



Physicochemical quality of brown soybean preserve in function of maceration time and ascorbic acid

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Abstract

The aim of the present study was to evaluate the physical and chemical quality of the brown soybean preserve in function of the ascorbic acid concentration and the maceration time, and also to evaluate the chemical composition, microbiological hazard, and acceptability of the best preserve, in addition verify its technological, nutritional, functional and sensory viability. The hardness of the grain ranged between 8.6 and 23N and was significantly affected by the maceration time. The ascorbic acid concentration influenced antioxidant activity (ranged between 4.3 and 183.8 mg100g⁻¹) and total phenolic compounds (ranged between 176.4 and 466.2 mg100g⁻¹). The best brown soybean preserve was obtained with a maceration time of 23 min and an ascorbic acid concentration of 0.15 g100g⁻¹. This shows high nutritional value and essential amino acid content, absence of trypsin inhibitor and microbiological risk, and also sensory acceptance. Therefore, the brown soybean preserve is viable, and its consumption is recommended.

Keywords: texture; colour; antioxidant capacity; proximal composition; amino acids profile.

Practical Application: The food industries have been looking for food products, which provide nutritional function for consumers, like antioxidant activity. The brown soybean preserve is an interesting product, with a similar appearance to beans, which present antioxidant activity and phenolic compounds. Besides that, brown soybean preserve it's a way to improve the acceptance of the soybean by the Brasilians consumers.

1 Introduction

The soybean (*Glycine max* (L.) Merrill), a leguminous crop originating in Asia and cultivated in almost all regions of the planet, is a grain of great interest in the worldwide economy and plays an important role in human nutrition because of its nutritional and functional properties (Kim et al., 2014).

Although Brazil is the world's second-largest soybean producer, consumption is low among Brazilians. Researchers have expended great efforts and investments in obtaining cultivars with more acceptable sensory characteristics in order to introduce this leguminous plant into the Brazilian diet. Moreover, the development of soybean cultivars for human food (with free or low-lipoxygenase content, which improves the flavor), health benefits divulgation, and consumer interest has increased. The brown tegument soybean is an alternative for increasing consumption in Brazil, relying on its similarity to Pinto beans, a typical Brazilian cuisine (Ciabotti et al., 2016).

Due to its nutritional superiority among beans, the brown soybean is an excellent option for governmental programs against malnutrition, as it can be introduced into school meals or basic-needs grocery packages. In this context, develop physical and chemical quality of the brown soybean preserve in function of the ascorbic acid concentration and the maceration time of a brown soybean preserve might create new consumption opportunities because the product's appearance is similar to be

an broth, a dish that plays an integral part in the Brazilian diet. This similarity to a familiar food could help avoid consumer resistance, besides preserving the product for a longer period (Mozzoni et al., 2009).

Hydration time of the grains is important point for the production of canned soybeans. The hydration or maceration is widely used in the processing of soybean products because this grain usually contains anti-nutritional compounds and substances that give odor and unpleasant taste, which can be minimized or excluded through this operation (Khatab & Arntfield, 2009). Maceration reduces phytate levels in soybeans; however if the grains remain for a long time in the hydration process, it can cause significant losses of soluble protein and total solids because of unabsorbed grains (Bayram et al., 2004a).

The common use of additives in canned soybean production aims to keep the colour and improve sensory characteristics. However, additives can also be used in order to maintain or increase the antioxidant activity of the food, in which ascorbic acid has been used in food conservation (Sogvar et al., 2016).

The aim of the present study was to evaluate the physical and chemical quality of the brown soybean preserve in function of the ascorbic acid concentration and the maceration time, and also to evaluate the chemical composition, microbiological

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hazard, and acceptability of the best preserve, in addition verify its technological, nutritional, functional and sensory viability.

2 Materials and methods

2.1 Raw materials

The brown soybean (BRM line 04-4382-4) used in the preserve was cultivated in a conventional system at the Federal University of Goiás – Samambaia Campus, Goiania - GO, Brazil, season 2013/2014. The material was donated by the Empresa de Pesquisa Agropecuária de Minas Gerais (EPAMIG). After cultivation, the grains were harvested and selected manually. Ascorbic acid P.A (Sigma®), fresh garlic and virgin olive oil (Galo®) were purchased in local shops in Goiania.

2.2 Brown soybean preserve processing

Response surface and the Central Composite Rotational Design, with four factorial points, four axial, and three central points, totaling eleven experiments, was utilized (Box et al., 2005). The independent variables were maceration time (0, 13.6, 50, 85.4 and 100 min) and concentration of ascorbic acid solution in the brine (relative to the mass of the grains) (0, 0.029, 0.1, 0.171 and 2 g100g⁻¹). Brown soybean preserved processing is presented in the following diagram (Figure 1).

