







## Article

# Chemical Characterization, Antioxidant Capacity, and Antimicrobial Activity of a New Fresh Cheese Added with Guabiroba Pulp

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## Abstract

Fresh cheeses are dairy products that are highly valued by consumers, and they are frequently added with ingredients with functional properties. For the first time, this study aimed to characterize fresh cheeses added with guabiroba pulp (5, 10, 15%) by evaluating their physical–chemical properties, concentration of bioactive compounds, and in vitro antioxidant and antimicrobial activities. Based on our previous studies, adding 10–15% guabiroba pulp to dairy products is enough to enhance their prebiotic activity, in addition to increasing the levels of bioactive compounds, antioxidant activity, and promoting an evident and natural orange color to the dairy product. Adding guabiroba pulp decreased the water activity, pH value, luminosity, and the products' texture properties (firmness, elasticity, cohesiveness, and gumminess). At the same time, it increased the concentration of bioactive compounds (carotenoids, amino acids, phenolic compounds, and fatty acids), organic acids, sugars (sucrose and fructose), and antioxidant activity. Antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli* was observed for fresh cheese samples with guabiroba pulp addition. In conclusion, fresh cheeses with guabiroba pulp presented an improved concentration of bioactive compounds and functional properties, demonstrating that they are innovative products for the dairy industry.

**Keywords:** *Campomanesia xanthocarpa* O. Berg; bioactive compounds; functional food; dairy products; innovative food; cheeses

## 1. Introduction

Cheese is one of the most popular dairy products consumed worldwide and has a high commercial value [1]. Fresh cheese shows excellent yield, is easily elaborated, and has a low cost [2]. Furthermore, it has been recognized as an excellent matrix for incorporating value-added ingredients obtained from fruit, improving their nutritional, functional, and technological properties by enhancing the profile of bioactive compounds and boosting their antioxidant properties [3]. In this way, dairy products with fruit-derived products may offer a range of health benefits due to their antioxidant, antimicrobial, and anti-inflammatory properties, which in turn are associated with controlling the development and progression of most chronic diseases, such as obesity, diabetes, cardiovascular diseases, neurodegenerative disorders, and cancer [4].

Fruits of the *Myrtaceae* family, such as *Campomanesia xanthocarpa* O. Berg, popularly known as guabiroba, are considered functional fruits [5]. Guabiroba is a fruit species commonly found in the forests of the South, Southeast, and Central-West of Brazil, where the Cerrado biome predominates. It can also be found in Argentina and Uruguay [6].

Guabiroba stands out for its bioactive compound content, polyphenols, and carotenoids. Thus, the phytochemicals of guabiroba are elucidated regarding its high antioxidant activity, which is related to human health benefits when introduced into a dietary routine [7]. These properties of guabiroba make its pulp suitable for consumption in nature or food compositions; however, guabiroba pulp is not yet an ingredient used on a large commercial scale. Amorim et al. [4] reported that fruits and pulps contributing to Brazilian biodiversity remain rarely explored as ingredients by the food industry, presenting enormous potential for research and development of new products.

Guabiroba pulp has already been evaluated as a functional ingredient in fermented milk [8,9] and petit suisse cheese [10]. These results suggest that fresh cheese may also serve as an interesting matrix to incorporate guabiroba pulp. However, as the authors mention, no studies use this fruit pulp in fresh cheese.

Fresh cheeses have a high moisture content, which makes them more porous and capable of readily absorbing bioactive compounds. Thus, due to their structure, fresh cheeses can be formulated to incorporate several bioactive compounds, such as antioxidants, polyphenols, and carotenoids. In addition, cheese proteins (casein) and lipids would be excellent vehicles for the controlled release of bioactive compounds [3]. Finally, bioactive compounds can be effectively preserved in fresh cheeses since the production process usually involves lower temperatures than ripened cheeses, which could minimize the thermal degradation of temperature-sensitive substances, such as certain bioactive compounds. Therefore, we hypothesized that fresh cheeses could be an interesting option to include guabiroba pulp.

The present work sought to explore the use of guabiroba pulp as an ingredient in an innovative fresh cheese, with the purpose of verifying its influence on product quality and functionality. It was expected that incorporating different levels of pulp (5, 10, and 15%) could modify the physicochemical profile of the cheeses, and at the same time, increase the supply of bioactive compounds. To address this question, analyses were carried out to characterize the main physicochemical parameters, quantify bioactive metabolites, and examine in vitro antioxidant and antimicrobial responses. In this way, the study aimed to establish whether the enrichment of fresh cheese with guabiroba pulp could represent a viable alternative for generating dairy products that combine technological quality with added nutritional and functional value.

## 2. Materials and Methods

### 2.1. Materials

Guabiroba fruits (*Campomanesia xanthocarpa* O. Berg) were collected in Irati (Paraná State, Brazil) (25°27'56" S; 50°37'51" W). The fruits were selected, sanitized, and subsequently processed in a pulp machine (model DES-20, Braesi, Caxias do Sul, RS, Brazil) to obtain the pulp, yielding 31% residue and 69% guabiroba pulp. The composition of the pulp was 1.4 g of protein/100 g, 7.5 g of total sugars/100 g, and 6.5 g of dietary fiber/100 g. The milk used in processing was pasteurized whole milk (Tirol<sup>®</sup>, Treze Tílias, SC, Brazil), with a composition of 3.2 g of proteins/100 g, 4.5 g of total sugars/100 g, and 4.7 g of carbohydrates/100 g. A commercial rennet (Ha-La, Chr. Hansen, Valinhos, Brazil) coagulated the milk. All chemicals used were of analytical grade.

### 2.2. Samples Preparation

Four cheese formulations were developed, with different percentages of guabiroba pulp added. The cheeses were produced in vats containing 10 L of pasteurized whole milk previously heated to 37 ± 1 °C, with the addition of commercial rennet (0.9 mL/L). This mixture (milk + rennet) was kept at 37 ± 1 °C for 40 min. The gel formed was delicately cut into cubes, the drained whey was removed, and the curd was drained. The fruit pulp was added to the cheese mass and mixed. The cheese curd containing the fruit pulp was placed in perforated circular containers (with an approximate capacity of 500 g) and kept refrigerated (5 ± 1 °C). The cheeses were salted (0.07%), placed in plastic bags, and stored and refrigerated during 24 h until the analyses were performed. The control sample had no addition, while the others contained 5%, 10%, and 15% of guabiroba pulp, called sample 0, sample 5, sample 10, and 15, respectively.

### 2.3. Physicochemical Analysis

The fresh cheese samples' titratable acidity, pH, moisture, protein, and ash contents were determined according to the Analytical Standards Manual of Institute Adolfo Lutz [11]. Titratable acidity was expressed in g of lactic acid per 100 g of cheese. The pH was measured using a digital pH meter (Kasvi<sup>®</sup>, São Paulo, SP, Brazil), calibrated with pH 4.0 and 7.0 buffer solutions, under ambient temperature conditions (Tecnaltec-7<sup>®</sup>, São Paulo, SP, Brazil). Moisture content (g/100 g) was evaluated by drying in a forced-air oven (TECNAL<sup>®</sup>, Piracicaba, SP, Brazil) at 105 ± 1 °C until reaching a constant weight. The protein content (g/100 g) was quantified using the Kjeldahl method (TECNAL<sup>®</sup> equipment, Piracicaba, SP, Brazil) by measuring the total nitrogen content multiplied by factor 6.38. The water activity (Aw) was measured instrumentally using an AquaLab meter (model CX-2, Decagon Devices, Pullman, WA, USA).

