Impact of Thermal Processing Parameters During Black Soymilk Extraction and Pasteurization on Key Bioactive Compounds and Antioxidant Capacity

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Abstract Heat treatments applied to soymilk during extraction and pasteurization may affect, among other aspects, the content of bioactive compounds. In this study, we identified favorable processing conditions during black soymilk production (extraction and pasteurization) to preserve key bioactive compounds and the antioxidant capacity (AC) of the black soybean. Temperature (80-98°C) and time (5-15 min), conditions for black soymilk extraction from (unpeeled) milled beans followed a 2² factorial design. Anthocyanins and isoflavones were analyzed by HPLC-PDA-reverse phase system. AC was measured by ORAC and DPPH assays. Results were treated by ANOVA and Fisher test at p ≤ 0.05. Soymilk samples were treated by batch pasteurization (98°C for 10 and 5 min, respectively) and ultra-high-temperature (UHT) (134°C for 6 sec). The best pasteurization parameters for high flavonoid contents and AC were chosen for the preparation of a chocolate-soymilk-beverage which was tested for sensory acceptability. Changes in temperature and time affected significantly the content of anthocyanins and AC. The best soymilk extraction and pasteurization conditions for anthocyanin and AC preservation were 80°C for 5 min and 98°C for 5 min, respectively, with 80% anthocyanins, 100% isoflavones, and 100% AC recovery compared to non-pasteurized control. The developed functional black soymilk beverage presented similar acceptance to a commercial organic yellow soy-based beverage.

Keywords: Glycine max (L.) Merrill, black soybean, pasteurization, anthocyanin, isoflavone, flavonoids

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1. Introduction

For hundreds of years, black soybean has been used both as healthy food and for the production of herbal extracts in oriental medicine [1], given its functional properties, which include strong antioxidative, anticancer, and glucose-lowering effects, to name a few. Such effects have been attributed to the joint action of several bioactive compounds, including the estrogen-like isoflavones, typically found in yellow soybeans, and, especially, the anthocyanins to which the black color of the beans is attributed [2,3,4,5].

The chemical composition of black soybeans may be affected by a number of variables, for example, genotype, agricultural practices, environmental factors, and processing [6,7,8]. In soymilk production, heating conditions are the most important variables. In addition to destroying spoilage microorganisms and reducing antinutritional factors, the heat treatments applied during extraction and subsequent pasteurization (low heat pasteurization) or ultrahigh-temperature pasteurization (UHT, also called sterilization), may influence the yields, color, flavor, nutritional value, the profile of bioactive compounds, and the medicinal properties of the milk [9,10,11,12]. In the case of black soymilk, the fact that anthocyanins are more susceptible to destruction compared to isoflavones must be considered. If on one hand low temperatures do not favor anthocyanins extraction, on the other hand, high temperatures can promote intense degradation of these compounds [13]. In
addition to the temperature issue, physical procedures commonly used to obtain yellow soymilk might not be suitable in the case of black soybean milk. For example, differently from yellow soymilk, which can be obtained both from unpeeled and peeled seeds, black soymilk must be prepared using unpeeled or whole seeds, given that anthocyanins are exclusively present in the seed coat [1]. Therefore, finding proper processing conditions is vital to preserve black soybean bioactive compounds and, consequently, its functional properties.

Another particularity about black soymilk is that, as with some other foods, the high amounts of anthocyanins tend to affect the visual appearance and consumer acceptance [14,15] and, therefore, this aspect has also to be considered when developing a functional product containing this ingredient. Chocolate seems to be an adequate ingredient to mask the grayish color of the milk and also tends to improve flavor [16].

Considering all aspects aforementioned, the present study aimed to identify favorable thermal conditions during black soymilk extraction and pasteurization, to preserve key bioactive compounds and the antioxidant capacity (AC) of black soybean. Additionally, we tested the sensory acceptability of the black soybean milk by preparing a chocolate-milk beverage and comparing it with similar traditional yellow chocolate-milk beverages available in the Western market.

2. Materials and Methods

The study’s experimental design is shown in Figure 1.

![Figure 1. Study experimental design](image-url)
2.1. Raw Materials

Black soybean [Glycine max (L.) Merrill], line BRM09-50995, a bean with yellow cotyledon with a black seed coat, was developed by Embrapa, Brazil, as part of the Soybean Breeding Program for Human Consumption. The beans were harvested in Passo Fundo-RS, Brazil. For the chocolate-milk beverage preparation, a commercial powdered chocolate mix, containing sugar, powdered cocoa, malt extract, salt, powdered whey, non-fat powdered milk, vitamins, flavorings, and soy lecithin, was used. This mixture was comparable to those used in similar commercial products. Filtered water was used for milk extraction and chocolate drink beverage preparation.

