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Changes in the Chemical, Technological, and Microbiological Properties of Kefir-Fermented Soymilk after Supplementation with Inulin and *Acrocomia aculeata* Pulp

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Abstract: Soymilk has received a lot of attention due to its nutritional value, especially its high protein and isoflavone contents. The objective of this study was to develop a beverage fermented with kefir biomass from soymilk supplemented with 3.5 or 7.0% of *Acrocomia aculeata* (Jacq.) Lodd. powder-pulp (also known as the bocaiúva or macaúba) (BO3.5 or BO7.0, respectively), 3.5% of inulin (IN3.5), or 3.5% of each ingredient (BO + IN). The beverage was produced from soymilk (9 °Brix) by fermentation with kefir (4 g:100 mL) for 12 h at 25 °C. The characteristics of the beverages (pH, titratable acidity, soluble solids, color, syneresis, sedimentation, and the microbiological counts of *Lactococcus*, *Lactobacillus*, and yeasts) were evaluated during 16 days of storage (0, 6, 11, and 16 days) at 7 °C. The addition of bocaiúva powder-pulp and/or inulin did not change the pH value of the beverage, which remained the same at a safe level throughout storage (pH < 4.5); it increased the soluble solids, especially when compared to supplementation of the studied ingredients (BO3.5, BO7.0, and IN3.5); and decreased syneresis when increasing the supplementation (CONT to other treatments), regardless of treatment. Kefir-fermented soymilk was classified as hypotonic (<270 mOsmol/kg) before supplementation and isotonic (270–330 mOsmol/kg) after supplementation with inulin and/or bocaiúva powder-pulp (or both). Evaluation of the microbial populations in the fermented beverages showed that this substrate could maintain viability above 10⁷ CFU/mL throughout the storage period. The supplementation improved the technological characteristics of kefir-fermented soymilk without altering the viability of the beneficial microorganisms present in kefir.

Keywords: bocaiúva; kefir; antioxidant activity; storage; fermented beverages; macaúba

1. Introduction

Brazil is a country rich in biodiversity, and much concern has been focused on the preservation of its five native biomes, as degradation of these biomes could have serious consequences, such as contributing to climate change, for the whole world. The Cerrado biome, which is located in the midwestern region of the country, currently contains approximately 22% of the natural vegetation [1]. Thus, efforts have been made to enhance awareness and better use of the native species to ensure their preservation [1–3]. In this biome, there are several species of native fruits that are considered to be sensorially and nutritionally attractive but are commercially devalued, mostly consumed in nature, and rarely processed and used technologically [4].

Acrocomia aculeata (Jacq.) Lodd., known as macaúba or bocaiúva, is one of the species that occur naturally in the Cerrado biome and has shown potential as a major source of daily nutrient intake. The epicarp of the fruit has a thin, hard, brittle, fibrous structure,

and light brown color, while the mesocarp has a yellowish color due to the presence of bioactive color compounds such as carotenoids (~200 µg/g) [5], mainly β-carotene [6]. Bocaiúva pulp is also rich in lipids (~29%) [5], especially fatty acids such as oleic (~53%), palmitic (~25%), and linoleic (~14%) [7,8]. Deficiency of oleic and linoleic acid has been associated with morbidity and mortality outcomes, including all causes, coronary heart disease, stroke, and cardiovascular disease [9], while the presence of palmitic acids has demonstrated a hypercholesterolemic effect [10]. In addition, the pulp of bocaiúva has a large amount of fibers (~20%) [5], which can contribute to the recommended daily intake for this nutrient. Fibers can promote rapid gastric emptying, decrease intestinal transit time, and increase fecal bulk, promoting actions beneficial to the health of the human body [11]. *A. aculeata* (Jacq.) Lodd. fruits have been used in regional cuisine; are consumed by local people in preparations such as ice cream, cakes, and sweets; and have been used in cosmetics and energy production [5,7,8,12].

Soybean is a legume that has been used to develop food products because of its high nutritional value, mainly its protein contents, and its health benefits, mainly due to the presence of phenolic compounds and isoflavones, as well as its low potential for adverse health effects [13]. Although this chemical composition in terms of health potential is known, investigating forms of processing that provide a greater amount of these nutrients, such as fermented soy products, is still a priority. Soymilk is one soybean product, which, with fermentation, can be a source of bioactive peptides as well as genistein and daidzein, which are responsible, in part, for the beneficial effects of soy products [14].