The color analyses of the fresh cheese samples were performed using a Minolta Chroma Meter CR-400 (Konica Minolta, Osaka, Japan) colorimeter, adjusted to operate with D65 lightning and a 10° observation angle. The colorimeter was calibrated with a white standard plate, and the CIELab color scale was used to measure the L\*, a\*, and b\* parameters. The L\* parameter ranges from 0 to 100, indicating luminosity (variation from black to white). The b\* axis is the variation from yellow (+b\*) to blue (−b\*), and the a\* axis shows the variation from red (+a\*) to green (−a\*). The total difference in color (ΔE\*) between the measured values of each fresh cheese (5, 10, 15% of guabiroba pulp) with the control sample (cheese without guabiroba pulp) was determined as described in Equation (1).

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (1)$$

where  $\Delta L^*$  is the difference in luminosity,  $\Delta a^*$  represents the intensity of the red color, and  $\Delta b^*$  is the intensity of the yellow color for each fresh cheese sample.

#### 2.4. Texture Analysis

A TA-XT plus texturometer was used for the texture analysis of the samples (Stable Micro Systems, Texture Exponent software (Version 6.2), Surrey, UK). An aluminum probe of 25 mm diameter was used to compress the fresh cheese samples (50 mm diameter and 20 mm height). The measurements were made at  $5 \pm 1$  °C, with a test speed of 1.0 mm/s and distance of 10.0 mm [12]. The data for force as a function of time were obtained for the two compression–decompression cycles using the TA-XT plus software (Version 6.2). The following parameters were obtained: firmness (N), elasticity (N.s), cohesiveness, and gumminess (N).

#### 2.5. Total Phenolic Compounds

An amount of 0.5 g of the sample was weighed into a plastic centrifuge tube with a screw cap, to which 50 mL of boiling water was added. The tube was subjected to ultrasound treatment (Biovera<sup>®</sup>, Rio de Janeiro, RJ, Brazil) for 30 min. After extraction, the solution was filtered through fast filtration paper (0.45  $\mu$ m) into a 100 mL volumetric flask, and the volume was completed with distilled water. Total phenolic compounds (TPC) were determined using a colorimetric analysis with the Folin–Ciocalteu reagent, according to Singleton and Rossi [12] and Gan et al. [13] protocols, with adaptations. The reaction occurred in the dark, at room temperature, for 90 min, and absorbance was measured at 725 nm using a Shimadzu-1800 UV–VIS<sup>®</sup> spectrophotometer (Kyoto, Japan). The results were expressed as gallic acid equivalents (GAE) micrograms per 100 g of dry sample ( $\mu$ g GAE/100 g). The gallic acid calibration curve was constructed with concentrations ranging from 0 to 100 mg/L.

#### 2.6. Carotenoid Content

Carotenoid content was analyzed according to Rodriguez-Amaya [14] with modifications. For carotenoid extraction, 1 g of the sample was weighed, and 20 mL of acetone was added into a Falcon<sup>®</sup> 50 mL tube. After vortex mixing (Biomixer<sup>®</sup>, Jacareí, São Paulo, Brazil), the tube with the mixture was subjected to ultrasound for 30 min. The extract was filtered using filter paper and a funnel. In a burette, 4 mL of petroleum ether was added, followed by the extract and 3 mL of ultrapure type 2 water. The burette was then left to stand until phase separation occurred. If separation did not happen, a few drops of NaOH solution were added, waiting for phase division. The lower and colorless phase was discarded, while the colored phase was kept in the burette. This colored phase was transferred to a volumetric flask, passing through a filter with sodium sulfate to eliminate any aqueous residue. The burette was washed with petroleum ether to avoid extract loss. Carotenoid levels were measured using a UV-Vis spectrophotometer (Shimadzu<sup>®</sup>, Barueri, SP, Brazil), with the following wavelengths: 450 nm for  $\beta$ -carotene, 444 nm for  $\alpha$ -carotene, 452 nm for  $\beta$ -cryptoxanthin, and 462 nm for  $\lambda$ -carotene.

The concentration of carotenoids was determined using the Lambert–Beer law, as shown in Equation (2).

$$A = \epsilon \times b \times c \quad (2)$$

Relating absorbance (A) to the specific absorption coefficient ( $\epsilon$ ), concentration (c), and optical length (b).

### 2.7. Sugar Analysis

An ICS 3000 Ionic Chromatograph Dionex (San Donato Milanese, Italy) conducted chromatographic analyses of lactose, galactose, sucrose, glucose, and fructose. The chromatographic separation was performed with a CarboPac PA20 column (3 mm × 150 mm, Dionex) equipped with a guard column (CarboPac PA20, 3 mm × 30 mm, Dionex) according to the methodology proposed by Neri et al. [15], with some modifications. The following work condition was used: NaOH  $5.0 \times 10^{-2}$  mol/L as mobile phase, a flow rate of 0.5 mL/min, a 35 min run at a column temperature of  $30 \pm 1$  °C, and a volume of injection of 10 µL. As the Dionex technical manual recommended, sugar detection was performed using the time/potential waveform A. Sugar identification and quantification were performed using retention times. The related sugar calibration curve was performed.

### 2.8. Gas Chromatography–Mass Spectrometry (GC–MS) Analysis

The gas chromatography–mass spectrometry (GC-MS) analysis (Agilent®, Santa Clara, CA, USA) was determined in the fresh cheeses with guabiroba pulp addition (5, 10, 15%). According to Lima et al. [16], the sample extraction processes were first realized. After these processes, the samples containing the hydrophilic and lipophilic compounds were analyzed using gas chromatography coupled to an ion trap mass spectrometer in split mode (1:25). The samples were submitted to DB-5 column (30 m × 0.25 mm × 0.25 µm) at a temperature of  $250 \pm 1$  °C, using helium gas at 1.5 mL/min for transportation. The GC oven was set at  $70 \pm 1$  °C, 1 min isotherm, and heated to  $320 \pm 1$  °C at a rate of 8 °C/min, with a final 5 min isotherm. The mass spectrometer was operated in positive mode with electron impact ionization at 70 eV and ion source temperature at  $200 \pm 1$  °C. The compounds were identified through the AMDIS® software using the Golm Metabolome Database reference collection [11] for hydrophilic compounds, and a library was built in the AMDIS software with samples analyzed for lipophilic compounds.

### 2.9. Antioxidant Activity

The antioxidant capacity was determined through the DPPH free radical method (2,2-diphenyl-1-picrylhydrazyl), following the procedure of Brand-Williams et al. [17]. The reaction was conducted in the dark, at room temperature, for 30 min. The absorbance was measured using a UV/VIS spectrophotometer (Shimadzu, Kyoto, Japan) at 515 nm, with results expressed as micrograms of Trolox equivalent antioxidant capacity (TEAC) per 100 g (µg TEAC/100 g).

The ABTS<sup>+</sup> 2,2'-azinobis(3ethylbenzothiazoline6sulfonic acid) radical inhibition activity was performed according to Re et al. [18], using a UV/VIS spectrophotometer (Shimadzu, Kyoto, Japan) at 734 nm. The results were expressed as µg TEAC/100 g.

