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# Impact of pH adjustment on the characteristics of processed cheese from sheep milk and cow milk

C.V. Raimundi[1], B.C. Scopel[1], A.N. Ito[1], A.A.L. Tribst[2], G.A.R. Sehn[1], D. Cavalheiro[1] and E. Rigo[1]*

[1] Departamento de Engenharia de Alimentos e Engenharia Química, Universidade do Estado de Santa Catarina, BR 282, km 573, Linha Santa Teresinha, Zip Code 89870-000, Pinhalzinho, Santa Catarina, Brasil

[2] Núcleo de Estudos e Pesquisas em Alimentação, Universidade Estadual de Campinas, Avenida Albert Einstein, 291, Zip Code: 13083-852, Campinas, São Paulo, Brasil

## PRELIMINARY COMMUNICATION

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## ABSTRACT

The effects of milk from different species (sheep/cow) and pH adjustment in the production of *Requeijão cremoso,* a kind of processed cheese, were investigated. The results showed that the sheep's *Requeijão cremoso* had higher pH (~3%), lower yellowness index (~11%), and lower brightness (~12%) after 5 days of storage, and at least 40% lower hardness compared to the *Requeijão* made from cow milk. The pH adjustment did not change the visual appearance of the samples but affected their hardness in different ways, with an increase of up to 16% for the cheese from cow milk and a reduction of up to 39% for the cheese produced from sheep milk. The results suggested that the protein-protein interactions were favoured in the *Requeijão cremoso* from sheep milk, while a protein network with higher water holding capacity and increased hardness was observed for the samples from cow milk.

## KEYWORDS

*Requeijão cremoso* processed cheese, pH adjustment, hardness, luminosity, dairy matrix

* Corresponding author. Tel.: +55 54 999789339. E-mail: elisandra.rigo@udesc.br

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# 1. INTRODUCTION

Sheep milk has a higher content of proteins, lipids, minerals, vitamins, conjugated linolenic acid, short and medium-chain fatty acids, and n-3 fatty acids compared to bovine milk or goat milk, favouring the manufacture of dairy products, especially cheeses (Ochoa-Flores et al., 2021; Dias et al., 2022; Ramos and Poveda, 2022). Dairy products from sheep milk have increasing market interest and are attracting new producers due to their nutritional/sensory characteristics and low allergenicity (Dalziel et al., 2018). The lower allergenicity compared to cow milk can be explained by the $\alpha_{S1}$-casein and $\alpha_{S2}$-casein configuration/amino acid sequence of sheep milk, which is closer to goat milk (Malta et al., 2021).

*Requeijão cremoso* is a processed cheese widely consumed in Brazil, obtained by mixing cheese curd with emulsifying salts and other dairy ingredients. During processing, the replacement of calcium in the protein matrix by ions from the emulsifying salts leads to a reduction in hardness and an increase in spreadability. These changes depend mainly on the characteristics of the raw material used (protein and moisture contents), the pH of the product, and the type/concentration of the emulsifying salt, the latter affecting the mineral balance and the hydration capacity of proteins (Malta et al., 2021). Traditional *Requeijão cremoso* cheese made from cow milk should be acidified until reaching pH 5.5–5.8 to obtain a creamy and firm consistency (Bianchi et al., 2021), however, no studies evaluated the effect of the acidification steps on the production of processed cheese from sheep milk.

Considering the growing interest in sheep milk products, the demand for new food products, and the impact of different protein matrices on the characteristics of processed cheese, this study aimed to compare the characteristics of *Requeijão cremoso* processed cheese produced with sheep milk and cow milk, with and without pH adjustment.

