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DEVELOPMENT AND CHARACTERIZATION OF AN ECO-FUNCTIONAL FERMENTED DAIRY BEVERAGE: COMBINING WHEY VALORIZATION WITH NATIVE FRUITS

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Abstract: This study developed an eco-functional fermented dairy beverage based on whey valorization (30%) combined with milk (70%) and enriched with 15% native Brazilian fruit pulps (araçá, butiá, uvaia, and guabiro-ba). Fruit incorporation significantly reduced pH (4.79 to 4.48–4.67), enhancing system acidity. Protein content ranged from 0.49 to 3.03 g/100 g, while moisture remained ~88%, except for butiá (93.49 g/100 g), resulting in lower total solids (6.51 g/100 g). Color parameters were affected, with decreased luminosity (L* 84.47 to 48.75–66.30) and in-creased yellowness (b* 6.89 to 11.15–16.87). Bioactive compounds increased significantly, especially in the guabiroba formulation, which showed the highest carotenoid content (β-carotene: 1178.25 μg/100 g) and total phenolics (58.20 mg/100 g). Antioxidant capacity increased, with DPPH values rising from 146.56 to 763.46 μmol TE/g and ABTS reaching 1661.39 μmol TE/g. Ash content remained stable (~0.65–0.75 g/100 g), while calcium decreased (0.96 to 0.80–0.84 mg/g) and potassium increased (up to 1.73 mg/g). Antimicrobial assays against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* showed high inhibition (~99%), indicating synergistic effects between acidification, fer-mentation metabolites, and fruit-derived bioactives. These findings highlight the potential of combining whey valorization with Brazilian biodiversity to develop functional fermented beverages with enhanced nutritional, antioxidant, and natural biopreservative properties.

Keywords: dairy products; bioactive compounds; carotenoids; polyphenols; by-product; biodiversity; Brazilian fruits.

Highlights:

- Whey valorization enabled sustainable functional dairy beverage development.
- Native fruit pulps increased carotenoids and phenolic compounds.
- Guabiroba formulation showed the highest antioxidant activity.
- Fruit addition enhanced antimicrobial activity against foodborne pathogens.
- Brazilian biodiversity improved nutritional and technological properties.

1. Introduction

The growing demand for healthy, sustainable foods with regional appeal has driven the development of innovative fermented dairy products, particularly those that repurpose industrial by-products and valorize native Brazilian fruits. One of the most abundant by-products of the dairy industry is whey, which represents approximately 85 to 90% of the total volume of milk used in the manufacture of cheese and contains approximately 55% of the original nutrients of milk, including high biological value proteins, lactose, minerals, and vitamins (Amaral & Silva, 2021; Buchanan et al., 2023). In Brazil, it is estimated that the annual production of whey exceeds 8 billion liters, of which a significant portion is still improperly discarded, causing severe environmental impacts, such as increased organic load in water bodies and micro-biological imbalance (Buchanan et al., 2023).

The reuse of whey in fermented foods, such as dairy beverages, is a promising strategy to mitigate environmental impact while simultaneously adding nutritional value to the final product. Fermented dairy beverages are defined as products obtained by fermenting milk or its derivatives with specific microorganisms, often of the Lactobacillus and *Streptococcus* genera, and may contain additional ingredients such as fruits, pulps, or flavorings [3]. According to current Brazilian legislation, the use of a whey fraction in the formulation of these beverages is permitted provided that its addition does not compromise the product's nutritional quality (Brasil, 2007). These beverages stand out for their balanced macronutrient composition and functional benefits, including probiotic activity, improved lactose digestibility, and antioxidant, immunomodulatory, and hypocholesterolemic effects (Grispoldi et al., 2022; Tamang et al., 2016).

The addition of native fruits to the development of fermented dairy beverages represents an advance in terms of technological innovation and market differentiation (A. A. ; Prestes et al., 2023). Species such as araçá (*Psidium cattleianum*), butiá (Butia odorata), uvaia (*Eugenia pyriformis*), and guabiroba (*Campomanesia xanthocarpa* O. Berg) are fruits with a characteristic yellow color and recognized for their high content of bioactive compounds, such as phenolics, flavonoids, and carotenoids, in addition to presenting expressive antioxidant and anti-inflammatory activities (da Fonseca Antunes et al., 2024; Tischer et al., 2023). Despite their functional and sensory potential, these fruits remain commercially underutilized due to their high perishability, seasonality, and the fact that their production is mostly concentrated in small family farmers and through extractivism (Backes et al., 2021).

The incorporation of these native fruits into fermented dairy matrices not only promotes appreciation of Brazilian biodiversity but also strengthens family farming and the sustainable use of local resources. Recent studies indicate that the sensory acceptance of products derived from native fruits can be high, if they are combined with a food base familiar to the consumer, such as yogurts or dairy beverages (Bianchini et al., 2020; Pereira et al., 2024; Prestes et al., 2023; Prestes et al., 2024). The synergy between fermented whey and pulps of yellow native fruits can also result in improvements in the technological and rheological characteristics of the product, in addition to increasing its microbiological stability and extending its shelf life, which represents an additional attraction for the food industry (Priyashantha et al., 2025) .

Thus, the development of a fermented milk beverage made from recycled whey and enriched with pulp from native yellow fruits in Brazil represents an innovative, sustainable, and functional alternative with high potential for market acceptance. In addition to contributing to waste reduction and the rational use of natural resources, this proposal strengthens bioprospecting of native ingredients and expands the technological possibilities of the dairy industry. The main objective of this study is to develop and characterize a fermented whey-based dairy beverage with the addition of araçá, butiá, uvaia, and guabiroba, and to evaluate its physicochemical, functional, and bioactive properties.