### 2.10. Multielement Profile

Multielement profile measurements were performed using an inductively coupled plasma optical emission spectrometer (ICP-OES) (model iCAP 6000®, Thermo Analytical, Tewksbury, MA, USA). The analytes and respective monitored wavelengths were Al (308.215 nm), As (189.042 nm), Ca (422.673 nm), Cd (228.802 nm), Co (228.616 nm), Cr (283.563 nm), Cu (324.754 nm), Fe (238.204 nm), K (769.886 nm), Mg (279.553 nm), Mn (257.610 nm), Na (589.592 nm), P (214.914 nm), Pb (220.353 nm), S (182.034 nm), Se (196.090 nm), Sr (407.771 nm), and Zn (213.856 nm). The ICP-OES included a Miramist nebulizer (Burgener Research®, Mississauga, ON, Canada) and a cyclonic spray chamber. Operational parameters included radial vision, a pump rate of 50 rpm, a plasma gas flow of 12 L/min, radio frequency power of 1150 W, and an auxiliary gas flow rate of 1.0 L/min. Argon was the main and auxiliary gas, with a minimum purity of 99.95% (Air Liquide, Rio

de Janeiro, RJ, Brazil). Calibration curves were prepared for the analytes, with concentrations ranging from 0.05 to 2.5 mg/L for Al, As, Cd, Co, Cr, Cu, Fe, Mg, Mn, Pb, Se, Sr, and Zn, and from 0.5 to 20 mg/L for Ca, K, Na, P, and S.

Sample preparation was conducted according to the methods described by Melo et al. [19]. In each experiment, 0.570 g of the cheese sample was weighed directly into Teflon<sup>®</sup> microwave vessels (Microwave Model Reaction System/Multiwave PRO<sup>®</sup>, Anton Paar, Graz, Austria). Subsequently, 1.58 mL of 14 mol/L HNO<sub>3</sub> and 1.42 mL of ultrapure water were added. The vessels were sealed and subjected to the following temperature program: heating to 200 ± 1 °C for 8 min, holding at 200 ± 1 °C for 14 min, and cooling to 65 ± 1 °C for 23 min. The samples were then transferred to 50.0 mL polyethylene flasks, and ultrapure water was added up to 30 mL. Individual standard solutions with a concentration of 1000 mg/L of the analytes were used for the calibration curves.

The limits of detection (LOD) and quantification (LOQ) were determined for each element based on calibration curve parameters. The results were expressed in µg/g of sample. The LOD values obtained were Al = 0.015 µg/g; As = 0.031 µg/g; Ca = 0.004 µg/g; Cd = 0.002 µg/g; Co = 0.006 µg/g; Cr = 0.005 µg/g; Cu = 0.001 µg/g; Fe = 0.007 µg/g; K = 0.017 µg/g; Mg = 0.0002 µg/g; Mn = 0.001 µg/g; Na = 0.003 µg/g; P = 0.019 µg/g; Pb = 0.021 µg/g; S = 0.070 µg/g; Se = 0.033 µg/g; Sr = 0.0001 µg/g; and Zn = 0.001 µg/g.

The LOQ values were Al = 0.047 µg/g; As = 0.094 µg/g; Ca = 0.013 µg/g; Cd = 0.008 µg/g; Co = 0.019 µg/g; Cr = 0.015 µg/g; Cu = 0.004 µg/g; Fe = 0.021 µg/g; K = 0.052 µg/g; Mg = 0.0007 µg/g; Mn = 0.003 µg/g; Na = 0.011 µg/g; P = 0.057 µg/g; Pb = 0.065 µg/g; S = 0.21 µg/g; Se = 0.10 µg/g; Sr = 0.0003 µg/g; and Zn = 0.004 µg/g.

### 2.11. Antimicrobial Activity

An amount of 3.0 ± 0.2 g of each fresh cheese sample (without control and with 5, 10, and 15% guabiroba pulp) was weighed and stored in Falcon<sup>®</sup> tubes, then homogenized with 3 mL of sterile distilled water with constant vortex agitation (Biomixer<sup>®</sup>, Jacareí, São Paulo, Brazil) for 5 min. The homogenized samples were filtered through filter paper and then filtered again through 0.22 µm thick filters to sterilize each extract. The pathogenic microorganism strains used were *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 to screen for activity against Gram-positive and Gram-negative microorganisms. To ensure that the method has worked well and to compare the activity of the extract or isolated compound, a non-positive control of a standard antibiotic was included. The non-positive control was tested at the same concentration as the plant pulp.

The broth microdilution assay was performed using the incubation time and inoculum concentration parameters, according to the method described by De Bona et al. [20]. Traditional methods, such as broth dilution, also rely on visual assessment of bacterial growth to determine susceptibility. In the present study, the traditional method (broth dilution), with a visual readout, was used for potency characterization, as determined by MIC (minimum inhibitory concentration) and MBC (minimum bactericidal concentration).

Additionally, pH neutralization is important because acidic or basic extracts can independently inhibit bacterial growth, thereby confounding the assessment of true antimicrobial activity. Therefore, standardized protocols often recommend adjusting the pH to neutral before testing.

Standardized inocula on the McFarland scale 0.5 ( $1 \times 10^8$  to  $2 \times 10^8$  colonies-forming unit, CFU/mL) using brain–heart infusion broth (BHI) were prepared and reduced to an approximate concentration of  $1 \times 10^3$  to  $2 \times 10^3$  CFU/mL by serial dilution. One hundred microliters of the inoculum were added to a sterile microtube, and 100 µL of each extract was previously produced using fresh cheese samples. A control using only 100 µL of the inoculum and 100 µL of BHI was performed. Microtubes containing  $1 \times 10^3$  to

$2 \times 10^3$  CFU/mL were incubated for 6 h in a shaker-type incubator with 200 rpm rotation at  $37 \pm 1$  °C. After incubation, the inocula in contact with the extract was diluted in sterile saline and plated on nutrient agar for colony counting. For microtubes containing 1 g of the sample plus the inoculum, plating was performed on Baird–Parker agar selective medium for *S. aureus* and tryptone bile glucuronide (TBX) agar selective medium for *E. coli*. The plates were incubated at  $35 \pm 1$  °C in aerobic conditions for  $18 \pm 2$  h. The results were expressed as a percentage of reduction (%) of *S. aureus* and *E. coli*, as in Equation (3).

$$Reduction(\%) = 100 \times \left( \frac{(GLPCC - GLPCS)}{GLPCC} \right) \quad (3)$$

GLPCC is the growth log of a pathogen in the control cheese, and GLPCS is the growth log of a pathogen in the cheese sample (with 5, 10, or 15% guabiroba pulp).

### 2.12. Statistical Analysis

All cheese samples were produced in triplicate, which are biological replicates (independent cheese batches), and three parallel measurements were made for each replicate. Data analysis was performed using STATISTICA 13.3 software (TIBCO® Software Inc., Palo Alto, CA, USA). The results were expressed as mean  $\pm$  standard deviation. To determine significant differences ( $p < 0.05$ ), analysis of variance and Tukey's HSD test (95% confidence level) were performed.

## 3. Results and Discussion

### 3.1. Chemical and Physical Analysis

Table 1 presents the physicochemical and colorimetric parameters of the fresh cheese samples without (Sample 0, control) and with guabiroba pulp (5, 10, 15%). The addition of guabiroba pulp (5, 10, 15%) practically did not influence ( $p > 0.05$ ) the protein, moisture, ash, and titratable acidity contents in the modified fresh cheeses. Therefore, no effect was observed for gross chemical composition. On the other hand, the progressive increase in the guabiroba pulp content in the fresh cheese samples resulted in a decrease ( $p < 0.05$ ) in the  $A_w$  values. Another decrease ( $p < 0.05$ ) was verified for the pH value of the fresh cheese sample added with 15% guabiroba pulp. The lower pH values are related to the acidity in the guabiroba pulp, which only changed the pH values when added at the highest concentrations.

The parameters  $L^*$ ,  $a^*$ , and  $b^*$  are part of the CIE Lab\* color space, a colorimetric system that describes all perceptible colors. It was observed that adding guabiroba pulp contributed to the decrease in the lightness values ( $L^*$ ) and the increase in the  $a^*$  and  $b^*$  values of the fresh cheese samples ( $p < 0.05$ ). Thus, the increase in the guabiroba pulp content resulted in fresh cheeses with a darker yellow coloration (Figure 1).