Several classes of microorganisms can be used for soymilk fermentation, including acidophilic bacteria, especially lactic acid bacteria such as *Lactobacillus* and *Leuconostoc*, and yeasts, such as *Kluyveromyces*, *Pichia*, and *Saccharomyces* in their isolated form [15–18] or symbiosis association from kombucha [19] or kefir [20]. Kefir has been used for the production of functional products for people with special dietary needs (lactose intolerant individuals and vegans) [20] because it can also be used to ferment non-dairy substrates, such as fruits and vegetables, and produce functional beverages with distinct sensory characteristics [21–24].

The use of kefir in the soymilk fermentation process has been presented in the literature [25–29], and one of the benefits of the fermentation process using microorganisms is the release of aglycone isoflavones, as well as the glycosylated phenolic compounds present in soymilk or soybean [30,31], which increases the bioavailability of these compounds for the human organism. Recently, this product (kefir-fermented soymilk) demonstrated potentially inhibited pancreas lipase and α-amylase activities in vitro, and also decreased the activity of intestinal lipase and pancreas, which led to a reduction in total triglycerides and LDL-c and body weight and increased HDL-c in animals fed a high-fat diet [32]. This demonstrates how important the fermentation process is to ameliorate the beneficial effects of fermented soymilk.

Regarding the development of fermented beverages from soymilk, much has been discussed regarding the changes in appearance and texture that occur after fermentation. The low pH of fermented beverages interferes with the precipitation of soy proteins, thus changing the appearance and texture of these products [27]. Therefore, the use of body agents, such as inulin, is indicated [33]. Inulin is a polysaccharide consisting of 10 or more fructose units connected with β-(2→1) linkages and frequently terminating with a glycosyl moiety. Because humans lack the digestive enzymes to break down the β-(2→1) linkages of fructan, inulin is an indigestible fiber that is hydrolyzed by bifidobacteria and *Lactobacillus* species in the digestive tract; therefore, it is considered to be a prebiotic [34,35]. In recent decades, the food industry has begun to more rigorously exploit these ingredients to encourage increased fiber intake by consumers [36].

Before this work, our group demonstrated that fermented-kefir soymilk can demonstrate phase separation and high syneresis, even with the addition of inulin [27]. Thus, we hypothesize that supplementing this beverage with bocaiúva powder-pulp may contribute to the decrease in phase separation and syneresis behavior. To the best of our knowledge,

published data about the proposed inclusion of bocaiúva in kefir-fermented soymilk beverage is lacking. The objective of this study was to assess the changes in the chemical and microbiological properties of kefir-fermented soy-based beverages supplemented with inulin and/or bocaiúva powder-pulp.

2. Materials and Methods

2.1. Vegetable Material and Kefir Preparation

Bocaiúva powder-pulp was obtained in the Cuiabá region (Mato Grosso, Brazil), sanitized, frozen at -18°C , and then cooled in a refrigerator (8°C). Lipoxigenase-free soybean (BRSGO 8061 variety) was donated by the Goiás Agency for Technical Assistance, Rural Extension, and Agricultural Research (Emater, Rio Verde, Brazil). Kefir biomass starter was obtained from a local producer (Rio Verde, Brazil). The biomass was increased by growth in a brown sugar solution (10:1, v/m) and frozen until use. Before use, it was activated with a brown sugar solution, filtered, and mixed with sterilized water (1:10, m/v) with continuous exchange every 24 h at a controlled temperature (25°C) for 3 days [27].

2.2. Kefir-Fermented Soymilk Characterization

Soymilk was produced according to the method described by Baú, Garcia, and Ida [25], and the soluble solid concentration was corrected to 9 °Brix after pasteurization. The kefir biomass was activated 3 d before the experiment and then inoculated at a ratio of 4 g of kefir to 100 mL of soymilk and fermented at 25°C in a BOD incubator for 12 h, as previously described [27].

After fermentation, the biomass was separated from the beverage. The beverage was prepared and homogenized according to Table 1. No treatment contained more than 3.5 g of inulin per 100 mL of beverage to keep the content below the maximum daily intake limit established by the Brazilian government [37]. Comparatively, the same amount of bocaiúva powder-pulp was used, as well as double the amount of bocaiúva powder-pulp in an attempt to increase the use of the pulp.

Table 1. Treatment of kefir-fermented soymilk.