# 2. MATERIALS AND METHODS

## 2.1. Raw materials, cheese curd, and *Requeijão cremoso* processed cheese

Sheep and cow milk were provided by Casa Bianchi (Lajeado Grande, Brazil) and Laticínios Frizzo (Planalto, Brazil), respectively. Sheep and bovine curds were prepared with pasteurised milk (processed at 65 °C for 30 min in an agitated jacketed tank), 0.04% calcium chloride (v/v), and 0.14% commercial chymosin rennet of *Aspergillus niger* var. *awamori* (Ha-La, Christian Hansen, Valinhos, Brazil). These mixtures were allowed to rest for 30 min to form the curd, then the mass was cut, slowly agitated, and the whey was drained to obtain the final curds (Malta et al., 2021). For each curd, pasteurised cow cream (45% fat) was used to obtain a minimum fat content of 55% in dry matter (FDM) and 61% of moisture (Brasil, 1997). Cow cream was used in the formulation of sheep *Requeijão cremoso*, as sheep cream is not available, since the partial or total skimming of milk before manufacturing is not typical by sheep milk producers. Then, 0.7% sodium chloride and 1.6% emulsifying salt mixture containing sodium hexametaphosphate and tetrasodium pyrophosphate (ICL, São Paulo, Brazil) were added to the mixture, according to the manufacturer's recommendations. The mixture was melted at 90 °C for 15 min at 48 *g* in a Thermomix (Vorwerk, Cloyes-sur-le-Loir, France) (Dalmina et al., 2022). For samples produced with pH adjustment, the same mixture was added with 80% lactic acid (1 mL reduces 0.2 pH units per kg of curd) until a final pH of 5.7–5.9 (Bianchi et al., 2021). After processing, *Requeijão* *cremoso* samples were packed in polypropylene containers (50 g), cooled in a conventional refrigerator (convection air), and stored at 4.0 ± 0.1 °C for 21 days. The analyses were performed after 1, 3, 5, 7, and 21 days of storage. Each formulation was produced in duplicate.

### 2.2. Determination of pH, brightness, yellowness index, and texture

The pH was measured using a pH meter (MS Tecnopon, LUCA 210, Piracicaba, São Paulo). Colour was measured using a colorimeter (HunterLab, MiniScan EZ 4500 L, Reston, USA) and, from the measured raw data, the lightness ($L^*$), $b^*$ (blue-yellow), and yellowness index (YI) were determined according to Equation (1), as per the method described by Belsito et al. (2017).

$$\text{Yellowness Index} = 142.86\,\frac{b*}{L*} \tag{1}$$

Hardness was evaluated in a texture analyser (Brookfield, CT3 Texture Analyzer; Middleboro, USA) using an acrylic cylindrical probe of 25.4 mm diameter (Dalmina et al., 2022). All analyses were performed in triplicate. For statistical analysis, ANOVA and Tukey's tests were performed at a 95% confidence level (STATISTICA 14, StatSoft Inc®, Tulsa, USA).

## 3. RESULTS AND DISCUSSION

### 3.1. Physicochemical characterisation of cheese curd and *Requeijão cremoso* processed cheese

Different dairy matrices were used for the manufacture of *Requeijão cremoso*, which led to the production of cheese curd with different compositions (Table 1). The curd from sheep milk showed higher protein and fat contents and had lower whey retention ($P < 0.05$). This is explained by the lower hydration of sheep milk proteins due to their higher mineralisation (Park, 2007), which directly affects the formation and final characteristics of *Requeijão cremoso* (Ramos and Juarez, 2011). Both curds had similar pH (6.6–6.5), explained by the similarity of

*Table 1.* Determination of pH, moisture content, fat in dry matter (FDM), and crude protein of the cheese curd and *Requeijão* made from cow milk and sheep milk

| Product | | Parameters | | | |
|---|---|---|---|---|---|
| | | pH | Moisture g/100 g | FDM % | Crude protein g/100 g |
| Curd | Sheep | $6.6 \pm 0.0^{A}$ | $51.0 \pm 0.3^{B}$ | $57.2 \pm 0.3^{A}$ | $41.3 \pm 0.4^{A}$ |
| | Cow | $6.5 \pm 0.0^{B}$ | $62.1 \pm 0.4^{A}$ | $50.2 \pm 0.5^{B}$ | $46.5 \pm 0.5^{B}$ |
| *Requeijão cremoso* processed cheese | Sheep n.a | – | $57.6 \pm 0.3^{a}$ | $66.1 \pm 0.5^{a}$ | $27.1 \pm 0.6^{a}$ |
| | Sheep w.a | – | $57.3 \pm 0.1^{a}$ | $65.6 \pm 0.2^{a}$ | $27.0 \pm 0.5^{a}$ |
| | Cow n.a | – | $57.7 \pm 0.3^{a}$ | $66.2 \pm 0.3^{a}$ | $19.8 \pm 0.3^{b}$ |
| | Cow w.a | – | $57.1 \pm 0.1^{a}$ | $66.4 \pm 0.2^{a}$ | $19.8 \pm 0.4^{b}$ |

$^{A–B}$: indicate the difference ($P < 0.05$) between sheep and cow milk curd; $^{a–b}$: indicate significant difference ($P < 0.05$) between *Requeijão* made from sheep milk and cow milk, both with and without pH adjustment; n.a: no pH adjustment; w.a: with pH adjustment.

sheep and cow milk (pH ~6.6) and the absence of acidification/fermentation step in the curd obtaining.

