regarding sensory evaluation of soymilk using the specialty and LOX free soybean cultivars developed in Brazil as well as for the presence of anti-nutritional factors. This study aimed to investigate the performance of different conventional soybean cultivars, LOX-free and other specialty cultivars regarding the nutritional characteristics, presence of anti-nutritional factors and consumer acceptance of soymilk to contribute to the development of soymilk.

2. Material and Methods

2.1. Material

The soybean cultivars were selected based on previous studies [22] [23] [28] that investigated their nutritional characteristics and absence of LOX. The cultivars were provided by Embrapa Soybean (Londrina, PR, Brazil) at fully mature stage (R8), and their characteristics are described in **Table 1**.

Table 1. Soybean cultivars from the genetic breeding program at Embrapa Soybean.

Cultivars	Characteristics
BRS 213	Lipoxygenases free (LOX1, LOX2, LOX3), yellow hilum color
BRS 257	Lipoxygenases free (LOX1, LOX2, LOX3), light brown hilum
BRS 267	Specialty type for edamame, pleasant flavor, large grains, yellow hilum
BRS 216	Specialty type for soybean sprouts, yellow hilum, high protein and isoflavone contents
BRS 284	Conventional soybean cultivar, light brown hilum color
BRS 317	Conventional soybean cultivar with a light brown hilum

2.2. Processing

Soymilk processing was carried out according to Felberg *et al.* [15] and Antoniassi *et al.* [29]. The soybean was dehulled, cooked for 10 minutes in sodium bicarbonate solution (0.25%) at a ratio of 1:3 (dehulled soybean: solution) and drained. Then, the grinding with boiling water using a Waring® heavy-duty blender at a ratio of 1:8 (blanched soybeans: water) was performed for two minutes, followed by centrifugation using an IEC® K7165 centrifuge with a 150 µm nylon filter at 4000 rpm (~2500 g) and pasteurization (95 a 98˚C/10 min). The soymilk presented 7% of total solids and was formulated by adding 3% sucrose and 0.2% salt (w/w) (**Figure 1**). The process was carried out three to five times for each cultivar. The soymilk yield was measured as liters of soymilk resulting from the processing of one kg of dehulled soybeans.

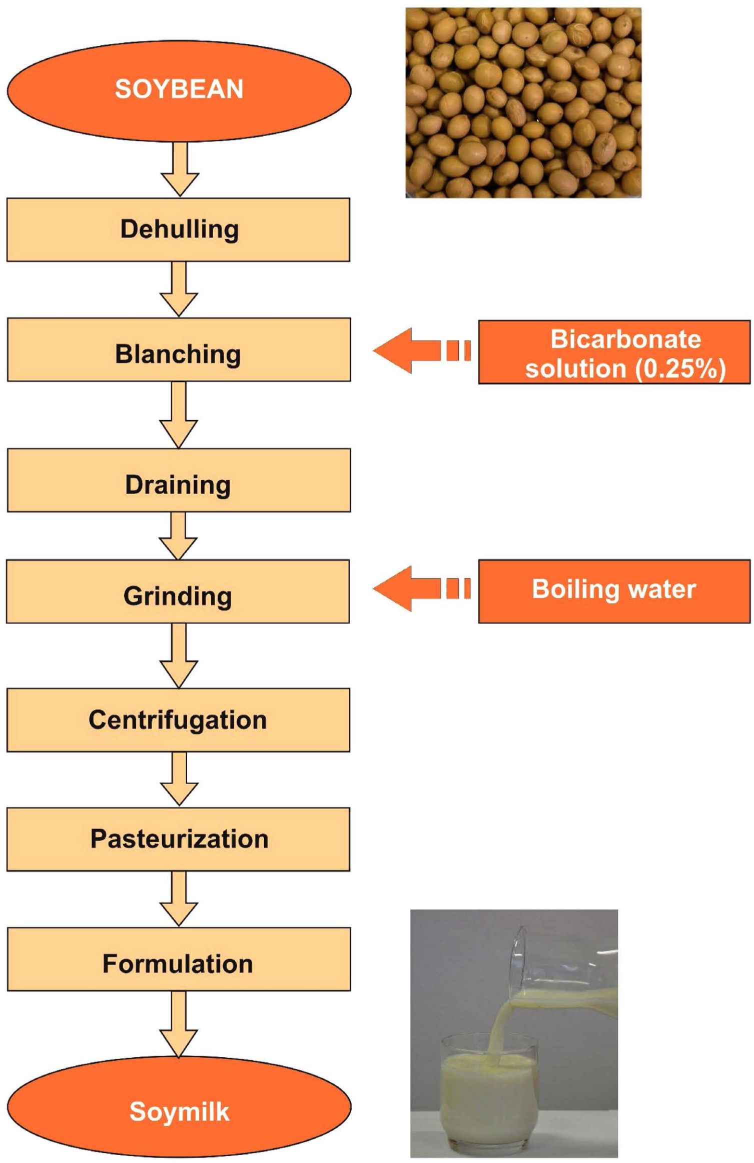

Figure 1. Flowchart of soymilk processing.