2. Materials and Methods

2.1 Reagent

All chemicals used were of analytical grade or higher purity. Ultrapure water (18.2 MΩ·cm) was obtained using an ultrapure water system (MS3000, Master System, Gehaka, São Paulo, SP, Brazil) and used to prepare all solutions and dilutions. Sample preparation was carried out using acetone, sodium hydroxide (NaOH), petroleum ether, nitric acid (HNO_3), and hydrochloric acid (HCl) (Quimis, São Paulo, SP, Brazil), as well as tetramethylammonium hydroxide (TMAH, 25% w/w in H_2O) supplied by Sigma Aldrich (Germany). Nitric acid was further purified by sub-boiling distillation using a polytetrafluoroethylene (PTFE) system (Distill Acid BSB-939-IR, Berghof, Germany). Individual standard solutions (1000 mg L^{-1}) of Ca, Cu, P, Zn, and Sc (Specsol®, Jacareí, SP, Brazil), K (Merck, Darmstadt, Germany), Cr, Fe, and Mg (SCP Science, Quebec, Canada), and Na (Vetec, Duque de Caxias, RJ, Brazil) were used to construct calibration curves and to perform recovery tests.

2.2 Raw Material

Native fruit pulps, including butiá, araçá, and uvaia, were supplied by Encontro de Sabores (Passo Fundo, RS, Brazil). Guabiroba pulp was provided by EMBRAPA Florestas (Colombo, PR, Brazil). These fruit pulps were used to formulate fermented dairy beverages. Whey obtained from the production of Minas Frescal cheese was used as a dairy by-product (a clotting enzyme HA-LA®, with a coagulation strength of 1:3000, was obtained from Chr. Hansen -Valinhos, SP, Brazil), along with whole

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UHT (ultra-high temperature) milk (Tirol®, Nova Trento, SC, Brazil), containing 3.2 g 100 g^{-1} protein, 3.0 g 100 g^{-1} lipids, and 4.5 g 100 g^{-1} carbohydrates. The fermented dairy beverages were prepared using a thermophilic culture of *Streptococcus salivarius* subsp. *thermophilus*, and *Lactobacillus acidophilus* LA-5 (BioRich®, Chr. Hansen, Valinhos, São Paulo, Brazil).

2.3.1 Preparation of fermented dairy beverages

Fermented dairy beverages were prepared using a formulation consisting of 70% whole milk and 30% whey, a proportion selected based on preliminary trials (data not shown) and adapted from the methodology proposed by Almeida et al. (Almeida et al., 2001). The milk–whey blend was heated to 42 ± 2 °C and subsequently inoculated with 0.05 g 100 g^{-1} of a thermophilic starter culture composed of *Lactobacillus acidophilus* LA-5 and *Streptococcus thermophilus*, in accordance with the manufacturer's instructions. The fermentation process was carried out at 42 ± 2 °C with continuous pH monitoring. Upon reaching the target pH, five formulations were produced by incorporating fruit pulp at 15%, as established in previous studies [12]. Four formulations contained native Brazilian fruit pulps—butiá, guabiroba, uvaia, and araçá—identified as BB15, BG15, BU15, and BA15, respectively, while a control formulation without fruit pulp was also prepared (Figure 1). After fermentation, the beverages were rapidly cooled and stored at 4 ± 2 °C until further analyses.

2.3.2 Physicochemical analysis

Color measurements of the samples were performed using a sphere spectrophotometer (SP60 Series, X-Rite Inc., Grand Rapids, MI, USA). The results were recorded in the CIELAB color space as L*, a*, and b* values. The L* coordinate represents lightness on a scale from 0 (black) to 100 (white), whereas the a* axis indicates the chromatic variation from green (–a*) to red (+a*), and the b* axis corresponds to the transition from blue (–b*) to yellow (+b*).

2.3.3 Total Carotenoids content

Carotenoids were quantified based on the method proposed by Rodriguez-Amaya (Rodriguez-Amaya, 2001), with slight modifications. Initially, 1 g of the sample was mixed with 20 mL of acetone in a 50 mL Falcon® tube. The mixture was homogenized using a vortex mixer and then submitted to ultrasonic extraction at 25 °C for 30 min to enhance pigment solubilization. After extraction, the suspension was filtered through filter paper to remove insoluble residues. The filtrate was transferred to a separatory burette containing 4 mL of petroleum ether, followed by the addition of 3 mL of ultrapure water (Type 2). The system was allowed to stand until complete phase separation. When necessary, a few drops of NaOH solution were added to promote separation. The lower aqueous and colorless phase was discarded, while the upper organic phase, containing the carotenoids, was collected. The organic extract was passed through an anhydrous sodium sulfate filter paper to remove residual moisture, then transferred to a volumetric flask. The burette was rinsed with petroleum ether to ensure quantitative recovery of the pigments. Carotenoid content was determined by UV–Vis spectrophotometry at specific wavelengths:

Figure 1- Fermented dairy beverages with 15% pulp of Brazilian native fruits; Note: butiá, guabiroba, uvaia, and araçá—identified as BB15, BG15, BU15, and BA15, respectively.

450 nm for β-carotene, 444 nm for α-carotene, 452 nm for β-cryptoxanthin, and 462 nm for λ-carotene. Results were expressed as micrograms of carotenoids per 100 g of sample (µg/100 g).

2.3.4. Total phenolic compounds and Antioxidant activity

Total phenolic compounds (TPC) were determined by the Folin–Ciocalteu colorimetric method, following the protocol described by Singleton and Rossi (Singleton & Rossi, 1965) with minor adaptations. This assay is based on the reduction of the Folin–Ciocalteu reagent by phenolic compounds under alkaline conditions, resulting in the formation of a blue-colored complex. For the analysis, the reaction mixture was incubated in the dark at room temperature for 90 min to ensure complete color development. Absorbance was then measured at 725 nm using a UV–Vis spectrophotometer (Shimadzu® UV-1800, Kyoto, Japan). Quantification was performed using a gallic acid calibration curve, and the results were expressed as micromoles of gallic acid equivalents per gram of sample (µM GAE/g).