Regarding the *Requeijão cremoso* composition, similar moisture and fat contents in the dry matter were observed ($P > 0.05$). This result was expected, once the composition of *Requeijão* formulation (curd, cream, and water) was established to minimise the differences among the samples made from different milk matrices, following the requirements established by the Brazilian legislation for this product (Brasil, 1997). On the other hand, the *Requeijão cremoso* made from sheep milk presented ~7% higher protein ($P < 0.05$), due to the differences in the protein content of the curds.

## 3.2. Effect of pH adjustment on the *Requeijão* made from sheep and cow milks

Differences in pH, hardness (Fig. 1), brightness, and yellowness index (Fig. 2) were observed in the samples due to the curd matrix (sheep or cow cheese) and pH adjustment. In addition, some changes were also observed during product storage.

The *Requeijão cremoso* from sheep milk had higher pH than their counterparts (with/without pH adjustment) produced with cow milk, probably explained by the higher buffering capacity of the sheep curd due to its higher protein and mineral concentrations (Ramos and Juarez, 2011).

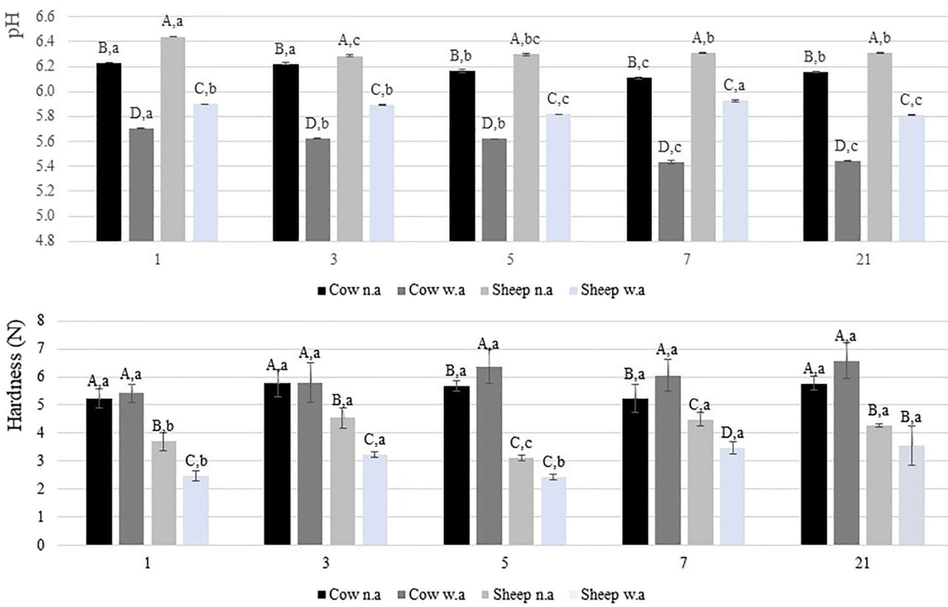


*Fig. 1.* Determination of pH and hardness (N) of *Requeijão cremoso* made from cow milk and sheep milk, with and without pH adjustment.

$^{A–D}$: indicate difference ($P < 0.05$) between *Requeijão cremos*o made from cow milk and sheep milk, with and without pH adjustment, evaluated at the same time. $^{a–c}$: indicate difference ($P < 0.05$) between the same sample evaluated at different times. The x-axis indicates the time of storage (days 1, 3, 5, 7, and 21). n.a: no pH adjustment; w.a: with pH adjustment