2.3. Physicochemical Analysis of Soybean and Soymilk

2.3.1. Proximate Analysis

The analysis of dehulled soybean and soymilk was performed in six replicates according to the Official Methods of Analysis of AOAC [30]. Moisture analysis was performed by oven-drying to constant weight at 105˚C (AOAC 925.45B). The ash content was measured at 550˚C in a muffle furnace (AOAC 923.03/32.1.05). The protein content was calculated based on the nitrogen content obtained by the Kjeldahl method (AOAC 4.2.11) using a 6.25 factor. Oil extraction was performed by acid hydrolysis followed by ethyl ether and petroleum ether extraction according to AOAC method 922.06 [30]. The nitrogen solubility index (NSI) and the trypsin inhibitor activity were determined according to the Official methods of AOCS [31], BA 11-65 and Ba 12-75, respectively. The phytate content followed the official method AOAC 986.11 [30] but the phosphorus content was measured by ICP-OES (Inductively coupled plasma-optical emission spectrometry) (Optima 2100 DV, Perkin Elmer, Waltham, MA, EUA) [32].

2.3.2. Chromatographic Analysis

The analysis of mono-, di- and oligosaccharides was performed with six replicates based on the liquid chromatographic separation with using the HPLC system (Waters™, Waltham, MA, USA) and refractive index detector mode W2410 (Waters™) and amino column (Zorbax carbohydrate column, 4.6 × 250 mm; 5 μm—Agilent™). The chromatographic conditions employed isocratic elution mode with acetonitrile: water (75:25, v/v), at temperature of 45˚C, and a 20 μL injection volume. Sample extraction was conducted with 1 g of sample weighed in a 25-mL volumetric flask containing 10 mL of ultrapure water, followed by an ultrasonic bath for 10 min. Subsequently, 5 mL of acetonitrile was added, and the volume was completed with ultrapure water. Finally, the extract was filtered. The quantification was done by external standardization.

The isoflavone analysis of dehulled soybean was carried out according to AOAC method 2001.10 [30] using six replicates. The extractions were performed with methanol/water (80:20, v/v), followed by hydrolysis with NaOH solution, acidification, and filtration. The analyses were carried out by HPLC-PDA (Alliance™ 2695, Waters, Waltham, MA, USA) using a YMC-Pack Pro C18 column (5 μm, 4.6 mm × 250 mm, YMC, Kyoto, Japan) and a gradient with acetic acid solution and methanol running at 1.3 mL/min. The identification and quantification at 260 nm of isoflavones were performed by comparing the peak retention time with those of the respective standards. The peak identities were confirmed as well as by UV spectra. The conversion of the isoflavone concentrations (genistin, glycitin, and daidzin) into aglycon equivalents was calculated by multiplying the mass of each isoflavone form by the ratio of its aglycone molecular weight to the molecular weight of the individual form [33]. The total isoflavones as aglycon equivalents were determined by summing the concentrations of daidzein, glycitein, and genistein to the aglycon equivalent concentrations of daidzin, glycitin, and genistin.

2.3.3. SDS-PAGE Analysis

To evaluate the presence of lipoxygenases, the soybeans were ground and defatted by Soxhlet extraction with petroleum ether for 16 hours. Protein extraction for further electrophoresis analysis was performed by solubilizing the defatted sample in 0.05 M Na_2CO_3, 6 M urea, 12% sucrose and 1% SDS buffer and stirring for 1 h. Samples were centrifuged at 4000 rpm for 10 minutes after filtration on a cotton filter. For the present work, the Bio-Rad electrophoresis system was used, according to the gel preparation methodology [34]. The run was carried out in a 12% polyacrylamide gel for 7 h at 100 V. The polypeptide bands in the SDS-PAGE were visualized by staining with the color reagent "Coomassie brilliant blue R250", overnight and washed with mixture of containing: 40% methanol, 10% acetic acid and 50% water, for 3 h.

2.3.4. Consumer Acceptance Evaluation

The six soymilk beverages were processed at EMBRAPA Food Technology one day before the test, and kept refrigerated (6˚C ± 2˚C). The samples were taken to a supermarket in Rio de Janeiro, Brazil following the requirements of a central location test. The study was approved by the Federal State University of Rio de Janeiro Ethical Committee (UNIRIO)—(TTDD:232/2011).

Consumers were invited to participate in the study at the entrance of the supermarket and, when agreed, they were taken to an area where the test was performed. Tables and chairs were adequately arranged to avoid close contact between participants. Ninety-nine consumers of non-alcoholic beverages but not usual consumers of soymilk, aged between 18 and 65 years agreed to take part in this study. Participants were asked to try the samples and to indicate their overall liking. Around 40 mL of each soymilk were placed in 50 mL plastic cups, coded with three-digit numbers and served to participants at refrigerator temperature (8˚C ± 2˚C). Tap water at room temperature was provided to wash their mouths between samples. The order of samples' presentation was balanced, and followed a complete block design [35]. Samples were presented monadically. The seven-point Hedonic Scale was used, ranging from (1) "I really disliked it" to (7) "I really liked it". After expressing their level of overall liking, consumers were given the option to freely state what they liked and disliked about each soymilk. This task was not mandatory, and they had the opportunity to express only likes, only dislikes, both or none for each product [36]. The test was carried out between 10:00 am - 16:30 pm.

