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Physicochemical properties of Butter cheese from Marajó manufactured with buffalo milk and cow milk

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Abstract: *This study aimed to evaluate the physicochemical characteristics, texture and color parameters of the artisanal Butter cheese from Marajó, manufactured with partial substitution of buffalo milk for cow milk. Four formulations were elaborated: B_{100} (100% buffalo milk); B_{80} (80% buffalo milk + 20% cow milk); B_{70} (70% buffalo milk + 30% cow milk) and B_{60} (60% buffalo milk + 40% cow milk). Three replicates were performed. The partial substitution of 20%, 30% and 40% of buffalo milk caused the reduction of fat in 16.2%, 21.6% and 25.4%, and protein in 5.2%, 5.7%, and 6%, respectively, with decrease of elasticity, cohesiveness, gumminess and chewiness, but had no influence on the hardness. Moisture had significant and strongly negative correlation with elasticity, cohesiveness, gumminess and chewiness. Fat and protein had significant and strongly positive correlations with elasticity, cohesiveness, gumminess and chewiness. Significant differences were found for color variables L*, a*, b* and h°. The increase in cow milk concentration increased the yellow-greenish tonality, with an emphasis in yellow tones.*

Keywords: *color, Marajó "Butter cheese", physicochemical composition, texture TPA.*

I. Introduction

The artisanal Butter cheese, are cheeses produced for almost two centuries on Marajó Island, the largest fluvial-marine island in the world, located in the northern region of Brazil, is the result of a centennial tradition passed down through the generations as a way to use the milk production of buffalos bred on small rural farms, and a source of income and work for the considerable number of producers on the island.

Before the introduction of buffalo in the Island, at the end of XIX century, the production of this cheese was exclusively made with bovine milk. However, with the expressive growth of the buffalo herd, due to the presence of natural pastures and floodplains, the production of buffalo milk increased, and it started to be mixed with bovine milk in cheese production [1]. Thus, since this time, the artisanal cheeses of Marajó started to be produced almost exclusively with buffalo milk.

Today, this cheese is produced from the spontaneous fermentation of raw buffalo milk and/or buffalo added with bovine milk at a maximum proportion of 40% [2], being obtained by fusion and intended for immediate consumption. However, the mixture of those milks may bring alterations in physicochemical characteristics, texture and color parameters, caused by bovine milk composition that has a lower amount of fat, lactose, protein, ashes, Ca and A and C vitamins, and higher concentration of β-carotene, absent in buffalo milk [3] [4]. Buffalo milk has higher levels of fat with a minimum and maximum of 6.6 g 100 g^{-1} and 8.8 g 100 g^{-1}, while cow milk average is 3.3 g 100 g^{-1}[5]. Protein concentration in buffalo milk range from 3.66% to 5.40% and in cow milk average is 3.2% [5].

Cheese is a visco-elastic material formed by a net of casein where dispersed globules of fat and water determine its texture [6]. The texture is identified by consumers as one of the most important attributes of cheese quality [7] and the most important sensorial characteristics, because it allows the consumer to identify specific varieties and their qualities, even before evaluating the flavor [8]. The texture characteristic of cheese is influenced by several factors, one of the most relevant being the raw-material composition [8] and changes on these parameters are caused by concentrations of protein, salt, water, pH and fat, in this order [9]. In addition, color parameters acting as a differential in characterization of different types of cheese [10].

The purpose of this work was to evaluate physicochemical characteristics, texture and color parameters of artisanal Marajó Butter cheese produced with different concentrations of buffalo milk and cow milk.

II. Material and Methods

2.1 Formulations of Butter Cheese

In this study, four formulations of the Butter Cheese were produced with buffalo and cow milk; all were processed in a cheese factory located in the municipality of Cachoeira do Arari, on the Marajó Island. The milk (buffalo and cow) came from the same rural property. The four formulations are designed by: B_{100} (100 % buffalo milk); B_{80} (80 % buffalo milk + 20 % cow milk); B_{70} (70 % buffalo milk + 30 % cow milk) and B_{60} (60 % buffalo milk + 40 % cow milk). Three replicates were performed. One cheese from each formulation was selected for analysis. The cheeses were produced according to process used by Marajó's producers, as shown in the workflow diagram depicted in Fig. 1.

