





Article

Development and Chemical, Physical, Functional, and Multi-Element Profile Characterization of *Requeijão* with Guabiroba Pulp

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Abstract: Five requeijão samples, classified as Brazilian cream cheeses, were developed: one control (without guabiroba pulp (*Campomanesia xanthocarpa* O. Berg) and four with 5, 10, 15, and 20% (*m/m*) guabiroba pulp. They were evaluated for pH, water activity (*aw*), color, texture, multi-mineral composition, carotenoid content, and microstructure. The addition of guabiroba pulp reduced pH and maintained *Aw*. The samples with 5%, 10%, 15%, and 20% guabiroba pulp presented a yellow–reddish coloration. The formulation with 5% had the lowest values of firmness, resilience, texture, and spreadability. From 10% onwards, an increase in cohesiveness and a reduction in creaminess were observed. The sample with 15% presented better spreadability, while the 20% sample had adhesiveness similar to the control. No traces of Al, As, Cd, Co, Cr, Cu, Fe, Mn, Pb, or Se were detected. The detected elements, in descending order, were Na, Ca, P, S, K, Mg, Sr, and Zn. β -carotene was predominant, with guabiroba pulp enhancing α -carotene, β -carotene, β -cryptoxanthin, and λ -carotene levels, especially at 20% pulp. Microstructure analysis by scanning electron microscopy (SEM) showed no significant differences. These findings highlight the potential of guabiroba pulp as a functional ingredient in requeijão, enhancing its carotenoid profile while maintaining desirable textural and physicochemical properties.

Keywords: creamy cheese; *Campomanesia xanthocarpa* O. Berg; texture analysis; multi-mineral composition; carotenoids; SEM

1. Introduction

Dairy products are essential for nutrition, as they are rich in proteins, minerals, and vitamins. Among dairy products, soft or creamy cheeses, such as cream cheese, stand out for their accessibility and convenience in relation to their consumption. Requeijão is a dairy product widely consumed in Brazil, characterized by its soft texture and mild flavor, obtained from the mixture of coagulated mass, cream, and additives, followed by a melting process [1]. Although it is a source of protein, calcium, and other minerals, its functional value can be limited, and many commercial formulations use artificial colors to improve their appearance. These additives, when consumed in excess, can pose health risks, such as allergic reactions and adverse effects on metabolism [2,3]. In this context, the present study presents an innovative approach by incorporating a native Brazilian fruit into requeijão formulation, aiming not only to replace artificial colors with a natural source of pigments and bioactive compounds, but also to enrich the product with functional nutrients. The proposal represents an original contribution to the national and global challenges of promoting healthier, more natural, and sustainable foods, valuing ingredients native to Brazil and aligning with the guidelines of technological innovation and public health [4,5].

The development of a dairy product with a claim of functional properties and that is highly acceptable to consumers has demonstrated economic importance and can add value to the product [6]. The importance of foods with functional claims has resurfaced due to the emergence of new diseases and the recent COVID-19 pandemic [3]. According to Shabbir et al. [7], many researchers have reformulated commonly available and widely consumed foods using natural ingredients with health-enhancing and immune-boosting properties.

Well-informed consumers are generally willing to pay for foods with desirable natural additives that promote health. Cheese is one of the most consumed foods, and therefore, improving its functional value by using a natural ingredient such as a native fruit pulp could further increase its acceptability and reach among consumers [6].

Guabiroba (*Campomanesia xanthocarpa* O. Berg), a native Brazilian fruit belonging to the *Myrtaceae* family, is widely recognized for its functional potential. Traditionally consumed fresh or in the form of juices and jellies, guabiroba has a phytochemical profile rich in bioactive compounds, such as carotenoids (especially α -carotene, β -carotene, and β -cryptoxanthin), phenolics, and vitamin C [4]. The phytochemicals of guabiroba pulp are elucidated regarding its high antioxidant activity, which is related to benefits to human health when routinely introduced into the diet. In addition, its antioxidant property can act as a natural preservative against oxidative, enzymatic reactions, and microbiological deterioration, extending the shelf life of foods [5,8]. These components make guabiroba a promising candidate for the formulation of functional foods, with potential benefits to human health, especially in combating oxidative stress and preventing chronic diseases.

Dairy products have already been produced using guabiroba pulp, with emphasis on probiotic fermented milk obtained from concentrated whey [9] and probiotic fermented milk [10]. Guabiroba pulp in fermented milk has a prebiotic effect, i.e., it contains fibers that favor beneficial bacteria in the gut (probiotics) and protects against *Bifidobacterium* BB-12, a probiotic microorganism, when subjected to in vitro gastrointestinal conditions [4].

Studies related to encouraging the use of guabiroba pulp and juice in food formulations have become the basis for improving its application in the food industry [5,9,11,12]. Thus, this study aimed to develop an innovative requeijão enriched with guabiroba pulp (5–20% *m/m*), combining the creamy matrix of requeijão with the functional potential of a native fruit rich in bioactive compounds to enhance health benefits and promote technological innovation in dairy products.