2.2. Soymilk Extraction and Freeze Drying

Unpeeled black soybeans were milled (Hammer Mill, Perten Laboratory Mill 3100) to pass a 0.8 μm sieve. Based on extraction and cooking conditions reported for yellow and black soymilk in different studies [8,17], the milled beans were immersed in water (ratio 1:10 w/w, seed: H2O) at different temperatures (80 - 98°C) and times (5- 15 min) defined by a 2 factorial design, with three replicates at the central point, resulting in seven randomized assays (Figure 1). After cooking, the slurry was homogenized in a Waring® blender for 2 min, at low (1700 rpm) speed, and centrifuged (IEC Model K7165, 150 μm nylon filter) at 4000 rpm; for 5 min, to obtain the soluble extract. Black soymilk was freeze-dried (K120 Liobras) at – 97°C and 20 μHg vacuum, and stored at – 18°C, for analyses of isoflavones, anthocyanins, and AC.

2.3. Flavonoids Extraction for Chromatographic Analyses

For anthocyanin extraction, the freeze-dried samples were reconstituted for chemical analyses. The powder (1 g) was reconstituted with 20 mL methanol/water/hydrochloric acid (60:39:1) in duplicate, and placed in a water bath (IKA HEIZBAD HB-250), at 50°C, for 1 h, followed by centrifugation (SORVALL LEGEND Centrifuge XRT) for 10 min at 12,000 rpm, room temperature [18]. The supernatant volume was completed to 50 mL, with the extraction solvent mixture, filtered through a 0.45 μm of pore size PTFE membrane (IPRO, Hangzhou, China), and stored in the dark at –20°C.

Extraction of isoflavones was performed according to AOAC [19], method 2001.10. The compounds were separated in a C18YMC column (5 μm, 4.6mm × 250 mm), kept at 30°C. A gradient of acetonitrile and aqueous formic acid (2 mL/100mL of acetic acid (1.3 mL/min). UV-Vis spectra were obtained between 200 and 400 nm, and the chromatograms were processed at 520 nm. Anthocyanins were identified considering the chromatographic performance and UV-Vis spectra data as compared to authentic standards analyzed using the same conditions. Anthocyanin identification was also confirmed by accurate molecular mass data obtained by mass spectrometry, using a Synapt G1 spectrometer (Waters), equipped with electrospray ionization source and quadrupole in series, with a time-of-flight (TOF) as a mass analyzer (ESI-qTOF) [20]. Quantification was performed by external calibration, using delphinidin-3-O-glucoside (PubChem CID:102513539), cyanidin-3-O-glucoside (PubChem CID: 12303203), and petunidin-3-O-glucoside standards (PubChem CID: 443651) (all with purity higher than 99%), which were isolated from açaí and confirmed by mass spectrometry [21].

Isoflavones were analyzed according to the AOAC [19] method 2001.10. The compounds were separated in a C18YMC column (5 μm, 4.6mm × 250 mm), kept at 45°C, by using a gradient of water/methanol, both with 2 mL/100mL of acetic acid solution (1.3 mL/min). UV-Vis spectra were obtained between 200 and 400 nm, and the chromatograms were processed at 260 nm. Identification of isoflavone aglycones (daidzein-PubChem CID: 5281708; genistein-PubChem CID: 5280961, and glycitein - PubChem CID: 5317750) and glycosides (genistin - PubChem CID: 5281377, daidzin - PubChem CID: 107971; and glycitin-PubChem CID: 187808), all from Sigma-Aldrich, St. Louis, MO, USA, was performed considering the chromatographic behavior and UV-Vis spectra, as compared to authentic standard analyzed at the same conditions. Quantification was carried out by external calibration using isoflavone aglycones, and glycosides standards. Results of total isoflavones were expressed as aglycones equivalents, by summing concentrations of genistin, daidzin, and glycitein and of the aglycones of the respective glucosides genistin, daidzin, and glycitin, as described in AOAC[19].

