Article

Application of a Multiscale Approach in the Substitution and Reduction of NaCl in Costeño-Type Artisan Cheese

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Abstract: The effects on the texture, rheology, and microstructure of costeño-type artisan cheese caused by the substitution and reduction of NaCl and the increase in cooking temperature during cheese production were studied using a multiscale approach that correlates responses at the macroscopic and microscopic levels. The decrease in the NaCl content, the partial substitution by KCl, and the increase in the cooking temperature before the serum drainage showed physicochemical, textural, and rheological differences between the cheeses. The microstructure was not affected by the reduction in salt or by modifications in the cheese making. The cheeses with an increase in the cooking temperature before the whey drainage stage and reduced NaCl by 5% and 7.5% (Q_2 and Q_3 , respectively) showed similarity with the physicochemical composition and textural attributes of the control cheese (Q_C). Overall, this study contributes to the design of cheeses with specific functionalities through multiscale modeling.

Keywords: costeño-type cheese; sodium chloride; texture; rheology; microstructure

1. Introduction

Salt (NaCl) is the primary source of sodium in the human diet. Its high consumption has been linked to hypertension and, consequently, an increased risk of stroke and premature death due to cardiovascular diseases [1,2]. Reducing sodium chloride intake represents one of the most important goals for advancing public health worldwide [2]. The maximum recommended daily intake of salt is 5 g per day, equivalent to 2 g of sodium per day [3]. However, approximately 80% of the ingested salt is added to food during manufacturing [4,5]. Cheese is perceived as a highly concentrated food source of sodium [6]. Therefore, importance should be given to reducing salt intake in this dairy product.

Sodium reduction in cheese is complex and challenging due to salt's multifunctional behavior in the product [5]. It could be achieved by reducing NaCl's amount in the product or using substitutes for mineral salts such as potassium chloride (KCl) [7–9]. The addition of KCl in cheeses has given good results concerning the rheological, textural, sensory, and stability properties of the product, and it has been considered a salt compound chemically similar to NaCl when compared to other substitute salts, such as $CaCl_2$ and $MgCl_2$ [6–10]. In addition, the dietary intake of KCl can decrease the effect of sodium-induced hypertension and can reduce calcium excretion in the urine [6]. However, completely replacing NaCl with KCl is not recommended because the latter gives food a bitter taste [11,12].

Reducing the salt content in cheeses accelerates protein hydration, significantly influencing the cheese's physical properties and quality [5,10,13]. Lucey et al. (2003) [14] indicated that the cheese's texture

would generally remain smooth even when the moisture content is adjusted to the typical sodium content. The texture is also influenced by the chemical composition of the cheeses and the manufacturing process. Some studies have even attempted to manufacture reduced-salt cheese by standard methods and have confirmed that moisture retention increases with salt reduction in cheese [5,9].

In Colombia, the *costeño*-type cheese is one of the fresh artisan cheeses made on the Colombian Caribbean coast, characterized by its high salt content from approximately 10% (*w/w*) [15–17]. Consequently, it leads to increased hardness and dryness [13]. *Costeño*-type cheese is considered crumbly, allowing it to be crushed and sprinkled on food [17].

Considering that the potential reduction of NaCl depends on many factors associated with the nature of the product—its composition, the type of processing, and the manufacturing conditions [6]—it is crucial when varying salt concentrations to understand the interactions existing between the production process, the cheese properties, and the product. Studies have been carried out on the rheological, textural, and microstructural properties of cheeses, but many were limited to the analysis of product–property relationships [18] and product–process [5] without considering the relationship as a whole (product–process–property).

Multiscale modeling visualizes food structure at various scales, creating a geometric model that predicts macroscale behavior consistent with the structure of matter at underlying scales without requiring excessive computing resources [19]. This methodology allows an understanding of the link between the macroscopic and microscopic properties of cheese. This method has also been used in other studies and has produced promising results [20,21].

Reducing the NaCl content in the *costeño*-type cheese may reduce salt consumption in the country. However, there is no information on the existing phenomenology when making a *costeño*-type cheese with low salt concentrations or a NaCl substitute. We hypothesized that by increasing the cooking temperature during the cheese-making procedure, one could reduce the curd's moisture content. This is because higher temperatures increase hydrophobic interactions, causing more syneresis and contraction of the curd. This decreases the amount of moisture retained in the matrix of cheese [14,22,23], which would create a *costeño*-type cheese with characteristic hardness with lower salt content.

