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Article

Formulation of Black Soybean Yogurt and Evaluation of Changes in the Bioactive Profile and Other Compositional Aspects During Fermentation and Storage

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Abstract

Black soybean is known for its antioxidant and anti-inflammatory properties that help prevent several degenerative diseases, but in the Western diet, it is poorly used, despite the interest in foods rich in bioactive compounds. This study aimed to formulate a black soybean yogurt (BSY) fermented by a probiotic culture of *L. acidophilus* and evaluate the nutritional and bioactive profiles, the total antioxidant capacity, and complementary parameters during fermentation and storage for one month. We also evaluated the potential for acceptance by Rio de Janeiro consumers ($n = 103$). The final BSY water content was 92.8%. The dry matter contained 50.2% protein, 20.1% lipid, 5.9% ashes, 23.8% carbohydrates, and other constituents, including 1% sucrose, 5.9% α-galactosides, 26.9 mg/100 g anthocyanins (mainly cyanidin-3-glucoside), 140.5mg/100 g isoflavones (mainly genistin and daidzin). Titratable acidity was 0.44% and pH 4.5. In the sensory test, 12% sucrose and fruit extracts (strawberry, prune, and grape) were added individually to the product to evaluate the acceptability. The sweetened strawberry extract offered the highest acceptability, with a 7.6 score in a nine-point hedonic scale, against a 5.6 of the sweetened control with no fruit extract. Furthermore, all products scored well in the clusters with assessors who consumed soy products often and daily (total $n = 26$), with the strawberry-flavored one scoring, on average, 8 or 9. One month storage at 8 ± 2 °C caused a 22% decrease in the anthocyanins content and no significant change in isoflavones, titratable acidity, and pH. Fermentation and the addition of a sweetened fruit extract proved to be promising tools to increase the consumption of black soy milk in the West.

Keywords: black soybean; *Glycine max* (L.) Merrill; fermentation; probiotic; flavonoid; isoflavone; anthocyanin; antioxidant activity; sensory acceptance; α-galactosides

Academic Editor: Adriana Dantas

Received: 30 April 2025
Revised: 13 June 2025
Accepted: 25 June 2025
Published: 2 July 2025

Citation: Esteves, T.C.F.; Felberg, I.; Farah, A.; Faria-Machado, A.F.d.; Walter, E.H.M.; Santiago, M.C.P.d.A.; Pacheco, S.; Antoniassi, R.; Deliza, R.; Carrão-Panizzi, M.C.; et al. Formulation of Black Soybean Yogurt and Evaluation of Changes in the Bioactive Profile and Other Compositional Aspects During Fermentation and Storage. *Beverages* **2025**, *11*, 103. https://doi.org/10.3390/beverages11040103

1. Introduction

Soy foods combine basic nutritional functions with beneficial health effects due to several functional components, including isoflavones and other polyphenols, peptides, fiber, and additional compounds [1]. Soybeans contain various colors of seed coat, including yellow, green, brown, and black, due to the presence of chlorophyll, anthocyanins, and other pigments [2,3]. Black soybean (*Glycine max* (L.) Merr.) has a black seed coat because of the anthocyanins concentration, which increases soybean antioxidant property compared to other colored soybeans [3]. This variety has been widely utilize for different purposes, including detoxification and anti-inflammatory effects, particularly in Asian countries such as China, Japan, Korea, Indonesia, and India [4–7]. Its regular consumption potentially promotes several health-related benefits, including anticancer, anti-atherosclerosis, and coronary heart disease, anti-diabetic, and anti-obesity properties, among others [1,4,8]. Despite the potential health benefits, black soybeans are still not widely used in the West [9]; however, more recently, their health benefits have been gaining attention. Black soybeans can be processed into different products, including soymilk, resulting in a nutritious and popular beverage, which is suitable for vegetarians, vegans, and lactose-intolerant individuals [10].

During the production of soymilk, the generated residue, called okara, is also reported to have a good amount of nutrient content and phytochemicals with antioxidant activity. In addition, according to Anjum et al. [2], black okara has higher nutritional and economic value than yellow okara and is suitable for use in different food products, oil extraction, and nutraceutical, pharmaceutical, and cosmetic formulations. A prebiotic effect on the gastrointestinal tract in adults and other positive effects on the cardiovascular system and brain function have been demonstrated [11].

Fermentation is known to bring nutritional and sensory advantages to soybean products. Fermented soy-based products contain microbial hydrolytic enzymes, such as α-galactosidase, β-glucosidase, and proteases, which contribute to better nutritional profiles. Free sugars (fructose, glucose, and galactose) and organic acids (acetic and lactic acids) are produced during fermentation, conferring pleasant sensory characteristics and improving the acceptability of soymilk [12–14]. Moreover, in soybeans, isoflavones are present mainly in the glycosylated form, while their biological activity occurs as aglycones. The bioavailability of glycosidic isoflavones depends on the hydrolysis of the sugar by the intestinal β-glucosidases and by β-intestinal microbial glycosidases. The removal of the sugar moiety by microorganisms promotes quicker and more efficient absorption than in the form of glucoside conjugates [15,16]. In this context, fermentation to produce soybean yogurts is a promising treatment to improve the functional value of the beverage. Only rare studies have been published on fermented black soybeans [9,17–20]. These studies have mainly evaluated the content of polyphenols and oxidative parameters. More recently, Hasan et al. [21] studied the changes in the chemical composition, bacterial viability and sensory profiling of fermented soymilk during 28 days storage at 4 °C. Rahmawati et al. [22] determined the effect of fermentation on the isoflavone profile of black soy yogurts and on their acceptance by a sensory panel. Suhaimi et al. [23] studied the effect of different extraction methods on the antioxidant and sensory properties of pasteurized black soymilk. The growing number of studies, including those on black soybean products, has resulted in recently published reviews [7,14,24,25].

The objective of the present study was to formulate a black soybean yogurt and evaluate the nutritional and bioactive profiles, as well as the antioxidant capacity of the product, in addition to complementary parameters, during fermentation and storage for a month. The potential for acceptance by Rio de Janeiro consumers was also evaluated.

2. Materials and Methods

2.1. Experimental Design

The study's experimental design is shown in Figure 1. Black soymilk (BSM) was extracted from black soybeans (BS), and the black okara (BO) was collected for analysis. BSM was pasteurized, and a probiotic culture was added, which was fermented to produce a black soymilk-based yogurt (BSY). BSY was characterized by pH, acidity, carbohydrates, and colony count. An aliquot of the yogurt was freeze-dried and stored for chemical and physicochemical analyses. Different sweetened fruit extracts were also added to other aliquots of the soybean yogurt for consumer acceptance evaluation. Unflavored BSY was stored for 0, 14, and 28 days. Samples were collected for chemical and physicochemical analyses and antioxidant activity measurement.

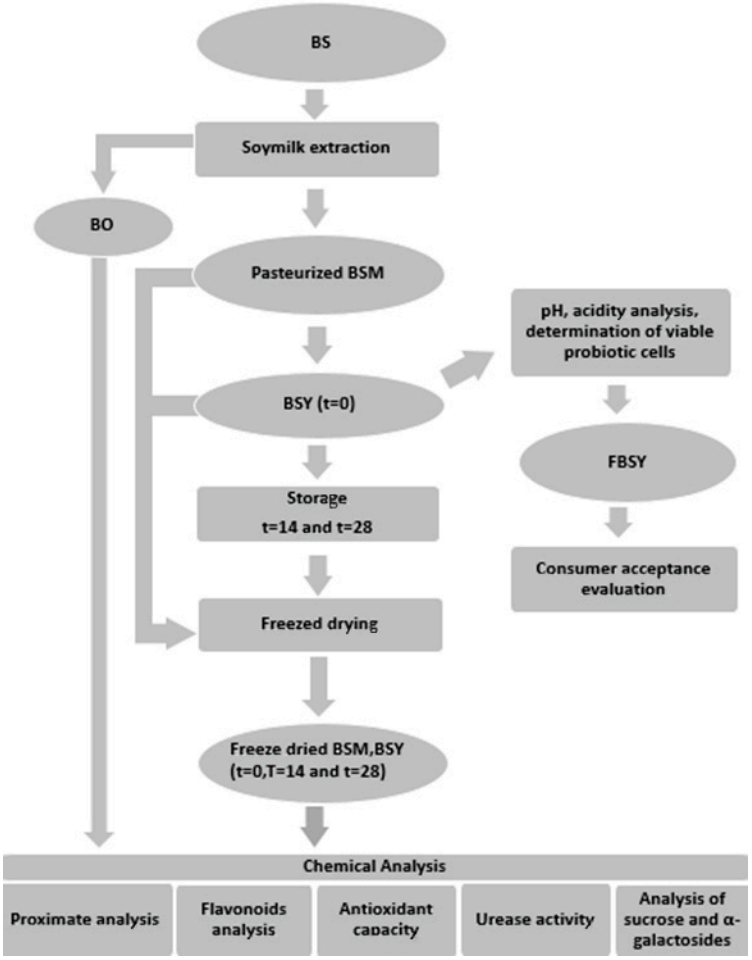

Figure 1. Study design. BS-black soybean; BO-black okara; BSM-black soymilk; BSY-black soybean yogurt; FBSY-flavored black soybean yogurt.