2. Materials and Methods

2.1. Reagents

All reagents used were of analytical grade or higher. Ultrapure water (with a resistivity of 18.2 MΩ) was obtained from an ultra-purifier system (MS3000, Master System, Gehaka, São Paulo, SP, Brazil) to prepare all sample and reagent dilutions. The reagents used for the sample preparations were acetone, NaOH, petroleum ether, HNO₃, HCl (Quimis, São Paulo, SP, Brazil), and tetramethylammonium hydroxide (TMAH) 25% *w/w* in H₂O (Sigma-Aldrich, Steinheim, Germany). Nitric acid was purified in a polytetrafluoroethylene (PTFE) sub-boiling system, model Distill acid BSB-939-IR (Berghof, Germany). Individual standard solutions with a concentration of 1000 mg L⁻¹ of the analytes Ca, Cu, P, Zn, Sc (Specsol[®], Jacareí, São Paulo, Brazil), K (MERCK, Darmstadt, Germany), Cr, Fe, Mg (SCP Science, Quebec, QC, Canada), and Na (VETEC, Duque de Caxias, RJ, Brazil) were used for the analytical calibration curves and recovery tests.

2.2. Raw Materials

The guabiroba fruit was provided by EMBRAPA Florestas, located in Colombo, PR, Brazil. This fruit was obtained from the harvest carried out in 2023 in Pinho de Baixo city, Irati, PR, Brazil (25°23'8" south and 50°41'45" west). It was used in the preparation of the cream cheeses: fresh salted ricotta (Sulfrios[®], Criciúma, SC, Brazil) containing 13.33 g/100 g of protein, 13.33 g/100 g of lipids, and 0 g/100 g of carbohydrates; salted butter (Batavo[®], Carambeí—PR, Brazil) containing 1 g/100 g of protein, 80 g/100 g of lipids, and 0.7 g/100 g of carbohydrates; and whole UHT (ultra-high-temperature) milk (Tirol[®], Nova Trento, SC, Brazil) containing 3.2 g/100 g of protein, 3 g/100 g of lipids, and 4.5 g/100 g of carbohydrates.

2.3. Sample Preparation

2.3.1. Guabiroba Pulp Production

The guabiroba fruits were selected, washed in running water, immersed in a chlorine solution (99.99 mg/L) for 15 min, washed again in running water, and taken for pulping. Using a pulper (MB—Braesi[®], model: DES-60, Itapipoca, CE, Brazil), the fruits were passed through this equipment, where the pulp residue was separated. The pulp was stored in sealed polyethylene packages and frozen (-18 ± 1 °C). At the end of these steps, the guabiroba pulp had the following composition: 1.4 g/100 g of protein, 0.6 g/100 g of lipids, 7.5 g/100 g of total carbohydrates, and 6.5 g/100 g of fiber.

2.3.2. Production of Requeijão

A requeijão mixture was prepared according to the formulation described in Table 1.

Table 1. Standard formulation of requeijão.

Ingredients	Amount (g)
Salted fresh ricotta	760
Slated butter	300
Whole UHT (ultra-high-temperature) milk	600

All ingredients were weighed, the fresh ricotta was cut into 1 cm cubes, and the milk was heated to boiling. All ingredients were mixed in a blender (Philco[®], São Paulo, SP, Brazil), placed in a stainless-steel pan, and heated until it reached a creamy consistency. The requeijão was cooled to a temperature of 50 ± 1 °C and separated into 5 equal parts, resulting in a control sample (without guabiroba pulp) and four samples to which 5, 10,

15, and 20% (*w/w*) of guabiroba pulp, called sample 5, sample 10, sample 15, and sample 20, respectively, was added. The steps involved in preparing the cream cheeses are shown in Figure 1.

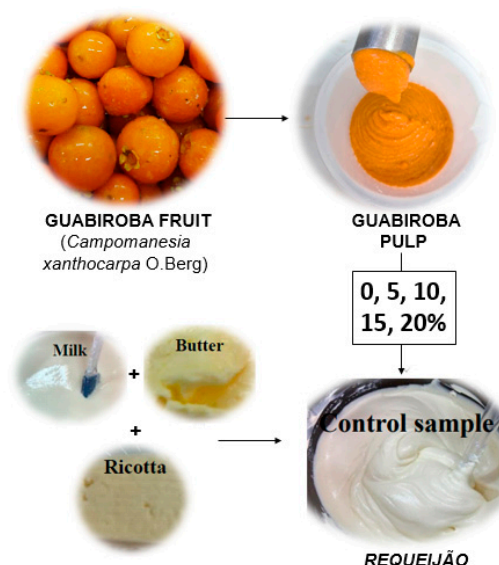


Figure 1. Flowchart of the steps involved in the preparation of requeijão. Note: the control sample is the requeijão without guabiroba pulp, while samples 5, 10, 15, and 20 contain 5, 10, 15, and 20% of guabiroba pulp, respectively.

2.4. Physicochemical Analysis

The pH values of the requeijão samples were determined using a digital pH meter (Kasvi[®], São Paulo, SP, Brazil). The water activity (*a_w*) of the curd samples was measured at a temperature of 25 ± 1 °C using the Aqualab[®] 4TE analyzer (Decagon Devices, Washington, WA, USA), after stabilizing the curd samples for a period of 15 min.