2.3.5. Statistical Analysis

The data was evaluated by one-way analysis of variance (ANOVA) followed by the Tukey at a significance level of 95% using SAS (Statistical Analysis System Institute).

For the consumer acceptance data, ANOVA was used and the sample was specified as fixed effect, whereas consumer as a random effect, and Tukey's test was used for post hoc pairwise comparisons of sample means, at a significance level of 5%. In addition, the data from the acceptance study were also analyzed using Cluster Analysis and Internal Preference Mapping [37] to better understanding the consumer preference. The analyses were carried out using XLSTAT.

3. Results and Discussion

3.1. Physicochemical Analysis of Soybean Cultivars

Analysis of the protein identity pattern by SDS-PAGE showed the absence of lipoxygenases in the molecular mass range of 100 kDa in the BRS 213 and BRS 257 confirming them as LOX free cultivars, different from the other specialty and conventional cultivars that presented the enzymes.

There were significant differences among cultivars evaluated regarding the proximate composition (dry weight), presence of anti-nutritional factors (phytate and trypsin inhibitor activity) and NSI (nitrogen solubility index) ($p < 0.05$) (**Table 2**). The levels of protein were in the range presented by Silva *et al.* [22] and Felberg *et al.* [17] but higher than those reported by Ciabotti *et al.* [25] for Brazilian cultivars. The protein content ranged from 38.8 to 44.8 g·100 g^{-1} and highest figure was obtained for cultivar BRS 216 recognized by its higher protein content [22] [23]. The oil content varied from 19.8 to 26.7 g·100 g^{-1} agreeing with the range presented by Felberg *et al.* [17] but higher than the results obtained by Silva *et al.* [22], Ciabotti *et al.* [25] and Seibel *et al.* [28] for Brazilian soybean cultivars. The highest oil content was obtained for BRS 284 that showed the lowest protein content, similar as observed by Felberg at al. [17]. The ash content ranged from 5 to 5.8 g·100 g^{-1}, similar to Felberg *et al.* [17] and Ciabotti *et al.* [25], and BRS 267 presented the highest content. No trend regarding proximate composition was observed among the specialty, conventional and lipoxygenase-free soybean cultivars.

Table 2. Proximate composition (g·100 g^{-1}, dry weight), anti-nutritional factors and Nitrogen Solubility index of Brazilian soybean cultivars.

Cultivar	Protein	Ash	Oil	Phytate (mg·g^{-1})	NSI (%)	Trypsin inhibitor activity (TIU/mg)
BRS 216	44.8^{a}	5.0^{c}	20.4^{e}	13.8^{c}	59.5^{b}	20.3^{c}
BRS 267	42.5^{c}	5.8^{a}	19.8^{f}	16.9^{a}	57.3^{bc}	25.6^{b}
BRS 213	41.8^{d}	5.6^{b}	23.2^{d}	14.2^{b}	67^{a}	34.3^{a}
BRS 257	43.3^{b}	5.5^{b}	23.5^{c}	13.8^{c}	59.2^{b}	24.0^{b}
BRS 317	39.3^{e}	5.0^{c}	24.4^{b}	11.2^{d}	52.7^{c}	NP
BRS 284	38.8^{f}	5.5^{b}	26.7^{a}	8.7^{e}	71.4^{a}	32.4^{a}

NSI: Nitrogen Solubility index; Trypsin inhibitor activity—TIU; NP—not performed. Means with different lowercase letters in the same column are significantly different ($p < 0.05$) by Tukey test. Results expressed as the average of six replicates.

The trypsin inhibitor activity (TIU) was significant higher for the BRS 284 (conventional) and BRS 213 (LOX free) while the lowest content was obtained for the specialty one BRS 216 ($p < 0.05$). The results obtained were in the range obtained by Galão *et al.* [38] for conventional and transgenic soybean cultivars grown in Brazil and higher than the results for Brazilian cultivars reported by Seibel *et al.* [28]. TIU elimination or reduction through genetic improvement techniques are valuable tools to enhance the digestibility and nutritional value of soy-based products [38]. The trypsin inhibitors are protease inhibitors that reduce the protein digestibility but can be eliminated by thermal processing. However, the conditions may reduce the nutrients (such as isoflavones) and decrease amino acid availability [3]. The blanching of soybeans with sodium bicarbonate inactivated the trypsin inhibitors according to Nelson *et al.* [13] which employed similar conditions of this work.