Selected grains were bleached in boiling water at a ratio of 1:5 for 5 min, immediately drained and washed in cold water for approximately 1 min, then macerated in mineral water for different times, which had been defined in the experiment. After the maceration stage, the preserve was processed and packed in a transparent glass recipient (30 mL), containing 1 g of garlic (chopped and fried in virgin olive oil) and a covering liquid (approximately 15 mL) composed of mineral water and NaOH (1.5 g100g⁻¹).

Then, an exhaustion was performed for 10 min. After the exhaustion, ascorbic acid was added in different concentrations, which had been defined in the experiment, and the recipient was immediately sealed and taken to the pre-heated autoclave. The heat treatment was performed for 15 min at 120 °C. Immediately after the gradual addition of cold water into the open autoclave, cooling was performed. The final products were stored at a refrigeration temperature of 5 °C until the analyses. The responses analyzed were instrumental colour parameters (luminosity, chroma a*, and chroma b*), instrumental hardness, antioxidant activity (DPPH*) and total phenolic compounds. Regression models were validated by a test with three repetitions of the preserve with higher desirability (optimized).

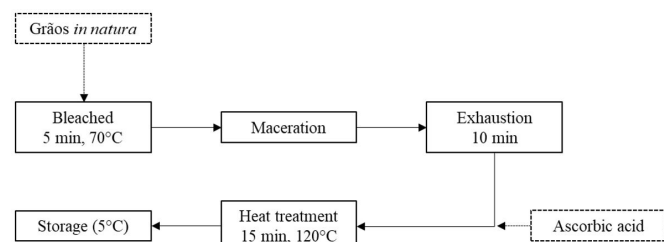


Figure 1. Diagram of the brown soybean preserved processing.

2.3 Instrumental hardness

Analyses were performed on six whole grains per experiment, using a texturometer (TA, XT2, Halesmere, England) with an aluminum cylindrical probe that was 20 mm in diameter (P₂₀). Pre-test, test and post-test speeds were set at 2 mms⁻¹, with a height of 15 mm.

2.4 Total phenolic compounds

Extracts for quantification of total phenolic compounds (TPC) and antioxidant activity were obtained according to the method reported by Hung et al. (2009). Quantification of TPC was performed according to the method proposed by Singleton et al. (1999). The absorbance was measured at 760 nm, using a spectrophotometer (BEL Photonics, S 2000 UV, Osasco, Brazil). The results were expressed as mg gallic acid equivalent per gram of sample (mgGAEg⁻¹) on a dry basis. The analyses were performed in triplicate.

2.5 Antioxidant activity

Antioxidant activity was determined by the 2,2-diphenyl-1-picryl-hidrazil (DPPH) radical-scavenging method, according to Thaipong et al. (2006). The absorbance was measured at 517 nm, using a spectrophotometer (BEL photonics, S 2000 UV, Osasco, Brazil). Total antioxidant activity was expressed as mgGAEg⁻¹ on a dry basis.

2.6 Colour

Colour instrumental parameters were quantified using a colorimeter (Color Quest, XE, HunterLab, Reston, USA). Grains were placed directly on the colorimeter sensor (35 mm of diameter) and measured by the Cielab Colour System. For each treatment, between 20 and 30 measurements were taken. The operations parameters were: viewing angle 10° and illuminant D65 (corresponding to natural daylight).

2.7 Proximate composition

The moisture content (AOAC 925.45b) was determined in an oven at 105 °C. Total nitrogen was quantified by the micro-Kjeldahl method (N×6.25) (AOAC 960.52) in a nitrogen distiller. Fat (AOAC 920.39) was separated in a Soxhlet apparatus with petroleum ether P.A. (Tecnal, TE-044, Piracicaba, Brazil). Ashes (AOAC 923.03) were determined by incineration in a muffle furnace (EDG, Oven Economic, São Carlos, Brazil), according to the methods recommended by the Association of Official Analytical Chemistry (2012). All analyses were performed in triplicate. Carbohydrates were determined by difference. All values were expressed in g100g⁻¹, on a wet basis.