Name	Inulin (%)	Bocaiúva Powder-Pulp (%)
CONT	-	-
IN3.5	3.5	-
BO3.5	-	3.5
BO7.0	-	7.0
BO + IN	3.5	3.5

The beverage was individually packaged (40 mL) in sterile polyethylene containers and stored at 7°C in a BOD incubator (TE-402; Tecnal, Piracicaba, Brazil). In a previous study, we evaluated the shelf life of kefir-fermented soymilk for 28 days and established that the product was stable for up to 14 d [27]. Therefore, in the present work, we used 16 d as the maximum storage time. The characteristics of the beverage were evaluated, including pH, titratable acidity, soluble solid content, color, syneresis, osmolality, and microbiological viability on days 1, 6, 11, and 16 of storage. Through these analyzes, we verified that the conservation of kefir-fermented soymilk occurs for up to 16 days (discussed in Section 3.2) and, therefore, at the end of the shelf life we evaluate vitamin C, total carotenoid, total phenolic compounds, and antioxidant activity.

2.3. Chemical Composition

Physicochemical analyses were performed according to methods established by the Association of Official Analytical Chemists [38] as follows: pH was determined using pH meter, titratable acidity was determined by titration with 0.1 N NaOH, and total soluble solid was determined using a digital refractometer.

Vitamin C content was determined using oxalic acid, dichlorophenol indophenol (DCFI), and a standard ascorbic acid solution as the extracting solvent, as described by Benassi and Antunes [39]. Briefly, 5 g of the sample was homogenized in 50 mL oxalic acid 2% and filtered through Whatman No. 4 filter paper. A total of 10 mL of aliquots were titrated with 0.2% dichlorophenol–indophenol. Results were expressed as milligrams of reduced ascorbic acid per 100 g of sample.

Carotenoid content was determined by extracting 5 g of the macerated sample and 3 g of celite with 30 mL of acetone and filtering the sample. This extract was mixed with 50 mL of petroleum ether and placed in a separating funnel, and the mixture was washed with distilled water three times. The lower phase (water) was discarded, and the upper phase was collected and mixed with anhydrous sodium sulfate. The volume was adjusted to 25 mL, and the absorbance was read on a spectrophotometer at 450 nm [40].

The crude extract used to determine total phenolic compounds and antioxidant activity was determined according to the method described by Larrauri et al. [41]. Briefly, 1 g of the sample was homogenized with 40 mL of 50% methanol and incubated in the dark for 60 min. Then, the mixture was filtered and the supernatant was transferred to a 100 mL volumetric flask. The residue from the first extraction was mixed with 40 mL of 70% acetone and incubated in the dark for 60 min. The mixture was filtered, the supernatant was combined with the first extraction, and the volume was adjusted to 100 mL with distilled water.

Total phenolic compounds were determined by mixing 200 µL of the prepared crude extract with 1.9 mL of Folin–Ciocalteu reagent diluted 10 fold with distilled water and 1.9 mL of aqueous sodium carbonate (Na_2CO_3) solution. After incubation for 120 min in the dark, the absorbance was measured at 725 nm using ferulic acid as a standard. The results were expressed in grams of ferulic acid equivalents per gram of sample.

Antioxidant activity was determined using the ABTS and DPPH methods. The DPPH method was performed as described by Brand-Williams et al. [42], with the modifications reported by Rufino et al. [43]. Briefly, a 0.1 mL aliquot was mixed with 3.9 mL of DPPH radical (0.06 mM). Methyl alcohol was used as a blank to calibrate the spectrophotometer and as a control. Absorbance was determined at 515 nm.

In the ABTS assay, ABTS radical cations ($\text{ABTS}^{\bullet+}$) were generated in the reaction between 5 mL ABTS aqueous solution (7 mM) and 88 mL potassium persulfate solution (140 mM) to reach the final concentration of 2.45 mM. The mixture was left in the dark for 14 h at room temperature and diluted with ethanol until absorbance of 0.7 ± 0.02 at 734 nm. Sample extracts (30 mL) were added to 3 mL ABTS radical solution in the dark, and absorbance was measured at 734 nm after 6 min.

The results of the ABTS and DPPH assays were expressed as µM TEAC (Trolox equivalent antioxidant capacity) per 100 g of sample.

2.4. Technological Characterization

Syneresis of the soy beverages was evaluated as the percentage of liquid released after 2 h of inversion of the containers at room temperature (25 °C) divided by the total mass of the beverage [44].

Color determination was performed using a Color Quest II color spectrophotometer (HunterLab, VI, USA) using the CIELAB system, where the L^* coordinates correspond to luminosity or brightness, which range from black (0) to white (100), and a^* and b^* refer to the green (−60)/red (+60) and blue (−60)/yellow (+60) chromaticity coordinates.