There were significant differences for sugars ($p < 0.05$) (**Table 3**). Glucose was not detected and fructose varied from 0.36 to 0.89 g·100 g^{-1}. Sucrose content ranged from 3.8 to 11 g·100 g^{-1} and the highest content was quantified for BRS267 followed by BRS216 and BRS284. The raffinose content ranged from 1 to 2.8 g·100 g^{-1}, and the highest value was obtained for BRS267. Stachyose content ranged from 5.8 to 11.8 g·100 g^{-1} and the highest content was found in BRS216. Silva *et al.* [22] evaluated five Brazilian soybean cultivars as well as BRS213, BRS216, BRS267 and BRS133 and the ranges observed for sucrose, raffinose and stachyose were 3.4 to 4.3; 0.4 to 1.0 and 2 to 3.5 g·100 g^{-1}, respectively. Oliveira *et al.* [23] evaluated 28 Brazilian, American and Japanese cultivars and observed very wide ranges of values for sucrose (2.4 to 6 g·100 g^{-1}), stachyose (2.2 to 4.4 g·100 g^{-1}), raffinose (0.4 to 1.2 g·100 g^{-1}) and fructose was detected up to 0.05 g·100 g^{-1}. According to Kumar *et al.* [39], the content of sucrose of soybeans was significantly higher at cooler growing location (up to 16%) while differences for raffinose and stachyose were genotype-dependent. The results showed different pattern of the specialty cultivars BRS 216 and BRS 267 related to sugars content. Although the oligosaccharides are commonly described as flatulence factor, currently the oligosaccharides from soybean have been reported as prebiotics that promote competitive exclusion of pathogens and the consumption of these prebiotics may be useful for maintaining populations of beneficial bacteria in the gut [40].

Table 3. Sugar content of six Brazilian soybean cultivars (g·100 g^{-1}, dry weight).

Cultivars	Fructose	Glucose	Sucrose	Raffinose	Stachyose
BRS 216	0.49^{c}	ND	8.67^{b}	2.33^{ab}	11.77^{a}
BRS 267	0.71^{b}	ND	11.04^{a}	2.77^{a}	5.92^{b}
BRS 213	0.36^{d}	ND	3.82^{c}	1.69^{c}	5.82^{b}
BRS 257	0.47^{cd}	ND	4.54^{c}	1.67^{c}	5.83^{b}
BRS 317	ND	ND	4.36^{c}	1.82^{bc}	5.85^{b}
BRS 284	0.89^{a}	ND	8.02^{b}	1.04^{d}	6.26^{b}

Means with different lowercase letters in the same column are significantly different ($p < 0.05$) by Tukey test. Results expressed as the average of six replicates. ND—Not detected.

There were significant differences among soybean cultivars ($p < 0.05$) for the total isoflavones content and the individual isoflavones (**Table 4**). The total isoflavones content varied from 65 to 150 mg/100g and the higher amount was observed for BRS 317 (conventional grain type) followed by BRS 216. The main isoflavones were genistin and daidzin, while lower amounts of genistein, daidzein and glycitin were observed. Glycitein was detected only for BRS 317. The results were in the range obtained for Brazilian cultivars by Carrão-Panizzi *et al.* [41] and Felberg *et al.* [17]. The variation of the isoflavones from different countries and cultivars was reported by Bhagwat *et al.* [42], for daidzein (2.64 to 191.43 mg·100 g^{-1}), genistein (5.56 to 276.21 mg·100 g^{-1}), glycitein (0 to 121.69 mg·100 g^{-1}) and for the total isoflavones (10.04 to 440.72 mg·100 g^{-1}). The isoflavone contents in soybean varies according to genetic differences, geographic location, soil, year, and environmental conditions during growth [43]. No trend regarding isoflavone contents was observed among the specialty, conventional and lipoxygenase-free soybean cultivars.

Table 4. Isoflavone content (mg·100 g^{-1}, dry basis)* of soybean from different Brazilian soybean cultivars.

Cultivar	aglycone equivalents (mg/100 grams, dry weight)						
	Daidzein	Glycitein	Genistein	Daidzin*	Glycitin*	Genistin*	Total
BRS 216	0.83^{b}	ND	1.40^{b}	40.49^{b}	2.16^{a}	60.16^{b}	105.04^{b}
BRS 267	0.91^{b}	ND	1.66^{a}	27.8^{c}	1.08^{e}	42.76^{e}	74.20^{d}
BRS 213	0.77^{b}	ND	1.46^{b}	28.49^{c}	1.39^{c}	52.91^{c}	85.02^{c}
BRS 257	0.61^{bc}	ND	1.20^{c}	22.94^{d}	2.00^{b}	46.30^{de}	73.05^{d}
BRS 317	2.48^{a}	0.17	0.74^{d}	68.99^{a}	0.93^{f}	76.78^{a}	150.08^{a}
BRS 284	ND	ND	1.08^{c}	12.92^{e}	1.24^{d}	49.91^{cd}	65.16^{e}

Means with different lowercase letters in the same column are significantly different ($p < 0.05$) by Tukey test. *Individual isoflavone glycosides were normalized for their molecular weight differences into aglycone forms and summed to obtain the total content. Results expressed as the average of six replicates for soybean; ND—not detected.