The antioxidant capacity of fermented dairy beverage samples was evaluated using two complementary radical scavenging assays, which assess different mechanisms of antioxidant action. The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was carried out according to Brand-Williams et al. (Brand-Williams et al., 1995) and is based on the ability of antioxidant compounds to donate hydrogen atoms or electrons to neutralize the DPPH free radical, thereby decreasing absorbance. The reaction was conducted in the dark at room temperature for 30 min, and absorbance was measured at 515 nm using a UV–Vis spectrophotometer (Shimadzu®, Kyoto, Japan). Antioxidant capacity was quantified using a Trolox standard curve and expressed as micromoles of Trolox equivalent antioxidant capacity per gram of sample (µM TEAC/g).

In addition, antioxidant activity was determined using the ABTS•+ [2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)] radical cation decolorization assay, as described by Re et al. (Re et al., 1999). This method evaluates the ability of antioxidants to quench the ABTS•+ radical, resulting in a reduction in absorbance proportional to antioxidant concentration. Measurements were performed at 734 nm using a UV–Vis spectrophotometer (Shimadzu®, Kyoto, Japan), and results were expressed as µM TEAC/g.

2.3.5 Multi-element profile

Fermented dairy samples were previously digested using a microwave-assisted system (Multiwave PRO, Anton Paar, Graz, Austria) with closed vessels, operating at microwave power up to 1200 W, internal temperature of 200 ± 1 °C, and pressure of 20 bar. Sample preparation involved different approaches. Ultrasound-assisted extraction was performed in an ultrasonic bath (Nova Instruments, Piracicaba, SP, Brazil) at 50 Hz and room temperature (25 ± 1 °C). Alkaline solubilization was carried out in a thermostatic water bath with a heating plate (IKA, Campinas, SP, Brazil). In parallel, dry ashing was conducted in a muffle furnace (Jung, Blumenau, SC, Brazil) at 550 ± 1 °C, followed by dissolution of the ashes in concentrated HCl at 80 ± 1 °C. After preparation, aliquots corresponding to 2.218 g of sample were centrifuged in a bench centrifuge (Fanem, Guarulhos, SP, Brazil).

Elemental composition was determined by inductively coupled plasma optical emission spectrometry (ICP OES; iCAP 6000, Thermo Scientific, Waltham, MA, USA). The elements were quantified at the following emission wavelengths: Ca (315.887 nm), Cr (267.716 nm), Cu (324.754 nm), Fe (259.940 nm), K (766.490 nm), Mg (279.553 nm), Na (589.592 nm), P (213.618 nm), and Zn (213.856 nm), using scandium (Sc, 361.384 nm) as the internal standard. Element selection considered both nutritional relevance in dairy products and instrument performance. The ICP OES system was equipped with a V-groove nebulizer and a cyclonic spray chamber, enabling reliable analysis of solutions with relatively high dissolved solids content when adequate sample pretreatment was provided.

Measurements were carried out in radial view mode, with a pump speed of 60 rpm, plasma gas flow of 12 L/min, RF power of 1300 W, auxiliary gas flow of 1 L/min, and nebulizer gas flow of 0.4 L/min. High-purity argon (≥ 99.95%, Air Liquide, Brazil) was used for plasma generation and aerosol transport. Quantification was based on external calibration curves prepared from multi-element standard solutions in the range of 0.1 to 10 mg/L. Ultrapure water (Master System, Gehaka, Brazil) was used throughout sample preparation and dilution procedures. Nitric acid and hydrochloric acid (Quimis, Brazil) and tetramethylammonium hydroxide (25%, w/w; Sigma-Aldrich, Germany) were used as reagents. Nitric acid was additionally purified by sub-boiling distillation (Berghof, Germany). Certified elemental standards (1000 mg/L) were used for calibration and recovery evaluation.

2.3.6 Antimicrobial activity

Approximately 5.0 ± 0.2 g of each fermented dairy beverage sample were weighed into Falcon tubes, 5 mL of distilled water was added, vortexed for 5 min, and kept under continuous agitation for 6 h at room temperature (25 ± 2°C) in a shaker (Novatecnica, Brazil). The extracts were then filtered through filter paper to remove coarse particles, and subsequently through a 0.22 µm membrane filter for sterilization. The sterile extracts were packaged in microtubes and stored at −80 °C until antimicrobial assays were performed.

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Antimicrobial activity was evaluated using the broth microdilution method, adapted from Martins at al. (Martins et al., 2020). The strains used were *Staphylococcus aureus* NCTC 12981, *Escherichia coli* NCTC 12241, and *Candida albicans* ATCC 10231, representing Gram-positive microorganisms, Gram-negative microorganisms, and fungi, respectively.

The bacterial strains were reactivated on nutrient agar (NA- Nutrient Agar; Himedia, India) and incubated for 24 h at 37 ± 2 °C. The inocula were standardized to approximately 1–2 × 10^8 CFU/mL (McFarland scale 0.5) and subsequently diluted in brain-heart infusion broth (BHI; Scharlau, Spain) to a final concentration of 1–2 × 10^2 CFU/mL. Confirmation of the microbial count was performed by plating on nutrient agar. For the assay, 100 µL of each extract were mixed with 100 µL of the microbial inoculum in sterile microtubes. The negative control consisted of 100 µL of the inoculum and 100 µL of BHI. The samples were incubated for 6 h at 37 ± 2 °C under agitation at 200 rpm. After incubation, the inoculum was diluted in sterile saline solution and plated on nutrient agar for colony counting.

The results were expressed in CFU/mL, according to Equation 1:

$$CFU = CN\, x \frac{1}{V} x \frac{1}{D}$$

Where:

CN: Number of colonies grown on the plate (between 30-300 colonies)

V: Pipetted volume of inoculum for plating (0.1 mL)

D: Dilution performed

The calculation of the microbial reduction caused by the extracts in percentage (%) was calculated as follows, using the microbiological counts obtained after the contact time (6h) (Equation 2):

Microbial reduction (%)=

$$100\, x \left(\frac{negative\ control\ count - sample\ count}{Negative\ control\ count} \right)$$

2.3.7 Statistical analysis

Data are presented as mean values accompanied by their respective standard deviations. Statistical differences among treatments were assessed by one-way analysis of variance (ANOVA), followed by Tukey's post hoc test, adopting a significance level of $p < 0.05$. All statistical procedures were carried out using STATISTICA software (version 13.3; TIBCO Software Inc., Palo Alto, CA, USA). All experiments and analyses were conducted in triplicate.