The osmolality of the beverages was determined according to the method of Musara and Pote [45]. The freezing temperature of the beverage (1.5 mL sample) was assessed using a microprocessor electronic cryoscope (ITR, MK 540, Esteiro, Rio Grande do Sul, Brasil). The osmolality of the beverages was calculated using Equation (1).

$$\text{Osmolality} \left(\text{mOsm.kg}^{-1} \right) = \frac{\Delta T \times 100}{K} \quad (1)$$

where K is $1.86\text{ }^{\circ}\text{C (mol/kg)}^{-1}$ (the water cryoscopic constant) and T is the cryoscope lowering value ($^{\circ}\text{C}$), i.e., $T\text{ (}^{\circ}\text{C)} = (\text{sample (}^{\circ}\text{C)} - 0\text{ }^{\circ}\text{C)}$.

2.5. Viability of Kefir Microorganism

Each species of microorganism was counted according to previously described methodology [46] in Petri dishes containing M17 agar (Sigma–Aldrich, São Paulo, São Paulo, Brazil) incubated at $37\text{ }^{\circ}\text{C}$ for *Lactococcus* species (thermophilic microorganisms), acidified MRS agar (Sigma–Aldrich, São Paulo, São Paulo, Brazil) at $30\text{ }^{\circ}\text{C}$ for *Lactobacillus* (mesophilic microorganisms) for 72 h under anaerobiosis, and Peptone Dextrose YPD agar (Sigma–Aldrich São Paulo, São Paulo, Brazil) incubated at $30\text{ }^{\circ}\text{C}$ for 72 h for yeasts. To acidify the MRS agar, a 1 M hydrochloric acid solution was added until the pH reached 4.58–5.20 [47]. A 1 mL aliquot of triplicate samples of each fermented beverage was homogenized in 9 mL of saline solution (8.5 g/1000 mL), and the dilutions were inoculated on Petri dishes containing agar and incubated in BOD. For *Lactobacillus* and *Lactococcus*, the plates were placed in anaerobic jars containing an atmospheric carbon dioxide (ATM CO_2) solution. At each time point, the colonies in each Petri dish were counted and recorded as colony forming units (CFU) per mL of fermented beverage.

2.6. Statistical Analysis

All analyses were performed in triplicate, and each experiment was repeated three times. Data were subjected to an analysis of variance to detect significant differences among the time of storage between treatments, whereas means were compared by Tukey's test using Sisvar 5.6 program (Lavras, Minas Gerais, Brazil). Statistical differences were considered significant at p values less than 0.05.

3. Results and Discussion

3.1. Bocaiúva Powder-Pulp Characterization

Table 2 shows the chemical composition of the bocaiúva pulp. The pulp had low moisture (6.22 g/100 g) and protein (2.94 g/100 g) contents and high lipid (9.15 g/100 g) and ash (3.15 g/100 g) contents, similar to that previously described (3.26 g/100 g) [48]. Furthermore, the bocaiúva powder-pulp had a low soluble solid content (3.10 °Brix) and a pH close to that reported by Mooz et al. [49] (5.48), which corroborates the low titratable acidity (0.24 mg/mL) found in the present study. Bocaiúva powder-pulp showed high values for a^* and b^* , indicating the presence of yellow and red colors, respectively. In addition, the hue value was between 0° and 90° , which indicates a red to yellow color, indicating that the color of the bocaiúva powder-pulp is orange, with low saturation (a Chroma value close to 0).

The vitamin C content of the fruit can decrease following pulp processing, depending on the method used. This was observed in our study, as the fresh pulp contained ~34 mg of vitamin C per 100 g (results not shown), while the dehydrated pulp contained ~13 mg/100 g. A 72% reduction in vitamin C between fresh and dehydrated apples was also reported by Egea et al. [50]. In contrast, dehydration via water evaporation can concentrate other compounds in the pulp, such as carotenoids and phenolic compounds. This was observed in this study, as the unprocessed pulp contained ~1 mg and ~81 mg of carotenoids and phenolic compounds per 100 g, respectively (results not shown), and the processed bocaiúva powder-pulp contained 6.34 mg and 216.58 mg per 100 g, respectively. The carotenoid content in the pulp was close to that reported for dehydrated acerola residue (~8 mg/100 g) [51], which is considered to be a good source of these compounds.

Antioxidant activity, as measured using the ABTS method, was also higher (557 $\mu\text{M TROLOX/g}$) than previously reported in another study by our group in which we analyzed fresh pulp (2.6 $\mu\text{M TROLOX/g}$) expressed on a dry basis [52]. This difference may be because the ABTS method measures the antioxidant activity of both hydrophilic and lipophilic compounds [53], which may be more available after the water has evaporated after dehydration.