The increasing attention that consumers pay to healthy foods is considered an essential factor influencing products' development of health benefits. Therefore, by its very nature, a multiscale model can potentially provide a more accurate description of how foods change during processing operations so that new products can be designed [24].

Based on the preceding, this research's objective was to study the effect of sodium reduction and partial substitution by KCl and to increase the cooking temperature in the production of *costeño*-type cheese through a multiscale approach. This approach studied the relationships between composition, cooking temperature, and properties (microscopic and macroscopic) when reducing NaCl concentration.

2. Materials and Methods

2.1. *Costeño*-Type Cheese-Making

Experimental cheeses were prepared according to Ballesta (2014) [25], with some modifications. Each trial was made using 2 L of raw milk, pasteurized at 63 °C for 30 min. The milk was cooled to 32 °C, and calcium chloride was added at a rate of 0.02% (*w/w*) with stirring for 2 min. The coagulant (Microclerici, 1 g per 150 L) was added to the milk with slow stirring. After 60 min, the curd was cut into cubes of 1 cm³ and held for another 5 min, followed by stirring. Before the whey drainage, the curd was cooked to a standard cooking temperature of 45 °C for 15 min. An increase in temperature was made for some experimental tests at this stage, as indicated in Table 1.

The curd was transferred to cheesecloth to drain the whey and obtain a percentage of lactic acid of 0.1% (*v/v*). The salt was added depending on the amount of milk used during the cheese making. It was made with dry salt crystals, either single NaCl or a combination of salt (NaCl and KCl), according to

the weight (Table 1). The percentages of NaCl substitution by KCl were chosen based on the maximum decrease of sodium in the cheeses and its possible effects on the protein structure.

Table 1. Stage drainage of the whey and salting modified during the production of the costeño-type cheese.

Experimental Cheese	Cook Temperature (°C)	Added Salt (%)	
		NaCl (w/w)	KCl (w/w)
Qc (Control)	45	10.00	0.00
Q ₁	45	7.50	0.00
Q ₂	55	5.00	0.00
Q ₃	55	2.50	0.00
Q ₄	45	5.00	5.00
Q ₅	45	2.50	7.50

All the cheeses were molded and pressed at 1.5 bar in a pneumatic press, making an initial unmold after 4 h. Subsequently, the pressure was increased to 3 bars, keeping these conditions constant for 3 h. Finally, the cheeses were stored at a temperature of 4 °C. For each formulation, the production and analysis of cheeses were done in duplicate and carried out after processing over the course of one day.

2.2. Physicochemical Analysis

The physicochemical characterization of the milk (pH, titratable acidity, and fat analysis) was carried out according to AOAC (1995) [26]. The density was determined using a lactodensimeter and the moisture content using a digital thermobalance XM 60-HR (Precisa Gravimetrics AG, Dietikon, Switzerland). For the characterization of costeño-type cheese, moisture content was determined by oven drying [27]. Fat content was analyzed by the Gerber method [27]. Fat-in-dry-matter (FDM, %) was calculated as $\text{fat}\% / (100 - \text{moisture}\%) \times 100$, and moisture-in-nonfat-substance (MNFS, %) was calculated as $\text{moisture}\% / (100 - \text{fat}\%) \times 100$ [28]. All physicochemical determinations were made in duplicate.

2.3. Texture Profile Analysis

The texture profile was determined using a TAHD plus texturometer (Stable Micro Systems, Godalming, UK). The analysis was conducted on cylindrical pieces of cheese (14 mm height, 13 mm diameter). A 35 mm diameter stainless steel probe was fitted to the texture analyzer, calibrated using a 5 kg load cell. The samples were analyzed in duplicate at 18 ± 2 °C and a compression of 70% using two compression cycles [29,30]. This was used to determine texture attributes such as hardness (force necessary to achieve a given deformation) and cohesiveness (resistance of the internal ties that make up the body of a product, $\text{area}_2/\text{area}_1$) [31,32].