3. Results and Discussion

3.1 Physicochemical parameters

The incorporation of 15% native Brazilian fruit pulps significantly affected the physicochemical and color properties of the fermented dairy beverages formulated with 70% milk and 30% whey (Table 1). In general, the addition of fruit pulps modified parameters related to pH, composition, and the product's optical characteristics, reflecting both the intrinsic composition of each fruit and the interactions between its constituents and the fermented dairy matrix.

A consistent reduction in pH was observed in the formulations containing pulp compared with the control, which had the highest value (4.79). Among the fruit beverages, formulation BG15 presented the lowest pH (4.48), while BA15 and BB15 presented intermediate values. This decrease can be attributed mainly to the presence of or-ganic acids naturally present in the pulps, such as citric and malic acids, which directly contribute to the system's total acidity (de Oliveira Galdino et al., 2025). In a study with goat-milk fermented with tropical fruit blends, formulations containing more acidic fruits (e.g., jaboticaba; pH ≈ 3.25) showed significantly lower pre-fermentation pH and higher acidity than controls (de Oliveira Galdino et al., 2025). In fermented dairy matrices, the addition of fruits frequently results in a reduction in pH not only due to the introduction of these natural acids, but also be-cause components of the fruits can influence the metabolism of lactic acid bacteria during or after fermentation (Gutiérrez-Álzate et al., 2023a). Similar behaviors have been reported in fermented dairy beverages with added tropical fruits, in which the incorporation of acidic pulps intensifies product acidity without compromising the typical pH range of these fermented systems (Borgonovi et al., 2022; Nasution et al., 2025).

Differences were also observed in the composition of the beverages. The protein content ranged from 0.49 to 3.03 g 100 g^{-1}, with the control showing 1.63 g 100 g^{-1}. The variations between the formulations may be mainly associated with dilution effects resulting from the addition of the pulps, since fruits have much lower protein concentrations than milk (Yang et al., 2023). The significantly lower value observed for BB15 suggests a more pronounced dilution effect related to the composition of the butiá pulp. In contrast, BG15 showed the highest protein content among the samples, which may be due to analytical variations or interactions between fruit compounds and milk proteins that could influence quantification. Fruit polyphenols and other compounds can bind to milk proteins (especially caseins) and form larger aggregates or adducts, altering solubility and recovery in Kjeldahl/Bradford-type as-says (Mao et al., 2024). Similar results have been reported in dairy beverages enriched with fruit pulps or juices, in which the proportion of milk proteins may vary depending on the moisture content and soluble solids from the added vegetable ingredient (Prestes et al., 2023; Prestes et al., 2024).

The moisture and total solids values also reflected the compositional contribution of the pulps used. Most formulations showed moisture values close to the control (approximately 88%), while BB15 showed a significantly higher content (93.49 g 100 g^{-1}), resulting in the lowest total solids content (6.51 g 100 g^{-1}). These results suggest that butiá pulp contributed a greater water fraction to the system compared to the other fruits evaluated. In fermented dairy beverages, the balance between moisture and total solids is a relevant technological parameter, as it directly influences properties such as viscosity, colloidal stability, and sensory perception (Gutiérrez-Álzate et al., 2023b). Thus, the differences observed between the formulations may affect the texture and stability of the final product, especially in systems with higher water content and lower solids fraction.

On the other hand, the ash content remained relatively constant among the samples, indicating that the addition of the pulps at the evaluated concentration did not significantly alter the mineral fraction of the beverages. This can be explained by the fact

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that the dairy matrix already naturally presents relevant concentrations of minerals, such as calcium, phosphorus, and potassium, originating from the milk and whey, which reduces the relative impact of the mineral contribution from the fruits (Angelino et al., 2025).

The color parameters were strongly influenced by the addition of fruit pulps, highlighting the impact of the natural pigments present in the fruits (Figure 1). The control beverage showed the highest luminosity value (L* = 84.47), characteristic of dairy systems with a predominantly white appearance (de Oliveira Galdino et al., 2025). In contrast, all formulations containing fruit showed significantly lower L* values, indicating visually darker products. The most pronounced reduction was observed in formulation BG15, suggesting a higher pigment concentration in the guabiroba pulp. Furthermore, while the control showed a slightly negative a* value, typical of simple dairy beverages, the addition of fruit pulps shifted it to positive values, indicating the development of reddish hues. Simultaneously, a significant increase in the b* parameter was observed in all formulations with fruit, indicating a greater intensity of yellow coloration. This behavior is characteristic of many native Brazilian fruits, which have a high concentration of carotenoids and phenolic compounds responsible for yellow or orange coloration (da Fonseca Antunes et al., 2024; Gwozdz et al., 2023; Prestes et al., 2022; Taver et al., 2022). The presence of these pigments not only influences the visual appearance of the product, but may also be associated with the presence of bioactive compounds with potential antioxidant activity (Prestes et al., 2023; Prestes et al., 2024)(Table 2).

3.2 Total Carotenoids content

The incorporation of native Brazilian fruit pulps significantly influenced the carotenoid profile of the fermented dairy beverages. In the control formulation, α-carotene and cryptoxanthin were present only at low levels (8.10 and 4.20 μg 100 g^{-1}, respectively), while β-carotene was the predominant carotenoid (45.25 μg 100 g^{-1}). This behavior is expected, as bovine milk naturally contains β-carotene derived from the animal's diet, which is the major carotenoid present in dairy matrices. However, the addition of fruit pulps substantially increased both the diversity and concentration of carotenoids in the beverages, demonstrating the nutritional contribution of the native fruits (Nozière et al., 2006)