2.4. Chromatographic Analyses of Flavonoids

The analyses of flavonoids were performed in a high-performance liquid chromatography system (HPLC, Waters® Alliance 2695, Waltham - WA, USA), equipped with a photodiode array detector (PDA, Waters 2996). Data acquisition and processing were performed using Empower® software (Waters).

Anthocyanins were analyzed according to Gouvêa et al. [20]. Separation was performed in a Thermo® Scientific C18 BDS column (100 mm × 4.6 mm; 2.4 μm) kept at 30°C. A gradient of acetonitrile and aqueous formic acid (5 g/100g) was used as the mobile phase. The UV-Vis spectra were obtained between 200 and 600 nm, and the chromatograms were processed at 520 nm. Anthocyanins were identified considering the chromatographic performance and UV-Vis spectra data as compared to authentic standards analyzed using the same conditions. Anthocyanin identification was also confirmed by accurate molecular mass data obtained by mass spectrometry, using a Synapt G1 spectrometer (Waters), equipped with electrospray ionization source and quadrupole in series, with a time-of-flight (TOF) as a mass analyzer (ESI-qTOF) [20]. Quantification was performed by external calibration, using delphinidin-3-O-glucoside (PubChem CID:102513539), cyanidin-3-O-glucoside (PubChem CID: 12303203), and petunidin-3-O-glucoside standards (PubChem CID: 443651) (all with purity higher than 99%), which were isolated from açaí and confirmed by mass spectrometry [21].

2.5. Antioxidative Capacity (AC) Assays

The AC was estimated using two assays, oxygen radical absorbance capacity (ORAC) and DPPH, from the compound 2,2–diphenyl-1-picrylhydrazyl. The extracts used for anthocyanins analyses were also used for both ORAC and DPPH assays. The assays were performed in duplicate.

ORAC was conducted according to Huang et al. [22]. Briefly, 25 μL black soymilk extracts were diluted with phosphate buffer saline (pH 7.4) to proper concentrations to fit the linearity range of the standard curve. Blank and standard solutions were loaded to a 96-well plate added of 150 μL fluorescein (61.2 nM final concentration). The plate was kept at 37°C for 30 min. Following, 25 μL of
2.2'-Azobis(2-methylpropionamidine) dihydrochloride 97% (AAPH) (Sigma-Aldrich) (19.1 mM final concentration) were added to each well, and the fluorescence was monitored at 528 nm and 37°C, for 60 min. AAPH was used as the peroxyl generator and quercetin (PubChem CID 329823865, Sigma-Aldrich, 97% purity) as a reference standard because of its higher chemical similarity to anthocyanins as compared to Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), commonly used as a reference antioxidant compound in this method [22]. Both AAPH and quercetin solutions were prepared with phosphate saline buffer (pH 7.4).

The AC of black soymilk samples against DPPH radical was measured according to Brand-Williams et al. [23]. Briefly, 0.1 mL of different dilutions of the extracts reacted with 3.9 mL of DPPH (75 µM) methanolic solution for 15 min. The absorbance was measured at 515 nm, using an Agilent 8453 spectrophotometer. A decrease in sample absorbance indicated an increase in DPPH radical scavenging activity. AC was expressed as the amount of extract required to reduce 50% of the initial concentration of DPPH (EC50).

2.6. Pasteurization

Samples with lower losses of bioactive compounds and higher AC were submitted to three different pasteurization treatments (Figure 1). Treatments 1 (T1) and 2 (T2) were performed according to Felberg et al. [17], with modifications. Briefly, fresh prepared black soymilk was submitted to boiling temperatures, 98°C, for 5 and 10 min, respectively. For treatment 3 (T3), a heat exchanger (model FT25D Armfield, UK) was operated at 132°C for 6 sec (UHT), similar to the conditions used by Zhang et al. [24]. After these three treatments, samples were analyzed again for flavonoid contents and AC.

2.7. Black Soy Chocolate Beverage Product versus Commercial Product Based on Yellow Soybean Milk

This section of the study was approved by the Ethics in Research Committee from the Rio de Janeiro University (UERJ) (Reg #. 55617516.2.0000.5282).

A consumer acceptance test was carried out by 100 assessors (60 female and 40 male, aged 18-68 years old, randomly recruited among staff and students of Embrapa Food Technology who eventually or rarely consumed soy milk. Assessors were asked to evaluate three samples: (1) the experimental black soymilk formulated with a commercial powdered chocolate mix (2) the leading brand of chocolate-flavored yellow soymilk in Brazil, and (3) an organic commercial chocolate-flavored yellow soymilk. It is worth noting that commercial yellow soymilk beverages were used for comparison in this study because there are no black soy-milk products available in the Brazilian market.