2.8 Total amino acid profile

The samples were ground, homogenized and digested to release amino acids from the proteins by hydrolysis, using hydrochloric acid for 22h at 110 °C under a vacuum in the digester block. Then, they were derivatized in a pre-column with phenyl isothiocyanate (PITC). Separation and identification of amino-feniltiocarbamil

(PTC-aa) was performed in a high-performance liquid chromatography (Shimadzu Corporation, Tokyo, Japan) on a reversed column phase Phenomenex-Luna C18 (Phenomenex Inc., Torrance, CA, USA), 250 mm × 4.6 mm and 5 µm. The mobile phase consisted of an acetate buffer solution, pH 6.4, and an acetonitrile solution of 40 g100g⁻¹. Sample injection was performed automatically (50 µL), and detection was at 254 nm. Chromatographic separation was measured at a constant flow rate of 1 mLmin⁻¹ at 35 °C. The chromatographic run time was 45 in, and the results were expressed in mg100g⁻¹. Identification of amino acids was performed by comparison to an external standard (Pierce, PN 20088). To quantify it, an internal standard of α-aminobutyric acid was used (Sigma-Aldrich, St. Louis, MO, USA), according to Hagen et al. (1989).

2.9 Trypsin inhibitor

The quantification method for trypsin inhibitor activity was based on the hydrolysis of ester bond and amide of benzoyl-L-arginine p-nitroanilide (Bapa), by releasing a synthetic derivative of these amino acids through free trypsin action, which was not connected to the inhibitor present in the solution containing the sample (if trypsin inhibitor existed in the sample, it would inhibit the action of trypsin on the Bapa). During Bapa hydrolysis by trypsin, p-nitroanilide was released, which was measured in a spectrophotometer at 410 nm (Rackis et al., 1974).

2.10 Microbiological risk

The samples were evaluated according to the methods established by the American Public Health Association (2001). *Coliforms* at 45 °C, *Staphylococci coagulase-positive*, *Salmonella* sp., were evaluated after 10 days' incubation at 35-37 °C, and after 5 days of incubation at 55 °C, according to the RDC number 12 from January 2001, of the Brazilian Health Regulatory Agency – Anvisa (Brasil, 2001).

2.11 Consumer acceptance and consumption intent

To evaluate the acceptance of flavor, texture and appearance of the brown soybean preserve, a hedonic scale of nine points was used, with the extreme terms “like extremely” and “extremely disliked”; and for consumption intent the scale was 5 points

(1 = definitely not buy, 3 = maybe buy / may not buy and 5 = definitely buy) (Stone et al., 2012). An average grade of seven (7) was pre-established for consideration that the canned brown soybeans were accepted by the tasters. The panel was composed of 53 people with an age range of 20-40 years. (Number Ethic Committee 041/13).

2.12 Analysis of results

Data were evaluated by analysis of variance and multiple regressions, using Statistica 7.0 (Statsoft, Statistica 7.0, Tulsa, USA). The Response Desirability Profiling function was used to estimate the most desirable brown soybean preserve. The preserve with higher values of total phenolic compounds and chroma a*, and lower values of instrumental hardness and luminosity was considered the most desirable.

3 Results and discussion

3.1 Physicochemical quality

The hardness of the brown soybean preserves varied 157% (between 8.6 and 23.0 N) (Table 1), which was influenced only by the linear ($p < 0.05$) and quadratic effects of the maceration time ($p < 0.01$) (Table 2).

The increase in ascorbic acid did not influence hardness ($p < 0.05$). The longer maceration time, less was the instrumental hardness (Figure 2). The reduction in the hardness of the grains is due to the water content absorbed by the leguminous grain in the maceration process, which causes less resistance to the grain (Pan & Tangratanavalee, 2003). Brown soybeans (BRM line 04-4382-4), classified as large grains (15 mm diameter), which needed 50 min in the maceration process with cooking for 15 min at 121 °C, showed hardness values (10.8 to 12.2N) similar to the those of the commercial preserve (Tables 1 and 3).

The antioxidant capacity of the brown soybean preserve ranged between 4.3 and 183.8 mgGAE100g⁻¹ (Table 1). The antioxidant capacity was significantly influenced by the linear effect ($p < 0.05$) of the ascorbic acid concentration in the brine; however, the quadratic effects of the ascorbic acid and the maceration time were kept in the model to improve the fit (Table 2, Figure 2B). These results suggest that the maintenance of the antioxidant capacity

Table 1. Hardness (N), antioxidant activity (DPPH*) (mg GAE 100g⁻¹), total phenolic compounds (mg GAE 100g⁻¹), luminosity (L*), chroma a* and chroma b* of brown soybean preserve in function of maceration time (min) (X₁) and ascorbic acid (g 100g⁻¹) (X₂). Experimental design.