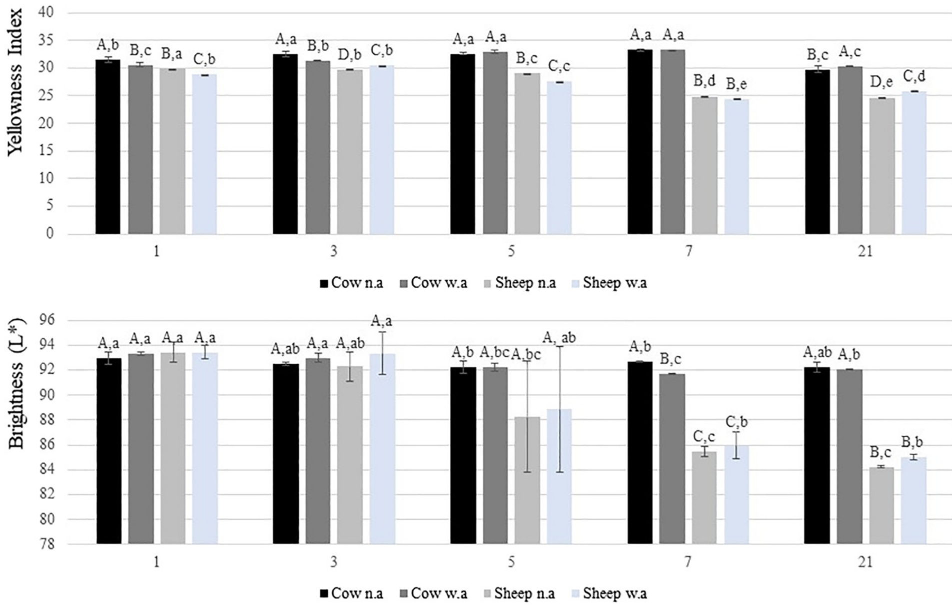


*Fig. 2.* Determination of yellowness index (YI) and brightness (L*) of *Requeijão cremoso* made from cow milk and sheep milk, with and without pH adjustment.
A–D: indicate difference ($P < 0.05$) between *Requeijão cremoso* made from cow milk and sheep milk, with and without pH adjustment, evaluated at the same time. a–e: indicate difference ($P < 0.05$) between the same sample evaluated at different times. The x-axis indicates the time of storage (days 1, 3, 5, 7, and 21). n.a: no pH adjustment; w.a: with pH adjustment

Even though, both the sheep and cow *Requeijão cremoso* with pH adjustment reached values within the desired range soon after the adjustment (pH between 5.9 and 5.6). Over time, a small pH reduction ($P < 0.05$) was observed (<5%, ΔpH <0.1) for the samples without pH adjustment and the one from sheep milk with pH adjustment. On the other hand, the cow *Requeijão cremoso* with pH adjustment had a threefold decrease in pH over storage (ΔpH = 0.3, $P < 0.05$), due to the lower buffering capacity of this sample along with acidification. According to Fox et al. (2017), the pH normally found in processed cheeses ranges from 6.0 to 5.5 and the buffering capacity of sodium salts decreases with increasing chain length, such as the long-chain polyphosphates used as the emulsifying salt in this study, which probably explains the pH decrease observed in the cow *Requeijão cremoso* with pH adjustment.

Regarding the textural parameter, the results of *Requeijão cremoso* produced with cow milk showed a constant hardness ($P > 0.05$) during storage (Fig. 1), suggesting that its structure was rapidly stabilised after manufacturing. Moreover, *Requeijão cremoso* from cow milk with adjusted pH was slightly harder ($P < 0.05$) than samples from the same matrix without pH adjustment after 5 and 7 days of storage. This is in accordance with the literature, which reported that cow-processed cheeses with lower pH have firmer characteristics, while those with higher pH have a more fluid texture due to weak protein-protein interactions (Silva et al., 2020).

On the other hand, samples produced with sheep milk had a large hardness variation during storage ($P < 0.05$), reaching the maximum difference (16%, $P < 0.05$) with *Requeijão cremoso* manufactured with cow milk after 5 days of storage. In addition, pH adjustment resulted in a 40% reduction in the hardness of this *Requeijão cremoso*, showing the opposite effect to that observed in cow milk samples. Furthermore, the sheep *Requeijão* showed hardness at least 20% lower when compared to the *Requeijão* from cow's milk ($P < 0.05$), despite its higher protein content.

Previous research has reported that microstructure formation in processed cheese is a critical process that defines the final textural properties of the product (Zhong and Daubert, 2004). The formation of this microstructure depends on the rate of cooling, protein content, kind/concentration of emulsifying salts, as well as the interaction between proteins and these salts (Zhong and Daubert, 2004), which is also affected by the pH system. Thus, considering that the rate of cooling and kind/concentration of emulsifying salts were equal for all samples, the differences observed can be attributed to (i) protein content (higher in sheep Requeijão Cremoso), (ii) differences in ovine and bovine casein structures, and (iii) the different interactions between the protein from the different matrix and the salts at different pHs.