Regarding the  $\Delta E^*$  parameter, it was possible to note that as the content of guabiroba pulp in the cheeses increased, the values for the color difference between the sample and the control were higher. According to Quintanilla et al. [21],  $\Delta E^*$  values between 1 and 3 are not perceptible to the human eye, while values above 3 are visible. Therefore, all fresh cheese samples had values greater than 3 for the color difference, indicating that the increased addition of guabiroba pulp (5, 10, 15%) resulted in greater visualization of the color difference.

The increase in the color parameters  $a^*$  and  $b^*$  can be explained by the high content of carotenoids generally found in guabiroba pulp [22]. The color of the fresh cheeses with guabiroba pulp may be interesting from the consumer point of view, as Tura et al. [23] stated that consumers prefer yellow cheeses, while El-Loly et al. [24] highlighted that the yellow-

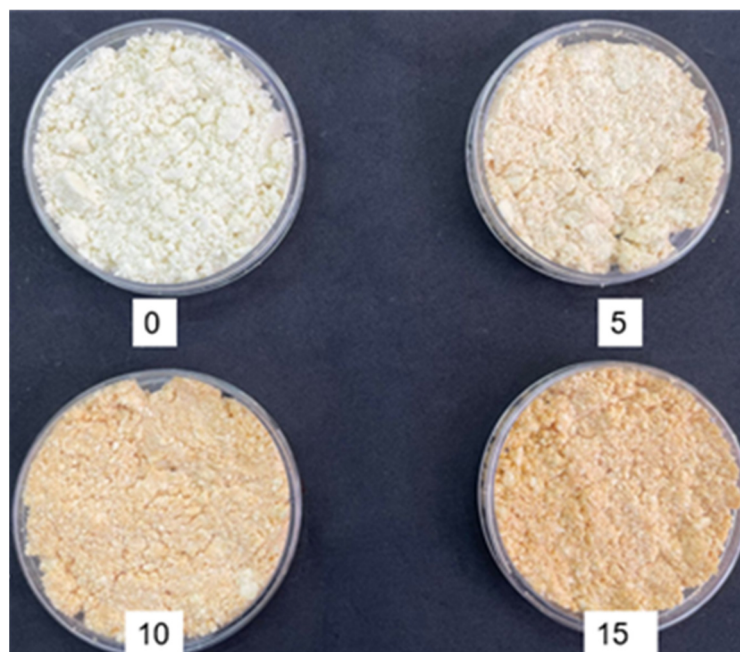
reddish color of some cheeses promoted a more attractive and appetizing appearance, which can enhance the overall taste of the cheese.

**Table 1.** Physicochemical, texture, and colorimetric parameters of fresh cheese formulation samples added with guabiroba pulp.

	Samples			
	0	5	10	15
<b>Physicochemical parameters</b>				
Protein (g/100 g)	20.18 ± 0.70 <sup>a</sup>	20.00 ± 0.70 <sup>a</sup>	19.84 ± 0.40 <sup>a</sup>	20.94 ± 1.60 <sup>a</sup>
Moisture (g/100 g)	41.81 ± 0.70 <sup>ab</sup>	40.77 ± 0.45 <sup>b</sup>	40.77 ± 0.10 <sup>b</sup>	42.69 ± 0.30 <sup>a</sup>
Ash (g/100 g)	2.94 ± 0.04 <sup>b</sup>	3.91 ± 0.53 <sup>a</sup>	3.50 ± 0.10 <sup>a</sup>	3.38 ± 0.06 <sup>a</sup>
Titratable acidity (g lactic acid/100 g)	2.03 ± 0.70 <sup>a</sup>	1.98 ± 0.10 <sup>a</sup>	1.98 ± 0.03 <sup>a</sup>	1.99 ± 0.03 <sup>a</sup>
Aw	0.886 ± 0.002 <sup>a</sup>	0.875 ± 0.002 <sup>b</sup>	0.864 ± 0.004 <sup>c</sup>	0.865 ± 0.019 <sup>c</sup>
pH	5.83 ± 0.02 <sup>a</sup>	5.84 ± 0.03 <sup>a</sup>	5.87 ± 0.05 <sup>a</sup>	5.76 ± 0.02 <sup>b</sup>
<b>Colorimetric parameters</b>				
L*	89.99 ± 0.50 <sup>a</sup>	81.00 ± 0.80 <sup>b</sup>	78.31 ± 0.30 <sup>c</sup>	77.51 ± 1.60 <sup>c</sup>
a*	4.21 ± 0.20 <sup>d</sup>	9.71 ± 0.10 <sup>c</sup>	11.83 ± 0.10 <sup>b</sup>	15.35 ± 0.30 <sup>a</sup>
b*	23.62 ± 0.40 <sup>d</sup>	38.34 ± 0.40 <sup>c</sup>	44.04 ± 0.30 <sup>b</sup>	54.00 ± 1.10 <sup>a</sup>
ΔE*	-	17.74 ± 0.50 <sup>c</sup>	24.74 ± 0.10 <sup>b</sup>	34.69 ± 1.30 <sup>a</sup>
<b>Texture parameters</b>				
Firmness (N)	7.94 ± 0.90 <sup>a</sup>	4.92 ± 0.11 <sup>b</sup>	3.29 ± 0.20 <sup>c</sup>	3.18 ± 0.30 <sup>c</sup>
Elasticity (N.s)	75.80 ± 0.19 <sup>a</sup>	67.84 ± 0.17 <sup>b</sup>	66.60 ± 0.41 <sup>b</sup>	49.70 ± 0.30 <sup>c</sup>
Cohesiveness	0.46 ± 0.06 <sup>a</sup>	0.28 ± 0.04 <sup>b</sup>	0.30 ± 0.20 <sup>b</sup>	0.27 ± 0.02 <sup>b</sup>
Gumminess (N)	3.65 ± 0.15 <sup>a</sup>	1.37 ± 0.24 <sup>b</sup>	1.02 ± 0.01 <sup>c</sup>	0.89 ± 0.14 <sup>c</sup>

Sample 0 is the control sample (without guabiroba pulp); Sample 5 is the sample with 5% guabiroba pulp; Sample 10 is the sample with 10% guabiroba pulp; and Sample 15 is the sample with 15% guabiroba pulp. L\*: represents luminosity, ranging from 0 (black) to 100 (white). a\*: represents the red color tone. b\*: represents the yellow tone. ΔE\*: represents the total difference in color between the measured values of each fresh cheese (5, 10, 15% of guabiroba pulp addition) with the control sample. Results are expressed as mean ± standard deviation (n = 3).  
<sup>a-d</sup> Within a row, different lowercase letters indicate significant differences between samples ( $p < 0.05$ ).

Table 1 also presents the results of the texture parameters of the modified fresh cheeses with and without the addition of guabiroba pulp (5, 10, 15%). Compared to the control cheese, it was observed that the firmness, elasticity, cohesiveness, and gumminess of the fresh cheese samples decreased with the addition of guabiroba pulp ( $p < 0.05$ ). The impact was more pronounced for 10 and 15% addition for firmness and gumminess and for 15% addition for elasticity ( $p < 0.05$ ). Fruit pulps promote a more marked hydration and a consequent weakness in the casein network [24,25]. Furthermore, fruit pulps may change the protein structure (casein network) [26,27], resulting in changes in firmness and gumminess, which are related properties [28], elasticity [27], and cohesiveness [29]. Elasticity may also be changed by new interactions between fruit pulp compounds and casein colloids [30]. Consumer preference for soft texture is a significant driver in the acceptance of fresh cheeses such as cottage cheese, petit suisse, and similar products. Studies consistently show that a soft texture is highly valued by consumers across different cheese types; texture is a key factor influencing purchase decisions [29,30]. Across various fresh cheese varieties, consumers consistently prefer a soft texture, often achieved using specific ingredients or processing methods. This preference is evident in cheeses, highlighting the importance of texture in product development and market success. Therefore, soft textures are interesting for consumer acceptance of fresh cheeses, demonstrating that adding guabiroba pulp could improve the texture properties.