Table 2. Proximate composition and properties of *Acromia aculeata* (Jacq.) Lodd. powder-pulp.

Analysis	Content
Moisture (g/100 g)	6.22 ± 0.30
Ash (g/100 g)	3.15 ± 0.06
Protein (g/100 g)	2.94 ± 0.30
Lipids (g/100 g)	9.15 ± 0.35
Carbohydrates	78.54 ± 0.25
Energy	408.27 ± 0.20
pH	5.57 ± 0.03
Soluble solids (°Brix)	3.10 ± 0.15
Acidity (mg/mL)	0.24 ± 0.01
L* parameter	53.24 ± 2.48
a* parameter	14.09 ± 1.14
b* parameter	39.20 ± 2.03
Chroma	37.97 ± 2.32
Hue	71.31 ± 0.89
Vitamin C content (mg/100 g)	13.12 ± 8.66
Total carotenoids (µg/g)	6.34 ± 1.50
Total phenolic compounds (g/100 g)	216.58 ± 7.12
Antioxidant activity using DPPH method (µM TEAC/100 g)	10.69 ± 0.003
Antioxidant activity using ABTS method (µM TEAC/100 g)	557.00 ± 36.56

3.2. Chemical and Technological Characterization and Microbiological Viability of the Fermented Beverages during Storage

Figure 1 shows the pH (1A), acidity (1B), soluble solids (1C), and osmolality (1D) of the kefir-fermented soymilk beverages supplemented with inulin and/or bocaiúva powder-pulp at 1, 6, 11, and 16 d. The pH of the CONT beverage (Figure 1A) showed no significant difference during storage, unlike what occurred with the other treatments where the values decreased from 4.22 to 3.75 for IN3.5, from 4.34 to 4.12 for BO3.5, from 4.43 to 4.07 for BO7.0, and from 4.19 to 3.93 for BO + IN, from day 1 to day 16. These values were lower than the values previously reported for kefir-fermented soy beverages containing inulin [25,27]. A pH < 4.5 guarantees the microbiological safety of food products due to it decreasing the development of pathogenic and spoilage microorganisms [54]. Similarly, we did not observe a significant difference for the titratable acidity during storage (Figure 1B), but we observed an increase in acidity in the other evaluated treatments ranging of 1.50 and 3.70% for IN3.5, 1.54 and 2.56% for BO3.5, 1.81 and 2.73% for BO7.0, and 2.87 and 3.76% for BO + IN from day 1 to day 16. A decrease in pH was observed over the 16 d of storage, which, together with the increase in acidity, demonstrated that these beverages became more acidic, probably due to the metabolism of the kefir microorganisms present in the beverage [20].

As expected, the addition of inulin and/or bocaiúva powder-pulp increased the soluble solid content of the kefir-fermented soymilk from ~2.3 °Brix for the CONT to ~5.3 °Brix for IN3.5, ~2.8 and ~3.4 °Brix for BO3.5 and BO7.0, respectively, and ~9.3 °Brix for BO + IN. Osmolality showed no significant difference during the storage of the CONT (~225.6 mOsmol/kg), BO3.5 (~279.3 mOsmol/kg), BO7.0 (~248.1 mOsmol/kg), and BO + IN (~292.3 mOsmol/kg) beverages. In contrast, for the IN3.5 beverage, the osmolality increased until day 11 (from 292.4 to 333.8 mOsmol/kg) and remained high until day 16 (337.4 mOsmol/kg). Based on the definitions of the European Food Safety Authority (EFSA) [55], we classified the beverage as hypotonic (<270 mOsmol/kg) before supplementation and isotonic (270–330 mOsmol/kg) after supplementation with inulin and/or bocaiúva powder-pulp (or both). Isotonic beverages can help preserve the body's hydration by being in equilibrium with body fluids and preserving water absorption [56].

Figure 2 shows the syneresis of kefir-fermented soymilk with and without inulin and/or bocaiúva powder-pulp supplementation. Syneresis data showed no significant difference in syneresis during the storage of CONT (~90.2%), BO3.5 (~71%), BO7.0 (~62.1%),

and BO + IN (~57%). In the IN3.5 beverage, there was no change in syneresis until day 11 (~75.3%), and then there was an increase on day 16 of storage to ~93.5%.