2.5. Colorimetric Analysis

The color analyses of the requeijão samples were carried out using a sphere spectrophotometer (Model SP60 Series, X-Rite Inc., Grand Rapids, MI, USA), and the color results were expressed in *L** (lightness), *a**, and *b**. The values were read directly from the equipment used. The *L** parameter ranges from 0 to 100 and indicates the lightness (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**).

2.6. Texture Analysis

Texture analysis of the requeijão samples was performed using a TA-XT plus texturometer (Stable Micro Systems, Texture Exponent software, Surrey, UK). A 25 mm diameter aluminum probe was used to compress the samples. Measurements were made at 5 ± 1 °C, with a test speed of 1.0 mm/s and 10.0 mm compression depth. Force data as a function of time were obtained for the two compression–decompression cycles using the TA-XT plus software.

2.7. Carotenoid Content

Carotenoid levels were assessed according to Rodriguez-Amaya [13], with modifications. For carotenoid extraction, 1 g of sample and 20 mL of acetone were weighed in a 50 mL Falcon[®] tube. After vortex mixing (Biomixer[®], Jacareí, São Paulo, Brazil), the tube containing the mixture was placed in an ultrasound for 30 min at 25 °C. The extract was separated with filter paper and a funnel. In a burette, 4 mL of petroleum ether was

added, followed by the extracted liquid and 3 mL of type 2 ultrapure water. The burette was left to stand, waiting for the phases to separate. When there was no separation, a few drops of NaOH solution were added, and the mixture was left to separate. After separation, the lower fraction (colorless) was removed for disposal, keeping only the colored phase in the burette. The colored phase was removed to a volumetric flask, passing through filter paper with sodium sulfate, retaining any aqueous residue. The burette was cleaned with petroleum ether, avoiding loss of the extract. The carotenoid content was obtained in a UV–Vis spectrophotometer (Shimadzu®, Barueri, São Paulo, Brazil), using a wavelength of 450 nm for β -carotene ($Abs = 0.092x + 0.005$; $R^2 = 0.9985$), a wavelength of 444 nm for α -carotene ($Abs = 0.089x + 0.0042$; $R^2 = 0.9971$), a wavelength of 452 nm for β -cryptoxanthin ($Abs = 0.081x + 0.0028$; $R^2 = 0.9968$), and a wavelength of 462 nm for λ -carotene ($Abs = 0.064x + 0.0017$; $R^2 = 0.9956$). The results were expressed in micrograms of carotenoids per 100 milliliters of sample ($\mu\text{g}/100\text{ mL}$).

2.8. Multi-Element Profile

Requeijão samples were previously microwave-digested using a microwave oven model Microwave Reaction System, Multiwave PRO (Anton Paar, Graz, Austria), with internal digestion vessels, operating at a maximum microwave power of 1200 W, a maximum internal temperature of $200 \pm 1\text{ }^\circ\text{C}$, and a maximum pressure of 20 bar. Ultrasound-assisted extraction was performed in an ultrasonic bath model 60/2 (Nova Instruments, Piracicaba, SP, Brazil) at a fixed frequency of 50 Hz at room temperature ($25 \pm 1\text{ }^\circ\text{C}$). In contrast, alkaline solubilization was performed using a water bath on a hot plate (model C-MAG HS 7, IKA, Campinas, SP, Brazil). Finally, for the dry ashing method, a muffle (model LF0613, Jung, Blumenau, SC, Brazil) was used at $550 \pm 1\text{ }^\circ\text{C}$, followed by ashing solubilization in concentrated HCl at $80 \pm 1\text{ }^\circ\text{C}$. In the sequence, a centrifuge (model 206 BL, Fanem, Guarulhos, SP, Brazil) was used to centrifuge 2.218 g samples.

The multi-element profile of the requeijão samples was adjustable in an ICP OES (inductively coupled plasma optical emission spectroscopy) (model iCAP 6000, Thermo Scientific, Waltham, MA, USA). The analytes (wavelength) were monitored—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), Zn (213.856 nm), and Sc (361.384 nm)—as an internal standard. These elements were selected due to their importance as elements in cheese samples and the instrumental capabilities of the ICP OES that had a V-Groove nebulizer and a cyclonic spray chamber. This nebulizer introduces samples with relatively high dissolved solids, but the samples obtained in the extraction procedures must be analyzed. The operational options used were radial view, pumping flow (60 rpm), plasma gas flow (12 L/min), radiofrequency power (1300 W), auxiliary gas flow (1 L/min), and nebulizer gas flow (0.4 L/min). Argon, with a minimum purity of 99.95% (Air Liquide, Rio de Janeiro, RJ, Brazil), was used as the main, auxiliary, and nebulizer gas for ICP OES. Analytical curves were prepared from 0.1 to 10 mg/L of each analyte.