2.2. Raw Materials

Black soybean (BS) (*Glycine max* (L.) Merrill), line BRM09-50995, a bean with yellow cotyledon and a black seed coat, was developed by Embrapa, Brazil, as part of the Soybean Breeding Program for Human Consumption. Beans were harvested in Passo Fundo-RS, located in the south of Brazil. Probiotic *L. acidophilus* culture Nu-trish® LA-5® (Chr. Hansen Indústria e Comércio, Valinhos, SP, Brazil), commercial natural extract of prune, strawberry, and grape (Duas Rodas Industrial Ltd., Jaraguá do Sul, SC, Brazil), and commercial sugar (Açúcar União, Camil Alimentos S/A, Barra Bonita, SP, Brazil) were also used in the study.

2.3. Product Development and Storage Conditions

2.3.1. BSM Processing

BSM was extracted according to Esteves et al. [26]. BS was selected, washed with tap water, dried in an oven at 70 °C for 5 min, and ground in a hammer mill (Perten Instruments Mill 3100, Huddinge, Sweden). Then, the ground beans were immersed and kept in a water bath (1:10 p/p) at 80 °C for 5 min. After cooking, the slurry was homogenized in a Waring® blender for 2 min., at low speed (1700 rpm), and centrifuged (International Equipment Company-IEC, Model K7165, Needham Heights, MA, USA) at 4000 rpm, for 5 min in a 150 µm nylon filter (IEC, USA), to obtain the soymilk. Following, soymilk was pasteurized at 98 °C for 5 min., cooled in an ice bath until reaching 45 °C, and kept still in a thermostatic bath at 42 ± 2 °C for fermentation. An aliquot of pasteurized black soymilk prior to fermentation was freeze-dried (K120 Liobras, São Carlos, SP, Brazil) at−97 °C and 20 µHg vacuum and stored at −18 °C for analyses of isoflavones, anthocyanins, and antioxidant capacity (Figure 1).

2.3.2. BSY and Flavored BSY Production

The first stage of the fermentation process consisted of preparing the lyophilized probiotic culture inocula, according to Walter et al. [27]. The culture was hydrated in sodium chloride solution (0.5% w/v), considering an initial concentration of 0.08% per mass of soymilk to be fermented. Then, the inocula were added to the soymilk at 42 ± 2 °C, homogenized for 60 sec., and kept at rest, in a thermostatic bath, until reaching a final pH of 4.8. After incubation time, the product was cooled in a cold room (8 ± 2 °C) to slow down the fermentation process and maintain the desirable sensory characteristics and suitable bacterial counts for probiotics. Three formulations of BSY using commercial prune, strawberry, and grape sweetened fruit extract (containing 12% sucrose, including the fruit sugar) at 20%, and a control formulation, with no fruit extract and 12% added sucrose for similar sweetness, were used to prepare the flavored BSYs before consumer acceptance tests, which were carried out on the following day after the formulations were prepared.

2.3.3. Storage Conditions and Freeze-Drying

BSY was stored over a period of 28 days in a cold room (8 ± 2 °C). Samples were then analyzed at t = 0, t = 14, and t = 28 days (Figure 1). Freeze-drying was performed in a freeze-dryer (K120 Liobras, São Carlos, SP, Brazil) at −97 °C and 20 µHg vacuum. Freeze-dried samples were stored at −18 °C for chemical analyses.

2.4. Chemical and Physicochemical Analyses

2.4.1. Determination and Viable Probiotic Cells of *L. acidophilus*, pH, and Titratable Acidity

The total count of lactic acid bacteria in BSY was carried out using the plate counting method, with quantification of viable cells only. Serial dilutions were performed for counting [28]. Briefly, 10 mL of each sample was transferred to a flask containing 0.1% peptone water (10^{-1} dilution) and from this initial dilution, six dilutions were performed in which each subsequent dilution tube contained a tenth of the number of bacteria present in the previous one, reaching up to 10^{-7} dilution. The 1 mL volume of the 10^{-5}, 10^{-6}, and 10^{-7}dilutions was poured into a Petri dish with MRS medium, using the seeding technique in depth (pour-plate) with the addition of an overlay. The plates containing *L. acidophilus* LA-5® were incubated at 37 °C for 72 h ± 3 h, without using anaerobiosis jars, as described by the International Dairy Federation—IDF [29].

The pH was measured in duplicate, using the AOAC [30] electrometric method (#981.12). Titratable acidity (TA) was determined in duplicate as in ISO/TS 22113:2012/IDF/RM204:2012 (2012) [31].

2.4.2. Proximate Analyses

The proximate analyses were determined in BS, BSM, BO, and BSY. Water, ash, and total lipids analyses were carried out in triplicate, according to AOAC [32] methods 925.45B, 923.03, 922.06, respectively. Protein content was calculated as nitrogen amount × 6.25 according to AACC [33], method 46-13. Carbohydrates and other macroconstituents were calculated by difference.

2.4.3. Urease Activity

The urease activity of BS, BSM and BSY was evaluated in duplicate, according to AOCS (method #Ba 9-58) [33], which is based on measuring the change in pH, caused by the formation of ammonia when the product is incubated with a buffered urea solution (Urea + H_2O + urease = CO_2 + NH_3).

2.4.4. Analysis of Sucrose and α-Galactosides

The determination of sucrose and α-galactosides (raffinose and stachyose) in BSM and in BSY was performed in triplicate by high performance liquid chromatography (HPLC), according to Macrae [34], using an Alliance 2690/5 HPLC system (Waters Corporation, MA, USA), including a refraction index detector (W 2410, Waters Corporation). One gram of sample was solubilized with 10 mL of Milli-Q water and taken to the ultrasound for 20 min. To extract the sugars. Then, 5 mL of acetonitrile wasadded to the flask, making up to 25 mL volume with Milli-Q water. The chromatographic separation was carried out in a Zorbax carbohydrate column (4.6 mm × 250 mm; 5 µm) (Agilent Technologies, Wilmington, DE, USA) with a mobile phase composed of acetonitrile/water (75:25) and a volumetric flow of 1.4 mL/min. The carbohydrates were identified based on comparison with analytical standards (Sigma Chemicals Co., St. Louis, MO, USA), under the same conditions as the samples. The quantification was performed using an external calibration curve with the same standards.

2.4.5. Analysis of Flavonoids

The extraction of anthocyanins was performed in duplicate, according to Pereira et al. [35,36], as thoroughly described in Esteves et al. [26]. Chromatographic analysis was performed according to Gouvêa et al. [37], also described in Esteves et al. [26]. Anthocyanins were identified considering the chromatographic performance and UV-Vis spectra data as compared to authentic standards (delphinidin-3-O-glucoside), cyanidin-3-O-glucoside, and petunidin-3-O-glucoside standards, all with purity higher than 99%, which were isolated from açaí and confirmed by mass spectrometry [38], under the same conditions. Anthocyanin identification was also confirmed by accurate molecular mass data obtained by mass spectrometry, using a Synapt G1 spectrometer (Waters Corporation, Milford, MA, USA), equipped with an electrospray ionization source and a quadrupole in series, with a time-of-flight (TOF) as a mass analyzer (ESI-qTOF) [37]. The quantification was performed by external calibration.

Extraction in duplicate and analyses of isoflavones were performed according to the AOAC method #2001.10 [30], also described in Esteves et al. [26]. Identification of isoflavone aglycones (daidzein, genistein, and glycitein) and glycosides (genistin, daidzin, and glycitin), all from Sigma-Aldrich, St. Louis, MO, USA, was performed considering the chromatographic behavior and UV-Vis spectra, as compared to authentic standard analyzed underthe same conditions. Quantification was carried out by external calibration, using isoflavone aglycone and glycoside standards. Results of total isoflavones were expressed as aglycone equivalents by summing concentrations of genistein, daidzein, and glycitein and

of the aglycones of the respective glucosides genistin, daidzin, and glycitin, as described in AOAC [30].