The soy beverages were evaluated for overall acceptability using the classical 9-point structured hedonic scale, where 1 referred to “dislike extremely” and 9 to “like extremely” [25]. Samples were served cold (8 ± 2°C) in 50 mL plastic cups coded with three-digit numbers, and the order of presentation was balanced to prevent carryover effects [26]. Spring water and cracker were provided for mouth rinsing between samples.

2.8. Statistical Analysis

STATISTICATM, version 13.0 for Windows (Tulsa, USA), was used for the experimental design and statistical analyses. Results from flavonoid content and AC assays were treated by analysis of variance (ANOVA) followed by Fisher’s test (LSD) to verify significant differences among means. Differences were considered when p ≤ 0.05. For sensory analysis data, in addition to ANOVA and LSD tests (p ≤ 0.05), hierarchical cluster analysis was used to identify consumers with different acceptance scores for soymilk beverages. Euclidean distances and Ward’s aggregation criterion were considered. Results are presented on a dry weight basis.

3. Results and Discussion

3.1. Anthocyanins

Three anthocyanins were identified in the black soymilk samples: cyanidin-3-O-glucoside (the main, anthocyanin), delphinidin-3-O-glucoside, and petunidin-3-O-glucoside, agreeing with the scarce literature data on both black soybean and soymilk [1,7,27-30].

The interaction between time and temperature conditions used for soymilk extraction affected (p = 0.0079) the anthocyanins contents. Extraction conditions of 80 °C/5 min and 98 °C/5 min provided the highest final content of anthocyanin while 80 °C/15 min and 89 °C/10 min, provided the lowest (Table 1).

| Black soymilk | Anthocyanins (mg/100g) | Isoflavones* (mg/100g) | ORAC (μmol QE/g) | DPPH EC50** (μmol QE/g) |
|--------------|------------------------|------------------------|------------------|------------------------|
| 80 °C/5 min  | 56.22 ± 0.35*          | 139.97 ± 2.53*         | 23.17 ± 0.00     | 0.44 ± 0.06            |
| 80 °C/15 min | 43.30 ± 1.25*          | 146.42 ± 2.34*         | 17.93 ± 0.32     | 0.33 ± 0.04             |
| 98 °C/5 min  | 52.70 ± 1.01**         | 148.28 ± 2.15*         | 18.45 ± 0.94     | 0.42 ± 0.03             |
| 98 °C/15 min | 49.19 ± 1.97**         | 138.74 ± 6.24*         | 19.23 ± 0.62     | 0.38 ± 0.02             |
| 89 °C/10 min | 43.10 ± 2.10           | 145.24 ± 5.19          | 17.04 ± 1.05     | 0.34 ± 0.03             |

Mean values ± standard deviation of the mean (mg/100 g of dry weight basis). Different letters in the same column are statistically different (p ≤ 0.05), by Fisher’s test. *Aglycone equivalent. **EC50 = concentration of extract (mg·mL⁻¹) required to scavenge 50% of the initial DPPH• concentration. ***QE = quercetin equivalent.
Heat temperature associated with processing time showed degrading effects on black soybean anthocyanins’ stability and content. This behavior is similar to those described for other anthocyanin-rich foods such as blackberry, cherry, and acai fruit, among others. [31-35]. The variety of existing methods used to obtain black soymilk makes it challenging to compare the content of bioactive compounds among published data. Xu and Chang [10] studied the effect of cooking time (120 minutes) at boiling temperature on the anthocyanin composition of black soybean and observed a great loss of these compounds. While in the raw material they reported 36.5 mg/100g of cyanidin-3-O-glucoside and 6.3 mg/100g of peonidin-3-O-glucoside, only 1.0 mg/100g of cyanidin-3-O-glucoside and no peonidin-3-O-glucoside were detected in cooked black soybeans after thermal processing.