Ultrapure water (with a resistivity of 18.2 M Ω) was obtained from an ultrapurification system (MS3000, Master System, Gehaka, São Paulo, SP, Brazil) to prepare all samples and reagent dilutions. The reagents used for the selective samples were HNO₃ (14.4 mol/L) and HCl (12 mol/L) (Quimis, São Paulo, SP, Brazil) and tetramethylammonium hydroxide 25% w/w (Sigma-Aldrich, Steinheim, Germany) in H₂O. Nitric acid was purified in a polytetrafluoroethylene sub-boiling system (model Distill acid BSB-939-IR, Berghof, Germany). The following individual standard solutions with a concentration of 1000 mg/L of the analytes were used for the analytical hunting curves and in the recovery tests: Al (aluminum), As (arsenic), Ca (calcium), Cd (cadmium), Co (cobalt), Cr (chrome), Cu (copper), Fe (iron), Mn (manganese), P (phosphorus), Pb (lead), S (sulfur), Se (selenium),

Sr (strontium), Zn (zinc) (Specsol[®], Jacareí, São Paulo, Brazil), K (potassium) (MERCK[®], Darmstadt, Germany), Mg (magnesium) (SCP Science[®], Quebec, QC, Canada), and Na (sodium) (VETEC[®], Duque de Caxias, RJ, Brazil).

2.9. Scanning Electron Microscopy (SEM)

The requeijão samples were prepared for scanning electron microscopy using a method adapted from that proposed by Lobato-Calleros et al. [14]. The samples were dehydrated in a Terroni[®] freeze-dryer (LD 3000, São Carlos, Brazil), and each sample was fractured, placed on stubs, and coated with a thin layer of gold using a Leica[®] sputter coating (EM SCD 500, Wetzlar, Germany). A scanning electron microscope (model VEGA[®] 3 SEM, Tescan, Tokyo, Japan) was used at 20 kV to visualize each sample at a magnification of 500×.

2.10. Statistical Analysis

The results were expressed as the mean \pm standard deviation. To determine significant differences ($p < 0.05$) between the results, one-way analysis of variance (ANOVA) and Tukey's test were used. All statistical analyses were performed using STATISTICA 13.3 software (TIBCO Software Inc., Palo Alto, CA, USA). The steps were performed in triplicate.

3. Results and Discussion

The guabiroba composition was 1.4 g/100 g of protein, 0.6 g/100 g of lipids, 7.5 g/100 g of total carbohydrates, and 6.5 g/100 g of fiber. Table 2 presents the pH and water activity (a_w) results and the color parameters L^* , a^* , and b^* of the requeijão produced. A decrease ($p < 0.05$) in pH values was observed after the addition of 10% (m/m) of guabiroba pulp. Thus, the influence of guabiroba pulp on pH values was only observed after adding 10% (m/m). According to Rigo et al. [15], guabiroba pulp has pH values ≤ 4.6 . However, no large decreases in pH values were observed when using between 10 and 20% (m/m) of guabiroba pulp in the cream cheeses. The values obtained for pH were close (5.4 to 5.8) to those obtained by Moura et al. [1] for Brazilian requeijão. The pH of all requeijões produced, including those with the addition of guabiroba pulp (5 to 20% w/w), according to Oliveira et al. [16], are as expected for a requeijão formulation, i.e., between 5.4 and 6.2. These authors also highlighted that pH is an important factor for the identity and quality of all cheeses because it directly affects their texture properties. pH values different from those expected for a given product could negatively affect the chemical interactions between the structural components, proteins, water, and mineral salts of the requeijão, compromising their texture. Adding 10% (m/m) guabiroba pulp to a product can lead to a greater decrease in pH compared to higher concentrations like 15% or 20% due to the specific interactions and concentration of bioactive compounds present at this level. Therefore, factors influencing pH decrease include bioactive compounds, acidic components, and interaction with other ingredients. Guabiroba pulp is rich in phenolic compounds, which can contribute to acidity. At 10% concentration, these compounds might be optimal to significantly impact pH without being buffered by other components at higher concentrations [9,10,17]. Natural acids in guabiroba pulp, such as citric acid, can lower pH. The specific balance of these acids at 10% might be more effective in reducing pH compared to higher concentrations where the effect could be diluted or counteracted by other factors [18]. The interaction between guabiroba pulp and other ingredients in the formulation can affect pH. At 10%, the pulp might interact more effectively with the matrix, enhancing the release or activity of acidic components [9,10]. The greater decrease in pH with 10% guabiroba pulp compared to 15% or 20% could be due to the optimal concentration of bioactive and acidic compounds at this level, which maximizes their impact

on pH. This effect might be less pronounced at higher concentrations due to dilution or interactions that buffer the acidity.

Table 2. Results of physicochemical and color analyses of requeijão with guabiroba pulp.

Parameters	Samples				
	Control	5	10	15	20
pH	5.08 ± 0.02 ^a	5.03 ± 0.04 ^a	4.24 ± 0.05 ^c	4.79 ± 0.02 ^b	4.76 ± 0.03 ^b
aw	0.91 ± 0.02	0.91 ± 0.01	0.91 ± 0.01	0.91 ± 0.01	0.91 ± 0.01
L*	88.53 ± 0.84 ^a	81.96 ± 0.59 ^b	77.00 ± 0.37 ^c	69.73 ± 0.74 ^d	63.54 ± 0.33 ^e
a*	4.95 ± 0.12 ^e	11.52 ± 0.18 ^d	14.90 ± 0.21 ^c	18.14 ± 0.29 ^a	16.89 ± 0.13 ^b
b*	28.89 ± 0.41 ^e	35.56 ± 0.80 ^d	42.17 ± 0.51 ^b	47.06 ± 0.51 ^a	39.47 ± 0.65 ^c

Notes: ^{a–e} Means accompanied by different letters in the same row present a significant difference ($p < 0.05$).