2.4.6. Antioxidant Capacity (AC) Assays

The AC was estimated as thoroughly described in Esteves et al. [26], using two assays, oxygen radical absorbance capacity (ORAC) [39] and DPPH [40], from the compound 2,2–diphenyl-1-picrylhydrazyl, both performed in duplicate. The extracts used for anthocyanins analyses were also used for both ORAC and DPPH assays. The antioxidant activity estimated by ORAC was expressed in quercetin equivalent (QE) and in Trolox equivalent (TE); in both cases, higher values were assigned to major activities [26]. Quercetin (Sigma-Aldrich) was also used 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 [39]. For DPPH, AC was expressed as the amount of extract required to reduce 50% of the initial concentration of DPPH (EC50) [40].

2.5. Consumer's Acceptance Test with Formulated BSY

This section of the study was approved by the Ethics in Research Committee ofthe Rio de Janeiro State University (UERJ) (Approval #1.576.379). The consumer acceptance test with FBSY was carried out with 103 consumer assessors (62 female and 41 male), aged 18–68 years old, randomly recruited among staff, students, and visitors of Embrapa Food Technology.

The BSY formulations were evaluated for overall acceptability, using the classical nine-point structured hedonic scale, where 1 referred to "dislike extremely" and 9 to "like extremely" [41]. 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 [42]. Spring water and crackers were provided for mouth rinsing between samples.

Assessors were asked to evaluate four samples: the BSY formulated with 12% added sugar and the FBSY added 20% of one of the three following commercial fruit mixes: black prune, strawberry, and grape. The panelists also filled out a questionnaire to provide general information about their age, gender, and frequency of consumption of soy products.

2.6. Statistical Analysis

Statistical analyses of physicochemical and microbiological data were performed by STATISTICA™, version 13.0 for Windows (StatSoft, Inc., Tulsa, OK, USA). Analytical results were treated by analysis of variance (ANOVA), followed by the Least Significant Difference (LSD) or Fisher's test, to verify significant differences among means at levels of significance lower than 0.05 ($p < 0.05$). Considering the differences among water contents of the evaluated samples, all results from physicochemical analyses (except for pH and titratable acidity) are presented on a dry weight basis, and the water contents were provided separately for the purpose of reconstitution. Sensory data were analyzed using the XLSTAT-MX software 2011 (Addinsoft SARL, Paris, Île-de-France, France) by analysis of variance (ANOVA) and Fisher's test (LSD) ($p < 0.05$). Hierarchical cluster analysis was used to identify consumers with similar acceptance ratings. Euclidean distances and Ward's aggregation criterion were considered.

3. Results and Discussion

3.1. Proximate Composition of BS, BSM, BSY, and BO

The proximate composition data on a dry basis are presented in Table 1. The results for BS are in accordance with the mean values available in the literature. Cho et al. [43] obtained

values of 41.4–44.3% for protein and 13.5–20.48% for oil. Felberg et al. [44] reported values of 41.2 g/100 g for protein, 19.8 g/100 g for lipids, 33.8 g/100 g for carbohydrates, and 5.3 g/100 g for ash, for the same black soybean line [44]. Ganesan and Xu [3] reported 32.0–43.6% of proteins, 31.7–31.9% of carbohydrates, 15.5–24.7% of lipids, and 5.6–11.5% of water. Leksono et al. [9] reported similar chemical parameters for black soymilk compared to the present data (Table 1) for different cultivars and processing. On the other hand, Lee et al. [45] reported 21.3% for proteins, 62.3% for carbohydrates, 12.5% for ashes, and 6.6% for lipids, and Hong et al. [46] reported 12.9% for proteins, 53.1% for carbohydrates, 5.2% for ashes, and 28.5% for lipids. Differences in the proximate composition of soy beverages generally derive from the use of different cultivars, maturation stage, environmental factors, and growing conditions. Pronounced differences may occur due to processing and the addition of supporting ingredients [47]; for example, soaking soybeans for hours before preparing the soymilk can reduce the soluble contents in the final product. Moreover, the black soybean used in this study was obtained from the ground grain. The particle size is one of the processing parameters that can influence the extraction and solubilization of proteins from the grain to the aqueous medium [48].

Table 1. Proximate composition of BS, BSM, BSY, and BO.

Component	BS	BSM	BSY	BO
	(g/100 g, dwb)			
Water	8.25 ± 0.22	92.45 ± 0.03	92.76 ± 0.16	74.17 ± 0.18
Proteins	43.34 ± 0.07	49.42 ± 0.17	50.22 ± 0.56	24.56 ± 0.06
Lipids	19.90 ± 0.13	17.35 ± 0.01	20.11 ± 0.13	10.97 ± 0.11
Ashes	5.43 ± 0.23	5.84 ± 0.01	5.85 ± 0.05	3.53 ± 0.00
Carbohydrates and other constituents, including dietary fiber **	31.34 ± 0.43	27.40 ± 0.17	23.82 ± 0.64	60.95 ± 0.05

Results are presented as means of triplicate analysis ± standard deviation; dwb = dry weight basis; BS = Black soybean; BSM = black soymilk; BSY = black soybean yogurt; BO = black okara; ** calculated as [100 − (proteins + ash + lipids)].

Still on Table 1, okara is a very nutritiousproduct. It is rich in dietary fiber (soluble and insoluble) and widely recognized for promoting physiological health [49,50]. Only a few studies were found reporting the black okara composition. Anjum et al. [2] reported values of 16.5% for lipids, 34.4% for proteins, 4.4% for ashes, and 38.5% for carbohydrates (excluding dietary fiber). Liu [47] reported 25.4–28.4% of proteins, 9.3–10.9% of lipids, 40.2–43.6% of insoluble fiber, 12.6–14.6% of soluble fiber, and 3.8–5.4% of carbohydrates. Yang et al. [51] reported 20.0% of protein, 10.5% of lipids, 63.2% of carbohydrates, and 13.8% of crude fiber. In this study, the content of macronutrients in BSM and BSY wassimilar (Table 1).

There was a reduction in the content of carbohydrates during fermentation, which was expected, given that microorganisms use them as a source of energy for their metabolism [52]. Changes observed in the proximate composition of black soymilk during fermentation were also reported by Lee et al. [45], who found for black soy beverage before and after fermentation with *L. acidophilus* 21.3% and 22.2% of protein, 62.3% and 59.3% carbohydrates, 12.5% and 12.0% ash, and 4.0% and 6.5% lipids. Hong et al. [46], on the other hand, reported 12.9% and 16.3% of protein, 53.1% and 46.7% carbohydrates, 5.2% and 5.5% ash, and 28.5% and 31.9% lipids, respectively. As previously mentioned, differences in the proximate composition of soy beverages are generally a function of the cultivar, processing, and addition of supporting ingredients.

3.2. Analysis of Flavonoids of BS, BSM, BSY, and BO

3.2.1. Anthocyanins

The contents of anthocyanins in BS, BSM, BSY, and BO are presented in Figure 2. The three anthocyanins identified in BS are in accordance with published data from different cultivars and regions [53,54]. Cyanidin-3-glycoside was the major anthocyanin, agreeing with data reported for other black soybean cultivars [3,6,55,56]. The values presented in Figure 2 for individual anthocyanins agree with Lee et al. [57], who evaluated 56 black soybean cultivars from Korea and found values ranging from 19.8 to 1420.4 mg/100 g of seed, and a similar profile to the one observed in this study for all cultivars analyzed.