3.2. Physicochemical Analysis of Soymilk

There were slight but significant differences ($p < 0.01$) related to the total solids (10 to 12 g·100 g^{-1}) of the soymilk (**Table 5**) then the results were presented as dry basis. There were significant differences for protein (28.8 to 38.6 g·100 g^{-1}), oil (19.9 to 27.6 g·100 g^{-1}) and ash contents (4.7 to 5.7 g·100 g^{-1}), of the soymilk from conventional, LOX free and specialty soybean cultivars ($p < 0.05$) (dry weight). Considering the nutritional value, the higher intake of protein and oil will be supplied, respectively, by the soymilk from LOX free cultivars (BRS213 and BRS257) (Supplementary data).

There were significant differences for sugars ($p < 0.05$) of soymilk (**Table 6**) but the variation among them were lower than for soybeans (**Table 3**). Glucose and fructose were not detected. Sucrose content ranged from 29.5 to 36.6 g·100 g^{-1}

because this sugar was added in the formulation step. The raffinose content ranged from 2.3 to 3.4 g·100 g^{-1}, and the stachyose varied from 5.7 to 7.8 g·100 g^{-1}. Some results of raffinose content of soymilk were higher than for soybeans because for soymilk the analysis accounted the extracted sugars and for soybeans the analysis extraction from dehulled soybeans depended on the grain hardness.

Table 5. Proximate composition (g·100 g^{-1}, dry weight), total solids (g·100 g^{-1} of the beverage), anti-nutritional factors and Nitrogen Solubility index of soymilk from Brazilian soybean cultivars*.

Cultivar	Protein	Ash	Oil	Phytate (mg·g^{-1})	NSI (%)	Yield (L·kg^{-1})**	Total solids (g·100 g^{-1})
BRS 216	33.4^{c}	5.1^{b}	23.0^{c}	13.0^{b}	81.2^{ac}	3.3^{b}	10.9^{cd}
BRS 267	33.1^{c}	5.6^{a}	19.9^{d}	15.2^{a}	82.9^{ab}	3.7^{ab}	10.9^{d}
BRS 213	36.1^{b}	5.1^{b}	21.7^{cd}	13.0^{b}	84.8^{a}	3.9^{a}	12^{a}
BRS 257	38.6^{a}	5.2^{b}	25.4^{b}	10.8^{c}	77.5^{cd}	3.9^{a}	11.6^{b}
BRS 317	28.8^{d}	5.7^{a}	27.6^{a}	10.3^{c}	79.4^{bcd}	4.1^{a}	10^{e}
BRS 284	31.4^{c}	4.7^{c}	21.5^{cd}	7.4^{d}	75.5^{d}	4.0^{a}	11.2^{bc}

NSI: Nitrogen Solubility index; *Results expressed as the average of six replicates; **Yield—Average Soymilk yield obtained from three to six processes (Liters of soymilk/kg of dehulled soybeans); Means with different lowercase letters in the same column are significantly different ($p < 0.05$) by Tukey test.

Table 6. Sugar contents of soymilk from six soybean cultivars (g·100 g^{-1}, dry weight).

Cultivars	Fructose	Glucose	Sucrose	Raffinose	Stachyose
BRS 216	ND	ND	36.56^{b}	2.65^{b}	7.79^{a}
BRS 267	ND	ND	36.21^{b}	3.37^{a}	7.15^{ab}
BRS 213	ND	ND	29.49^{c}	2.06^{c}	5.68^{c}
BRS 257	ND	ND	32.58^{bc}	2.56^{b}	7.48^{ab}
BRS 317	ND	ND	42.11^{a}	3.19^{a}	7.35^{ab}
BRS 284	ND	ND	35.24^{b}	2.27^{bc}	6.34^{bc}

Means with different lowercase letters in the same column are significantly different ($p < 0.05$) by Tukey test. Results expressed as the average of six replicates. ND—not detected.

3.3. Effects of the Processing

Regarding the composition of soymilk (Table 5), a different trend related the ratio of protein, oil, ash and phytate from soybean to soymilk was observed (Figure 2). There was difference among cultivars and for different nutrients moreover higher ratios were observed for oil and ash than for protein ratio. The protein content of the soymilk from BRS 216 (33.4 g·100 g^{-1} dry weight, Table 5) stood among the lower figures than the other ones, although this soybean presented the highest protein content among the cultivars evaluated. This behavior may be confirmed by the soymilk yield which ranged from 3.3 to 4.1 Liter·kg^{-1} of dehulled soybeans and the lower figure was observed for BRS 216 that did not differ from BRS 267 and no difference was observed from the former one the BRS213, 257, 317 and 284 ($p < 0.05$). The mass transfer of compounds from soybean to soymilk depended on the soybean cultivars as well as on the process conditions, such as the grain softening or tenderization during macerating and during grinding. Additionally, the centrifugation step may be relevant for the composition of soymilk. The Nitrogen Solubility index of soybeans varied from 52.7% to 71.4% (**Table 2**) while for soymilk ranged from 75.5% to 84.8% ($p < 0.05$) (**Table 5**). Then there was an increase of NSI in the soymilk, even after heat treatment (blanching, grinding and pasteurization). Rigo *et al.* [24] found NSI for Brazilian soybean cultivars varying from 60% - 63% and significant reduction was observed after heat treatment (98˚C for 5 minutes). The water extraction of soymilk followed by centrifugation favored the extraction of more soluble protein fraction that present higher NSI found in this work.