The water activity (aw) values of the requeijões did not differ from each other ($p > 0.05$). Thus, it can be verified that the addition of guabiroba pulp at the levels used (5 to 20% w/w) was not able to generate changes in Aw. According to Oliveira et al. [16], the thermal processes employed in the preparation of a requeijão would be more related to its Aw values. Vollmer et al. [19] reported that the thermal processes used in preparing cream cheeses would affect their Aw values through protein–protein interactions, fat–protein interactions, and fibril formation, which would drive their structure formation. In the requeijão, the water activity is represented by the concentration of sodium chloride in the aqueous phase and is important to prevent the multiplication and activity of certain microorganisms [20]. From the values obtained for Aw, it would be possible to predict future conditions and parameters to be used during the storage of a given cheese, avoiding changes in the quality of this cheese [21]. The values obtained for Aw of requeijões without or with the addition of guabiroba pulp (5 to 20% m/m) were similar to those obtained by Biegalski [15], which ranged from 0.91 to 0.96.

The results of the color analyses of requeijão samples with or without the addition of guabiroba pulp are expressed in terms of the parameters L*, a*, and b*. From the parameter L*, it was found that the requeijões with lower additions of guabiroba pulp presented lower values for the parameter L* ($p < 0.05$) and, therefore, a lighter color. The color parameters a* and b* indicated that all requeijão samples tended towards a reddish–yellowish hue. As expected, this trend was more evident in samples with the highest guabiroba pulp content. Prestes et al. [9] reported that guabiroba pulp is orange due to carotenoids and flavonoids. Moura et al. [1] highlighted that measurements of color parameters in cream cheeses are recommended because, depending on consumer preferences, changes in color can affect the perception and acceptance of the product.

Table 3 presents the results of the analysis of the texture parameters of the requeijões without (control) and with the addition of guabiroba pulp (5 to 20% w/w), where firmness, adhesiveness, resilience, and cohesiveness were evaluated. Gavahian et al. [22] highlighted that texture is one attribute affecting product acceptance. By evaluating the instrumental texture, it is possible to define the texture profile of a given product. Many instrumental methods have been developed to determine the texture properties of foods, with emphasis on instrumental texture analysis. This analysis applies successive deforming forces, simulating the compression and cutting action of the teeth during chewing using a measuring instrument, the texturometer. Determining the texture parameters is important in evaluating consistency and stability and providing information about the structure of the product [16].

Table 3. Results of texture parameters of control cheeses and those with the addition of guabiroba pulp.

Parameter	Samples				
	Control	5	10	15	20
Firmness (N)	14.3 ± 6.4 ^a	11.3 ± 0.3 ^a	10.8 ± 0.6 ^a	5.2 ± 2.2 ^b	9.9 ± 1.2 ^a
Adhesiveness (N.s)	−10.7 ± 0.5 ^a	−9.1 ± 0.5 ^b	−9.1 ± 0.1 ^b	−8.1 ± 1.0 ^b	−10.3 ± 1.8 ^{a,b}
Resilience *	3.7 ± 1.2	2.5 ± 0.1	3.0 ± 0.7	3.1 ± 0.9	2.4 ± 0.1
Cohesiveness *	0.6 ± 0.1 ^b	0.8 ± 0.1 ^{a,b}	0.9 ± 0.1 ^a	1.0 ± 0.1 ^a	1.0 ± 0.1 ^a

Note: ^{a,b} Means accompanied by different letters in the same row present a significant difference ($p < 0.05$).

* Dimensionless parameters.

The decrease in firmness with 15% guabiroba pulp compared to 10% or 20% could be due to the specific balance of mechanical and compositional interactions at this concentration. The presence of natural antioxidants and sugars in the pulp likely alters the film's mechanical properties, leading to a more significant reduction in firmness at this specific concentration, i.e., using 15% guabiroba pulp [17]. According to Brighenti et al. [23], increasing firmness values could reduce the spreadability of creamy cheeses, such as requeijão. Amaral et al. [24] stated that adhesiveness, as a texture parameter, is characterized by removing the product adhered to the material of the texturometer probe, which would represent the difficulty in being removed from a utensil used to spread the cream cheese. In general, it was found that the addition of up to 15% (w/w) of guabiroba pulp reduced ($p < 0.05$) the adhesiveness of the cream cheeses. However, when the pulp content used in the requeijão samples was 20% (w/w), it was observed that the adhesiveness was also similar ($p < 0.05$) to the control sample. One factor that could be related to the increased adhesiveness in the sample with 20% (w/w) of guabiroba pulp would be the fiber content present. According to Alves et al. [25], guabiroba pulp has a total dietary fiber content of 7 g/100 g, corresponding to approximately 23% of the daily recommendation for a healthy adult individual (30 g), constituting a food with a high fiber content. Szafrńska and Sołowiej [26] evaluated the effect of different fibers on the adhesiveness of processed cheese sauce and observed that increasing the fiber content would increase adhesiveness. These authors reported that the fibers would be responsible for forming a network with the product's other ingredients and could keep it adhered to the texturometer probe.