**Figure 1.** Fresh cheese formulations added with guabiroba pulp, where 0 = control sample (without guabiroba pulp); 5 = sample with 5% guabiroba pulp; 10 = sample with 10% guabiroba pulp; and 15 = sample with 15% guabiroba pulp.

### 3.2. Total Phenolic Compounds

The total phenolic compound results are shown in Figure 2A.

Adding guabiroba pulp increased ( $p < 0.05$ ) the total phenolic compound content of the fresh cheese samples by 1.4 to 2.5 times. The impact was more pronounced for 10 and 15% addition ( $p < 0.05$ ). The results are interesting, as dairy products with some bioactive compounds represent an up-and-coming line of research for the dairy industry when the objective is to increase functionality [31]. Previous studies have reported increased phenolic compounds concentration after adding fruit to cheeses [31–33]. However, this is the first study using guabiroba pulps.

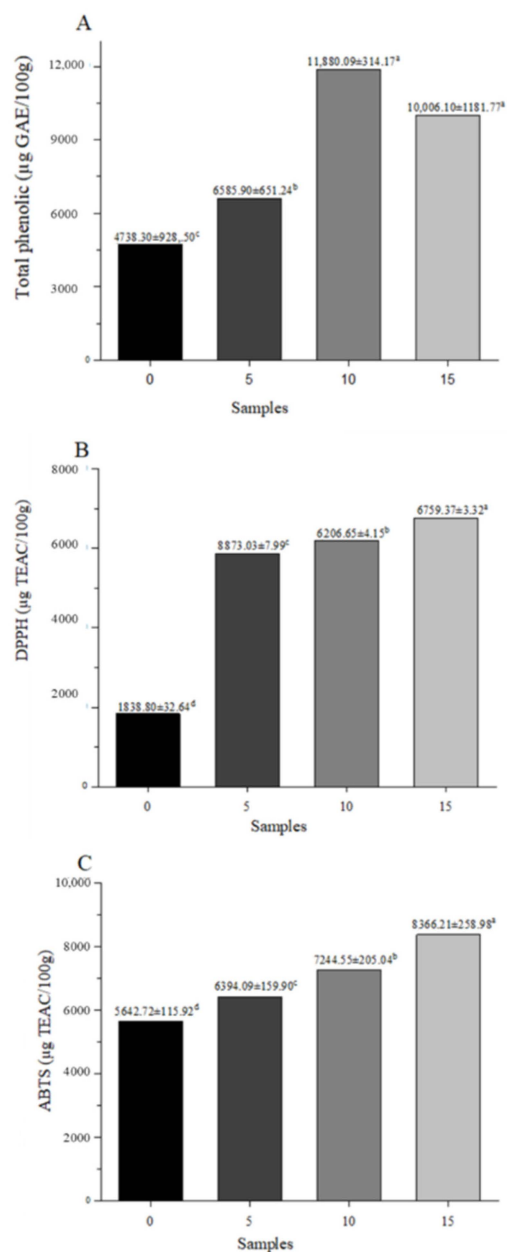
### 3.3. Carotenoid Content

Table 2 shows the carotenoid content in the fresh cheese samples (control and with guabiroba pulp). In the control cheese, only  $\beta$ -carotene was found, which can be attributed to its presence in the whole milk used in its preparation [34]. This carotenoid comprises about 90% of the total carotenoids in cow's milk, making it the predominant type found in whole milk [34].

**Table 2.** Carotenoid content of fresh cheese formulation samples added with guabiroba pulp.

Carotenoid Content	Samples			
	0	5	10	15
$\beta$ -carotene ( $\mu\text{g/g}$ )	$74.84 \pm 0.01^d$	$77.85 \pm 0.47^c$	$155.70 \pm 0.93^b$	$158.70 \pm 1.40^a$
$\alpha$ -carotene ( $\mu\text{g/g}$ )	<0.001	$27.51 \pm 0.04^c$	$55.03 \pm 0.09^b$	$82.54 \pm 0.13^a$
$\beta$ -cryptoxanthin ( $\mu\text{g/g}$ )	<0.001	$31.88 \pm 0.05^c$	$63.75 \pm 0.80^b$	$95.63 \pm 0.14^a$
$\lambda$ -carotene ( $\mu\text{g/g}$ )	<0.001	$20.50 \pm 0.03^c$	$40.99 \pm 0.06^b$	$61.49 \pm 0.09^a$

Sample 0 is the control sample (without guabiroba pulp); Sample 5 is the sample with 5% guabiroba pulp; Sample 10 is the sample with 10% guabiroba pulp; and Sample 15 is the sample with 15% guabiroba pulp. Results are expressed as mean  $\pm$  standard deviation ( $n = 3$ ). <sup>a-c</sup> Within a row, different lowercase letters indicate significant differences between samples ( $p < 0.05$ ).



**Figure 2.** Results from (A) total phenolic compounds and in vitro antioxidant capacity by the (B) DPPH free radical method (2,2-diphenyl-1-picrylhydrazyl) and (C) ABTS<sup>+</sup> (2,2-casino-bis acid (3-ethylbenzothiazolin-6-sulfonic acid) radical inhibition activity. Sample 0 is the control sample (without guabiroba pulp); Sample 5 is the sample with 5% guabiroba pulp; Sample 10 is the sample with 10% guabiroba pulp; and Sample 15 is the sample with 15% guabiroba pulp. Results are expressed as mean  $\pm$  standard deviation ( $n = 3$ ). <sup>a-c</sup> Different lowercase letters indicate significant differences between samples ( $p < 0.05$ ).

The greater the amount of guabiroba pulp added (5, 10, 15%), the higher ( $p < 0.05$ ) the content of carotenoids ( $\alpha$ -carotene,  $\beta$ -carotene,  $\beta$ -cryptoxanthin, and  $\lambda$ -carotene) that is found in the fresh cheese samples. These results showed an improvement in the nutritional value of fresh cheeses using guabiroba pulp.  $\beta$ -carotene is the precursor of ligands necessary for nuclear receptors' activity in regulating energy metabolism [35]. At the same time,  $\beta$ -carotene,  $\alpha$ -carotene, and  $\beta$ -cryptoxanthin are significant contributors to vitamin A intake, often surpassing the contribution of retinol. This contribution is crucial for maintaining adequate vitamin A content for vision, immune function, and skin health [36].

The results of carotenoid content corroborated those of color parameters. Therefore, the color of the guabiroba added in cheeses may be associated with the higher concentration of  $\beta$ -carotene.  $\beta$ -carotene may impart an appealing color for food applications to be used as a food colorant. Therefore, it may be an excellent candidate as an ingredient, given the increasing concern of consumers on the use of artificial food colorants [35]. Furthermore, they may be associated with antioxidant activity, as carotenoids have the potential to act as a natural antioxidant [36].