Experiment	Maceration Time	Ascorbic Acid	Maceration Time	Ascorbic Acid	Hardness	DPPH*	Total Phenolic	L*	a*	b*
1	-1	-1	13.6	0.029	18.3	33.1	204.2	41.5	13.6	11.6
2	1	-1	85.4	0.029	8.6	33.2	204.4	43.2	13.6	12.5
3	-1	1	13.6	0.171	20.7	183.8	445.6	45.6	14.0	14.6
4	1	1	85.4	0.171	12.2	143.5	445.0	47.4	12.0	12.4
5	-1.41	0	0	0.1	23.0	57.1	231.0	42.8	15.4	15.1
6	1.41	0	100	0.1	9.4	72.6	351.0	46.2	14.5	15.4
7	0	-1.41	50	0.0	16.1	4.3	176.4	41.9	12.1	9.2
8	0	1.41	50	0.2	10.8	114.6	466.2	46.0	14.8	15.6
9	0	0	50	0.1	11.2	41.2	319.4	43.1	15.7	15.0
10	0	0	50	0.1	11.8	32.7	318.3	45.0	15.7	14.3
11	0	0	50	0.1	12.2	49.6	310.6	43.2	17.1	15.6

Table 2. Multiple regression models and determination coefficient of hardness (N), total phenolic compounds (mgGAE 100g⁻¹), antioxidant capacity (mgGAE 100g⁻¹), luminosity, chroma a* and chroma b* from brown soybean preserve in function of the maceration time (X₁) and ascorbic acid concentration (X₂), in encoded values.

Characteristic	Model	R ²
Hardness	$y = 12.57^* - 4.70 x_1^* + 2.02 x_1^{2**}$	0.88
Antioxidant capacity	$y = 41.01^{**} + 21.07 x_1^{2ns} + 52.21 x_2^* + 18.37 x_2^{2ns}$	0.85
Total phenolic compounds	$y = 315.69^* + 21.14 x_1^{ns} + 111.66 x_2^*$	0.95
Luminosity	$y = 44.18^* + 1.04 x_1^* + 1.77 x_2^*$	0.91
Chroma a*	$y = 16.19^* - 0.85 x_1^{2ns} - 1.60 x_2^{2*}$	0.75
Chroma b*	$y = 14.89^* + 1.31 x_2^* - 1.71 x_2^{2*} - 0.78 x_1 x_2^{ns}$	0.87

*p < 0.01; **0.01 ≤ p < 0.05; ^{ns}p > 0.10, kept to improve the model fit.