Ningtyas et al. [27] emphasized that the perception of the texture of creamy cheeses is based on resilience as the first dominant attribute, which depends on the ingredient used. Wee et al. [28] reported that resilience is a property of food texture that correlates with the parameters of sensory analysis performed by judges. Based on the results obtained for resilience, it was observed that they did not present differences ($p < 0.05$) among all the requeijões produced. Thus, the guabiroba pulp did not influence the resilience of the samples. Using the data obtained for resilience, no differences would be noted between the texture of the requeijão samples (control and with 5 to 20% w/w of guabiroba pulp) when tasted by consumers. Since cohesiveness is defined as the extent to which a food can be deformed before rupture, it was observed that the use of 10% (w/w) of guabiroba pulp contributed to the increase ($p < 0.05$) in the cohesiveness of the requeijão. Souza et al. [29] presumed in their study that requeijão with the addition of pectin, a soluble fiber, showed increased cohesiveness. Thus, the increase in cohesiveness observed in the sample with 10% (w/w) of guabiroba pulp could be related to the high fiber content present in the guabiroba pulp. Inoue et al. [30] mentioned that cohesiveness represents how much the material can be compressed between the teeth before breaking; the more cohesive the material, the better it can maintain the same shape. Cheese samples with a more cohesive

texture were closely correlated with less pasty characteristics [30]. Thus, requeijão with the addition of guabiroba pulp $\geq 10\%$ (w/w) would tend to be less pasty, i.e., creamy.

Table 4 contains the results of the multi-element profile analysis of the requeijão samples. The following elements were not detected in any of the requeijão samples, including the control: Al (aluminum), As (arsenic), Cd (cadmium), Co (cobalt), Cr (chrome), Cu (copper), Fe (iron), Mn (manganese), Pb (lead), and Se (selenium). Elements like Cu, Fe, Mn, Co, and Se are essential for various physiological functions. They are crucial for maintaining healthy bodily functions and preventing deficiencies. However, Al, As, Cd, Pb, and Cr can lead to serious health issues.

Table 4. Multi-element profile results of requeijão samples with addition of 0 (control), 5, 10, 15, and 20% of guabiroba pulp.

Element (mg/g)	Requeijão Sample				
	Control	5	10	15	20
Al	<LOD	<LOD	<LOD	<LOD	<LOD
As	<LOD	<LOD	<LOD	<LOD	<LOD
Ca	1.89 ± 0.07^a	1.94 ± 0.02^a	1.73 ± 0.06^b	$1.68 \pm 0.07^{b,c}$	1.59 ± 0.07^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	0.88 ± 0.02^c	0.99 ± 0.01^b	0.97 ± 0.01^b	1.05 ± 0.03^a	1.08 ± 0.03^a
Mg	0.10 ± 0.01	0.10 ± 0.01	0.09 ± 0.01	0.09 ± 0.01	0.09 ± 0.01
Mn	<LOD	<LOD	<LOD	<LOD	<LOD
Na	3.03 ± 0.16^a	3.15 ± 0.03^a	2.70 ± 0.04^b	2.69 ± 0.17^b	2.60 ± 0.08^b
P	1.49 ± 0.02^a	1.46 ± 0.03^a	1.33 ± 0.09^b	1.29 ± 0.01^b	1.24 ± 0.06^b
Pb	<LOD	<LOD	<LOD	<LOD	<LOD
S	1.39 ± 0.04^a	1.33 ± 0.04^a	1.24 ± 0.05^b	1.23 ± 0.03^b	1.20 ± 0.02^b
Se	<LOD	<LOD	<LOD	<LOD	<LOD
Sr	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01
Zn	Presence	Presence	Presence	Presence	Presence

Note: LOD: limit of detection. ^{a-c} In the same line, means accompanied by different letters show a significant difference ($p < 0.05$). Al: aluminum; As: arsenic; Ca: calcium; Cd: cadmium; Co: cobalt; Cr: chrome; Cu: copper; Fe: iron; K: potassium; Mg: magnesium; Mn: manganese; Na: sodium; P: phosphorus; Pb: lead; S: sulfur; Se: selenium; Sr: strontium; Zn: zinc.

Al, As, Cd, and Pb are generally considered toxic and can pose health risks if consumed in significant amounts. Al and Pb have been associated with neurological issues, while As is a known carcinogen, and Cd can cause kidney damage [31–33]. Doroszkiewicz et al. [34] reported that Al, Pb, and Cd are toxic metals that can impair cognitive development and synaptic transmission, exacerbate neuroinflammation, and cause osteodystrophies by interacting with calcium in the skeletal system. These authors highlighted that Al is also associated with neurodegenerative diseases like Alzheimer's. As, Cd, and Pb elements pose significant health risks, including carcinogenic effects and damage to the nervous system [35]. The Cr element is essential in trace amounts for glucose metabolism; however, excessive intake can be toxic and lead to adverse health effects [31,32,36].

Among the elements detected in the results of the multi-element profile analysis, Na (sodium) was the element found in the greatest quantity, followed by Ca (calcium), P (phosphorus), S (sulfur), K (potassium), Mg (magnesium), Sr (strontium), and Zn (zinc). The World Health Organization (WHO) recommends a daily sodium intake of 2.0 g, corresponding to 5 g of salt per day [37]. Na intake recommendations for children are adjusted based on energy requirements and growth factors, ranging from 1.1 g per day for ages 1–3 to 2.0 g per day for ages 11–17 [38]. Therefore, the Na amount found in requeijão samples is under the indicated recommendations for children and adults.