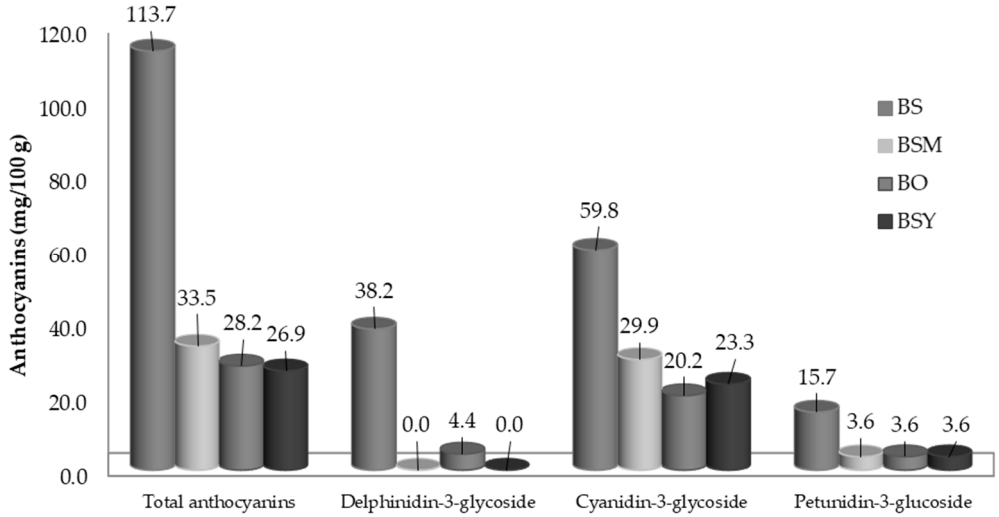

Figure 2. Anthocyanin content in BS, BSM, BSY, and BO (mg/100 g, dry weight basis). BS = black soybean; BSM = black soymilk; BSY = black soybean yogurt; BO = black okara. Results are means of duplicate analyses, with coefficients of variation ranging between 0.75% and 5.2%.

Processing to obtain BSM reduced the total anthocyanin content. Only 30% of the total anthocyanins remained in BSM and 25% in BO. The loss of anthocyanins during the extraction and thermal processing of black soymilk has been previously reported [10,58]. Leithardt et al. [59] reported an 83% reduction in total anthocyanins of black soybeans after cooking. Hsiao and Hsieh [10] compared the content of anthocyanins in non-heated and heated soymilk samples. The contents of delphinidin-3-glycoside and cyanidin-3-glycoside decreased by 76.0% and 82.4%, respectively, after heating at 90 °C for 1 h ($p < 0.05$). On the other hand, De Moraes Filho et al. [60] reported 60.41 mg of anthocyanins/100 g of black soybean and 384.69 mg/100 g of black soymilk, six-fold the initial content. Acidification of BSM due to fermentation to produce BSY caused a change in color from bluish gray to pink. After fermentation, 80% of the anthocyanins contained in black soymilk remained in BSY.

3.2.2. Isoflavones

The isoflavone profile found in the BS, BSM, BSY, and BO was predominantly genistein, daidzin, and glycitin, in order of abundance, containing only traces of the aglycone forms (genistein, daidzein, and glycitein) (Figure 3), agreeing with the literature [55]. Cyanidin-3-glycoside was still the major anthocyanin in BS products. When compared to BS, 50% of this anthocyanin remained in BSM, 39% in BSY, and 34% in BO. Delphinidin-3-glycoside showed greater degradation and could not be identified in BSM or BSY, and less than 8% was detected in BO. Petunidin-3-glycoside content was 23% the initial BS content in BSM, BSY, and BO.

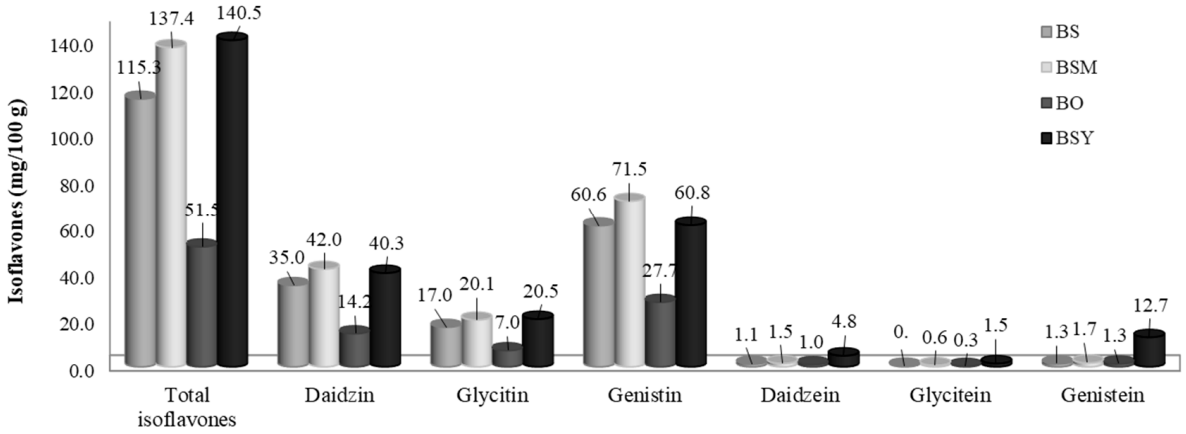

Figure 3. Isoflavone content in BS, BSM, BSY, and BO (mg/100 g, dry weight basis). BS = black soybean; BSM = black soymilk; BSY = black soybean yogurt; BO = black okara. Results are means of duplicate analyses, with coefficients of variation ranging between 0.8% and 2.9%.

The content of 115.3 mg/100 g (dry basis) of total isoflavone observed in BS agrees with the literature range [6,57,61]. Genistin and daidzin are the two major isoflavones found in BS, BSM, BSY, and BO, as also demonstrated by several other studies for non-fermented soybean products [6,46,47,62]. The isoflavone contents in BSM (137.4 mg/100 g), on a dry basis, were higher (p = 0.0007) than in BS (115.3 mg/100 g) (Figure 3). The same behavior was described by De Moraes Filho et al. [60], who observed a higher isoflavone content (>20%) in black soymilk (109 mg/100 g) than in black soybean (83 mg/100 g, dry basis) when producing black soybean cheese with similar processing for soymilk. This effect was observed in the present study, and there was greater amplitude in the conversion of genistin to genistein, the main isoflavone among those in black soybean. Fermentation promoted the hydrolysis of the glycosidic fractions of the isoflavones, converting part of them into aglycones through β-glucosidase activity. This behavior is widely reported in other studies with fermented soy foods [45,62–64]. There was no significant difference between the total isoflavone content in black soymilk (137.4 mg/100 g) and black soybean yogurt (140.5 mg/100 g). The isoflavone content in black okara (51.5 mg/100 g) was approximately 45% of the initial bean content. This content is higher than the values described by Hsiao and Hsieh [10], but lower than those reported by Jackson et al. [65] and Wang and Murphy [66]. Differences in cultivar and processing, such as the use of ground beans, can explain the differences among studies. Even though okara is a soybean by-product, it includes numerous nutritional and bioactive compounds and can be used as a valuable food ingredient to enrich other foods [25,67], including black soybean yogurt, to enhance health benefits.

3.3. Antioxidant Capacity Assays of BS, BSM, and BSY

Antioxidant capacity values estimated by ORAC and DPPH methods for BS and BSM are shown in Table 2.

There was no significant difference among the antioxidant capacities of the samples evaluated by the ORAC method. However, there were marginally significant differences between BS and BSY (p = 0.0583) and between BSM and BSY (p = 0.0773) when the antioxidant capacity was evaluated by the ORAC method, estimating values based on quercetin equivalent (QE). Similar behavior occurred for ORAC values estimated by Trolox equivalent (TE). There were marginally significant differences between BS and BSY (p = 0.0687) and between BSM and BSY (p = 0.0665). The ORAC values determined for BS agree with the values obtained by Slavin et al. [68] (75.0 to 250.0 μmol TE/g), who studied five cultivars of BS, and by Zhang et al. [54] (42.5 to 1834.6 μmol TE/g), who studied 60 Chinese cultivars of BS.

No studies were found comparing the antioxidant capacity of soy products measured by ORAC in QE/g. The antioxidant capacity estimated by the DPPH was expressed in EC50. In this method, BSM and BSY exhibited higher antioxidant capacity than BS (Table 2). This can be possibly explained by the formation of new compounds with stronger antioxidant activity in black soymilk and black soybean yogurt [61,69]. Few studies were found in the literature with DPPH values expressed as EC50 for black soybeans. Zhang et al. [54] evaluated the antioxidant activity of 60 black soybean cultivars and found EC50 values ranging from 0.048 mg/L to 0.65 mg/L, with lower values than the ones found for the BS used in this experiment (Table 2). These results show the importance of selecting a cultivar when developing black soybean products. No values expressed in EC50 were found in the literature for black soymilk.

Table 2. Antioxidant activity of BS, BSM, and BSY, estimated by ORAC and DPPH.