3.2. Isoflavones

The interaction between temperature and time affected the contents of isoflavones in black soymilk during extraction (p = 0.0361) (Table 2). Longer periods were inadequate for higher temperatures. There are no data available on the combined effect of these two parameters on isoflavones for black soybean, but previous studies on the influence of processing time and temperature on yellow soymilk’s isoflavones contents have shown no consensus. Baú and Ida [36] evaluated the effect of thermal treatment (97 ± 2°C) and time (0-25min) on soymilk isoflavones. The contents of daidzein and genistein aglycones were maintained, glycitein and β-glucosides contents increased, and malonyl glucoside content decreased, demonstrating that they were converted but not degraded. No difference was observed in the total content of isoflavones after 5 min. In another study, however, Tan et al. [37] characterized the effect of cooking (100°C for 20 minutes) on yellow soymilk isoflavones and other phenolic compounds and found that cooked soymilk presented lower total isoflavones contents than raw soymilk. In a similar study, Xu and Chang [32] observed different behaviors for two evaluated cultivars: one cultivar had a significant decrease in isoflavones content after cooking, while the other did not show a significant loss after processing.

3.3. Antioxidant Capacity

The AC of black soymilk sample was evaluated by ORAC assay and the values were expressed in quercetin equivalent (QE) per gram of freeze dried black soymilk. For the first time in black soybean literature, quercetin was used as a reference antioxidant owing to its structural similarity with anthocyanin. Both quercetin and anthocyanin are flavonoids that show the same basic structure derived from benzo-γ-pyrone, whereas Trolox, the most commonly used reference standard for relative AC measurement, is a synthetic water-soluble vitamin E analog, with a different chemical structure from flavonoids (Figure 2) [38,39].

| Thermal Treatments | Anthocyanins (mg/100g) | Isoflavones** (mg/100g) | ORAC (μmol QE***/g) | DPPH EC50** |
|-------------------|------------------------|-------------------------|---------------------|-------------|
| Control*          | 40.72 ± 2.74a          | 142.51 ± 8.16a          | 25.53 ± 0.26a       | 0.41 ± 0.04a |
| T1                | 34.02 ± 0.83b          | 140.71 ± 1.73a          | 24.45 ± 0.56a       | 0.37 ± 0.00a |
| T2                | 32.75 ± 0.67b          | 141.16 ± 1.07a          | 25.46 ± 0.82a       | 0.40 ± 0.00a |
| T3                | 32.76 ± 0.54b          | 144.79 ± 2.69a          | 19.58 ± 0.26b       | 0.21 ± 0.02b |

Mean values ± standard deviation of the mean (mg/100 g of dry weight sample ou mg/100g of dry sample). Different letters in the same column are statistically different (P < 0.05), by Fisher’s test. Treatments:*Not submitted to any treatment; **Aglycone equivalent.; T1: 98 °C/5 min; T2: 98 °C/10 min; T3: UHT (132 °C/6 s).

Figure 2. Comparative chemical structure of a: Cyanidin, b: Quercetin and c: Trolox. URL (http://www.chemspider.com).
The highest AC was observed in the extracts submitted to a lower temperature for a shorter time (Figure 3A). This is in agreement with that reported by Huang et al. [22], who compared the AC of various pure chemicals by ORAC values expressed as TE, and estimated 7.1 ± 0.2 TE for quercetin’s AC.

The ORAC value for quercetin (6.7 ± 0.8 Trolox equivalents-TE), was also determined to facilitate comparison with literature results. Considering the ORAC value of quercetin, the results obtained for black soymilk were also estimated in TE to enable comparison with previous results of black soybean and black soymilk. The estimated ORAC values varied from 105.9 to 155.4 μmol TE/g of freeze-dried black soymilk. Zhang et al. [29] evaluated sixty black soybean varieties and found ORAC values ranging from 424.0 μmol TE/g to 1274.0 μmol TE/g. In another study with black soymilk, Xu and Chang [32] found 55.1 μmol TE/g in raw soymilk, 42.8 μmol TE/g for milk cooked in a stove (100 °C/20 minutes), and 50.3 μmol TE/g for milk processed by UHT (143 °C/60s). Therefore, the AC values obtained for different processing conditions are within the range of values reported in previous studies on black soybean. In addition to analytical conditions, such wide variation in the values may be attributed to differences in cultivars, environmental conditions, and processing [6,7].

The AC of black soymilk sample was also evaluated by DPPH assay. Considering that DPPH radical scavenging activity was expressed as EC50, with lower values meaning higher AC, samples cooked for longer time (10 and 15 min) showed higher AC than those submitted to short cooking time (5 min) (Figure 3 B). These results suggest that isoflavones (which were resistant to cooking) contributed more significantly to the AC estimated by DPPH than anthocyanins. This is in accordance with the results from the study by Xu and Chang [32] who observed a positive correlation between isoflavones and DPPH levels in black and yellow soymilks obtained from different procedures [32,40]. The increase in AC with longer cooking time may be attributed to the formation or release of new compounds with high AC [32,40].

