Effect of Salt and Chymosin on the Physico-Chemical Properties of Feta Cheese During Ripening

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ABSTRACT

The physico-chemical properties during ripening of Feta cheese made from bovine milk were monitored at 7-d intervals for 63 d. Samples were analyzed for moisture, fat, protein and ash contents, salt uptake, pH, and hardness. Three percentages of salt in brine (8, 15, and 18%) and three concentrations of double-strength chymosin (0.5, 1, and 2.5 ml/10 L of milk) were investigated. Salt had a significant effect on moisture, uptake of salt, pH, and hardness. Results indicated that higher percentages of salt in brine developed a harder cheese with higher salt content and higher pH values but lower moisture content. High rennet concentration had a significant effect on the uptake of salt. Increasing the concentration of rennet gave a softer cheese with higher salt content. Rennet level had no significant effect on the moisture or protein contents or pH of the cheese.

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INTRODUCTION

Traditional Feta cheese is a white, brined cheese that has been consumed traditionally in the Middle East and in countries bordering the Mediterranean Sea. Feta cheese is made from a mixture of ovine and caprine milks or from a mixture of caprine and bovine milks; sometimes Feta cheese is made with bovine milk alone. Feta is a pickled cheese that is cured for 4 to 6 wk in an NaCl brine and has a characteristic soft, crumbly texture and a strong, salty flavor (20). Feta cheese from ovine or caprine milk accounted for approximately 51% of world production in 1992 (18), and cheese from bovine milk accounted for approximately 46%. These data indicate that the production of traditional Feta cheese (from caprine and ovine milks) is not sufficient to cover the demand; thus, Feta cheese is being manufactured with bovine milk.

Feta cheese production is in excess of 400,000 tonnes (30). Although the traditional method of manufacturing Feta cheese is simple, considerable variations exist among the processing conditions and procedures (3, 10, 20, 22). A comprehensive description of Feta cheese production at an industrial scale has been reviewed (4, 17, 19, 30).

A high salt content is a characteristic feature of Feta cheese. When cheese is placed in brine, there is a net movement of NaCl into the cheese and water into the brine (11, 35). Geurts et al. (14) concluded that the penetration of salt into cheese and the simultaneous outward migration of water in brine-salted Gouda-type cheeses could be described as an impeded diffusion process. The principal role of salt in cheese may be a direct contribution of flavor; however, salt is very significant in the ripening of cheese. Other effects of salt listed by Fox (11) are control of microbial growth and activity, control of various enzymatic activities in cheese, increased syneresis of the curd, and physical changes in cheese proteins that influence cheese texture, protein solubility, and probably protein conformation. The increased syneresis of curd results in whey expulsion and thus in a reduction of cheese moisture. The presence of moisture influences microbial and enzyme activities.

Rennet is used in cheese making and is important in the formation of the casein network during coagulation. Feta cheese is a rennet-set curd. Rennet is known to contribute to proteolysis in pickled cheese (8). The texture of the cheese is also affected by rennet (7). The network formation caused by rennet or acid set has been studied extensively (5, 6, 8, 9, 11, 15, 31, 34). These studies indicate that the initial stages of casein aggregation to form a network influence the final physical and chemical characteristics of the cheese.

The ripening of Feta is less complex than that of many other ripened cheese varieties. Feta cheese requires a short period of ripening in a salt brine, and a change in texture is easily visualized. Voudouris et al.
(38) and Veinoglou et al. (37) have previously reported physico-chemical changes during ripening in Feta cheese. There is, however, little information regarding the role of rennet or salt concentrations in brine on physico-chemical properties of Feta cheese.

The objectives of this study were to investigate the effect of different concentrations of salt and rennet on the physico-chemical properties of Feta cheese made from bovine milk during ripening.