### 3.4. Sugar Analysis

The sugar content in fresh cheese samples is shown in Table 3. The fresh cheeses presented lactose, galactose, glucose, sucrose, and fructose as sugars. Sugars are important because they are basic nutrients necessary for the human body [37]. Lactose is the primary sugar in milk, and consequently, in fresh cheeses [38]. Glucose plays a significant role in brain and muscle function [39]. Finally, fructose and sucrose play significant roles in foods as sweeteners, with fructose potentially offering benefits in glycemic control but posing risks to liver health when consumed in excess. Sucrose is a common dietary sugar with implications for energy intake and metabolic health, although its direct link to chronic disease is less clear when accounting for total caloric intake. Both sugars should be consumed in moderation, considering their metabolic effects and potential health impacts [40]. The present study's results demonstrate the low sugar concentration in the products.

**Table 3.** Sugar content in fresh cheese formulations samples added with guabiroba pulp.

Sugars Content	Samples			
	0	5	10	15
Lactose (g/100 g)	3.80 ± 0.20 <sup>a</sup>	4.10 ± 0.10 <sup>a</sup>	3.99 ± 0.10 <sup>a</sup>	3.80 ± 0.10 <sup>a</sup>
Galactose (g/100 g)	1.31 ± 0.10 <sup>a</sup>	1.29 ± 0.10 <sup>a</sup>	1.28 ± 0.10 <sup>a</sup>	1.02 ± 0.10 <sup>b</sup>
Glucose (g/100 g)	2.53 ± 0.90 <sup>a</sup>	2.95 ± 0.40 <sup>a</sup>	2.94 ± 0.40 <sup>a</sup>	2.74 ± 0.40 <sup>a</sup>
Sucrose (g/100 g)	1.12 ± 0.30 <sup>b</sup>	1.24 ± 0.40 <sup>ab</sup>	1.44 ± 0.10 <sup>a</sup>	1.64 ± 0.30 <sup>a</sup>
Fructose (g/100 g)	0.55 ± 0.01 <sup>b</sup>	1.13 ± 0.40 <sup>a</sup>	1.37 ± 0.11 <sup>a</sup>	1.71 ± 0.47 <sup>a</sup>

Sample 0 is the control sample (without guabiroba pulp); Sample 5 is the sample with 5% guabiroba pulp; Sample 10 is the sample with 10% guabiroba pulp; and Sample 15 is the sample with 15% guabiroba pulp. Results are expressed as mean ± standard deviation (n = 3). <sup>a-b</sup> Within a row, different lowercase letters indicate significant differences between samples ( $p < 0.05$ ).

Adding guabiroba pulp to the fresh cheeses did not influence the lactose and glucose contents ( $p > 0.05$ ). Increases in the sucrose (only for 10 and 15% guabiroba pulp addition) and fructose (all guabiroba added products) were reported ( $p < 0.05$ ). Finally, decreases in galactose (only for 15% guabiroba pulp addition) were observed. The increases in sucrose and fructose contents may be associated with the sugar content in the fruit pulps [41]. The lower galactose content in the 15% product could be related to changes in the lactose degradation during processing in this product, as the added ingredient may impact the cheese manufacturing process [42].

### 3.5. Hydrophilic and Lipophilic Compound Determination

Table 4 presents the fresh cheeses' acquired values of hydrophilic and lipophilic compounds. The increase in the guabiroba pulp increased ( $p < 0.05$ ) the levels of hydrophilic and lipophilic compounds. The hydrophilic compounds with the highest increases after guabiroba pulp addition were from the following classes: organic acids (citric acid, quinic acid, malic acid, and dehydroascorbic acid), cyclitol (myo-inositol), amino acids (4-hydroxy-

1-methyl-proline), and sugar acids (glucuronic acid and gluconic acid). The other classes were increased at lower expressivity.

**Table 4.** Content of the increase in hydrophilic and lipophilic compounds present in fresh cheese samples adding guabiroba pulp concerning the control sample.

Class	Hydrophilic Compounds	Content ( $\mu\text{g/g}$ )		
		Sample 5	Sample 10	Sample 15
Amino acid	Glutamic acid	$0.45 \pm 0.05^c$	$0.91 \pm 0.10^b$	$1.35 \pm 0.10^a$
	4-amino-butanoic acid (GABA)	$1.40 \pm 0.07^c$	$2.80 \pm 0.13^b$	$4.20 \pm 0.20^a$
	4-hydroxy-1-methyl-proline (dimer)	$19.10 \pm 0.70^c$	$38.00 \pm 1.40^b$	$57.15 \pm 1.04^a$
Cyclitol	Myo-inositol	$25.20 \pm 2.25^c$	$50.00 \pm 2.30^b$	$76.00 \pm 2.79^a$
Organic acid	Citric acid	$44.70 \pm 0.97^c$	$90.01 \pm 1.50^b$	$135.01 \pm 6.07^a$
	Dehydroascorbic acid	$2.75 \pm 0.01^c$	$5.04 \pm 1.00^b$	$8.31 \pm 1.45^a$
	Glycolic acid	$0.42 \pm 0.02^c$	$0.84 \pm 0.03^b$	$1.30 \pm 0.05^a$
	Lactic acid	$0.40 \pm 0.03^c$	$0.78 \pm 0.06^b$	$1.17 \pm 0.17^a$
	Malic acid	$6.29 \pm 0.09^c$	$12.60 \pm 0.09^b$	$19.00 \pm 0.09^a$
	Quinic acid	$10.81 \pm 0.06^c$	$21.50 \pm 0.06^b$	$33.01 \pm 0.07^a$
	Shikimic acid	$0.40 \pm 0.01^c$	$0.73 \pm 0.03^b$	$1.10 \pm 0.05^a$
Organic nitrogen	Succinic acid	$0.33 \pm 0.01^c$	$0.70 \pm 0.01^b$	$2.50 \pm 0.02^a$
	1-pyrroline-3-hydroxy-5-carbocyclic-acid	$0.91 \pm 0.01^c$	$1.80 \pm 0.01^b$	$2.71 \pm 0.06^a$
Sugar acid	Galacturonic acid	$1.35 \pm 0.06^c$	$2.69 \pm 0.02^b$	$4.03 \pm 0.04^a$
	Glyceric acid	$1.58 \pm 0.01^c$	$3.16 \pm 0.01^b$	$4.57 \pm 0.01^a$
	Gluconic acid	$2.19 \pm 0.01^c$	$4.40 \pm 0.01^b$	$6.60 \pm 0.08^a$
	Glucuronic acid	$5.58 \pm 0.05^c$	$12.01 \pm 1.00^b$	$16.75 \pm 1.00^a$
Sugar alcohol	Arabitol	$0.41 \pm 0.04^c$	$0.80 \pm 0.09^b$	$1.25 \pm 0.13^a$
Class	Lipophilic compounds			
Aromatic	Benzoic acid	$1.00 \pm 0.03^c$	$1.99 \pm 0.09^b$	$3.02 \pm 0.09^a$
Fatty acid	n-9-(Z)-hexadecenoic acid	$7.27 \pm 0.07^c$	$15.00 \pm 0.15^b$	$22.00 \pm 0.22^a$
	Oleic acid	$0.14 \pm 0.01^c$	$0.27 \pm 0.03^b$	$0.45 \pm 0.05^a$
	Eicosanoic acid	$0.45 \pm 0.02^c$	$1.00 \pm 0.02^b$	$1.40 \pm 0.02^a$
	Hexadecanoic acid	$0.58 \pm 0.02^c$	$1.17 \pm 0.02^b$	$1.75 \pm 0.03^a$
	Octadecenoic (C18:1)	$21.48 \pm 0.01^c$	$43.00 \pm 0.05^b$	$64.45 \pm 1.62^a$

Sample 0 is the control sample (without guabiroba pulp); Sample 5 is the sample with 5% guabiroba pulp; Sample 10 is the sample with 10% guabiroba pulp; and Sample 15 is the sample with 15% guabiroba pulp. Results are expressed as mean  $\pm$  standard deviation ( $n = 3$ ). <sup>a-c</sup> Within a row, different lowercase letters indicate significant differences between samples ( $p < 0.05$ ).