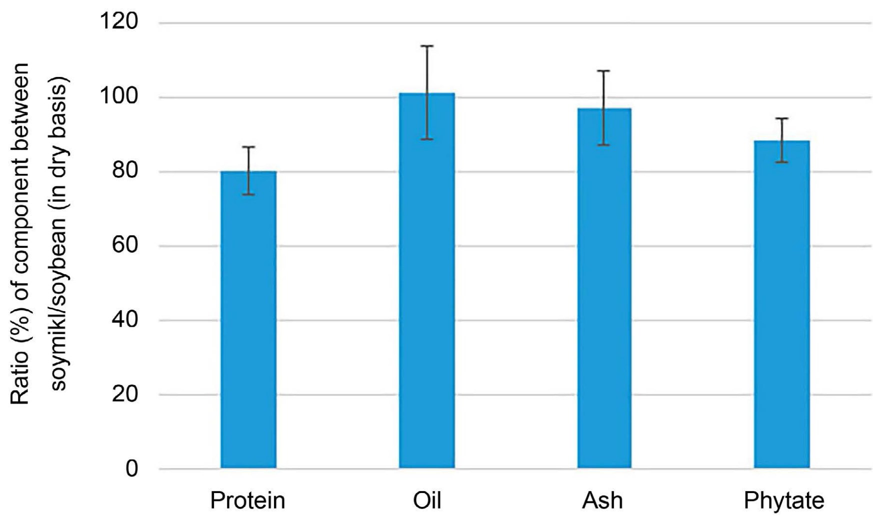

Figure 2. Average ratio (%) of the component between soymilk/soybean (content in dry basis) among cultivars.

In relation to phytate, the ranges observed for soybean and soymilk were 8.7 to 16.9 and 7.4 to 15.2 mg/g (dry weight) (**Table 2** and **Table 5**), respectively, but a similar ratio (78% - 84%) was observed indicating that the processing did not removed this anti-nutritional factor (**Figure 2**). The phytate content of Brazilian cultivars was evaluated by Karkle and Beleia [44] and Oliveira *et al.* [45] ranging from 11.9 to 18.3 and 7.7 to 11.8 mg/g, respectively. The phytate content from 116 different genotypes varied from 7.7 to 22.2 mg/g [46] while Kumar *et al.* [47] reported the range of 9 - 17 mg/g. Phytate has both beneficial and detrimental effects on human nutrition, due to ability to complex with several metal ions such as zinc, calcium, and iron reducing their bioavailability. However, it had been reported to be a potent natural plant antioxidant that plays a protective role against oxidative stress in seeds and preventive role in various human diseases [47] [48]. The higher phytate content was observed for BRS 267 followed by BRS

213 (LOX free) while the lower content was found for BRS 284 (conventional).

3.4. Consumer Acceptance Results

The results of the analysis of variance showed that there was a difference ($p \leq 0.05$) in consumer acceptance only between soymilk processed from BRS257 (LOX free) and BRS284 (conventional) soybean cultivar (Table 7). In addition, there was no average acceptance difference among beverages elaborated from the other cultivars. However, when the score of each consumer is observed, it was possible to notice that some participants liked the products, while others rated the beverages with very low scores. Results from the Preference Mapping (Figure 3) and cluster analysis allow the observation of this diversity and help identifying consumers who liked one beverage over another. Figure 3 shows the position of consumers (Figure 3(a)) and samples (Figure 3(b)).

Table 7. Average consumer acceptance scores and standard deviation of soymilk samples.

Acceptance§	Soymilk samples					
	BRS213	BRS216	BRS257	BRS267	BRS284	BRS317
Average	4.9^{ab}	4.5^{ab}	4.4^{b}	4.6^{ab}	5.0^{a}	4.7^{ab}
Standard deviation	1.56	1.76	1.67	1.63	1.58	1.66

§Evaluated in 7-point hedonic scales: 1—"I really disliked it" to 7—"I really liked it".

The first and second dimensions (F1 and F2) represented 51% of the total variation and can be considered adequate taking into account that samples were evaluated by consumers with no experience on the product. The results shown in Figure 3(a) revealed the consumer segmentation regarding the acceptance of the samples, and three segments were identified, which had different acceptance towards samples. The dimension 1 separated the sample BRS213 from the others, while in the dimension 2, soymilks from cultivars BRS317 and BRS284 were positioned apart. Participants were mainly spread across three quadrants of the Figure 3(b), indicating that soymilks made from BRS317, BRS284 and BRS213 cultivars were preferred, as they pleased a greater number of consumers and Table 8 presents the mean acceptance scores of the three consumer's segments.

The soymilk made from BRS317 (conventional) reached higher acceptance score only for segment one (37 consumers) while no difference was observed for this cultivar and BRS284, BRS257 (LOX free), BRS267 and BRS216 for the segment 1. The segment 2 with 43 consumers preferred the BRS284 and BRS213 (LOX free). No difference was observed for BRS216, BRS213, BRS284 and BRS257 for the segment 3 with 19 consumers.