2.4. Rheological Analysis

The rheological measurements were made on samples of 1 mm in height and 20 mm in diameter with an ARG2 rheometer (TA Instruments, New Castle, DE, USA). A corrugated surface was placed on the upper and lower plate to eliminate the slippage of the sample. The lower plate temperature of the measuring system was maintained by circulating water at 25 °C. The analysis in each experimental test was carried out at 1 Hz frequency and at a deformation of 0.1%, under which the properties of the cheeses remained within the linear viscoelastic region, where the product can still be recovered and where there is a linear relationship between the stress applied and the strain obtained [31–33]. The dynamic rheological data collected included the two components of the complex shear modulus: the storage module or elastic component (G') and the loss module or viscous component (G'') [31,32]. Results are presented as the average of two sweeps. The modules were plotted against frequency (Hz) for comparative purposes.

2.5. Scanning Electron Microscopy

The cheese samples (approximately 1 mm × 20 mm) were stored at a temperature of −80 °C. Then, they underwent a drying process using a lyophilizer. Later they were coated in gold with a DV-TSC metallizer (Denton Vacuum LLC, Moorestown, NJ, USA) to be observed in a Phenom Pro X Scanning Electron Microscope (Phenom-World, Thermo Fisher Scientific, Waltham, MA, USA) operated at 10 kV. The fields were randomly selected over the sample area. The images were recorded at 4500× magnification and were used to determine the average pore size using ImageJ v.1.43s software (National Institute of Health, Bethesda, MD, USA).

2.6. Statistical Analysis

In order to determine whether the samples differed in their physicochemical composition and textural attributes, the data were analyzed using the Student's *t* test for two samples, assuming equal variances. It was determined whether the samples' specific differences were significant, with a confidence interval (CI) of at least 95%. The software used for analysis was Microsoft Office Excel 2019.

3. Results and Discussion

3.1. Physicochemical Analysis of Milk

Table 2 shows the average physicochemical composition of the raw milk used in the production of costeño-type cheese.

Table 2. Physicochemical composition of milk used in the production of costeño-type cheese.

Composition	Raw Bovine Milk
Density (g/cm ³)	1.027 ± 0.010
Titrateable acidity (°D)	14.20 ± 1.17
pH	6.70 ± 0.12
Fat (% <i>w/w</i>)	3.20 ± 0.32
Total Solids (% <i>w/w</i>)	10.70 ± 0.44

Mean values ± standard deviation.

3.2. Physicochemical Analysis in Costeño-Type Cheese

The reduction in NaCl content and the increase in the cooking temperature did not significantly affect the physicochemical composition of the costeño-type cheese (Figure 1). Reduction in the salt content of cheese generally causes an increase in the moisture due to the higher capacity of water retention in the protein matrix [5,7,13]. Grummer and T. C. Schoenfuss (2011) [34] attempted to produce reduced-salt cheese by standard processes and confirmed that moisture retention increases with salt reduction in cheese; this effect was observed in cheese Q₁.

Conversely, the experimental cheeses Q₂ and Q₃ with NaCl addition of 5.0% and 2.5%, respectively, showed higher similarity with the physicochemical composition of the control cheese (Q_C). This could be due to the increase in cooking temperature before draining the whey, which could increase hydrophobic interactions. This would cause more syneresis and contraction of the curd, decreasing the amount of moisture retained in the matrix of cheese [14,22,23].

The effect of temperature on moisture retention has been previously reported by Ganesan et al. [5]. They achieved the same moisture content in mozzarella production by decreasing the temperature during stretching with reduced salt.

The fat content tends to decrease in Q₁ cheese (*p*-value < 0.01) made through the standard process and reduced NaCl content. This tendency contrasts with the cheeses made with increased cooking temperature and salt mixtures. Salt reduction increases the moisture content, thus decreasing the

fat content [31]. Moreover, within the standard cheese-making process, fat reduction also increases moisture content [32].