Among the evaluated formulations, the beverage containing guabiroba pulp (BG15) showed the highest levels of α-carotene (1090.72 μg/100 g) and β-carotene (1178.25 μg/100 g), indicating a strong enrichment in provitamin A compounds. This result is consistent with previous studies reporting that fruits from the Brazilian Atlantic For-est, particularly guabiroba and related *Myrtaceae* species, contain considerable amounts of carotenoids and other bioactive compounds that contribute to their functional potential (Prestes et al., 2022). These fruits are recognized as promising sources of nutritionally relevant phytochemicals with antioxidant activity and potential health benefits (da Silva et al., 2022; de Oliveira Raphaelli et al., 2021; de Paulo Farias et al., 2020)

In contrast, the beverages formulated with araçá (BA15) and butiá (BB15) presented intermediate carotenoid concentrations. Although lower than those observed for guabiroba and uvaia, the presence of these carotenoids still represents a relevant nu-

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	Samples				
	Control	**BA15**	**BU15**	**BG15**	**BB15**
pH	4.79 ± 0.01[a]	4.65 ± 0.01[b]	4.56 ± 0.01[c]	4.48 ± 0.01[d]	4.67 ± 0.01[b]
Protein (g/100g)	1.63 ± 0.37[b]	1.48 ± 0.36[c]	0.97 ± 0.02[d]	3.03 ± 0.31[a]	0.49 ± 0.08[e]
Moisture (g/100g)	88.30 ± 1.90[b]	88.24 ± 0.09[b]	88.24 ± 0.12[b]	88.08 ± 0.08[b]	93.49 ± 1.26[a]
Total Solids content (g/100g)	11.70 ± 1.90[a]	11.76 ± 0.09[a]	11.76 ± 0.12[a]	11.92 ± 0.08[a]	6.51 ± 1.46[b]
Ash (g/100g)	0.72 ± 0.36	0.70 ± 0.02	0.65 ± 0.04	0.75 ± 0.02	0.71 ± 0.04
L*	84.47 ± 0.10[a]	65,31 ± 0.20[c]	66.30 ± 0.01[b]	48.75 ± 0.10[e]	61. 04 ± 0.01[d]
a*	-0.42 ± 0.20[e]	0.97 ± 0.01[c]	1.22 ± 0.20[bc]	2.46 ± 0.10[a]	0.76 ± 0.01[d]
b*	6.89 ± 0.10[e]	11.15 ± 0.01[d]	13.84 ± 0.02[c]	16. 87 ± 0.10[a]	15.30 ± 0.01[b]

Table 1. Physicochemical and color parameters of fermented dairy beverages added with 15% native fruit pulps.

Notes: BG15 = 15% guabiroba pulp; BB15 = 15% butiá pulp; BA15 = 15% araçá pulp; BU15 = 15% uvaia pulp; Values with mean ± standard deviation; a-e Means accompanied by the same letters in the same column do not show a significant difference ($p < 0.05$).

	Samples				
	Control	**BA15**	**BU15**	**BG15**	**BB15**
α-caroten (μg/100g)	8.10 ± 1.60[e]	218.25 ± 41.96[c]	342.61 ± 61.50[b]	1090.72 ± 184.43[a]	158.52 ± 31.70[d]
β-caroten (μg/100g)	45.25 ± 9.00[e]	235.77 ± 153.36[c]	370.12 ± 74.00[b]	1178.25 ± 199.23[a]	171.24 ± 34.00[d]
Criptoxantin (μg/100g)	4.20 ± 0.85[e]	197.13 ± 128.23[c]	309.05 ± 61.80[b]	985.17 ± 166.58[a]	143.18 ± 28.00[d]
λ- caroten (μg/100g)	<0.01	256.13 ± 166.60[c]	402.06 ± 80.40[b]	1029.98 ± 216.44[a]	186.02 ± 37.00[d]
Total phenolic content (mg/100g	Present *	19.20 ± 0.9[bc]	7.25 ± 0.50[cd]	58.20 ± 2.5[a]	27.70 ± 2.4[b]
DPPH (μmolTE/g*)	146.56 ± 20.52[d]	421.46 ± 15.14[b]	414.13 ± 26.63[bc]	763.46 ± 74.57[a]	399.46 ± 15.16[c]
ABTS (μmolTE/g*)	61.45 ± 18.60[e]	547.89 ± 83.13[b]	448.88 ± 79.14[c]	1661.39 ± 73.15[a]	468.96 ± 88.54[d]

Table 2. Total carotenoid content, phenolic compounds, and antioxidant activity of fermented dairy beverages with 15% native fruit pulps.

Note: G15= 15% guabiroba pulp ; B15= 15% butiá pulp, A15= 15% araçá pulp; U15= 15% uvaia pualp; Values with mean ± standard deviation; [a-e] Means accompanied by the same letters in the same column do not show a significant difference. ($p< 0.05$).

tritional enhancement compared with the control formulation. Native fruits belonging to the *Myrtaceae* family, such as araçá and guabiroba, are recognized for their rich phytochemical composition, including phenolic compounds and carotenoids, which contribute to antioxidant capacity and may provide additional health benefits when incorporated into food systems(Gwozdz et al., 2023; A. Prestes et al., 2022)

The rise in carotenoid levels found in fruit-enriched beverages underscores the potential of these formulations as functional dairy products. Carotenoids like β-carotene and cryptoxanthin are known as provitamin A compounds and have important physiological roles, including antioxidant activity, immune support, and reducing oxidative stress. Therefore, adding native Brazilian fruits not only enhances the nutritional profile of fermented dairy beverages but also aids in developing value-added functional foods based on biodiversity resources (Rockett et al., 2020). Overall, the findings indicate that using native fruits such as guabiroba, uvaia, araçá, and butiá is a promising approach to increasing the carotenoid content in dairy drinks, thereby strengthening their potential as functional foods with added nutritional and bioactive benefits (Machado et al., 2025).

3.3 Total phenolic content and Antioxidant Activity

The incorporation of native Brazilian fruit pulps significantly influenced the total phenolic content and antioxidant capacity of the fermented dairy beverages. In the control formulation, phenolic compounds were detected only in trace amounts, as expected, since milk naturally contains low levels of phenolic compounds.