The soymilk processed with the conventional cultivar BRS284 achieved similar acceptance score for the three consumer's segments and was positioned between "liked slightly" and "liked moderately". On the other hand, it is worth

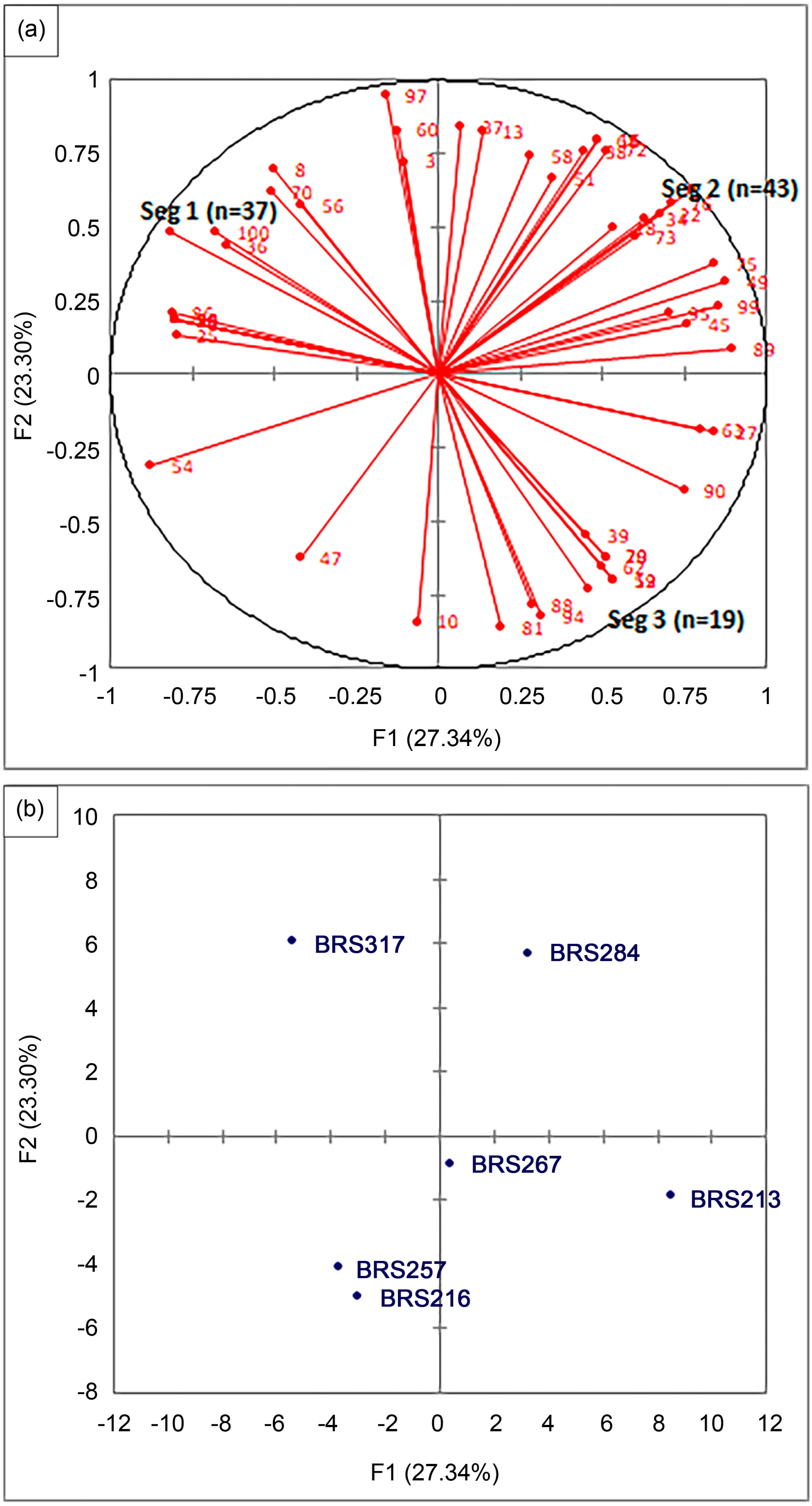

Figure 3. Internal preference mapping results the position of: (a) Consumers (b) Samples.

Table 8. Average soymilk acceptance§ scores and standard errors of consumer's segments.