Figure 1. The pH (A), acidity (B), soluble solid content (C), and osmolality (D) of kefir-fermented soymilk beverages supplemented with inulin and/or bocaiúva powder-pulp at 1 (black bar), 6 (dots), 11 (gray), and 16 (white) days. CONT, without adding ingredients; IN3.5, with the addition of 3.5% inulin (*m/v*); BO3.5, with the addition of 3.5% bocaiúva powder-pulp (*m/v*); BO7.0, with the addition of 7.0% bocaiúva powder-pulp (*m/v*); and BO + IN, with the addition of 3.5% inulin + 3.5% bocaiúva powder-pulp. Different letters indicate a statistical difference between the fermented beverages during the shelf life by the Tukey test ($p < 0.05$).

Figure 2. Syneresis (%) of kefir-fermented soymilk supplemented with inulin and/or bocaiúva powder-pulp at 1 (black bar), 6 (dots), 11 (gray), and 16 (white) days of shelf storage. CONT, without adding ingredients; IN3.5, with the addition of 3.5% inulin (*m/v*); BO3.5, with the addition of 3.5% bocaiúva powder-pulp (*m/v*); BO7.0, with the addition of 7.0% bocaiúva powder-pulp (*m/v*); and BO + IN, addition of 3.5% inulin + 3.5% bocaiúva powder-pulp. Different letters indicate a statistical difference between the fermented beverages during the shelf life by the Tukey test ($p < 0.05$).

This increase in the syneresis of the beverage containing inulin around the 11th day of storage has already been reported in another paper from our laboratory [27], in which we established day 14 as the end of shelf life, mainly due to the increase in syneresis and decrease in pH, which was related to an increase in phase separation (protein coagulation) caused by destabilization of the protein structure [57]. In the present work, an interesting observation was that the addition of bocaiúva powder-pulp led to a decrease in syneresis of ~19.2%, ~28.1%, and ~33.2% for the BO3.5, BO7.0, and BO + IN treatments, respectively. This may be due to the insoluble fiber present in the bocaiúva powder-pulp, which helps to form a network that traps water and prevents phase separation [58]. This is an interesting finding because, in addition to using a fruit pulp that is usually undervalued as it is unknown, we managed to improve the phase separation in the beverage, which is not an interesting feature from a commercial point of view [20].

Table 3 shows the results of the colorimetric evaluation (L^* , a^* , and chroma) of kefir-fermented soymilk supplemented with inulin and/or bocaiúva powder-pulp. The hue values were between 60 and 80, indicating that the color of the beverages was reddish-orange, with high luminosity (>50) and low saturation (<20), indicating that the color was muted and the saturation was low.

Table 3. Chromatic analysis of kefir-fermented soymilk supplemented with inulin and/or bocaiúva powder-pulp at 1, 6, 11, and 16 days of storage.

Time (d)	CONT	IN3.5	BO3.5	BO7.0	BO + IN
L^*					
0	69.48 ± 2.84 ^a	66.22 ± 4.27 ^a	65.36 ± 2.24 ^b	67.22 ± 0.89 ^b	63.00 ± 1.06 ^b
6	68.36 ± 1.79 ^a	67.95 ± 2.58 ^a	68.50 ± 1.31 ^a	66.13 ± 0.58 ^a	64.85 ± 1.79 ^a
11	66.00 ± 4.58 ^{ab}	60.30 ± 2.71 ^{bc}	66.62 ± 1.42 ^c	67.64 ± 1.33 ^d	64.75 ± 0.48 ^{bc}
16	60.9 ± 5.14 ^a	59.93 ± 3.48 ^a	65.17 ± 1.36 ^a	67.66 ± 0.91 ^c	63.87 ± 1.31 ^a
Chroma					
0	17.27 ± 1.22 ^a	8.62 ± 3.26 ^b	14.60 ± 3.16 ^{cab}	18.96 ± 1.69 ^d	25.15 ± 1.05 ^e
6	9.03 ± 1.84 ^a	8.46 ± 3.30 ^b	14.07 ± 1.99 ^c	20.23 ± 0.74 ^d	24.55 ± 1.06 ^e
11	10.68 ± 4.48 ^a	6.75 ± 2.33 ^{ac}	12.95 ± 2.16 ^{ab}	16.07 ± 1.59 ^c	26.00 ± 0.90 ^d
16	6.52 ± 3.35 ^a	6.26 ± 2.25 ^b	15.05 ± 2.22 ^c	18.22 ± 1.97 ^{ad}	24.49 ± 1.66 ^e
hue					
0	77.55 ± 3.82 ^a	73.15 ± 6.05 ^b	83.00 ± 3.14 ^c	88.32 ± 1.46 ^d	85.64 ± 1.08 ^e
6	74.32 ± 2.68 ^a	73.32 ± 3.30 ^b	83.56 ± 2.45 ^c	89.07 ± 0.58 ^d	86.62 ± 1.06 ^e
11	75.85 ± 7.01 ^a	68.49 ± 4.15 ^b	81.28 ± 2.45 ^c	84.87 ± 1.59 ^d	85.93 ± 0.90 ^e
16	63.49 ± 11.62 ^a	64.42 ± 8.02 ^b	83.43 ± 3.82 ^c	87.20 ± 1.97 ^d	87.11 ± 1.87 ^e