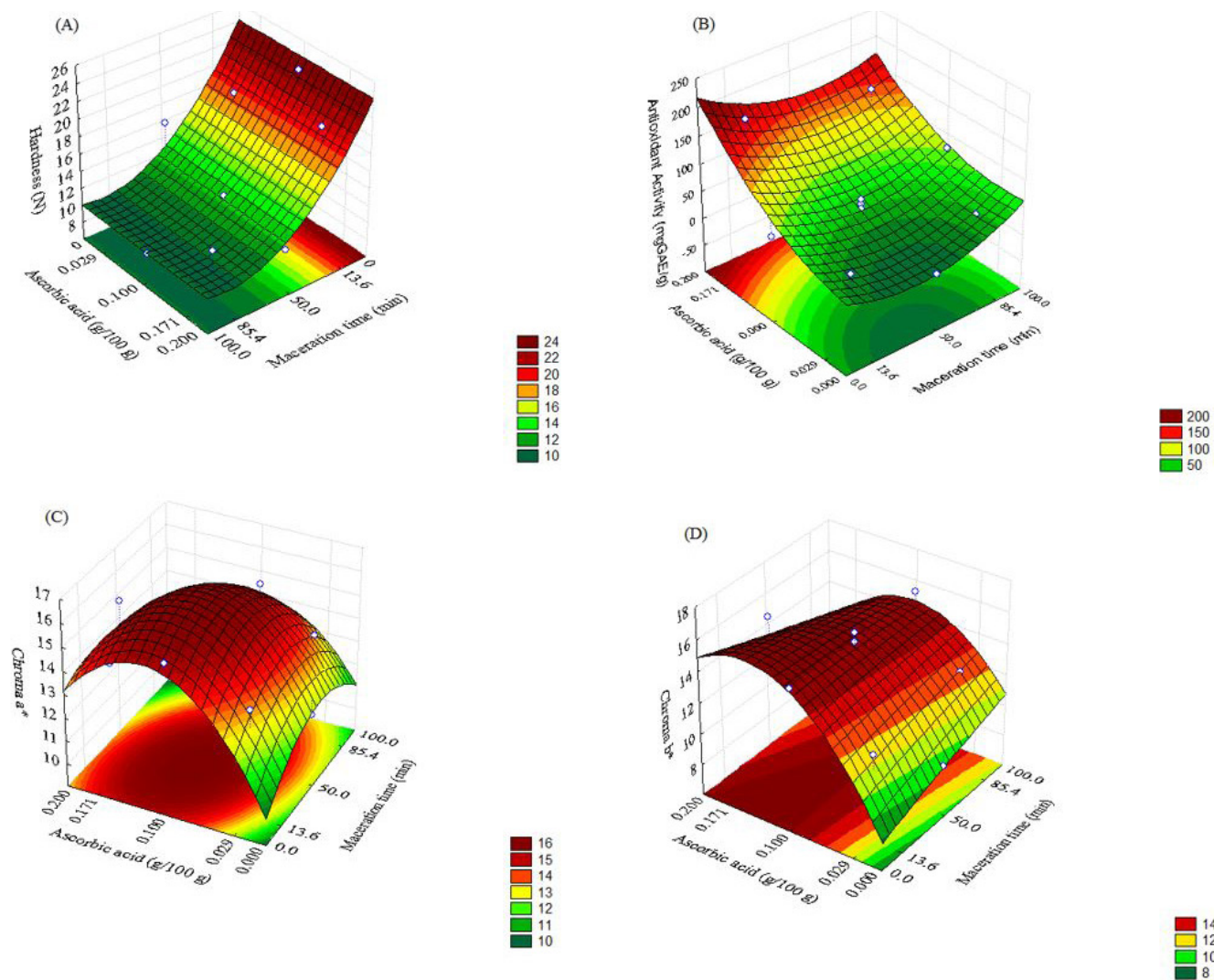


Figure 2. (A) Hardness, (B) Antioxidant activity, (C) Chroma a* and (D) Chroma b* from soybean grain (*Glycinemax*, BRM 04-4382-41 line), a function of the maceration time (mins) and ascorbic acid concentration (g 100g⁻¹).

of the brown soybean (antioxidant capacity of the brown soybean *in natura*: 200 mgGAE100g⁻¹) is due to the presence of ascorbic acid. It acts in the regeneration of primary antioxidants, and slows down lipid oxidation, which increases the antioxidant capacity of the product (Sogvar et al., 2016). The antioxidant capacity of these compounds is attributed to the reducing power of the aromatic hydroxyl group, which reduces reactive free radicals,

such as singlet oxygen, or decomposes peroxides to produce phenoxyl radical, a less reactive substance (Kim et al., 2015a).

The total phenolics of the brown soybean preserve ranged between 176.4 and 466.2 mgGAE100g⁻¹ (Table 1). These were also significantly influenced (p < 0.01) by the linear effect of the ascorbic acid concentration in the brine. However, to improve the model fit, the linear effect of maceration time was

maintained (Table 2). Brown soybean grain *in natura* presented 458.4 mgGAE100g⁻¹ of total phenolic compounds, which shows that in high concentrations, ascorbic acid contributes to the maintenance of these compounds in the preserve.

The maceration process contributes to the reduction of the antioxidant capacity of the grain and total phenolic compounds, since there is a loss of these compounds from leaching into the maceration solvent. This loss occurs because these are water-soluble compounds, which facilitates their extraction into the maceration water (Bayram et al., 2004a). In addition, heat treatment contributes to the increased loss of phenolic compounds by leaching of the phenolic hydrophilic compounds, degrading polyphenols, transforming phenolics and stimulating additional chemical reactions (Kim et al., 2015b). In particular, the loss of phenolic compounds significantly decreases when black soybeans are cooked rather than boiled, due to the amount of steam involved (Xu & Chang, 2008).

The luminosity of the grains varied 14.21% (Table 1). The linear effects of the maceration time ($p < 0.05$) and the ascorbic acid concentration ($p < 0.05$) in the brine significantly influenced the luminosity of the brown soybean preserve (Table 2). Water absorption of the soybean grains increased with the maceration process time, as did the loss of pigments into the maceration water, contributing to the increase in the luminosity of the brown soybean preserve. In addition, temperature also contributes to increasing the luminosity of soybeans; it causes changes in their structure due to the penetration of water and the gelling of the compounds (protein, starch, etc.) in soybeans (Bayram et al., 2004b).