Figure 1. Physicochemical composition in percent of the experimental costeño-type cheese: (A) Moisture; (B) Fat; (C) Fat-in-dry-matter (FDM); (D) Moisture-in-non-fat-substances (MNFS) in Q_C (black bar), Q₁ (gray bar), Q₂ (diagonal striped bar), Q₃ (point bar), Q₄ (horizontal striped bar) and Q₅ (vertical striped bar). Error bars represent one standard deviation. Student's t-tests were performed to assess significance compared to the control cheese (Q_C), where (*) corresponds to a *p*-value < 0.05, and (**) to a *p*-value < 0.01.

Cheeses with mixtures of NaCl and KCl showed an increase in fat content compared to cheeses with only NaCl. This behavior indicates a possible interaction between the added KCl and the amount of fat trapped in the Q₄ and Q₅ cheeses' protein networks. This phenomenon requires further research outside the scope of this study because several studies indicate that the fat content is not affected by the different salt mixtures in the cheeses compared to the control cheese and the cheeses made by standard process [7,13,35].

All the experimental cheeses could be classified as semi-fat, because their FDM content <25%, and hard cheese, because the MNFS content was between 49 and 56% [36]. In hard cheeses like cheddar, FDM content decreases when the moisture increases; this behavior is similar to the one observed in Q₁ cheese [26,36].

Overall, slight differences were observed in the cheeses' physicochemical properties, with changes in the salt content and the cheese-making process.

3.3. Texture Profile Analysis

Figure 2 shows the texture attributes evaluated instrumentally in the experimental costeño-type cheeses. Control cheese (Q_C) had the highest hardness and cohesiveness compared with reduced-salt cheese.

Figure 2. Texture attributes of costeño-type cheese: (A) hardness; (B) cohesiveness. Q_C (black bar), Q_1 (gray bar), Q_2 (diagonal striped bar), Q_3 (point bar), Q_4 (horizontal striped bar), and Q_5 (vertical striped bar). Error bars represent standard deviation. Student's t-tests were performed to assess significance compared to the control cheese (Q_C), where (*) corresponds to a p -value < 0.05.

Pastorino et al. (2003) [37] reported that the decrease in the salt content of muenster cheese decreased its hardness. This effect was seen in the hardness results reported in this study, except for Q_1 (p -value < 0.05). Studies report that the firmness of the cheese increases as the level of fat decreases. This phenomenon indicates that the moisture content present in Q_1 (Figure 1) and the adjustment of the fat globules within the protein matrix may have affected this cheese [18,37]. The hardness also significantly increases as the moisture content decreases, and the FDM content increases [14]. This phenomenon was observed in this study (Table 1).

On the other hand, Q_2 cheese with lower addition of NaCl compared to Q_1 had a hardness with values similar to those reported in the control cheese (Q_C) and the cheeses with partial substitution of NaCl for KCl (Q_4 and Q_5). These similarities could be due to the increase in cooking temperature applied during the making of these cheeses (Table 1) [10,14,24].

McSweeney (2007) [38] indicated that the cooking process increases the hydrophobic junction's relative strength, resulting in particles' aggregation into more extensive and easily processed curds with higher density and strength. Consequently, there is an increase in the degree of serum separation.

Concerning cohesiveness, Gunasekaran and Ak (2003) [32] reported that an increase in hardness creates more brittle and less cohesive cheese texture. Nevertheless, costeño-type cheese with maximum salt content (Q_C) had the highest cohesion than reduced-salt cheese and cheeses with NaCl and KCl. However, it is impossible to establish statistical differences in this texture variable due to the sample size.

Partial substitution of NaCl with KCl resulted in a difference in cohesiveness compared to control cheese (Q_C). Various studies have demonstrated that mixtures of NaCl and KCl affect the cheese's texture [7,8,13]. KCl has a decreased ionic strength towards NaCl, which results in a decrease in the "salinity" or solubility of proteins, with a direct effect on the cheese matrix [8].

3.4. Rheological Analysis

An increase in the storage and loss modulus was evident and proportional to the increase in frequency, demonstrating dominant viscoelastic properties (Figure 3A,B). These observations agree with previous rheological research in cheeses [31,39]. Figure 3 also shows a correlation between rheological behavior and NaCl's addition in the cheese—except for Q₁, which showed low storage modulus compared to Q₂, which had a smaller NaCl addition.