The ideal Ca intake for humans varies by age, gender, and life stage, but generally, adults should aim for around 750–950 mg per day to maintain bone health and prevent deficiencies. Huang et al. [39] reported that milk and dairy products are primary sources, contributing significantly to dietary Ca intake. After adding 10% guabiroba pulp to the requeijão, a decrease ($p < 0.05$) in the Ca content was observed due to the lower content of Ca in guabiroba pulp and because dairy products are rich sources of calcium. Concerning the dietary reference values, the European Food Safety Authority suggests a population reference intake (PRI) of 950 mg/day for adults over 25 years, with adjustments for children and young adults.

The P content of requeijão samples decreases ($p < 0.05$) after adding 10% guabiroba pulp. This behavior was expected because it is an important nutrient, particularly in dairy products, although the ideal P content in food for humans is not explicitly defined. A decrease ($p < 0.05$) in S content was also observed after adding 10% of guabiroba pulp. Thus, a minor amount of guabiroba pulp (5%) does not decrease the content of S when added to the requeijão. Milk and dairy products contain sulfur in the form of sulfur-containing amino acids like cysteine [40], favoring the higher S content found in requeijões. The P content of requeijão samples decreases ($p < 0.05$) after adding 10% guabiroba pulp. This behavior was expected because it is an important nutrient, particularly in dairy products, although the ideal P content in food for humans is not explicitly defined. Similarly, it is known that S is an essential element; however, its amount is not specified. Mitchell [41] related that S is involved in redox reactions and regulating oxidative processes, which are crucial for maintaining cellular health and preventing cardiovascular disorders.

The element K was the only one that showed an increase ($p < 0.05$) with the increase in adding guabiroba pulp to the requeijão samples. According to Toft et al. [42], fruits are also a major source of potassium, often contributing more to the overall potassium intake than milk. They are frequently listed alongside vegetables as primary sources of dietary potassium. K deficiency; toxicity is rare in healthy people. Observational studies show that a potassium intake above 3500 mg/day is associated with a reduced risk of stroke [42]. Similarly important is the role of the main intracellular ion-K, a synergist of Mg, especially concerning the effect on cardiovascular system function. Due to the versatility of its functions and participation in all types of metabolism, Mg can be considered the main cation in the human organism [43]. The present study verified that the Mg content remained unchanged ($p < 0.05$) with the addition of guabiroba pulp (5 to 20%). As verified for the Mg element, the Sr content did not vary ($p > 0.05$) with the addition of guabiroba pulp (up to 20%). Strontium plays a significant role in human health by promoting bone health and reducing the risk of osteoporosis [44] while potentially lowering the odds of type 2 diabetes and impaired glucose regulation [45].

Zinc determines more critical functions than any other micronutrient [46] (Lowe et al., 2024). However, all the requeijão samples produced in the present study contained only the presence of the element Zn. This result agrees with that selected by Manzi et al. [47]. First, these authors reported that food products of animal origin generally have a higher zinc content than vegetables. Manzi et al. [47] studied milk and cheeses from several animal species. They

observed that the variability in Zn content among the samples results from the influence of several factors, such as species (cow, sheep, goat, and buffalo) and cheese manufacturing. Manzi et al. [47] highlighted that cow's milk and cheese presented the lowest Zn contents. These authors recommended mixing cow's milk with milk from other species to obtain dairy products with higher Zn contents.

Table 5 shows the carotenoid levels present in the requeijão samples. It was possible to verify that the greater the amount of guabiroba pulp added, the higher the carotenoid level ($p < 0.05$) in the samples. Among the carotenoids evaluated (α -carotene, β -carotene, β -cryptoxanthin, and λ -carotene), the highest level was β -carotene, which can be attributed to the ingredients used in the preparation of the requeijões (ricotta, butter, and whole milk). However, except for β -carotene, the other carotenoids were not detected in the control sample, i.e., without adding guabiroba pulp. Guabiroba pulp contributed to the increase ($p < 0.05$) in α -carotene, β -carotene, β -cryptoxanthin, and λ -carotene in the samples, contributing to the improvement of the nutritional value of the requeijões. According to Rodriguez-Amaya [13], carotenoids are phytochemicals that are among the most important constituents of foods, as they are also considered bioactive substances that promote several health benefits. Pereira et al. [4] stated that carotenoids also protect biological systems, reacting mainly with the peroxide radical and molecular oxygen. β -carotene is an example of a carotenoid that plays an antioxidant role in the lipid phases, since it can inactivate reactive chemical species that cause damage to lipoprotein membranes. In addition, some carotenoids, such as β -carotene, can be absorbed and converted into vitamin A by the human body. Vitamin A plays an important role in the human body because it participates in the chemistry of vision, cell differentiation, the immune system, the reproductive system, and growth, as well as the formation of organs and bones [4].

Table 5. Results of carotenoid contents of control requeijão and with the addition of guabiroba pulp.