Chroma a* of the grain varied 42.5% (Table 1), indicating a reddish hue in the soybean tegument. The quadratic effects of the maceration time ($p < 0.05$) and the ascorbic acid concentration ($p < 0.05$) in the brine influenced the color of the grain of the brown soybean preserve. From the response surface (Figure 2C), it was found that the redder color (higher values of chroma a*) was obtained with intermediate concentrations of ascorbic acid and maceration time (maximum area); in other words, it was obtained in the center of the response surface plot.

Chroma b* of the brown soybean preserve ranged between 9.2 and 15.6 (Table 1). The linear and quadratic effects of the ascorbic acid concentration in the brine of the brown soybean significantly influenced the chroma b* ($p < 0.05$); however, the interaction effect between the concentration of ascorbic acid

and the maceration time was maintained in order to improve the model fit (Table 2). The combination of a shorter maceration time and an increase in the concentration of ascorbic acid showed higher chroma b* values (Figura 1D), resulting in soybean grains in preserve with a more yellowish colour. In maceration, longer times predominated the quadratic effect; in other words, the highest values of chroma b* were observed at intermediate concentrations of ascorbic acid.

The increase in the reddish hue is due to the degradation of chlorophyll. The increase in the yellowish hue occurs due to the degradation effect of the temperature on the chemical structure of carotenoids. Broken carotenoid structures turn a reddish hue to a slightly yellowish one (short chemical structure) (Bayram et al., 2004b).

In the desirability test, a brown soybean preserve with lower hardness, a higher percentage of phenolic compounds and antioxidant activity, and more intense colors (more reddish and yellowish) was selected, and these characteristics were obtained with a maceration time of 23 min and 49s (- 0.705) and a concentration of ascorbic acid of 15 mg100g⁻¹ (+ 0.705). The maceration time and ascorbic acid content used in order to obtain the brown soybean with greater desirability were also used to produce the preserve and model validation test.

The hardness of the brown soybean preserve obtained during the validation test was 15.6N, approximately 8.2% lower than expected by the model (17N), so acceptable, and 34.5% higher than the hardness value of the commercial yellow soybean preserve. The difference between the estimated value and the model value for the antioxidant activity was low (3.1%). Antioxidant activity in the brown soybean grains *in natura* was 9.1 times higher than the selected soybean preserve. For the concentration of total phenolics, the model also showed little difference (2.5%) between expected and obtained results, showing a loss of 24.0% of the total phenolic compounds present in the grain *in natura* (Table 3).

Study on bean preserves subjected to autoclaving found a loss in total phenolic compounds (90%) (Pedrosa et al., 2015). This loss was higher than the one verified in this study, probably due to the incorporation of ascorbic acid into the brown soybean preserve. Studies have demonstrated that the phenolic composition of dark grains is related to the binomial time-temperature employed during the cooking process, which directly reflects on their neuroprotective and anti-carcinogenic activity. The cooking of

Table 3. Expected and measured values for hardness (N), antioxidant capacity (mgGAE 100g⁻¹), total phenolic compounds (mg GAE 100g⁻¹), luminosity, chroma a* and chroma b* of brown soybean preserve (*Glycine max*, BRM 04-4382-41 line), and hardness of commercial yellow soybeans preserve. Amounts in average followed by standard deviations.

Characteristic	Grain <i>in natura</i>	Preservecommercial ²	Expected values	Measured values
Hardness	ND ¹	11.6 ± 1.5	17.0	15.6 ± 0.9
Antioxidant capacity	210.9 ± 9.2	ND	198.0	191.8 ± 7.6
Total phenolic	487.14 ± 18.2	ND	380.0	370.4 ± 12.3
Luminosity	44.0 ± 1.71	ND	45.0	45.4 ± 3.64
Chroma a*	9.08 ± 1.29	ND	15.0	17.1 ± 1.02
Chroma b*	3.43 ± 1.52	ND	15.0	12.8 ± 1.22

¹ND (not determined); ²Yellow soybeans commercial preserve.

dark grains also improves their digestion and absorption at the intestinal level, maintaining protective capacity in the oxidative process at the cellular level (López et al., 2013).

The luminosity of the soybean grains in preserve obtained in the validation models was 0.9% higher than the value estimated by the model and 3.2% higher than the grain *in natura* (Table 3). The chroma a* obtained in the test was 14.0% higher than estimated by the model and 88.3% higher than the grain *in natura*. The value of chroma b* was 14.7% higher than estimated by the model and 3.7 times higher than the grains *in natura*. Therefore, the models for hardness, antioxidant activity, TPC, and luminosity can be considered predictive, due to the presented acceptable variations (below 10%). The results of this test on the selected preserve, compared with the values obtained from the grain *in natura*, indicate that processing has increased lightness; in other words, it has decreased the dark color of the brown soybean and also promoted an increase in reddish and yellowish hues in the grains of the soybean preserve.