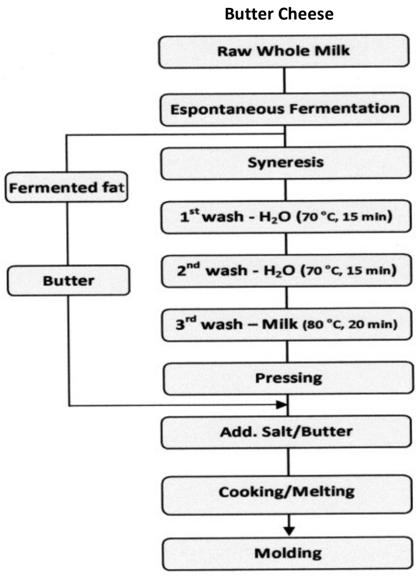

Figure 1 - Production flow chart of artisanal Marajó Butter cheese type.

During cheese preparation, raw milk was homogenized without the addition of a starter culture, submitted to spontaneous fermentation and kept at room temperature (30 °C) for twenty-four hours. The fermented cream on the surface was removed to produce butter. Afterwards, the curd mass went through a manual handling to induce syneresis, and the whey was extracted. Then it was washed twice with water at a room temperature of about 30 °C (2 L of water kg^{-1} of curd mass) under heating (70 °C, 15 min), for acidity reduction. A third wash with buffalo milk (2 L of milk kg^{-1} of curd mass) was performed, also under heating (70 °C, 15 min), to reincorporate flavor and components washed out by the water, that is, some soluble solids.

Posteriorly, to remove excess liquids, the mass was manually pressed. Salt (15 g kg^{-1} of curd mass) and butter (100 g of butter kg^{-1} of curd mass) from baking the fermented fat were added and submitted to heat treatment (80 °C, 20 min). In this stage the curd stretching occurs, which is performed by manual beating. The melted mass was distributed in polypropylene plastic packages (PP) and cooled to room temperature (30 °C). All cheeses were refrigerated (7 °C - 8 °C) until the analyses were carried out. A package of each formulation was used for this purpose.

2.2 Physicochemical analysis

The cheese fat was determined by the Mojonnier's method [11]. The total protein content was estimated by the micro-Kjeldal's method; moisture was determined by kiln drying at 105 °C until constant weight; and the fixed mineral residue was assessed by calcination in muffle furnace [11]. The factor used in conversion from nitrogen to total protein was 6.38. The cheese pH was determined by the potentiometric method (Metrohn Pensalab Instrumentação Analítica Ltda., São Paulo, SP, Brazil) and the acidity was estimated by the determination of the percentage of lactic acid [11]. Determination of fat content in dry matter was made indirectly, by calculating the ratio between the fat content and the total solid content of the cheese [12]. The analyses of physicochemical composition were evaluated seven days after the production of the formulations. Analyses were performed in duplicates.

2.3 Texture Profile Analysis (TPA)

The textural characteristics were evaluated two days after the production of the formulations, using a device from Stable Micro Systems, model TA.XT Plus (Surrey, England), equipped with a cell charge of 25 kg.

Cylindrical samples, with diameters and heights equal to 20 mm, were removed from random points in the cheese, in order to get uniform and homogeneous samples, and kept at room temperature (21 °C). The texture profile was obtained by a double compression test of the cheese cylinder, at room temperature, using a cylindrical compression probe with a 75 mm diameter (P75). Compression was performed at a constant speed of 2.0 mm s^{-1} with contact strength equal to 5 g until the sample height was reduced to 10 mm (that means 50% of the initial height). Data was obtained by software Texture Expert 1.20 for Windows (Stable Micro System). Five independent replicates were produced.