Sample	ORAC		DPPH (EC50)
	(μmol QE/g, dwb)	(μmol TE/g, dwb)	(mg/L, dwb)
BS	36.44 ± 2.26 [a]	156.15 ± 9.67 [a]	1.56 ± 0.24 [a]
BSM	35.91 ± 1.46 [a]	156.40 ± 1.45 [a]	0.58 ± 0.02 [b]
BSY	31.80 ± 0.06 [a]	139.07 ± 4.17 [b]	0.28 ± 0.07 [b]

Results are means of duplicate analysis ± standard deviation. dwb = dry weight basis. Different superscript letters on the same column indicate statistical difference by ANOVA, followed by Fisher ($p < 0.05$) significance level. QE = Quercetin equivalent; TE = Trolox equivalent; BS = black soybean; BSM = black soymilk; BSY = black soybean yogurt.

3.4. Analysisof Sucrose and α-Galactosides of BSM and BSY

There was a 52% reduction in total sugars during BSM fermentation (Table 3). Sucrose was reduced by 86%, raffinose by 51% and stachyose by 7%, respectively. Feng et al. [70] have reported similar behavior and results when analyzing sugars in black soy yogurts made with different bacterial cultures. This was expected because microorganisms use sugars for their growth and multiplication.

Table 3. Content of sucrose and α-galactosides in BSM and BSY.

Sample	Sucrose	Raffinose	Stachyose	Total
		(g/100 g, dwb)		
BSM	7.14 ± 0.06 [a]	1.42 ± 0.01 [a]	5.57 ± 0.01 [a]	14.13 ± 0.06 [a]
BSY t = 0	0.97 ± 0.01 [b]	0.69 ± 0.01 [b]	5.16 ± 0.03 [b]	6.83 ± 0.05 [b]

Results are means of triplicate analyses ± standard deviation. dwb = dry weight basis. Different superscript letters on the same column indicate statistical difference by ANOVA, followed by Fisher test ($p < 0.05$) significance level. BSM = black soymilk and BSY = black soybean yogurt.

3.5. Urease Activity (UA) of BS, BSM, BSY, and BO

The determination of urease activity measured by pH change between samples before and after treatment (delta pH) is used by the industry to evaluate the effectiveness of heat treatment on antinutritional factors, since the urease enzyme presents thermal resistance similar to the main antinutritional factors in soy, especially the trypsin inhibitor [71]. This method is based on the release of ammonia from urea by the action of the urease enzyme. This causes a change in the pH expressed as an index. In the present study, the urease activity values for BS, BSM, BSY, and BO were 1.78, 0.30, 0.30, and 0.36, respectively. Delta pH values between 0.02 and0.30 indicate adequacy of the heat treatment applied to soy products to inactivate trypsin inhibitor and other labile antinutritional factors [71,72]. Therefore, our results indicate the adequacy of soymilk processing. On the other hand,

as for the hull, cooking is needed in order to use okara as a rich fiber ingredient in the yogurt [73].

3.6. Consumers' Acceptance Test of BSY and Flavored BSY

Consumer acceptability for sweetened BSY (12% sugar) with different natural flavors by Rio de Janeiro assessors aged 18–68 years differed significantly. The mean acceptance values ranged from 5.6 (liked it slightly) to 7.6 (liked it very much) (Figure 4 and Table 4). The strawberry flavored BSY was most accepted (mean score 7.6), followed by the grape flavored one (mean score 7.0). The control BSY with only sugar added (mean score 5.6) and the prune flavored one (mean score 5.9) did not differ from each other, presenting the lowest acceptance among the four BSY evaluated. The present result for unflavored black soybean yogurt was similar to that reported by Chun et al. [74], who obtained a 5.2 mean score on a nine-point scale for overall acceptability of sweetened black soybean yogurt.

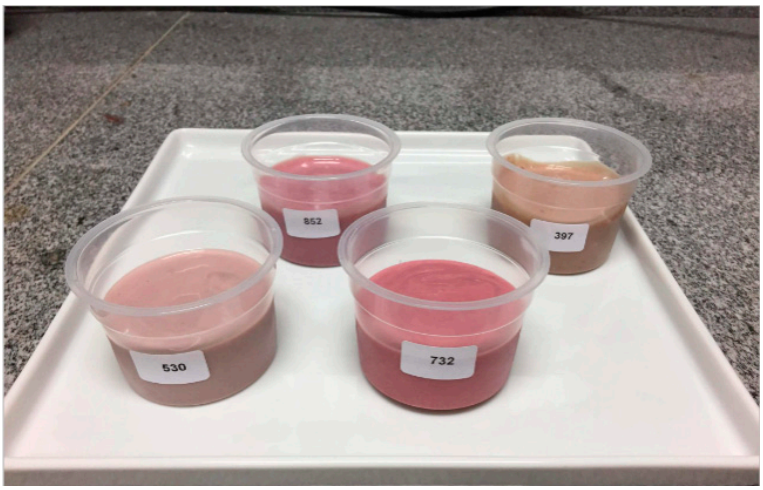

Figure 4. Black soybean yogurt samples formulated with the addition of sucrose (control) or with the addition of one of three commercial sweetened fruit extracts (prune, strawberry, and grape).

Table 4. Mean acceptance [§] of black soy yogurt with fruit extracts by consumers from Rio de Janeiro (n = 103).

Black Soybean Yogurt	Mean Acceptance ± SD
Unflavored	5.6 ±1.99 [c]
Prune flavor	5.9 ± 2.37 [c]
Grape flavor	7.0 ± 1.81 [b]
Strawberry flavor	7.6 ± 1.51 [a]

[§] Assessed by a nine-point hedonic scale ranging from 1 ('I disliked it extremely') to 9 ('I liked it extremely'). Different superscript letters mean values statistically different ($p < 0.05$) by Fisher's test. SD = standard deviation.

Hierarchical cluster analysis was used to identify consumers with different acceptance ratings for BSY. For segmentation by cluster analysis, three consumer segments were obtained (Table 5).

In Cluster 1, consumers assigned the lowest scores for all samples. In contrast, the consumers of Cluster 3 gave the highest scores for all samples. The strawberry flavored BSY was the most accepted in all threeclusters, with scores ranging from 6.4 to 8.3 among the segments. Consumers in Cluster 1 preferred strawberry and grape flavored SBY, followed by the unflavored one and prune flavored, which also did not differ from each other. In Cluster 2, the strawberry and prune black soybean yogurt were also accepted with the highest scores, followed by the grape flavored and unflavored ones. In Cluster 3, the

segment with the largest number of consumers showed similar perceptions for strawberry, grape, and unflavored yogurts, whose acceptance did not differ. These samples scored higher than the prune-flavored one.

Table 5. Mean acceptance [§] of black soybean yogurt for each of the three clusters based on consumers' likings.

Black Soybean Yogurt	Consumer's Segments		
	Cluster 1 (n = 30)	Cluster 2 (n = 33)	Cluster 3 (n = 40)
Unflavored	4.1 ^{ef}	4.7 ^{e}	7.4 ^{abc}
Prune flavor	3.2 ^{f}	7.0 ^{bcd}	7.0 ^{bcd}
Strawberry flavor	6.4 ^{cd}	7.7 ^{ab}	8.3 ^{a}
Grape flavor	6.5 ^{cd}	6.0 ^{d}	8.2 ^{a}

[§] Assessed using a nine-point hedonic scale ranging from 1 ('I disliked it extremely') to 9 ('I liked it extremely'). Different superscript letters comparing rows and columns simultaneously mean values statistically different (p< 0.05) by Fisher's test.

Regarding the influence of frequency of consumption on acceptance, the highest scores generally reflected the frequency of soy product consumption, with excellentresults for those who consumed soy products daily (Table 6).

Table 6. Mean acceptance [§] of BSY as a function of consumers' frequency of soy beverage consumption.

Black Soybean Yogurt	Consumer Assessors' Frequency of Consumption			
	Never (n = 19)	Rarely (n = 58)	Often (n = 24)	Daily (n = 2)
Unflavored	5.6 ^{cde}	5.3 ^{de}	6.0 ^{bcd}	8.0 ^{a}
Prune flavor	4.8 ^{e}	5.9 ^{cd}	6.7 ^{abc}	7.5 ^{a}
Strawberry flavor	6.7 ^{ab}	7.6 ^{a}	8.0 ^{a}	9.0 ^{a}
Grape flavor	7.0 ^{ab}	6.7 ^{ab}	7.5 ^{a}	8.5 ^{a}

[§] Assessed by nine-point hedonic scale ranging from 1 ('I disliked it extremely') to 9 ('I liked it extremely'). Different superscript letters comparing rows and columns simultaneously mean values statistically different (p < 0.05) by Fisher's test.