3.2 Chemical composition of selected brown soybean preserve

The brown soybean preserve presented a moisture content six times higher than the grains *in natura* (Table 4). The high moisture content of the preserve is due to the stages of the maceration process and cooking, during which the grains absorb water. Silva et al. (2013) evaluated the proximal composition of raw and cooked beans and found the moisture value of the cooked grain to be 5 times higher than the grain *in natura* (raw); these values were below those of the brown soybean preserve.

Considerable ash wins were observed in the soybean preserve in relation to the grain *in natura* (Table 4), in dry basis. On the other hand, studies reported the loss of minerals during the processing of peas and beans. This decrease in ash content is related to the leaching of minerals into the maceration water and/or the cooking water of these grains studies (Wang et al., 2010; Pedrosa et al., 2015).

No losses in lipid, protein and carbohydrate contents during the processing of the brown soybean preserve were observed, if consider the dry basis (Table 4). Moreover, when comparing this result with the commercial yellow soybean, it was found that the lipid content was similar, while the protein and carbohydrate contents of the brown soybean preserve were higher than the commercial yellow soybean preserve (26.6 and 21.6%, respectively), showing the higher quality of the product. Wang et al. (2010) observed that increases in the concentrations of carbohydrates

and proteins in beans and chickpeas were due to the loss of soluble solids in the cooking water, which would increase the concentration of these constituents in the cooked product. And also report that the type of processing influences the proximal composition of the cooked product.

The processing of the brown soybean preserve increased the protein quality of the grain because the amino acid content of the preserve was higher than the values obtained from the grains *in natura*, especially concerning cysteine (conditionally essential), with 90.6% growth, and methyl-cysteine, with an increase of 59.9% (essential) (Table 5).

Heat treatments for food, such as immersion, boiling, cooking in a microwave and autoclaving, increase the total essential amino acids in leguminous grains. In particular, autoclaving increases significantly the total essential amino acids when compared to other processing methods (Khattab et al., 2009). Increases in amino acid content after heat treatment is the result of protein degradation. This finding is consistent with the results of the present study, in which the total content of essential amino acids increased in the brown soybean preserve after cooking with the application of heat (Table 5). A similar result was observed in soybeans cooked with rice (Kim et al., 2015b).

It has been found that higher concentrations of amino acids in the grains *in natura* and in preserve were from glutamic acid, aspartic acid, arginine and phenylamine+tyrosine (Table 5). Higher values of essential amino acids, which need to be provided by the diet, were found for phenylamine+tyrosine, and lower values for methionine+cysteine, for grains *in natura* and in preserve. Higher values of conditionally essential amino acids, in other words, those that become essential under certain pathological conditions or organism development, were found for arginine and proline, where the highest values were obtained for the soybean preserve. The brown soybean preserve showed higher values for all essential amino acids, when compared to the standard value suggested for healthy children and adults by the Food and Nutrition Board (2005). In short, the amino acid composition of the brown soybean preserve met the amino acids standard proposed for ages higher than or equal to one year, as recommended by the Food and Nutrition Board.

Trypsin inhibitor was present in the brown soybean grains *in natura* (15.734.18UI g⁻¹ ± 5.6), but it was not found in the brown soybean preserve (zero); in other words, the processing of the preserve inactivated the trypsin inhibitor. In a study on the effect of some physical treatments on the nutritional quality of beans and peas, Khattab & Arntfield (2009) reported that

Table 4. Moisture, ashes, protein, lipids and carbohydrates of the *in natura* grain and selected brown soybean preserve (*Glycine max*, BRM 04-4382-41 line). Average followed by standard deviation and variation coefficient.

Parameter	<i>in natura</i> grain	Preserve grain	Commercial yellow preserve ²
Moisture ¹	10.14 ± 0.04 (0.43)	64.87 ± 1.02 (1.58)	-
Ashes ¹	6.44 ± 0.23 (3.61)	4.28 ± 0.19 (4.43)	5.9
Lipids ¹	15.20 ± 0.11 (0.74)	5.97 ± 0.20 (3.27)	6.0
Protein ¹	34.66 ± 0.75 (2.17)	13.93 ± 0.04 (0.26)	11
Carbohydrates ¹	33.56	10.95	9

¹g100g⁻¹ (on a wet basis); ²Source extracted from commercial canned package label (wet basis).