The beverage formulated with araçá pulp (BA15) showed a total phenolic content of 19.20 ± 0.9 mg/100 g, representing a substantial increase compared with the control sample. This enrichment resulted in a significant increase in antioxidant capacity, particularly in the DPPH assay (421.46 µmol TE/g). Fruits of the genus *Psidium* are recognized as important sources of phenolic compounds, including flavonoids and phenolic acids, which contribute strongly to antioxidant activity and have been associated with functional properties in food systems (Machado et al., 2025)

For the beverage containing uvaia pulp (BU15), the total phenolic content reached 7.25 ± 0.50 mg/100 g. Although lower than that observed for the araçá formulation, the antioxidant activity remained high, with values of 414.13 µmol TE/g for DPPH and 448.88 µmol TE/ g for ABTS. Uvaia (*Eugenia pyriformis*) has been described as a fruit rich in bioactive compounds, particularly phenolics and carotenoids, which contribute to antioxidant capacity and may promote additional nutritional benefits when incorporated into dairy matrices (Rodrigues et al., 2021; Taver et al., 2022)

Among the evaluated formulations, the beverage containing guabiroba pulp (BG15) presented the highest phenolic concentration (58.20 ± 2.5 mg/100 g) and the greatest antioxidant capacity in both assays (763.46 µmol TE/g for DPPH and 1661.39 µmol TE/g for ABTS). These results highlight the strong functional potential of guabiroba (*Campomanesia xanthocarpa*), which has been widely reported to be a rich source of phenolic compounds with high radical-scavenging capacity (Machado et al., 2025; Prestes et al., 2022).

The beverage formulated with butiá pulp (BB15) also showed a considerable increase in total phenolic content (27.70 ± 2.4 mg/100 g) compared with the control. Correspondingly, antioxidant activity values of 399.46 µmol TE/g (DPPH) and 468.96 µmol TE/g (ABTS) were observed, indicating that butiá contributes significant amounts of bioactive compounds to the beverage matrix. Fruits from the genus Butia have been recognized for their phenolic profile and antioxidant capacity, supporting their potential use in the development of nutritionally enriched foods (da Fonseca Antunes et al., 2024). The results show a clear link between adding native fruit pulps and increasing phenolic content and antioxidant activity in fermented dairy beverages. The presence of these bioactive compounds greatly enhances the functional value of the beverages, underscoring the potential of Brazilian native fruits as natural antioxidant sources for functional dairy products (Bianchini et al., 2020; Prestes et al., 2023; Prestes et al., 2021)

3.4 Multi- element profile

Although the ash content did not show significant differences between the formulations (Table 1), indicating that the total mineral fraction of the beverages remained relatively constant, detailed analysis of the multi-element profile revealed important variations in the concentration of individual minerals. This result suggests that incorporating native fruit pulps promoted redistribution of the beverage's mineral composition without significantly altering the matrix's total mineral content. In complex food systems, such as fermented dairy beverages, ash content represents only the total mineral content, not necessarily reflecting changes in the concentrations of specific elements. Thus, the addition of fruit pulps can increase the concentration of some fruit-specific minerals, while other elements from the dairy matrix remain constant or are slightly diluted, resulting in an overall balance in the total mineral fraction.

The evaluation of the multi-element profile of the dairy beverages formulated with the addition of 15% native fruit pulp is presented in Table 3, which indicates that only calcium (Ca), potassium (K), and magnesium (Mg) showed measurable concentrations above the limit of detection (LOD). The other elements analyzed (Al, As, Cd, Co, Cr, Cu, Fe, and Mn) were not detected and remained below the LOD. Calcium showed the highest concentration in the control bever-age (0.96 ± 0.02 mg g^{-1}), with a statistically significant reduction ($p < 0.05$) in the formulations containing native fruit pulp, whose values ranged from 0.80 to 0.84 mg g^{-1}. This reduction can be explained by the dilution effect of the dairy fraction, resulting from the partial replacement of milk and whey with plant-based pulps, which naturally have lower calcium levels than the dairy matrix. Similar behavior has been reported in fermented dairy beverages, processed cheese spreads, and fruit-enriched yogurts, in which the incorporation of plant ingredients leads to a proportional decrease in calcium content without compromising its nutritional relevance (Al-aswad & Shehata, 2025; Nunes et al., 2025; Prestes et al., 2023; Prestes et al., 2025; Toft et al., 2024). Nevertheless, the values observed remain within the ranges commonly reported for fermented dairy beverages, which are recognized as important sources of this mineral, indicating that the formulations maintain adequate nutritional potential even after the addition of native fruits (Nunes et al., 2025; A. A. Prestes et al., 2025).

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Elements (mg g-1)	Control	BB15	BA15	BG15	BU 15
Al	< LOD	< LOD	< LOD	< LOD	< LOD
As	< LOD	< LOD	< LOD	< LOD	< LOD
Ca	0.96 ± 0.02[a]	0.84 ± 0.02[b]	0.84 ± 0.02[b]	0.82 ± 0.01[bc]	0.80 ± 0.01[c]
Cd	< LOD	< LOD	< LOD	< LOD	< LOD
Co	< LOD	< LOD	< LOD	< LOD	< LOD
Cr	< LOD	< LOD	< LOD	< LOD	< LOD
Cu	< LOD	< LOD	< LOD	< LOD	< LOD
Fe	< LOD	< LOD	< LOD	< LOD	< LOD
K	1.49 ± 0.03[d]	1.61 ± 0.04[b]	1.73 ± 0.04[a]	1.58±0.08[c]	1.31±0.01[e]
Mg	0.099±0.003[c]	0.098±0.003[c]	0.106±0.002[b]	0.114±0.001[a]	0.098±0.001[c]
Mn	< LOD	< LOD	< LOD	< LOD	< LOD

Table 3. Multi-element profile in fermented lactic beverage with 15% native fruit pulp

Note: G15= 15% guabiroba pulp ; B15= 15% butiá pulp, A15= 15% araçá pulp; U15= 15% uvaia pulp; Values with mean ± standard deviation; a-c Means accompanied by the same letters in the same column do not show a significant difference. (p< 0.05); LOD= Limit of Detection

Among the minerals evaluated, potassium was the element pre-sent at the highest concentration in the analyzed beverages, with values ranging from (1.31 ± 0.01 mg g^{-1}) in the formulation containing uvaia (BU15) to (1.73 ± 0.04 mg g^{-1}) in the beverage prepared with araçá (BA15), showing statistically significant differences among the samples ($p < 0.05$). This behavior is associated with the mineral contribution of native fruit pulps, which are recognized for their high potassium content and directly influence the final composition of the beverages (Batista et al., 2025; Toft et al., 2024). Studies have shown that fortifying dairy beverages with fruits generally increases potassi-um content, contributing to an overall improvement in the product's nutritional profile. In this context, the results reinforce the potential of the developed beverages as complementary sources of this mineral, particularly in the formulations containing Araçá and Butiá (Al-aswad & Shehata, 2025; A. Prestes et al., 2023; A. A. Prestes et al., 2025).