Considering that most test participants (n = 58) rarely consumed soy beverages, the mean scores given by them for the black soy yogurt, with different formulations ranged from 5.3 (only added sugar) to 7.6 (strawberry flavor), which can be considered a high acceptance, especially for soy products [75].

3.7. Analyses of Chemical Components After Storage

3.7.1. Flavonoids Analyses

Table 7 presents the anthocyanin content in BSM and BSY (freshly prepared and during storage).

Table 7. Anthocyanin content in BSM and BSY during storage.

Anthocyanins	BSM	BSY t = 0	BSY t = 14	BSY t = 28
	(g/100 g, dwb)			
Delphinidin-3-glucoside	ND	ND	ND	ND
Cyanidin-3-glucoside	29.94 ± 1.59 ^{a}	23.26 ± 0.19 ^{b}	20.19 ± 0.25 ^{c}	19.10 ± 0.49 ^{c}
Petunidin-3-glucoside	3.61 ± 0.14 ^{a}	3.62 ± 0.02 ^{a}	2.78 ± 0.28 ^{b}	1.95 ± 0.19 ^{c}
Anthocyanins Total	33.55 ± 1.73 ^{a}	26.88 ± 0.21 ^{b}	22.97 ± 0.53 ^{c}	21.05 ± 0.30 ^{c}

Results are expressed as the mean of duplicate analysis ± SD. Different superscript letters on the same row indicate statistical difference by ANOVA, followed by Fisher test (p < 0.05) significance level. t = 0, 14, and 28 are days of storage; SD = standard deviation. ND = Not detected. BSM = black soymilk and BSY = black soybean yogurt.

The total anthocyanin content of BSM (on a dry weight basis) reduced during fermentation (p = 0.0019) and in the storage period of 14 days (p = 0.0133), after which it remained relatively stable throughout the remaining studied period (Table 7, Figure 5). The same behavior was observed for the main anthocyanin cyanidin-3-glycoside. Regarding petunidin-3-glycoside, there was no reduction in its content from BSM to BSY, but a reduction was observed after the first 14 days of storage (p = 0.0106) and from 14 to 28 days of storage (p = 0.0115). Delphinidin-3-glycoside was not detected in the BSM or BSY, having probably been degraded during soymilk production.

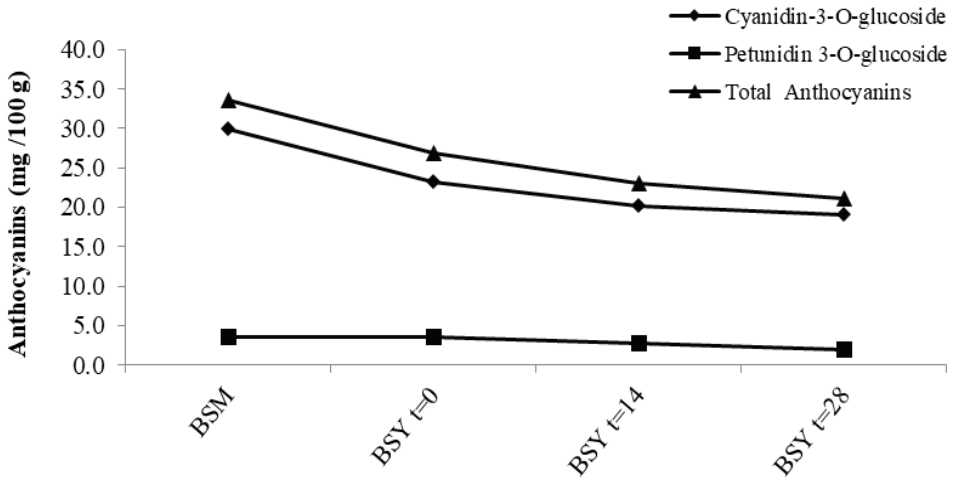

Figure 5. Anthocyanin contents in BSM and BSY during 28 days of storage. BSM = black soymilk and BSY = black soybean yogurt.

De Moraes Filho et al. [60] studied the behavior of anthocyanins from black soymilk fermented with lactic acid bacteria to obtain quark cheese. However, it was not possible to determine the loss of anthocyanins attributed to fermentation because the quantification of anthocyanins was performed only in the final product after syneresis. Several studies also reported reductionsin total anthocyanin content after fermentation [76–78]. Lan et al. [78] attributed the reduction in anthocyanin content to the polymerization and degradation reactions and interactions between these and other phenolic compounds during the process of obtaining pomegranate fermented beverage.

Regarding the isoflavones, there was no significant difference between the total isoflavone contents of BSM and BSY (Table 8). Furthermore, there was no significant difference between fresh BSY and BSY after 14 or 28 days of storage. The same behavior was observed between 14 and 28 days of storage (Figure 6). Fermentation promoted hydrolysis of the isoflavones' glycosidic fractions, converting part of them into aglycones through β-glucosidase activity. This effect was observed with greater amplitude in the conversion of genistin to genistein (Table 8, Figure 6). Hong et al. [46] and Lee et al. [45] also observed the conversion of glycosidic isoflavones to aglycones during the fermentation of black soy beverage. Furthermore, this event has been widely reported in studies with fermented soy foods [62–64]. Aglycones are the biologically active forms in the body, and studies have revealed that these forms are absorbed faster and in greater amounts than their glycosides. Isoflavones are converted into aglycones in the body by the action of β-glucosidases in the intestinal mucosa and microbiota [45,79]. Therefore, the increase in the aglycone forms in black soybean yogurt can be considered a benefit resulting from the fermentation process to which the soymilk was submitted.

Table 8. Isoflavone content in BSM and BSY during storage.

Isoflavones		BSM	BSY t = 0	BSY t= 14	BSY t = 28
		(mg/100 g, dwb)			
Glucosides	Daidzin	41.95 ± 0.50 [a]	40.27± 1.19 [a]	38.72 ± 2.42 [a]	40.33 ± 0.50 [a]
	Glyciti n	20.14 ± 0.23 [a]	20.52± 0.58 [a]	19.61 ± 1.28 [a]	20.48 ± 0.25 [a]
	Genistin	71.52 ± 0.56 [a]	60.84± 0.87 [b]	58.40 ± 2.39 [b]	59.91 ± 0.71 [b]
Aglicones	Daidzein	1.46± 0.02 [a]	4.78± 0.35 [b]	4.59 ± 0.44 [b]	5.11± 0.02 [b]
	Glycitein	0.63± 0.05 [a]	1.45± 0.05 [b]	1.40 ± 0.12 [b]	1.52± 0.00 [b]
	Genistein	1.69± 0.00 [a]	12.68± 1.09 [b]	12.16 ± 1.23 [b]	13.45 ± 0.14 [b]
	Total Isoflavones	137.39 ± 1.35 [a]	140.53 ± 4.14 [a]	134.86 ± 7.89 [a]	140.81 ± 1.61 [a]

Results are means of duplicate analysis ± standard deviation. dwb = dry weight basis. Different superscript letters on the same row indicate statistical difference by ANOVA, followed by Fisher test ($p < 0.05$) significance level. t = 0, 14, and 28 are days of storage; SD = standard deviation. BSM = black soymilk and BSY = black soybean yogurt.