Soymilk sample	Segment 1 (n = 37)	Segment 2 (n = 43)	Segment 3 (n = 19)
BRS317	$5.4^{aA} \pm 0.25$	$4.5^{bB} \pm 0.23$	$3.5^{cC} \pm 0.35$
BRS257	$4.8^{abA} \pm 0.26$	$3.8^{cB} \pm 0.34$	$5.1^{aA} \pm 0.36$
BRS284	$4.8^{abA} \pm 0.26$	$5.2^{aA} \pm 0.24$	$4.7^{abA} \pm 0.36$
BRS267	$5.0^{aA} \pm 0.27$	$4.5^{bA} \pm 0.25$	$4.2^{bcA} \pm 0.36$
BRS213	$4.2^{bB} \pm 0.24$	$5.1^{aA} \pm 0.23$	$5.5^{aA} \pm 0.34$
BRS216	$4.9^{abA} \pm 0.29$	$3.8^{cB} \pm 0.25$	$5.5^{aA} \pm 0.38$

§Evaluated in 7-point hedonic scales. Different lowercase letters on the same column indicate difference ($p \leq 0.05$), and different capital letters on the same line indicate difference ($p \leq 0.05$). n: number of consumers.

comment about the soymilks prepared with LOX free soybeans achieved an adequate performance for consumers in segments 2 and 3 for BRS213 and segments 1 and 3 for BRS 257.

Comparing the consumer acceptance of soymilks and its composition, the soymilk from the conventional cultivar BRS317 presented the lowest protein content (wet basis, supplementary data) which could negatively affect its acceptance because the sucrose content was the highest. Additionally, the soybean BRS317 showed the highest isoflavone content among the cultivars evaluated and this characteristic may be useful for nutrition claim if authorized. In contrast, the highest protein content was observed for LOX free cultivars BRS213 and BRS257 (wet basis, supplementary data). Regarding the phytate content the higher result was observed for BRS 267 followed by BRS 213 and lowest content was found for BRS 284. Although, the Trypsin inhibitor activity was higher for BRS 284 and BRS 213, the alkaline soaking is efficient for inactivation. The LOX free cultivars is interesting for avoiding the off flavors associated with lipoxygenases activity but the heat treatment is required for anti-trypsin factor inactivation. Then the cultivars LOX free with reduced anti-trypsin factor should be evaluated in order to reduce the conditions of heat treatment.

The soymilk from BRS267, specialty type for edamame called as vegetable type [22] presented high score only for one segment of consumers and performance similar was observed by Felberg *et al.* [17], indicating that this cultivar is not recommended for soymilk production. In the other hand, the BRS216 (specialty type for sprouts) reached high scores for segment 1 and 3.

Considering that the consumer has a key role on the food product development, the understanding of his/her preference is a key factor for success. Therefore, knowing how much consumers like a product is not enough, it is also important to understand the reasons of their evaluations [49], which is a quite challenging task for both marketing and sensory scientists. Taking into account the comments made by participants about the soymilks together with the scores on liking may contribute to the understanding. Consumers were asked to comment on what they liked and/or disliked about each soymilk after they had tasted and given their overall liking score. Looking at the comments it was possible to notice that "nice flavor", "tasty", "balanced flavor", "light", "low soybean flavor", "texture" were mentioned about those samples that were liked. On the contrary, "no flavor", "strong soybean flavor", "off flavor", "astringent" were the words that participants referred to the samples that were not liked. These comments were spontaneous and, therefore, important to identify drivers of liking, which can help on the beverage development process. Further studies can use these comments to deeply investigate soymilk beverages.

The acceptance results indicated that the conventional, LOX free as well as the specialty cultivar for soybean sprouts were suitable for soymilk production and the conditions of processing employed were appropriate to meet the expectations of Brazilian consumers of soymilk.

4. Conclusion

There was a difference among the cultivars related to the transfer rate of protein and oil from soybean to soymilk as well as the soymilk yield. The transfer rate of phytate from soybean to soymilk suggested that this anti-nutritional factor was not inactivated or removed. No trend was observed between sensory acceptance and nutrients of soymilk. The preference mapping and cluster analysis were useful tools to help identify soybean cultivars more adequate for soymilk production. However, a sensory descriptive analysis carried out with consumers or trained panel is recommended to make clear decision about the adequate cultivar for soymilk processing. One conventional soybean cultivar achieved higher and similar average acceptance score for the three consumer's segments. On the other hand, the LOX free and specialty cultivars evaluated showed higher score for two segments of consumers. The sensory evaluation of soymilk from different soybean cultivars could improve consumer uptake.

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Ethical Guidelines

The study was approved by the Federal State University of Rio de Janeiro Ethical Committee (UNIRIO)—(TTDD:232/2011).

Data Availability Statement

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Conflicts of Interest

The authors declare no conflicts of interest.

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Supplementary Data

Table S1. Soymilk composition (wet basis).

Cultivar	Total solids ($g \cdot 100\ g^{-1}$)	Nutrients ($g \cdot 100\ g^{-1}$ wet basis)					
		Protein	Oil	Ash	Sucrose	Raffinose	Stachyose
BRS216	10.9	3.64	2.51	0.56	3.99	0.29	0.85
BRS267	10.9	3.61	2.17	0.61	3.95	0.37	0.78
BRS213	12	4.33	2.60	0.61	3.54	0.22	0.68
BRS257	11.6	4.48	2.95	0.60	3.78	0.28	0.87
BRS317	10.0	2.88	2.76	0.57	4.21	0.35	0.74
BRS284	11.2	3.52	2.41	0.53	3.95	0.25	0.71