The casein micelle of sheep milk presents a larger size, with higher mineralisation and lower hydration when compared to the bovine micelle; in addition, these micelles present different percentages of casein fractions in their structures (Park, 2007). Such differences can affect the hydration process, protein mobility, intermolecular attraction, and water holding capacity, being the last parameter associated with lower protein mobility and increased consistency of spreadable processed cheese (Belsito et al., 2017).

Therefore, considering these differences and the samples' hardness results, it was suggested that strong protein-protein interaction instead of a uniform and open protein network (Lee et al., 2015) was favoured in sheep *Requeijão cremoso,* leading to whey release and lower water holding capacity that negatively affected its final hardness. This strong protein-protein interaction possibly means that the amount of emulsifying salt used in sheep *Requeijão* was insufficient for adequate replacement of colloidal calcium, explained by (i) the 27% higher protein content of sheep *Requeijão*, (ii) the 60% higher calcium content of sheep milk, and (iii) the higher mineralisation level of sheep milk micelle compared to cow one (Dalmina et al., 2022). Such a hypothesis is corroborated by the lower hardness of *Requeijão* from sheep milk at lower pH values, since the lower pH favours the interaction between non-emulsified protein chains (Tribst et al., 2018), with consequent lower water retention.

Regarding the brightness (Fig. 2), despite a similar initial value observed for the samples ($L^* \sim 93$), there was ~8% reduction in sheep *Requeijão cremoso* ($P < 0.05$) during storage, while *Requeijão* made from cow milk remained stable, with a reduction <1.7%. For yellowness index (YI), higher values were observed for *Requeijão cremoso* made from cow milk, reaching greater differences (~20%, $P < 0.05$) of sheep samples at the end of the storage, explained by the YI reduction for sheep *Requeijão cremoso* over time. On the other hand, brightness was not affected by the pH adjustment, with little or no consistent difference (<2%, even when significant) in the yellowness index of the samples produced with the same dairy matrix.

The yellow colour of *Requeijão cremoso* is mainly due to the presence of β-carotene in cow milk and, consequently, in cheese and cream (Walter et al., 2020). In turn, sheep milk does not contain β-carotene (Ramos and Juarez, 2011), which justifies the lower saturation of the yellow colour of sheep *Requeijão*. In processed cheeses, brightness is affected not only by the β-carotene concentration but also by the size of the fat globules of the different matrices (Walter et al., 2020; Dias et al., 2022), protein concentration, product moisture, and the interaction between proteins and water molecules (Silva et al., 2020). The *Requeijão cremoso* made from sheep milk showed lower brightness over time, suggesting a greater contraction of the protein network of these samples during storage, with the formation of a network with lower water retention capacity, as described by Tribst et al. (2018). Thus, the brightness of the samples in the present study may have been affected by the behaviour of the protein matrix rather than the presence of β-carotene.

The present results show that the manufacturing parameters of *Requeijão cremoso* made from cow milk should not be directly adopted for sheep *Requeijão cremoso* manufacture due to the great impact of the dairy matrix on the final product. Thus, further studies should address the effect of different concentrations of emulsifying salts, as well as the effect of pH adjustment on the characteristics of sheep *Requeijão cremoso*. In addition, the association between physicochemical parameters and sensory acceptance is required to establish effective processing parameters for the sheep dairy industry.

## 4. CONCLUSIONS

The results showed that different dairy matrices (cow and sheep milk) and pH adjustment affected the behaviour of *Requeijão cremoso* throughout the storage. *Requeijão cremoso* produced with sheep milk had lower hardness (up to 20%) than traditional *Requeijão* (made with cow milk), and pH adjustment negatively affected its texture, reducing 40% hardness. Brightness and yellow index of *Requeijão cremoso* were also affected by the dairy matrix, being stable in cow milk samples, while reduced up to 8 and 20%, respectively, in the sheep milk samples during storage. Therefore, the processing parameters used in the manufacture of cow milk-based products should not be directly adopted for the production of sheep milk-based products due to the differences in the dairy matrices. Consequently, specific studies are required to optimise the characteristics of *Requeijão cremoso* processed cheese made from sheep milk.

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