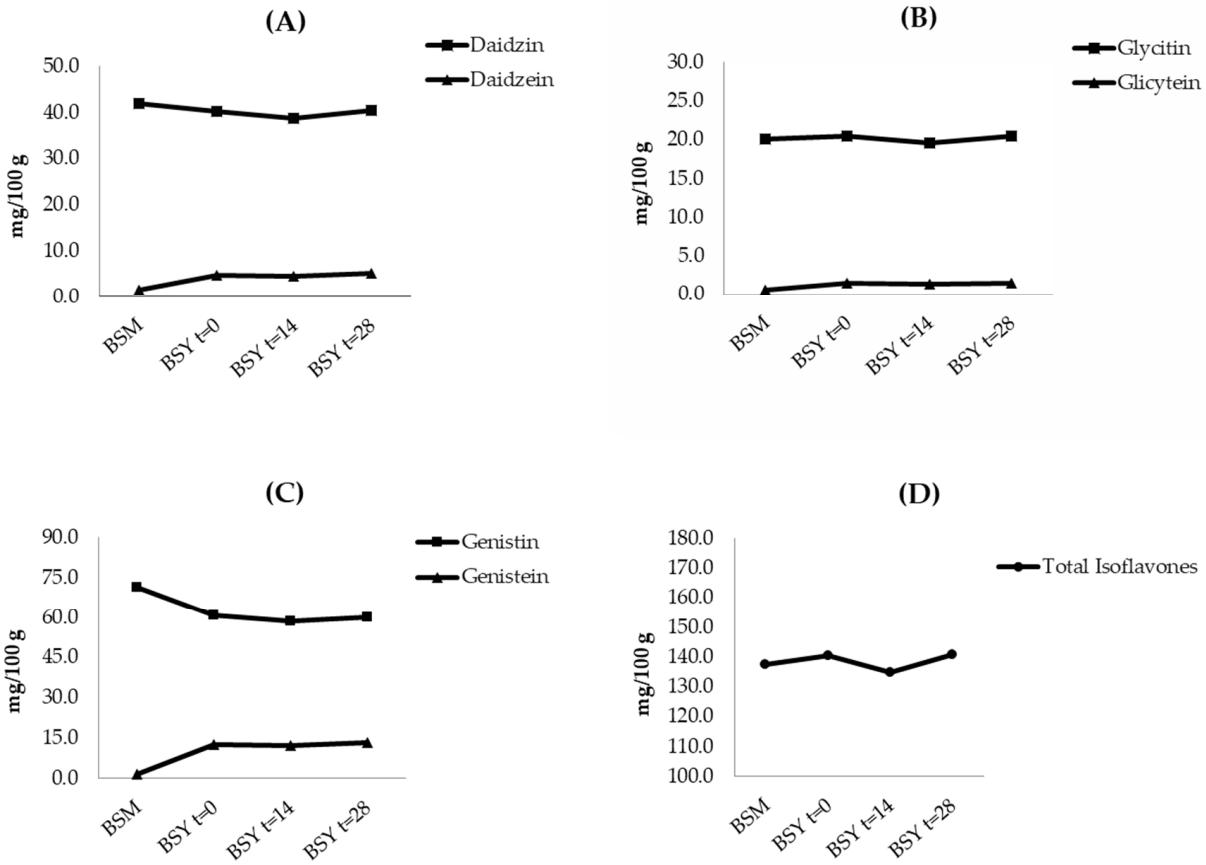

Figure 6. Isoflavones in the BSM and BSY during 28 days of storage. BSM = black soymilk and BSY = black soybean yogurt (dry basis). (**A**–**C**) represent the aglycones and glucosides; (**D**) represents the total concentration of isoflavones.

3.7.2. Antioxidant Capacity Assays

In the present study, fermentation decreased the antioxidant capacity of BSM by 45% ($p = 0.0181$) when evaluated by ORAC. Despite this apparent reduction in BSY t = 0, this result did not represent the behavior trend observed in BSY t = 14 and t = 28 (Table 9). No studies were found estimating the antioxidant capacity of fermented black soymilk using ORAC (*Glycine max* (L.) Merr) in the literature during storage, but a decrease in ORAC values was observed by Lim [80] when a fermented soymilk made from small black soybeans (*Rhynchosia Nulubilis*) was stored. Although popularly called small black soybeans, this legume belongs to another family.

Table 9. Antioxidant activity of BSM and BSY by ORAC and DPPH methods during storage.

Sample	ORAC (μmol QE/g, dwb)	ORAC (μmol TE/g, dwb)	DPPH (EC50) (mg/L, dwb)
BSM	35.91 ± 1.46 [a]	156.40 ± 1.45 [a]	0.58 ± 0.02 [a]
BSY t = 0	31.80 ± 0.06 [b]	139.07 ± 4.17 [b]	0.28 ± 0.07 [b]
BSY t = 14	36.78 ± 1.48 [a]	156.48 ± 4.73 [a]	0.22 ± 0.04 [b]
BSY t = 28	34.28 ± 0.45 [ab]	150.51 ± 2.49 [a]	0.18 ± 0.03 [b]

Results are means of duplicate analysis ± standard deviation. dwb = dry weight basis. Different superscript letters on the same column indicate statistical difference by ANOVA, followed by Fisher test ($p < 0.05$) significance level. BSM = black soymilk and BSY = black soybean yogurt. t = 0, 14 and 28 are days of storage; QE = Quercetin equivalent; TE = Trolox equivalent. The lower the EC50 value, the greater the antioxidant activity exerted by the sample [40].

When evaluating the antioxidant capacity by DPPH, a 107% increase ($p = 0.0020$) in BSM antioxidant activity was observed after BSM fermentation with *L. acidophilus*. The EC50 value for BSY did not differ during the storage period. The differences between the results observed in both methods support the idea that it is appropriate to measure the antioxidant capacity of foods using more than one method, considering different mechanisms (electron transfer-DPPH and hydrogen atom transfer-ORAC) and hydrophilic and lipophilic antioxidant compounds [39,40,81]. Considering both assays, fermentation and storage did not decrease the antioxidant capacity of BSM.

Lee et al. [45] also found higher antioxidant activity, estimated by the DPPH method, in the black soymilk fermented with *L. acidophilus* compared to the unfermented one. Zahrani and Shori [82] found the same results in soymilk fermented with *B. longum B. lactis* compared to control unfermnented. Chun et al. [13] evaluated the antioxidant activity of fermented yellow and black soymilks in different proportions by DPPH and observed a significant increase in the antioxidant activity, as the percentage of black soymilk increased in the beverage.In the work developed by Pyo et al. [83], it was demonstrated that the increase in the antioxidant activity by the DPPH method for a soy product fermented with lactic acid bacteria correlated with a higher concentration of aglycone isoflavones converted during the fermentation process. In addition, it has been reported that soy proteins and polyphenols that are less bioavailable *in natura* form can be converted into more bioavailable forms by fermentation, resulting in products with higher antioxidant activity [84]. Despite the apparent reduction inantioxidant activity soon after fermentation (BSY t = 0), this result did not represent the trend of behavior confirmed by BSY t = 14 and BSY t = 28 (Table 9). Despite the decrease in the content of anthocyanins during BSM fermentation, the antioxidant activity was maintained, possibly due to the conversion of isoflavone glycosides into aglycones, balancing the loss of activity caused by the decrease in anthocyanin content.

3.7.3. Determination of Viable Probiotic Cells of *L. acidophilus*

Regarding the viability of probiotic cells, at time t = 0 after fermentation, there was a high count of *L. acidophilus* LA-5®, followed by a reduction in the count during the storage period, initially going from 10^7 to 10^5 CFU/g at the end of the study (Table 10).

Lee et al. [45] evaluated the viability of probiotic lactic acid bacteria *L. acidophilus*, *L. plantarun* or *S. thermophilus* isolated and mixed cultures during the fermentation of black soymilk and found a significant increase ($p < 0.05$) in the viable cell count for all six samples of black soymilk during fermentation at 37 °C, for 24 h, compared to unfermented controls. The initial count of lactic acid bacteria averaged 10^7 CFU/mL and reached 10^8–10^9 CFU/mL after 24 h of fermentation.

Table 10. Determination of viable probiotic cells of *L. acidophilus* in BSM and BSY during storage.

Sample	*L. acidophilus LA-5*® (UFC/g)	*L. acidophilus LA-5*® (UFC/Portion of 200 g)
BSM	0.0	0.0
BSY t = 0	7.30×10^7	1.46×10^{10}
BSY t = 14	4.80×10^6	9.6×10^8
BSY t = 28	2.20×10^5	4.40×10^7

BSM = black soymilk and BSY = black soybean yogurt; t = 0, 14, and 28 are days of storage.

3.7.4. Analysis of Sucrose and α-Galactosides

A reduction in the carbohydrate content in BSY was observed compared to BSM (Table 11, Figure 7), indicating its consumption during fermentation. In fact, consumption of fermentable sugars was expected, as it is a characteristic of the lactic fermentation process [52]. For the culture used, sucrose was the preferred source, showing a significant reduction (p = 0.0000) during the 28-day storage. This result corroborates with those of Pithong et al. [85], who showed that *L. acidophilus* grows well in yellow soymilk due to the efficient use of sucrose. Raffinose and stachyose were also consumed by *L. acidophilus* from BSM to BSY t = 0 (p = 0.0000) (Table 11, Figure 7). While the raffinose content remained stable throughout the storage period, stachyose content reduced up to 14 days (p = 0.0000) and from 14 to 28 days of storage (p = 0.0000). This behavior agrees with the results reported by Mital and Steinkraus [86] and by Wang et al. [12], in which *L. acidophilus* cultures were capable of metabolizing stachyose and raffinose.