CONT, without adding ingredients; IN3.5: with the addition of 3.5% inulin (m/v); BO3.5: with the addition of 3.5% bocaiúva powder-pulp (m/v); BO7.0: with the addition of 7.0% bocaiúva powder-pulp (m/v); and BO+IN: addition of 3.5% inulin + 3.5% bocaiúva powder-pulp. Different letters indicate a statistical difference between the fermented beverages during the shelf life by the Tukey test ($p < 0.05$).

Table 4 shows the results of the microbiological viability for *Lactobacillus*, *Lactococcus*, and yeasts in the kefir-fermented soymilk supplemented with inulin and/or bocaiúva powder-pulp during storage. The minimum microbial counts detected during the storage of fermented beverages was 10^7 CFU/mL. According to FAO/WHO guidelines (2003), beverages that use kefir biomass must contain 10^7 UFC/g lactic acid bacteria and 10^4 UFC/g yeasts. This demonstrates that all the tested beverage formulations met these established guidelines for microorganism counts, for all tested genera. Kefir has become important in the production of fermented products, mainly for its ability to improve nutritional and sensory characteristics. In addition, the viable microorganisms in kefir are considered beneficial, and isolation, identification, and in vivo testing have shown that many of them are probiotics [20].

Fermentation of food products using kefir has been responsible for several beneficial health effects such as decreased body weight [59], increased anti-inflammatory [59,60], anti-ulcerogenic [59], hyperlipidemic, and anti-hyperglycemic [61] activities. These bene-

ficial properties to the human organism can be attributed both to the presence of viable microorganisms and to the production of metabolites by these microorganisms from the substrate that is being fermented [20,62].

Table 4. Microbiological viability of the *Lactobacillus* and *Lactococcus* genera bacteria and yeasts in kefir-fermented soymilk supplemented with inulin and/or bocaiúva powder-pulp at 1, 6, 11, and 16 days of storage.

Time (days)	<i>Lactobacillus</i> (UFC/mL)				
	CONT	IN3.5	BO3.5	BO7.0	BO + IN
0	2.70×10^7	1.70×10^7	2.45×10^7	3.65×10^7	1.00×10^7
6	3.00×10^7	1.00×10^8	1.60×10^7	3.63×10^7	1.20×10^7
11	1.70×10^8	1.20×10^8	1.10×10^8	2.66×10^7	8.00×10^7
16	3.78×10^7	1.40×10^7	2.73×10^7	1.50×10^7	1.20×10^7
	<i>Lactococcus</i> (UFC/mL)				
	CONT	IN3.5	BO3.5	BO7.0	BO + IN
0	4.80×10^7	4.60×10^7	4.25×10^7	5.02×10^7	1.60×10^7
6	2.73×10^7	5.00×10^7	3.05×10^7	1.00×10^7	5.00×10^7
11	3.00×10^7	8.83×10^7	4.95×10^7	2.00×10^7	4.23×10^7
16	1.36×10^7	2.80×10^7	2.56×10^7	4.50×10^7	4.43×10^7
	Yeast (UFC/mL)				
	CONT	IN3.5	BO3.5	BO7.0	BO + IN
0	5.70×10^7	1.90×10^8	3.50×10^8	4.12×10^7	1.60×10^7
6	5.28×10^7	1.50×10^8	1.30×10^8	2.70×10^8	1.00×10^8
11	3.00×10^7	1.60×10^7	1.10×10^7	2.00×10^7	4.23×10^7
16	2.26×10^7	5.65×10^7	3.46×10^7	2.50×10^7	1.20×10^7

CONT, without adding ingredients; IN3.5, with the addition of 3.5% inulin (*m/v*); BO3.5, with the addition of 3.5% bocaiúva powder-pulp (*m/v*); BO7.0, with the addition of 7.0% bocaiúva powder-pulp (*m/v*); and BO + IN, addition of 3.5% inulin + 3.5% bocaiúva powder-pulp. Different letters indicate a statistical difference between the fermented beverages during the shelf life by the Tukey test ($p < 0.05$).