Figure 3. Frequency sweep in experimental costeño-type cheese. (A) The storage modulus (G') for the cheeses Q_C (black circles), Q₁ (black squares), Q₂ (black diamonds), Q₃ (crosses), Q₄ (black diamonds) and Q₅ (asterisks), and (B) loss modulus (G'') for the cheeses Q_C (white circles), Q₁ (white squares), Q₂ (white diamonds), Q₃ (short dashes), Q₄ (white triangles) and Q₅ (long dashes).

The effect of salt on cheese protein hydration affects the cheese's viscoelastic character [18]. Some studies have held compositional parameters constant and shown a role for salt in cheese rheology primarily because of its effect on protein hydration [13].

The temperature at which the curd is heated affects the rheological properties to a certain extent [32]. Therefore, increasing the cooking temperature before draining the whey could influence the rheological behavior of the cheeses Q₂ and Q₃. Lucey et al. (2003) [14] stated a more rapid relaxation of protein bonds at high temperatures and a change to a more liquid character, indicating a decrease in the modules and a greater probability that the chemical bonds will break.

As part of the cheese-making process, the cooking temperature correlates with the rheological behavior and the previously mentioned texture attributes.

3.5. Scanning Electron Microscopy

Figure 4A–F shows the microstructure of the experimental costeño-type cheeses. The cheese microstructure can be viewed as a continuous protein gel network disrupted with interspersed fat globules [37,40]. The scanning electron micrographs showed that all the experimental costeño-type cheese had a discontinuous structure with numerous irregular cavities. Similar observations have previously been reported by Tunick and Van Hekken [28] in fresh Mexican cheese.

From a rheological viewpoint, the structural discontinuities may result in the lack of tensile strength in many kinds of cheese, which in practical terms may be reflected as crumbliness, shortness, and fracturability, as occurs in cheeses such as Feta, Stilton, and Cheshire [41].

The average pore size of the cheese microstructures evidences a variability in the pore distribution of the microstructures (Figure 4G). The cheeses Q_C, Q₄, and Q₅ had more homogeneous pore sizes (Figure 4A,E,F) compared to cheeses with less NaCl addition (Q₁, Q₂, and Q₃) (Figure 4B–D). This pore variability can be attributed to changes in the texture of the cheeses.

Figure 4. Scanning electron micrograph of costeño-type cheese at 10 μm scale at 4500×. Qc (A), Q₁ (B), Q₂ (C), Q₃ (D), Q₄ (E), Q₅ (F), and average pore size (G), where error bars represent one standard deviation. The light areas in the micrograph are the protein phase, and the dark areas are the fat or serum phase. Arrows indicate coalesced fat globules.

Lamichhane et al. (2018) [22] indicated that temperature influences the cheese structure through its effect on the components of cheese and their interactions, including changes in the physical state of fat. The molecular interactions between the casein show a relationship between the influence of the increase in cooking temperature on the microstructure of these cheeses. However, Q₂ and Q₃ cheeses' microstructures do not establish a relationship between increasing the cooking temperature on the cheese's microstructure.

Cheese Q₁ showed a microstructure with coalescences of fat globules observed as larger and irregularly shaped openings and discontinuities in the para-casein matrix (Figure 4B) [41]. Coalescence of fat globules occurs during manufacturing because of the combined effects of shear stress on the fat globule membrane and shrinkage of the surrounding para-casein matrix, which forces the occluded globules into close contact [41].

4. Conclusions

Cheeses with salt reduction generally show alterations in their structure. Increasing the cooking temperature during the Q₂ and Q₃ cheese production influenced their physicochemical composition and textural and rheological properties. These cheeses, with a reduction in NaCl of 5% and 2.5%, respectively, showed similarities with the physicochemical composition and textural properties of control cheese (Q_C). This could be possible without partial substitution of NaCl with KCl. This study established that the increase in cooking temperature before the whey drainage stage during the cheese making can also influence macrostructural properties (rheology and texture).

Furthermore, applying a multiscale approach allowed the macro and microstructural properties' responses to be correlated with the process, the product, and the properties. The results evidenced existing changes between the texture variables and the rheological behavior when making variations in the NaCl addition and the manufacturing process of the costeño-type cheese.

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