MATERIALS AND METHODS

Cheese Making

All equipment was washed using clean-in-place technology and sanitized with 50 ppm of chlorine. Fresh, whole milk at 5°C from The Ohio State University Dairy Farm was used to make Feta cheese. Milk was collected in 80-L containers and stored at 5°C. Cream was separated in a separator (Model 29-AE/60; Alfa-Laval, Warminster, PA). The fat content of the cream was evaluated using the Babcock method (24). Standardized milk (3.25%) was homogenized at 100 kg/cm² at 60°C and then pasteurized at 72°C for 30 s. Three replicate batches of cheese were made, each batch consisting of nine vats of 25 L of milk. Milk was heated in a vat to 32°C and then inoculated with a lactic starter culture (Lactococcus lactis ssp. lactis and Lactococcus lactis ssp. cremoris; DVS R604, Chr. Hansen Inc., Milwaukee, WI) at the rate of 0.015% (wt/wt). Rennet (double-strength, fermentation-derived chymosin; Chr. Hansen Inc., Milwaukee, WI) was added (0.5, 1, or 2.5 ml/10 L) 45 min after addition of the starter culture. The curd was allowed to set for 30 min and then was cut into approximately 1-cm² cubes with vertical and horizontal knives. The metal knives were made of 0.06-cm wire placed 1.0 cm apart. Upon being cut, the curd was left to settle for 10 to 20 min. Whey was collected and stored at 5°C for making brine. Cut curd was allowed to drain into molds (30 cm × 15 cm × 15 cm) and was left at approximately 22°C. Molds were turned every hour to help form a firm curd. After five turns, the molds were incubated at 22°C for about 22 to 24 h.

The curd was cut into two 15 cm × 15 cm × 15 cm blocks and packed into plastic containers with whey brine containing 8, 15, or 18% salt. The brine was adjusted to pH 4.7 using a 5% (wt/vol) solution of food-grade lactic acid. Containers were stored in a refrigerator at 5°C for 9 wk. Titratable acidity and pH were monitored during the entire cheese-making process at 30 min interval.

Sample Preparation and Collection

Samples for analysis were collected in duplicate at 7-d intervals from two different containers made within the same trial. Samples for physico-chemical analyses were obtained from two different 15-cm × 15-cm × 15-cm cheese blocks for each data point. Two slices were cut from each side of the blocks. The 1-cm outside slices were separated and kept for the chemical analyses because they were softer than the rest of the cheese and produced inaccurate measurements during texture analysis. The 2-cm inside slices were used to measure hardness on a texture analyzer. The chemical analyses were conducted by combining and mixing the crumbled slices from textural analyses and the outside slices. Physico-chemical analyses were conducted on the same day as sample collection.

Texture Analysis

Samples (4 cm × 4 cm × 2 cm) from the cheese stored in brine were analyzed for hardness. The texture analysis was conducted on a Texture Analyzer® (TA-XT2 Texture Analyzer; Texture Technologies Corp., New York, NY). Testing conditions were as follows. A cylindrical probe was used that was 1 cm in diameter and penetrated to a depth of 1 cm into the sample with a speed of 0.05 cm/s. A force versus time plot was generated with data acquisition of 200 pps. The highest peak on the graph was reported as the hardness value of the cheese. The textural hardness or fracturability was measured in duplicate and expressed in shear force as Newtons.

Chemical Analyses

Chemical analyses during ripening of the cheese were conducted following the Standard Methods for the Examination of Dairy Products (25). The fat content of cheese was determined using the Babcock method, and pH was measured as described by Ling (21) using a pH meter (Fisher Accumet® pH meter model 630; Fisher Scientific, Pittsburg, PA). Sodium chloride content was determined by the modified Volhard method (25). Moisture content was determined by heating to a constant weight as reported by Marshall (25). Protein content was determined by the micro-Kjeldhal method (25) using the Kjeltech™ Auto Sampler system (Tecator, Perstrop Analytical Co., Hoganas, Sweden).

Statistical Design and Analysis

Statistical analyses were performed using the procedure PROC MIXED of SAS software (33), a
procedure designed to analyze unbalanced repeated measures data. A repeated measures experimental design, with a two-way whole-plot treatment structure, in a completely randomized whole-plot design structure was used. Repeated measurements were taken of the batches over time. Salt and rennet, the whole-plot factors, were applied to batches in a $3 \times 3$ factorial treatment structure. Batches were taken from different vats, which were designated as the blocks in the design. The variables were ash, fat, hardness, moisture, pH, protein, and uptake of salt. A probability of <0.05 was used to establish statistical significance for fixed effects and interactions.

**RESULTS AND DISCUSSION**

Physico-chemical Properties of Feta cheese expressed as the mean and standard deviations of duplicate samples from each of three batches are given in Table 1. The data are for cheese samples made with 1 ml of rennet/10 L of milk and cured in a 15% brine solution. Data of samples made with 0.5 and 2.5 ml rennet/10 L of milk were similar. The specific variations and values are included and discussed in the corresponding sections. Ash content was not affected by salt or rennet concentrations. The changes measured are the result of the differences in NaCl.