Citric, quinic, malic, and dehydroascorbic acids are hydrophilic semi-volatile compounds in fruits and other foods that enhance nutritional value and functional properties [43]. Furthermore, they contribute to the flavor and preservation of foods, aid in nutrient absorption, and have potential therapeutic roles in managing conditions like diabetes [43]. At the same time, myo-inositol can improve metabolic parameters, potentially protecting the cardiovascular system, and cyclitol acts as a precursor for inositol triphosphate, which regulates hormones like insulin, contributing to its role in diabetes management [44]. The amino acid class represented by the hydrophilic compound 4-hydroxy-1-methyl-proline showed emphasis because this amino acid is involved in protein synthesis and may play a role in collagen stability [45], while glucuronic and gluconic acids support detoxification and metabolic processes [46].

The lipophilic compounds with the highest increases after guabiroba pulp addition were from the class fatty acids (octadecenoic (C18:1) and n-9-(Z)-hexadecenoic acid). The other classes were increased at a lower expressivity. Octadecenoic acid and its isomers are important as they can serve as precursors to essential fatty acids like linoleic acid, which is vital for maintaining cell membrane integrity and producing signaling molecules. The n-9-(Z)-hexadecenoic acid and its isomer is a predominant form of hexadecenoic acid found in human depot fat. It is part of a mixture of positional isomers that contribute to the overall fatty acid profile in the body, potentially influencing metabolic processes and energy storage. Both octadecenoic and hexadecenoic acids are part of a broader group of fatty acids that exhibit bioactive properties [47]. Therefore, adding guabiroba pulp to fresh cheeses enhances their potential health benefits and may improve the sensory properties of the products.

### 3.6. Antioxidant Activity

Figure 2B,C show the in vitro antioxidant capacity results using the DDPH and ABTS methods. By the DPPH method, it was observed that the fresh cheese samples containing 5, 10, and 15% of guabiroba pulp presented a gradual increase ( $p < 0.05$ ) of 220, 238, and 268% in antioxidant activity when compared to the control sample (without the addition of guabiroba pulp), respectively. Similar behavior was also observed through the ABTS method, whose increases ( $p < 0.05$ ) in antioxidant activity for fresh cheese samples added with 5, 10, and 15% of guabiroba pulp were 13, 28, and 48%, compared to the control sample. The total phenolic compounds of guabiroba (*Campomanesia xanthocarpa*) offer several health benefits, mainly due to their antioxidant, antidiabetic, anti-inflammatory, and hypotensive properties [7]. Fatty acids may have antioxidants, anti-inflammatory, and antimicrobial activities, which are crucial for preventing and managing various diseases [47]. Ingredients with antioxidant activities have attracted considerable attention as potential compounds to prevent or delay oxidative stress-related diseases [48]. Therefore, adding guabiroba to develop new products may reduce the use of chemical additives [7], and this presents an opportunity for future studies.

Therefore, our results demonstrate that the bioactive compounds of the guabiroba pulp were transferred to the cheeses during processing and positively impacted the products' antioxidant activity. However, it is important to note that the increase in the antioxidant activity for the cheeses after guabiroba pulp addition in the present study was lower than those observed for yogurt [9] and fermented milk [41]. Fermented milk and yogurt retain more bioactive compounds than cheeses due to increased proteolytic activity and the microbial fermentation process that releases bioactive peptides [49]. Furthermore, some bioactive compounds may be lost in the whey during cheese processing.

### 3.7. Multielement Profile

Table 5 presents the minerals identified in the fresh cheeses. The minerals considered toxic to some extent are As (arsenic), Cd (cadmium), Pb (lead), Cr (chromium), and Al (aluminum). These minerals were found below the detection limit for all fresh cheese samples (with or without adding guabiroba pulp). As, Cd, and Pb are toxic minerals commonly found in dairy products, including cheeses [50–52]. Pb concentrations in cheese often exceed permissible limits, posing potential health risks to children, as it can adversely affect the nervous system and kidneys [53]. Therefore, continuous monitoring of these toxic elements ensures food safety and minimizes exposure to these toxic elements. Our results demonstrate that the addition of guabiroba pulp did not result in the inclusion of toxic minerals in the fresh cheeses.

**Table 5.** Multi-element profile of fresh cheese formulations samples added with guabiroba pulp.

Elements (mg/g)	Samples			
	0	5	10	15
Al	<LOD	<LOD	<LOD	<LOD
As	<LOD	<LOD	<LOD	<LOD
Ca	5.85 ± 0.75 <sup>ac</sup>	6.09 ± 0.11 <sup>a</sup>	5.47 ± 0.40 <sup>abc</sup>	4.92 ± 0.50 <sup>c</sup>
Cd	<LOD	<LOD	<LOD	<LOD
Co	<LOD	<LOD	<LOD	<LOD
Cr	<LOD	<LOD	<LOD	<LOD
Cu	<LOD	<LOD	<LOD	<LOD
Fe	<LOD	<LOD	<LOD	<LOD
K	1.00 ± 0.12 <sup>bc</sup>	1.20 ± 0.02 <sup>a</sup>	1.16 ± 0.04 <sup>ab</sup>	1.01 ± 0.06 <sup>c</sup>
Mg	0.22 ± 0.01 <sup>a</sup>	0.30 ± 0.08 <sup>a</sup>	0.24 ± 0.01 <sup>a</sup>	0.23 ± 0.01 <sup>a</sup>
Mn	<LOD	<LOD	<LOD	<LOD
Na	3.30 ± 0.52 <sup>d</sup>	6.59 ± 0.11 <sup>b</sup>	7.08 ± 0.38 <sup>a</sup>	4.59 ± 0.25 <sup>c</sup>
P	4.39 ± 0.53 <sup>ab</sup>	4.65 ± 0.25 <sup>a</sup>	4.10 ± 0.29 <sup>ab</sup>	3.86 ± 0.13 <sup>b</sup>
Pb	<LOD	<LOD	<LOD	<LOD
S	1.89 ± 0.19 <sup>ab</sup>	2.05 ± 0.10 <sup>a</sup>	1.80 ± 0.15 <sup>b</sup>	1.76 ± 0.02 <sup>b</sup>
Se	<LOD	<LOD	<LOD	<LOD
Sr	0.003 ± 0.001 <sup>a</sup>	0.004 ± 0.001 <sup>a</sup>	0.003 ± 0.001 <sup>a</sup>	0.003 ± 0.001 <sup>a</sup>
Zn	0.03 ± 0.01 <sup>a</sup>	0.03 ± 0.01 <sup>a</sup>	0.03 ± 0.01 <sup>a</sup>	0.03 ± 0.01 <sup>a</sup>

Sample 0 is the control sample (without guabiroba pulp); Sample 5 is the sample with 5% guabiroba pulp; Sample 10 is the sample with 10% guabiroba pulp; and Sample 15 is the sample with 15% guabiroba pulp. Results are expressed as mean ± standard deviation (n = 3). <sup>a-d</sup> Within a row, different lowercase letters indicate significant differences between samples ( $p < 0.05$ ). LOD, limit of detection.

Other minerals were also found below the limit of detection, such as Co (cobalt), Cu (copper), Fe (iron), Mn (manganese), and Se (selenium). These minerals have already been reported in cheeses [54], but they are not typically detected in fresh cheeses [50]. Their presence depends on the cheese type and manufacturing process [54]. This result demonstrates the importance of evaluating the chemical composition of each cheese variety.