2.4 Color Measurement

Two days after the production of the formulations, the cheese color parameters were evaluated using a colorimeter Hunter Lab, model Color Quest XE (Reston, EUA), according to definitions proposed by the Commission Internationale de l'Éclairage. The value L* represents luminosity and indicates how bright or dark the product is; it refers to the object's capacity to reflect or transmit light and varies from zero (totally black) to one hundred (totally white), the higher the value of L* is, the brighter the object is. The chromaticity coordinate a* is an indicator of green (-) and red (+), while b* is an indicator of blue (-) and yellow (+). The values a* and b* were used to calculate Hue (h^o), tone angle or color, and Chroma (C*), color intensity (chromaticity of color saturation), using the following formulas: $h = \tan^{-1}(b^*/a^*)$ and $C^* = (a^{*2} + b^{*2})^{1/2}$. The determinations were performed in triplicate, with the calibrated equipment, using 10 samples, 5 from the center and 5 from the periphery of the cheeses from each formulation [10].

2.5 Statistical analysis

In order to compare the physicochemical properties, the averages of experimental data were submitted to analysis of variance (One Way-ANOVA). When significant differences were observed ($P \leq 0.05$), Tukey's test was applied. The correlation between texture and physicochemical parameters was expressed as Pearson's correlation coefficient. All analyses used a significance level of 5% ($P \leq 005$). Values of P were obtained with software BioEstat 5.0.

III. Results and Discussion

3.1 Physicochemical Composition

Table 1 displays the results of the compositions of each cheese formulation. Statistical differences were found for values of pH, moisture, minerals, protein and fat; however, acidity in the four formulations did not present significant. As expected, cheese made with 100% of buffalo milk (B_{100}) had the higher values of protein, fat and minerals and the lower values of pH and moisture [13].

Table1. The physicochemical properties of the cheeses (g 100 g^{-1}).[a]

Formulation	Physicochemical Parameters						
	pH	Moisture (%)	Ashes (%)	Fat (%)	Protein (%)	FDM[b] (%)	Acidity[c] (%)
B_{100}	5.51 ± 0.01^d	47.38 ± 0.06^d	2.48 ± 0.01^d	37.00 ± 0.01^d	26.40 ± 0.14^d	75.04 ± 0.10^d	0.18 ± 0.01
B_{80}	5.60 ± 0.01^e	49.93 ± 0.13^e	2.37 ± 0.01^e	31.00 ± 0.01^e	$25.05 \pm 0.21^{e.}$	59.54 ± 0.15^e	0.16 ± 0.01
B_{70}	5.97 ± 0.01^f	50.25 ± 0.14^f	2.19 ± 0.01^f	29.00 ± 0.01^f	24.90 ± 0.01^e	57.29 ± 0.16^f	0.16 ± 0.01
B_{60}	5.59 ± 0.01^e	50.70 ± 0.12^f	2.17 ± 0.01^f	27.60 ± 0.01^g	24.80 ± 0.01^e	57.29 ± 0.14^f	0.18 ± 0.01
P value*	0.0004	0.0007	0.0004	0.0001	0.0019	0.0002	0.5518

[a] Each value represents mean ± standard deviation; data represent means of two replicates ($n = 6$).
[b] FDM – Fat in dry matter.
[c] Lactic acid.
[d-g] Means with different superscript letters within the same column are significantly different ($P < 0.05$).

The lower moisture in B_{100} formulation may be explained by the higher amount of fat present in buffalo milk. This result is according to reports by Souza et al., 2012 [14] that also reported in their studies an increase in the moisture as the fat rate was reduced. In other formulations, the decrease of protein, fat and mineral amounts was due to the lower amounts of those components in cow milk, which was added in larger proportions.

According to Brazilian legislation, ''cottage" cheese must have a maximum moisture content of 65% and a minimum fat content on a dry weight basis of 55% [15]. Analysis of moisture and fat concentrations in the four formulations shows that those products may be classified as Butter Cottage Cheese, a melted cheese also called processed cheese, typically from the northern region of Brazil.

The partial substitution of 20%, 30% and 40% of buffalo milk for cow milk caused the reduction of fat in 16.2%, 21.6% and 25.4%, and protein in 5.2%, 5.7%, and 6%, respectively. Thus, Butter cheeses manufactured with the partial addition of cow milk have more moisture and lower concentration of protein, minerals and calories.

3.2 Texture Profile Analysis (TPA)

Results for Texture Profile Analysis (TPA) are compiled in Table 2.

When there is a reduction of fat, the microstructure of the protein net is changed; normally hardness and elasticity increase, while adhesiveness and cohesiveness decrease [16]. On the other hand, cheeses with high amounts of fat are characterized by attributes such as more softness, cohesiveness and good flavor.