The magnesium contents observed in the formulations ranged from (0.098 to 0.114 mg g^{-1}), with the bever-age containing Guabiroba (BG15) standing out for presenting a significantly higher concentration compared to the other samples ($p < 0.05$). The higher magnesium content in this formulation can be attributed to the specific characteristics of the mineral profile of guabiroba, as de-scribed in studies on the characterization of Brazilian native fruits (Batista et al., 2025; Preste s et al., 2022). Similar results have been re-ported for fermented beverages supplemented with plant-based pulps, in which the vegetal fraction contributes to maintaining or slightly in-creasing magnesium content in the final product. Therefore, the incorporation of native fruit pulps proved to be an effective strategy to complement the magnesium content (Al-aswad & Shehata, 2025; Nunes et al., 2025; Prestes et al., 2025).

The absence of detectable levels of potentially toxic elements, such as Al, As, Cd, Co, Cr, Cu, Fe, and Mn, in all analyzed samples indicates that the beverages exhibited low levels of contamination. When present at elevated concentrations, these elements are associated with risks to human health, including neurotoxic and carcinogenic ef-fects (Doroszkiewicz et al., 2023). Thus, the results of the multi-element profile indicate that the incorporation of native fruit pulps constitutes a technically viable alternative for the formulation of fermented dairy beverages with improved nutritional value, while preserving the mineral balance and quality of the dairy matrix.

3.5 Antimicrobial activity

The antimicrobial activity observed in fermented dairy beverages can be interpreted in an integrated way when correlated with physicochemical parameters, total phenol content, antioxidant capacity, and mineral profile (Tables 1–3) (Figure 2 a,b,c). The data indicate that the inhibitory effect results from a synergy between acidification of the medium, the presence of fermentation metabolites, and the incorporation of bioactive compounds from native pulps, which simultaneously modulate acid, oxidative, and structural stress on microorganisms (Ahansaz et al., 2023).

The progressive reduction of pH in pulp-based formulations, especially in BG15 (pH 4.48) and BU15 (pH 4.56), compared to the control (pH 4.79), is a primary factor for antimicrobial activity. More acidic environments increase the undissociated fraction of organic acids, favoring their diffusion through the cell membrane and causing intra-cellular acidification, protein denaturation, and collapse of microbial energy metabolism (Ababouch et al., 2024; Ng et al., 2024). Recent studies reinforce the finding that fermented systems containing *Lactobacillus acidophilus* and *Streptococcus thermophilus* exhibit high antimicrobial capacity, precisely due to the combination of low pH and the production of bioactive metabolites (Girma & Aemiro, 2021; Liu et al., 2024). In this context, the "ceiling" effect observed for Escherichia coli, with reductions close to 99.99% in all samples, suggests that the fermented matrix itself already establishes a highly hostile environment for this Gram-negative microorganism, limiting the visualization of additional effects from the pulps (Aguirre-Ramírez et al., 2025; Gir-ma & Aemiro, 2021)(Aguirre-Ramírez et al., 2025).

On the other hand, the statistical differences observed for *Staphylococcus aureus* and *Candida albicans* when comparing the control with the samples containing pulp indicate that acidification, although relevant, does not act in isolation. The incorporation of fruits substantially increased the total phenol content and antioxidant activity (DPPH and ABTS), with BG15 and BU15 standing out, showing the highest values of phenols and antioxidant capacity. Dietary phenolics are widely described as natural antimicrobial agents, acting through mechanisms such as disruption of the cytoplasmic membrane, increased permeability, inhibition of essential enzymes, and induction of oxidative stress in cells (Davidova et al., 2024; De Rossi et al., 2025). In acidic matrices, such as fermented dairy beverages, these effects tend to be enhanced, as the reduced pH increases microbial sensitivity to phenolic compounds (Allerberger & Wagner, 2019).

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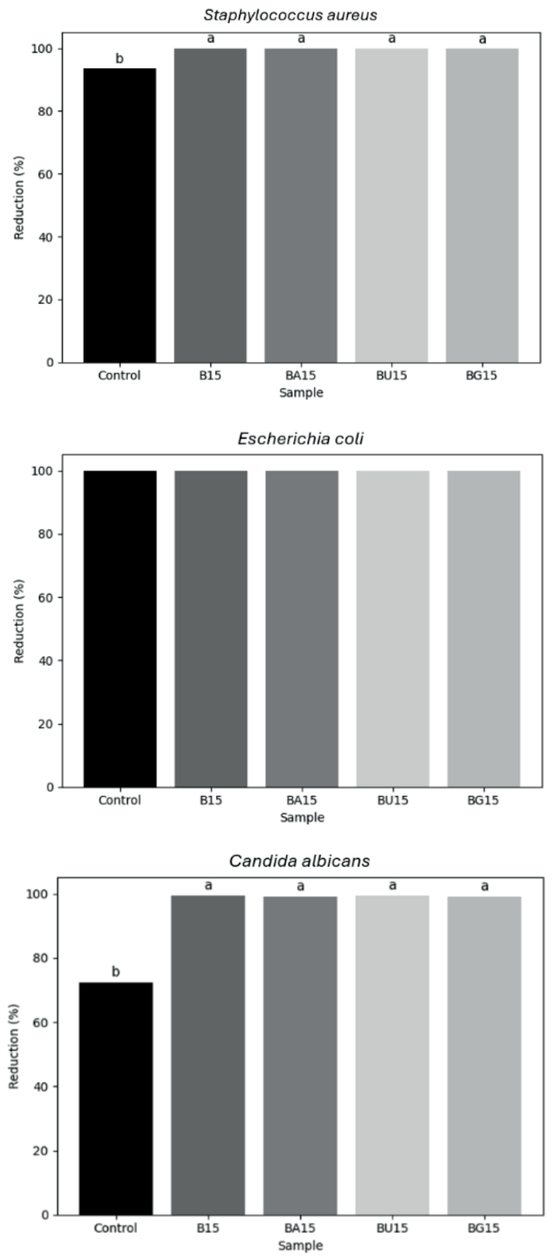