3.4. Soymilk Pasteurization Treatments x Bioactive Compounds and Total Antioxidant Capacity

After extraction, black soymilk was pasteurized by three methods (Figure 1). The anthocyanin contents were significantly lowered by all heat treatments (p = 0.0002) when compared to control. This result is consistent with the fact that anthocyanins are sensitive to heat [33]. Isoflavones, however, were not degraded by batch pasteurization nor UHT conditions (Table 2). As previously described, isoflavones are quite stable to heat, although heat exposure can change the profile of aglycones and glucosides [41,42,43].

ORAC assay results also showed that two pasteurization treatments (T1: 98°C/5 minutes and T2: 98°C/10 minutes) did not differ from control, while UHT (T3: 132°C/6 seconds) presented lower (p = 0.0000) AC, probably because of the ultra-high temperature, despite the lower time. Xu and Chang [10] also observed a reduction in ORAC values, while processing black soybean in boiling water for 120 minutes. In the present study, similar results were obtained for DPPH assay, except for the fact that the sample treated by UHT (T3 - 132°C/6s) showed a lower EC50 value and consequently, higher AC by this method. Interestingly, Xu and Chang [32] also observed this behavior in AC for UHT-treated black soymilk (1.73 μmol TE/g) compared to control (1.19 μmol TE/g) and attributed this result to the formation and changes in antioxidant compounds, as well as to the generation of bioactive peptides capable of increasing the AC estimated by DPPH and not by ORAC.

3.5. Consumer Acceptance Test

Figure 4 contains the overall consumer acceptance scores of the three tested beverages. Because of the grayish tone of black soybean caused by high levels of anthocyanins, chocolate was used to improve the visual appearance of the beverage. The acceptability of the beverage was tested compared to a leading commercial brand of yellow soybean milk beverage in the Brazilian market and with a similar commercial organic beverage (Figure 4). The leading commercial brand reached the highest mean acceptance value (6.9 ± 1.8, p = 0.0001), while the organic brand and the black soymilk formulation
scored similarly (4.5 ± 2.4 and 4.3 ± 2.3, respectively). Differences in acceptance are attributed to the product formulation and characteristics of assessors. Deshpande et al. [44] evaluated 28 chocolate-flavored peanut-soy beverages using a 9-point hedonic scale and the overall acceptability was between less than 4 to less than 6.5. Although Wang et al. [45] demonstrated that chocolate flavoring enhanced the overall sensory quality of soy beverages, usually, soy products do not present high hedonic scores in the literature, especially when assessors are not regular consumers [46]. In the present study, only 14% of assessors were habitual soymilk consumers (of which only 2% were daily consumers), 40%, occasionally consumed and 44% never or rarely consumed it. When three clusters were produced based on the frequency of consumption, in the cluster with the higher frequency of consumption (24% of assessors) the black soymilk product scored, on average, 6.7.

Figure 4. Percentage distribution of Acceptance test scores for chocolate-flavored soymilk beverages (1 = leading commercial brand, 2 = black soymilk beverage, 3 = organic commercial brand). Scores refer to a 9-point hedonic scale (Stone & Sidel 2004)

Considering that no improvement was performed in the formulation other than the addition of commercial powdered chocolate mix, and that it presented similar acceptability compared to the organic commercial soy beverage established in the market, the present results suggest that this and other functional black soymilks can be introduced in the Brazilian health food market. It is worth emphasizing that the tested formulation can be improved to obtain a black soymilk beverage with sensory attributes according to consumer preferences.

This scenario among assessors certainly contributed to low scores. The authors strongly believe that by improving the beverage formulation the acceptance among regular soy milk consumers could be comparable to the leading product and with the bonus of anthocyanins.

3.5. Conclusions

For the first time, it was shown that temperature and time can negatively impact anthocyanins content during black soymilk extraction, which did not occur for isoflavones. The best black soymilk extraction condition for anthocyanin and antioxidant capacity preservation was 80°C for 5 min and the best pasteurization condition was 98°C for 5 min, with 80% anthocyanins, 100% isoflavones and 100% AC recovery compared to non-pasteurized control. The developed black soymilk beverage presented similar acceptance to the commercial organic yellow soy-chocolate beverage.

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Statement of Competing Interests

The authors declare no competing interests.

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