3.3. Bioactive Compounds in Kefir-Fermented Beverages Supplemented with Inulin and/or Bocaiúva Powder-Pulp

Table 5 shows the chemical composition of kefir-fermented soymilk supplemented with inulin and/or bocaiúva powder-pulp. There was no significant difference between the values found for vitamin C for the different beverages developed in the present work. The vitamin C value found for kefir-fermented beverages supplemented with inulin and/or bocaiúva powder-pulp was close to what had been reported for cranberry (25 mg/100 g) [63] and strawberry (17 mg/100 g) [64] juices.

Table 5. Chemical composition of soymilk fermented with kefir and supplemented with inulin and bocaiúva powder-pulp.

Analysis	CONT	IN3.5	BO3.5	BO7.0	BO + IN
Vitamin C (mg/100 g)	28.56 ± 5.96^a	20.64 ± 4.20^a	12.18 ± 1.49^a	17.94 ± 7.99^a	13.62 ± 2.47^a
Total carotenoids ($\mu\text{g/g}$)	0.115 ± 0.02^b	0.206 ± 0.03^b	0.146 ± 0.11^b	0.563 ± 0.09^a	0.231 ± 0.05^b
Total phenolic compounds (mg/100 g)	37.17 ± 1.17^c	39.40 ± 0.50^{bc}	39.79 ± 1.64^{bc}	45.80 ± 4.36^{ab}	49.52 ± 3.08^a
Antioxidant activity using ABTS method ($\mu\text{M TEAC/g}$)	335.00 ± 5.27^b	376.75 ± 2.93^{ab}	406.59 ± 20.38^a	378.75 ± 12.69^{ab}	388.61 ± 10.21^a

CONT, without adding ingredients; IN3.5, with the addition of 3.5% inulin (*m/v*); BO3.5, with the addition of 3.5% bocaiúva powder-pulp (*m/v*); BO7.0, with the addition of 7.0% bocaiúva powder-pulp (*m/v*); and BO + IN, addition of 3.5% inulin + 3.5% bocaiúva powder-pulp. Different letters indicate a statistical difference between the fermented beverages during the shelf life by the Tukey test ($p < 0.05$).

Higher content of carotenoids was found for the formulation containing the highest amount of bocaiúva powder-pulp as this ingredient is the biggest contributor to this bioactive compound. De Oliveira Gonçalves et al. [65] demonstrated that bocaiúva pulp

contains β -carotene (24.3 $\mu\text{g/g}$) and α -caroteno (22 $\mu\text{g/g}$). Alpha-carotene produces 50% of vitamin A, while beta-carotene is the most powerful carotene, which produces vitamin A in the small intestine. Vitamin A is an essential micronutrient that plays an important role in a wide array of physiologic processes, including vision, immune response, cell differentiation and proliferation, intercellular communication, and reproduction [66].

Higher levels of phenolic compounds were detected in the BO + IN (49.52 μM TEAC/100 g) and BO7.0 (45.80 μM TEAC/100 g) beverages, which were probably derived from the bocaiúva powder-pulp. All phenolic compound contents in the beverages of the present study were higher than those reported for soymilk supplemented with pineapple, mango, and passion fruit (18.9 μM TEAC/100 g) [67]. The quantification of total phenolic compounds suggests the compounds produced by the shikimic acid route including simple phenylpropanoids, phenolic acids, and isoflavones. In the present work, the content of phenolic compounds can be associated with both the presence of soybean [68] and the presence of bocaiúva [52].

The antioxidant activity of the beverages appeared to be related to supplementation of inulin and/or bocaiúva powder-pulp because all formulations containing these ingredients had a higher value compared to CONT beverage, demonstrating that it may be related to the presence of phenolic compounds that demonstrated the same behavior.

4. Conclusions

Fermented soymilk supplemented with inulin and/or bocaiúva powder-pulp appears to be a good substrate for maintaining the number of viable fermenting microorganisms in kefir (*Lactobacillus*, *Lactococcus*, and yeasts) above 10^7 CFU/mL. The supplementation improved the technological characteristics, mainly syneresis and phase separation, of kefir-fermented soymilk without altering the viability of beneficial microorganisms present in kefir. The kefir-fermented beverage containing 3.5% of inulin and bocaiúva powder-pulp, which showed good technological characteristics, seems to be an alternative for the use of *Acrocomia aculeata* pulp, increasing the added value of these fruits and promoting the valorization of the Cerrado biome.

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