Figure 2. Reduction (%) of (a) *Staphylococcus aureus*, (b) *Escherichia coli,* and (c) *Candida albicans* in fermented dairy beverages formulations. Different lowercase letters indicate significant differences among samples ($p < 0.05$). Note: B15= 15% butiá pulp ; BA15= 15% araçá pulp ; BU15= 15% uvaia pulp ; BG15= 15% guabiroba pulp

The strong correlation between high levels of total phenols and greater microbial reduction, especially against C. albicans, suggests an important role for these compounds in antifungal activity. Related studies demonstrate that phenolics, such as phenolic acids and flavonoids, can induce mitochondrial dysfunction, oxidative stress, and programmed cell death in yeasts of the genus Candida, going beyond simple inhibition by acidification (Khan et al., 2011; Kim & Lee, 2021). This mechanism helps explain the striking difference between the control (72.40% reduction) and the formulations with pulp (~99%), showing that the addition of fruit was decisive for controlling the fungus.

In addition to phenolics, the high antioxidant activity observed in the pulp samples, especially in BG15, indicates a greater capacity to neutralize free radicals and modulate redox reactions. Although antioxidant activity is not antimicrobial in itself, there is evidence that antioxidant compounds can interfere with the microbial redox balance, intensifying oxidative stress when associated with other factors, such as organic acids and fermentation metabolites (Fernandes et al., 2024; Kumar et al., 2025; Yahyaoui et al., 2025). Thus, the positive correlation between elevated DPPH/ABTS and greater microbial reduction reinforces the hypothesis of a synergistic effect between pulp antioxidants and postbiotics produced during fermentation.

The mineral profile may also have contributed, albeit secondarily, to the antimicrobial activity. Samples with pulp showed significant variations in K, Mg, and Ca compared to the control. Minerals such as Mg and Ca play a structural role in the cell membrane and in enzyme stability (Kolodkin-Gal et al., 2023; Wang et al., 2019). Alterations in their availability can affect cell integrity and the response to acid stress. Furthermore, high concentrations of K, as observed in BA15 and BG15, can intensify osmotic imbalances in microorganisms already subjected to low pH and the action of phenolics, reducing their viability(Do & Gries, 2021). Recent reviews discuss that the interaction between minerals, organic acids, and phenolic compounds can influence microbial survival in fermented foods, although this effect is highly dependent on the matrix and the microorganism evaluated (Auchtung et al., 2025; Yang et al., 2023).

Therefore, the results indicate that the high antimicrobial activity of fermented dairy beverages enriched with native pulps cannot be attributed to a single factor. It is a combined effect involving the reduction of pH and the action of organic acids, antimicrobial metabolites from lactic fermentation, a significant increase in total phenols and the antioxidant capacity of the pulps, and modifications in the mineral profile of the matrix. This synergy explains both the high percentage reductions observed and the differences in sensitivity between *E. coli*, *S. aureus*, and *C. albicans*, reinforcing the technological potential of these formulations as fermented foods with functional properties and a natural biopreservative effect.

4. Conclusions

The present study confirms that the use of whey combined with native Brazilian fruit pulps represents a feasible strategy for developing fermented dairy beverages with improved functional and technological attributes. The incorporation of 15% fruit pulp resulted in consistent changes in physicochemical parameters, particularly reduced pH and modified color characteristics, while maintaining the overall stability of the dairy matrix. The enrichment with fruit pulps markedly enhanced the content of bioactive compounds, leading to higher levels of carotenoids, phenolic compounds, and antioxidant capacity, with the guabiroba formulation showing the most pronounced effect. Although a reduction in calcium was observed due to partial substitution of the dairy fraction, the mineral profile remained nutritionally relevant, with a noticeable contribution of potassium from the fruit matrices.

The antimicrobial results against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* indicate that the combination of acidification, fermentation metabolites, and fruit-derived compounds can act synergistically, supporting their potential application as natural biopreservative systems in fermented foods.

From an industrial perspective, these findings reinforce the potential of whey valorization associated with Brazilian biodiversity as a sustainable approach to obtain functional fermented beverages with enhanced nutritional, antioxidant, and technological properties

Author Contributions

Conceptualization, A.A.P, C.V.H, T.C.P, C.K.S, and E.S.P; methodology, A.A.P, A.C.F.C, D.R.M.A, F.F, M.A.P, M.M, and C.V.H.; software, A.C.F.C, K.M; validation, A.A.P, A.C.F.C, F.F, and M.A.P ; formal analysis, A.A.P, A.C.F.C, D.R.M.A, M.A.P, F.F, M.M, and J.S.G; investigation, A.A.P, E.S.P; resources, C.K.S, E.S.P, J.S.G; data curation, A.A.P, A.C.F.C, K.M, D.R.M.A, M.A.P; writing—original draft preparation, A.A.P, A.C.F.C, K.M; writing—review and editing, A.A.P, E.S.P, C.V.H, C.K.S, T.C.P; visualization, E.S.P. T.C.P ; supervision, E.S.P, T.C.P; project administration, E.S.P; funding acquisition, E.S.P, C.K.S, J.S.G. All authors have read and agreed to the published version of the manuscript.

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