Table 2. Textural characteristics of the cheeses[a].

Formulation	Texture Characteristics						
	Hardness (N)	Fracturability (N)	Adhesiveness	Springiness (mm)	Cohesiveness	Gumminess (N)	Chewiness (N. mm)
B_{100}	28.20 ± 4.32	30.80 ± 2.94	-2.257 ± 0.549	7.24 ± 0.40^{b}	0.66 ± 0.05^{b}	19.93 ± 3.07^{b}	143.97± 23.27^{b}
B_{80}	27.23 ± 4.98	28.83 ± 3.86	-2.329 ± 1.567-	5.87 ± 0.66^{c}	0.51 ± 0.02^{c}	11.73± 1.33^{c}	69.28 ± 10.57^{c}
B_{70}	26.87± 3.27	28.60 ± 4.56	-1.951 ± 1.066	5.47 ± 0.49^{c}	0.45 ± 0.04^{c}	11.30 ± 2.66^{c}	62.50 ± 16.44^{c}
B_{60}	26.10 ± 2.53	27.83 ± 3.97	-1.662 ± 0.300	5.37 ± 0.98^{c}	0.45 ± 0.05^{c}	10.08 ± 2.08^{c}	54.80 ± 16.51^{c}
P value	0.596	0,075	0.7178	< 0.0001	< 0.0001	< 0,0001	< 0.0001

[a] Each value represents mean ± standard deviation; data represent means of five replicates (*n* = 15).
[b-c] Means with different superscript letters within the same column are significantly different (P < 0.05).

However, in this study, the reduction of fat, caused by the increase of cow milk concentration, reduced the values of elasticity cohesiveness, gumminess and chewiness while not significantly changing the values of hardness, brittleness and adhesiveness.

Hardness, the measure of the strength needed to compress a sample producing a deformation, is explained by resistance of the protein matrix of cheese; in other words, lower concentrations of protein reduced the hardness. However, the joint action of protein and fat reduction, caused by the cow milk, kept the cheese's hardness. In this case, lower values of hardness, produced by the lower concentration of proteins, possibly were balanced by the lower amount of fat that caused higher resistance to deformation, an antagonistic effect [17].

Values of hardness were lower when compared with other types of cheese, for example, Cheddar, Gouda, Mozzarella and rennet cheese; they were respectively 47 N, 77 N, 68 N and 36 N [18]. On the other hand, the values of hardness were higher than the ones found in fresh cheese [19].

Elasticity, or recovery after compression, as seen in Table 2, went from 7.24 mm to 5.37 mm, showing that the increase of cow milk concentration reduced the elasticity of Butter Cheese, with formulation B_{100} significantly differed from others which had similar elasticity. In cheese, the protein matrix produces elasticity [8] and is the main factor responsible for flexibility and recovery after tension removal. Thus, as there was a decrease in the amount of protein with the increase of cow milk concentration, TPA values for elasticity decreased. In comparison to Cheddar, Gouda, and Mozzarella, that have elasticity values from 8.5 to 10.0 mm, Butter Cheese does not recover well after compression [20].

Finally, for the parameters cohesiveness and chewiness the values decreased with the decline in the concentration of added cow milk. The cohesion was between 0.45 and 0.66, contrasting with 0.21, 0.28 and 0.41 for cheeses like fresh *Cheddar, Gouda* and *Mozzarella* [20]. Therefore, artisanal Marajó Butter cheese is quite cohesive. The values for chewiness were higher to those reported for cheese of the *Feta* type, elaborated from buffalo milk [21].

Thus, Butter cheeses manufactured with the partial addition of cow milk kept the same hardness, fracturability and adhesiveness, but lower elasticity, cohesiveness and chewiness.

3.3 Correlation between physicochemical and texture parameters

Pearson's correlation coefficients between texture parameters and physicochemical characteristics are presented in Table 3. Analyzing the values found, it was observed that moisture had significant and strongly negative correlation with elasticity, cohesiveness, gumminess and chewiness. On the other hand, fat and protein had significant and strongly positive correlations with elasticity, cohesiveness, gumminess and chewiness. The pH did not significantly influence alterations of texture parameters. The meaning and importance of correlation coefficients may be related to the type of formulation and, in this experimental model, the increase of cow milk concentration caused the decrease of protein and fat concentration, and also, the increase of moisture, which played an important role on the decrease of elasticity, cohesiveness, gumminess and chewiness of cheeses.