Carotenoids Content	Samples				
	Control	5	10	15	20
α -carotene ($\mu\text{g/g}$)	<LD	0.24 ± 0.01^d	0.51 ± 0.02^c	0.70 ± 0.02^b	0.94 ± 0.03^a
β -carotene ($\mu\text{g/g}$)	8.11 ± 0.02^e	8.41 ± 0.02^d	8.72 ± 0.03^c	8.96 ± 0.07^b	9.25 ± 0.05^a
β -criptoxantin ($\mu\text{g/g}$)	<LD	0.31 ± 0.01^d	0.58 ± 0.07^c	0.84 ± 0.11^b	1.13 ± 0.06^a
λ -carotene ($\mu\text{g/g}$)	<LD	0.22 ± 0.04^d	0.42 ± 0.01^c	0.64 ± 0.12^b	0.84 ± 0.02^a

Note: ^{a–e} Means accompanied by the same letters in the same row do not present a significant difference ($p < 0.05$).

Figure 2A–E shows the micrographs of the control sample (without the addition of guabiroba pulp) and with the addition of 5, 10, 15, and 20% (w/w) of guabiroba pulp. From these micrographs, it was observed that there were no major differences in the microstructure of the requeijões. According to Prudencio et al. [48], the characteristics presented by the microstructures of the samples would be related to the texture properties. Prudencio et al. [48] verified this behavior in fresh cheeses. Another important factor for the similarity of the microstructure of the requeijões observed in the micrographs would be the lack of differentiation between the Aw values of the processed creamy cheeses.

Finally, the study presented in this work allowed the development of requeijões with 5 to 20% guabiroba pulp. In addition, the first characterization analyses of these products were performed, highlighting their physical, chemical, and nutritional properties. Therefore, it is recommended that other analyses, such as determining the centesimal composition, including fiber, and sensory analysis, be performed in the future.

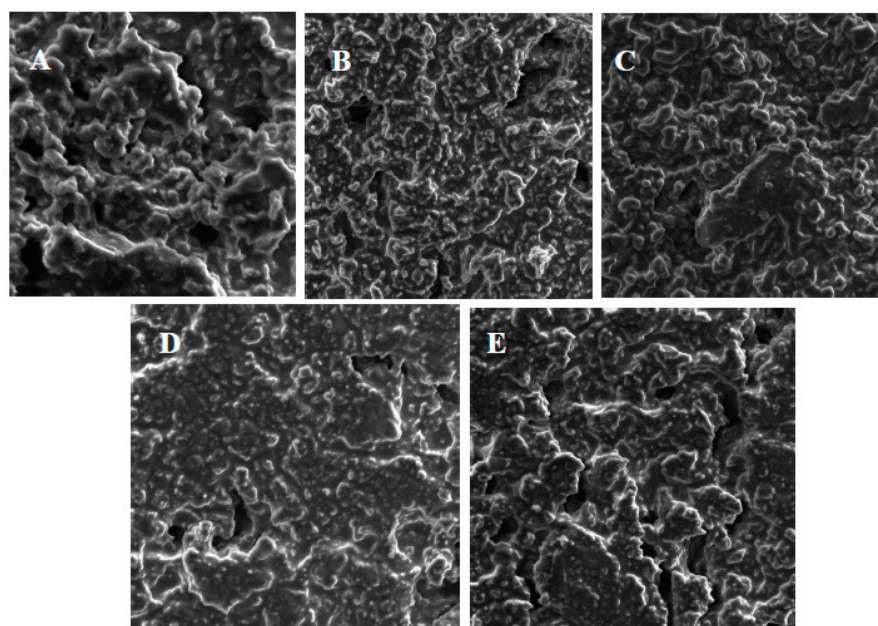


Figure 2. Micrographs resulting from scanning electron microscopy (SEM) requeijão samples at 500× magnification: (A) control sample is the requeijão without addition of guabiroba pulp, (B) sample 5 is the requeijão with 5% (*m/m*) of guabiroba pulp, (C) sample 10 is the requeijão with 10% (*m/m*) of guabiroba pulp, (D) sample 15 is the requeijão with 15% (*m/m*) of guabiroba pulp, and (E) sample 20 is the requeijão with 20% (*m/m*) of guabiroba pulp.

4. Conclusions

This study demonstrated the viability of using guabiroba pulp in different proportions in the development of requeijão, a dairy product widely consumed in Brazil, promoting not only the appreciation of a fruit native to Brazil, but also the manufacture of a dairy product with functional appeal and innovative potential. Formulations with up to 20% pulp were successfully developed, maintaining technological characteristics compatible with the expected profile for this type of dairy product.

The addition of guabiroba pulp contributed to the nutritional enrichment of the product, with emphasis on the increase in carotenoids such as α -carotene, β -carotene, β -cryptoxanthin, and λ -carotene, in addition to providing essential minerals in relevant concentrations. The stability of the texture and microstructure of the samples across the different formulations indicates that the addition of the ingredient does not compromise the integrity of the product and can even improve spreadability in intermediate concentrations.

The results obtained serve as a basis for future industrial applications, indicating that cream cheese with the addition of guabiroba pulp can meet the demands of consumers seeking healthier, more functional, and sustainable foods. In addition, this work reinforces the importance of using regional ingredients as a strategic alternative for diversifying dairy products and stimulating the circular economy in the food sector.

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