In a general view, the addition of guabiroba pulp did not change the mineral composition of the fresh cheeses ( $p > 0.05$ ). Therefore, Ca, Mg, P, S, and Zn content was maintained. Ca, Mg, and P are major minerals in fresh cheeses essential for various bodily functions [55]. S is also present in fresh cheeses, often as part of amino acids and proteins. At the same time, Zn is a trace element found in fresh cheeses, contributing to various enzymatic functions in the body [56]. Finally, the small contents of the mineral Sr in the cheese samples agree with the result observed by Herman-Lara et al. [55]. These authors affirmed that, while Sr can be present in some cheeses, it is not typically highlighted as a common mineral in fresh cheeses, suggesting it may be negligible in many fresh cheese varieties.

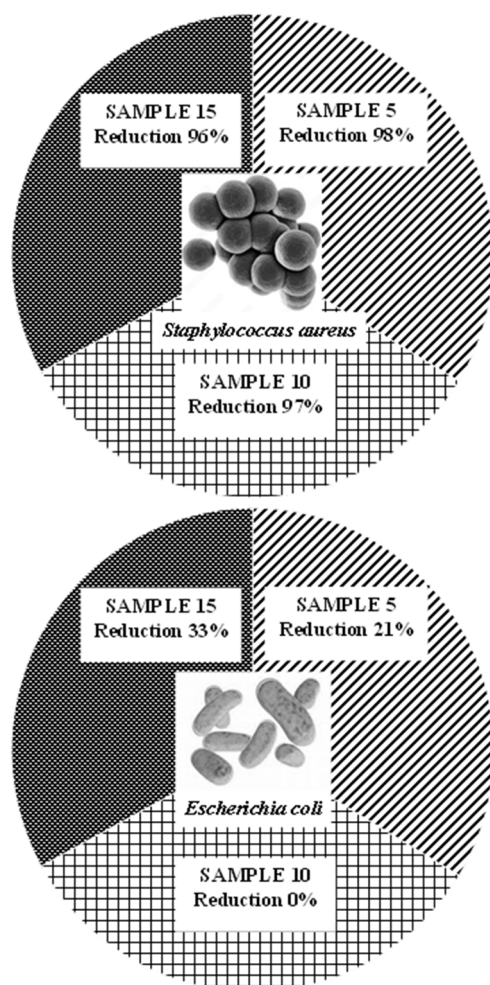
Only a slight decrease in K (after 15% guabiroba pulp addition) and increase in Na (for all products with guabiroba pulp) were noted ( $p < 0.05$ ). Guabiroba pulp addition may have modified the interaction of Na in the cheese matrix, resulting in changes in the concentration of Na in the product. According to Tidona et al. [57], the variability in sodium content in fresh cheeses is primarily due to the multifaceted role of salt in cheese-making.

The sodium content in all cheeses was low (3.30–7.08 mg/g), representing less than the recommended daily intake. Therefore, although this mineral content was observed after guabiroba pulp addition, this increase would not affect consumer health. According to the WHO [58], Na is an essential nutrient for maintaining plasma volume, acid–base balance, transmission of nerve impulses, and normal cell function; sodium deficiency is extremely unlikely in healthy individuals [58]. However, excess sodium is linked to adverse health outcomes, including increased blood pressure [58].

Despite guabiroba pulp presenting a modest contribution concerning the multielement profile of the fresh cheeses produced, it was possible to note that this information is useful for dietary planning and understanding the nutritional profile of fresh cheeses, as well as the safety of consuming them about the presence of the main toxic minerals.

### 3.8. Antimicrobial Activity

Figure 3 shows the results for reducing *Staphylococcus aureus* and *Escherichia coli* in fresh cheese samples. A representative reduction (96–98%) of *S. aureus* in all samples containing guabiroba pulp (5, 10, 15%) was observed, while the reduction in *E. coli* was less pronounced, i.e., 21% and 33% for sample 5 and sample 15, respectively. However, for sample 10, the *E. coli* reduction result was of 0%.



**Figure 3.** Reduction (%) of *Staphylococcus aureus* and *Escherichia coli* in fresh cheese samples added with 5% guabiroba pulp (Sample 5), 10% guabiroba pulp (Sample 10), and 15% guabiroba pulp (Sample 15) (n = 3).

The genus *Staphylococcus* is made up of 52 species. *S. aureus* is one of the species most regularly associated with human pathologies, and it causes nosocomial infections, such as pneumonia and other respiratory and cardiovascular infections [59]. Some strains of *S. aureus* produce enterotoxins that cause staphylococcal food poisoning, and the foods mainly involved are milk and dairy products [59,60]. *E. coli* is a major cause of diarrhea and is responsible for extraintestinal infections in humans and animals [61]. Fruit pulps and grains have demonstrated the ability to effectively inhibit *S. aureus* in dairy products, such as açai pulp [62] and pinhão [63], while pineapple peel extract and pomegranate fruit

exhibited antimicrobial activity against *E. coli* [64,65]. However, this is the first study to report on the antimicrobial activity of guabiroba-pulp-added fresh cheeses.

The antimicrobial activity of guabiroba pulp is mainly due to the presence of phenolic compounds and other bioactive compounds with antimicrobial activity. The mechanisms of action of phenolic compounds and other bioactive compounds on bacterial cells have been partially attributed to damage to the bacterial membrane, inhibition of virulence factors such as enzymes and toxins, and suppression of bacterial biofilm formation [61]. Furthermore, phenolic hydroxyl groups, which have a high affinity for proteins, and microbial enzyme inhibition may enhance the antibacterial effects [66]. However, in studies of Brazilian fruit pulps, some extracts with high phenolic content did not always correlate with antimicrobial activity, indicating that other factors, such as pH or salt, may be responsible for observed effects. The relationship between pulp addition; changes in physicochemical properties such as pH, water activity, and salt/mineral content; and observed antimicrobial outcomes is complex. It is crucial to distinguish whether the antimicrobial effects are due to these shifts or to specific bioactive compounds in the pulp. The addition of fruit pulps, such as blackberry or uvaia, often lowers the pH and water activity of the matrix, creating less favorable conditions for microbial survival. For example, uvaia pulp has a pH of 3.45, classifying it as acidic; however, its extracts did not show antimicrobial activity against the tested bacteria, suggesting that a low pH alone may not always be sufficient for antimicrobial effects, or that other factors are at play [67].

On the other hand, studies do not explicitly report the use of pH- or water-activity-matched controls (e.g., neutralized extracts or pH-matched matrices) to separate the effects of physicochemical changes from those of specific bioactives. This fact is a significant limitation, as it makes it difficult to attribute antimicrobial outcomes solely to pulp-derived compounds [68,69].

#### 4. Conclusions

This study was the first to characterize fresh cheeses added with guabiroba pulp (5, 10, 15%). Guabiroba pulp changed the color (to a darker yellow) and reduced the products' texture parameters properties (firmness, elasticity, cohesiveness, and gumminess). At the same time, it increased the concentration of bioactive compounds (carotenoids, amino acids, phenolic compounds, and fatty acids), organic acids, sugars (sucrose and fructose), and antioxidant activity. Antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli* was observed for fresh cheese samples with guabiroba pulp addition. In conclusion, fresh cheese with guabiroba pulp presented an improved concentration of bioactive compounds and functional properties, demonstrating an innovative product for dairy industries. However, while innovation is vital for the dairy industry, product readiness claims should be tempered unless supported by comprehensive sensory acceptability and stability data. Ongoing research, larger-scale consumer studies, and shelf-life determination of the product are essential to ensure that innovative products meet both industry standards and consumer expectations.

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