Table 3. Linear correlation between physicochemical and texture parameters.[a]

Formulation	Texture Characteristics						
	Hardness	Fracturability	Adhesiveness	Springiness	Cohesiveness	Gumminess	Chewiness
Moisture	-0.705^{NS}	-0.843^{NS}	0.627^{NS}	-0.997^{S}	-0.983^{S}	-0.986^{S}	-0.992^{S}
FDM[b]	0.674^{NS}	0.765^{NS}	-0.548^{NS}	0.996^{S}	0.983^{S}	0.986^{S}	0.967^{S}
Protein	0.675^{NS}	0.796^{NS}	-0.569^{NS}	0.998^{S}	0.982^{S}	0.985^{S}	0.999^{S}
pH	-0.632^{NS}	-0.065^{NS}	0.272^{NS}	-0.541^{NS}	-0.612^{NS}	-0.607^{NS}	-0.571^{NS}

[a] Results expressed as Pearson's correlation coefficient (r).
[b] Fat in dry matter.
S - Significant ($P < 0.05$).
NS - Not significant ($P \geq 0.05$).

3.4 Color Analysis

Table 4 shows the average values of color parameters of cheeses obtained by instrumental measures. For the four formulations, parameter a* had negative values, appearing slightly green in color, while parameter b* had positive values, appearing slightly yellow in color. For variables L*, a*, b* and h^o significant differences were found ($P<0.05$) between the four formulations.

Table 4. Mean composition and Hunter color (L*, a*, b*, C*, h^o) values of cheeses"[a].

Formulation	Hunter Color				
	L*	a*	b*	C*	h^o
B_{100}	88.67 ± 4.90^b	-5.41 ± 0.48^b	17.47 ± 1.04^b	19.53 ± 0.89	106.07 ± 1.86^b
B_{80}	86.71 ± 3.20^b	-4.41 ± 0.42^c	18.29 ± 1.06^c	18.02 ± 0.85	103.14 ± 0.48^c
B_{70}	86.56 ± 5.52^b	-3.67 ± 0.68^d	$18.85 \pm 1.75^{c,d}$	18.86 ± 2.05	100.70 ± 2.46^d
B_{60}	84.85 ± 4.96^c	-3.53 ± 0.56^e	21.38 ± 1.54^e	21.68 ± 1.15	99.41 ± 1.63^e
P value	0.0228	< 0.0001	< 0.0001	0.8660	< 0.0001

[a] Each value represents mean ± standard deviation; data represent means of five replicates (*n* = 30).
[b-e] Means with different superscript letters within the same column are significantly different ($P < 0.05$).

As expected, cheese produced with 100% of buffalo milk had a higher value for luminosity (white component), differing only in B_{60}. In the same way, formulation B_{100} reached higher values of the chromaticity component of green color, caused by the presence of the blue-green pigment (biliverdin), which is present in buffalo milk and absent in cow milk [3]. However, the presence of β-carotene pigment in cow milk caused an increase in the yellow chromaticity component in formulations produced with this type of milk [3].

Values of C* that quantify saturation or color intensity, were not significantly affected by the addition of cow milk. However, the tone angle (h^o), indicated the existence of significant variations in formulation tonalities. In this case, the increase in cow milk concentration did not cause an increase in color intensity but did cause a change in the yellow-greenish tonality, with an emphasis in yellow tones.

IV. Conclusion

Partial addition of cow milk improved some properties of artisanal Marajó Butter cheese. Moisture and pH shown a significantly increased, however, smaller amounts of protein, minerals and fat were found. Similarly, the instrumental elasticity, cohesiveness and chewiness decreased. The increase of moisture played an important role on the decrease of elasticity, cohesiveness, gumminess and chewiness of these cheeses. The increase in cow milk concentration did not cause an increase in color intensity but changed the yellow-greenish tonality, with an emphasis in yellow tones.

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