Table 5. Essential, conditionally essential, non-essential and total amino acids of brown soybean fresh and pickled in mg amino acid/g protein and the proposed pattern of amino acids for infants, preschool children and adults based on estimated needs for protein and essential amino acids.

Essential Amino acids	Grain <i>in natura</i>	Preserve grain	Range %	Standard ¹		
				Infants ^a	1 to 3 years ^b	≥ 18 years ^b
Leucine	71.32	78.79	10.5	101	55	52
Lysine	60.97	64.27	5.4	69	51	47
Fenil + tirosina	80.14	89.56	11.8	87	47	41
Valine	45.59	51.79	13.6	56	32	29
Isoleucine	41.96	46.82	11.6	57	25	23
Threonine	40.42	44.13	9.2	47	27	24
Metio+Cisteína	26.42	42.25	59.9	38	25	23
Conditionally essential amino acids						
Tyrosine	33.57	35.13	4.6	-	-	-
Cysteine	13.87	26.44	90.6	-	-	-
Arginine	79.48	89.74	12.9	-	-	-
Glycine	40.27	44.06	9.4	-	-	-
Proline	45.9	51.8	12.9	-	-	-
Non-essential amino acids						
Glutamic acid	184.85	202.09	9.3	-	-	-
Aspartic acid	116.57	124.58	6.9	-	-	-
Serina	50.06	55.88	11.6	-	-	-
Alanine	41.49	44.00	6.0	-	-	-
TAA ^c	972.88	1091.33	12.2	-	-	-
TEAA ^d	366.82	417.61	13.8	455.00	262.00	239.00
TCEAA ^e	213.09	247.17	16.0	-	-	-
TNEAA ^f	392.97	426.55	8.5	-	-	-

¹Source: Adapted from Food and Nutrition Board (2005, p. 687); ^aStandard based on the amino acid composition of human milk; ^bStandard derived from the EAR to amino acid/EAR for protein; for group of 1 to 3 years protein EAR = 0.88 g/kg day and for adults protein EAR = 0.66 g/kg day. EAR is the estimated average requirement; ^cTAA= total amino acid; ^dTEAA= total essential amino acids; ^eTCEAA = total conditionally essential amino acids; and ^fTNEAA= total non-essential amino acids.

the maceration process and heat treatment participate in the reduction and inactivation of the trypsin inhibitor, respectively. Inactivation of trypsin inhibitors by heat treatment, due to protein denaturation, depends on the applied temperature and grain exposure time (Wang et al., 2010).

3.3 Microbiological risk and sensory evaluation

The microbiological counts of the brown soybean preserve were below the maximum limits established by Brazilian law: Thermotolerant *Coliforms* (10^2 CFU g^{-1}), *Staphylococci coagulase positive* (5×10^2 CFU g^{-1}) and *Salmonella sp.* (absence in 25 g). Furthermore, the brown soybean preserve, after 10 days of incubation at 35-37 °C and after 5 days of incubation at 55 °C, did not change the results obtained for the three evaluated microorganisms. The results suggest that the brown soybean preserve that was produced was a microbiologically safe product, reinforcing the hypothesis that raw materials and proper handling methods were used. The average scores and standard deviations obtained for the selected brown soybean preserve were 7.1 ± 1.5 for appearance, 7.2 ± 1.6 for texture and 7.1 ± 1.7 for flavor. All averages were between moderately liked and enjoyed. Therefore, the scores were above the preset limit for acceptance of the preserve, which was greater than or equal to six. Scores for purchase intent ranged between 3 (maybe buy / may not buy) and 4 (possibly buy). The tasters also reported a lack of soybean preserve consumption, which might have negatively affected the results.

4 Conclusions

The hardness of the grains in preserve is not affected by the addition of ascorbic acid, but only by the maceration time, reaching the same texture as the commercial soybean. Antioxidant activity and total phenolic compounds are preserved by the addition of ascorbic acid. The best brown soybean preserve is obtained with a maceration time of 23 min and an ascorbic acid concentration of $0.15 \text{ g}100\text{g}^{-1}$. The brown soybean preserve shows a high nutritional value, higher than the commercial yellow soybean preserve, amino acid contents above the standards recommend for ages higher than or equal to one year, absence of trypsin inhibitor and microbiological risk, as well as sensory acceptance. Therefore, the brown soybean preserve is viable, and its production and consumption are recommended.

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