Table 11. Content of sucrose and α-galactosides in BSM and BSY during storage.

Sample	Sucrose	Raffinose	Stachyose	Total
	(g/100 g ± SD, dwb)			
BSM	7.14 ± 0.06 ^{a}	1.42 ± 0.01 ^{a}	5.57 ± 0.01 ^{a}	14.13 ± 0.06 ^{a}
BSY t = 0	0.97 ± 0.01 ^{b}	0.69 ± 0.01 ^{b}	5.16 ± 0.03 ^{b}	6.83 ± 0.05 ^{b}
BSYt = 14	0.61 ± 0.01 ^{c}	0.71 ± 0.01 ^{b}	4.74 ± 0.01 ^{c}	6.06 ± 0.01 ^{c}
BSYt = 28	0.30 ± 0.01 ^{d}	0.70 ± 0.01 ^{d}	4.27 ± 0.02 ^{d}	5.27 ± 0.03 ^{d}

Results are means of triplicate analyses ± standard deviation, dwb = dry weight basis; different superscript letters on the same column indicate statistical difference by ANOVA, followed by Fisher test (p< 0.05 significance level). t = 0, 14, and 28 are days of storage; SD = standard deviation. BSM = black soymilk and BSY = black soybean yogurt.

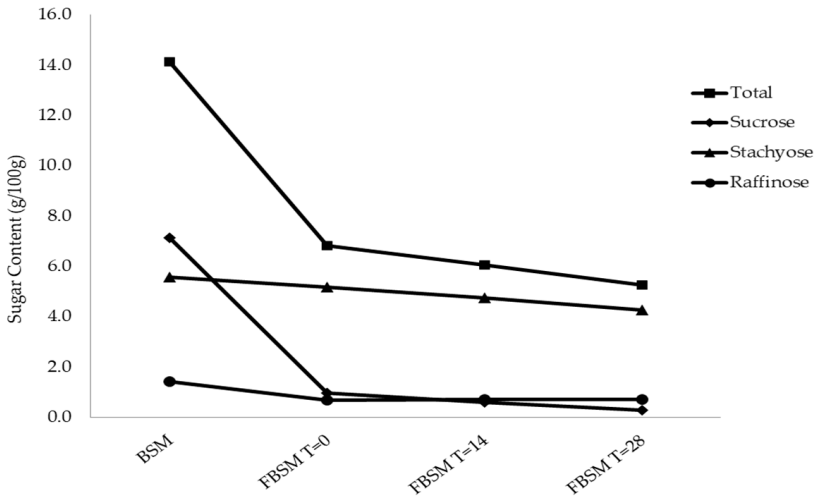

Figure 7. Content of sucrose and α-galactosides during fermentation and 28 days of storage. Results are means of triplicate analysis.

3.7.5. Determination of pH and Titratable Acidity

Parallel to the sugar consumption, the decrease in pH and the increase in titratable acidity (Table 12) occurred in BSY, a characteristic of the fermentation process [87,88]. After 4 h of fermentation, BSM pH decreased from 6.46 to 4.50 and the total acidity increased from 0.10% to 0.44%, remaining stable during the storage period studied, which indicates low post-acidification of the product. Lee et al. [45] evaluated the fermentation of black soymilk with different lactic acid bacteria (*L. acidophilus*, *L. plantarun*, or *S. thermophilus*). They found a significant increase ($p < 0.05$) in the viable cell count for all samples of black soymilk during fermentation at 37 °C for 24 h, compared to unfermented controls.

Table 12. Determination of pH and titratable acidity (wet basis) in BSY during storage.

Sample	pH	Total Acidity in Lactic Acid (g/100 g)
BSM	6.46 ± 0.00	0.10 ± 0.00
BSY t = 0	4.50 ± 0.00	0.44 ± 0.00
BSY t = 14	4.56 ± 0.00	0.44 ± 0.01
BSY t = 28	4.59 ± 0.00	0.44 ± 0.00

Results are means of duplicate analyses ± standard deviation; t = 0, 14, and 28 are days of storage; BSM = black soymilk and BSY = black soybean yogurt.

The initial count of lactic acid bacteria averaged 10^7 CFU/mL and reached 10^8–10^9 CFU/mL after 24 h of fermentation. The pH value decreased from 6.05–6.28 to 4.15, and the titratable acidity increased from 0.10–0.11% to 0.61–0.65%, after 24 h of fermentation due to the culture's growth and production of lactic acid. Regarding the storage period, Rinaldoni et al. [89] reported similar results. They did not observe significant variations ($p < 0.05$) in the pH and acidity values of yellow soybean fermented beverages evaluated over 21 days.

4. Conclusions and Final Considerations

Plant-based food consumption has been increasing in recent years, as a healthier food choice both for humans and for the environment. Legume seeds, especially soybeans, are among the main sources of protein for human fed, as long as the sustainability principles are respected. The investigated black soybean cultivar BRM 09-50995 showed a content of protein and other macronutrients, polyphenols, and antioxidant capacity in accordance with the ranges of variation found in the literature for black soybeans cultivated in other countries. The black soybean yogurt composition showed a reduction in the total content of carbohydrates and an increase in the content of lipids, compared to black soymilk, which hassimilar protein content. *L. acidophilus* LA-5® was able to ferment oligosaccharides (stachyose and raffinose) in black soymilk and produce yogurt, using them as sources of carbon in addition to sucrose. A probiotic black soybean yogurt was obtained with 10^8 CFU/serving probiotics after 14 days of storage. There was a 20% reduction in the anthocyanin content in BSM after fermentation, with a considerable residual amount remaining. Fermentation promoted the conversion of isoflavone glycosidic compounds into aglycones, with no change in total anthocyanins content. Fermentation preserved or improved the antioxidative capacity of the developed black soymilk and contributed to better sensory acceptance of soybean products. Among the flavors evaluated, the strawberry-flavored yogurt obtained the highest mean acceptance by the Rio de Janeiro consumer assessors, corresponding to "I liked it a lot" on the rating scale, with grape flavor following. Furthermore, for those who consumed soybean products often or daily, all yogurts were highly accepted, including the unflavored one, with mean acceptance reaching 8 and 9, on a scale of 9, for the strawberry-flavored one. Black okara presentsnutritional and bioactive potential and can be used to enrich the produced yogurt or as an ingredient

in other foods. In conclusion, black soybean probiotic yogurt is an excellent source of nutrients and a functional product, with great market potential in Rio de Janeiro. In future studies, additional markets should be evaluated, alternatives to sucrose should be tested, and the flavonoids' bioaccessibility before and after the product fermentation should be compared. This study reminds us that soybeans should be thought of in a broadersense beyond the production of protein derivatives and oil.

Author Contributions: T.C.F.E.: Conceptualization and Formal Analysis; I.F.: Concep-tualization, Supervision, Funding acquisition, Data Curation and Writing (original draft and review-editing); V.C.: Conceptualization, Data Curation, Formal Analysis, Funding Acquisition and Supervision; A.F.: Data Curation, Writing (original draft and review editing); A.F.d.F.-M., E.H.M.W., M.C.P.d.A.S., S.P., R.A., R.D. and M.C.C.-P.: Formal Analysis. All authors have read and agreed to the published version of the manuscript.

Funding: Funding for research and scholarship was provided by the National Research Development Council (CNPq, Brazil (409855/2016-2), CAPES Coordination for the Improvement of Higher Education Personnel (CAPES), and Carlos Chagas Filho Foundation for Research Support in the State of Rio de Janeiro (FAPERJ, E-26/110.783/2013, E-26/204.113/2024, CNPq 304102/2022-9, FAPERJ E-26/204.248/2024 (299489).

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki for studies involving humans and approved by the Institutional Review Board (or Ethics Committee) of Rio de Janeiro State University (approval protocol code #1.576.379, approved on 6 June 2016).

Informed Consent Statement: Informed consent for data publication was obtained from all subjects involved in the study.

Data Availability Statement: Additional data generated during this study will be available upon request.

Conflicts of Interest: The authors declare no conflicts of interest.

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