**Effect on Moisture Content**

As salt concentration increased from 8 to 15%, there was no change in moisture, but from 8 or 15 to 18% in brine, moisture decreased (Figure 1). At 18% concentration, salt diffusion was faster and accelerated the salt uptake and, thus, reduced the final moisture, which suggests that salt penetration was influenced by salt concentration. These observations are similar to previous studies (3, 16), except at the beginning of salting in 8 and 15% brine. Moisture content initially increased from the 0-d level through

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**TABLE 1.** Physico-chemical properties of Feta cheese cured in brine at different salt concentrations at 0 and 63 d.

<table>
<thead>
<tr>
<th></th>
<th>8% Salt</th>
<th>15% Salt</th>
<th>18% Salt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture, %</td>
<td>48.61</td>
<td>47.55</td>
<td>48.75</td>
</tr>
<tr>
<td>SD</td>
<td>1.06</td>
<td>1.22</td>
<td>0.36</td>
</tr>
<tr>
<td>Fat, %</td>
<td>26.38</td>
<td>26.32</td>
<td>25.88</td>
</tr>
<tr>
<td>SD</td>
<td>2.45</td>
<td>1.57</td>
<td>0.45</td>
</tr>
<tr>
<td>Protein, %</td>
<td>19.08</td>
<td>19.14</td>
<td>17.58</td>
</tr>
<tr>
<td>SD</td>
<td>0.32</td>
<td>1.38</td>
<td>1.95</td>
</tr>
<tr>
<td>Salt, %</td>
<td>3.26$^3$</td>
<td>4.36$^3$</td>
<td>5.13$^3$</td>
</tr>
<tr>
<td>SD</td>
<td>0.23</td>
<td>0.41</td>
<td>0.18</td>
</tr>
<tr>
<td>pH</td>
<td>5.09</td>
<td>4.81</td>
<td>5.09</td>
</tr>
<tr>
<td>SD</td>
<td>0.10</td>
<td>0.04</td>
<td>0.08</td>
</tr>
<tr>
<td>Ash, %</td>
<td>1.72</td>
<td>1.83</td>
<td>1.72</td>
</tr>
<tr>
<td>SD</td>
<td>0.15</td>
<td>0.04</td>
<td>0.07</td>
</tr>
<tr>
<td>Hardness, (N)</td>
<td>10.88</td>
<td>11.35</td>
<td>10.53</td>
</tr>
<tr>
<td>SD</td>
<td>3.0</td>
<td>2.00</td>
<td>0.73</td>
</tr>
</tbody>
</table>

$^1$Values are the means of duplicate determinations from three batches.
$^2$Cheese was made using 1.0 ml rennet/10 L.
$^3$Salt (percentage) measured after 7 d of ripening in brine; at d 0, salt content was <0.15%.
$^4$Newtons.

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Figure 1. Effect of different concentrations of salt on the percentage of moisture during the ripening of Feta cheese. Each data point is a mean of treatment combinations replicated in triplicate. Significance was established at $P < 0.05$. 
the 14- and 21-d levels for cheeses treated with 8 and 15% salt brine. Several factors that influence the rate of salt penetration and outward migration of water in cheese have been reported. These factors include fat globules and protein structures, the amount of bound water and viscosity of the aqueous phase, and pH (16). We cannot offer an explanation for the increase of moisture at the beginning of salting because we did not monitor all of these parameters. Limitations in the method of sampling may be another cause of these results. In this study, we may assume that as salt uptake increased, moisture content decreased during ripening. An inverse relationship between moisture and salt content is well documented in the literature (24, 28, 32). This decrease in moisture has been explained by Geuerts et al. (14). In their model experiments on brining of cheese, the penetration of NaCl into cheese and the outward migration of water have been reported as an impeded mutual diffusion process to restore the osmotic pressure equilibrium. A common defect in Feta cheese, rotting appears to be due to high moisture content and low salt content (30). Different percentages of salt had an effect ($P \leq 0.05$) on moisture. However, different rennet levels did not have an effect ($P \geq 0.05$) on moisture content of the cheese during ripening (Table 2).

**Effect on Final Salt**

An increase in salt concentration caused a proportional increase in the final salt-in-moisture content of the cheese. Most of the increase occurred during the 1st wk of ripening (Figure 2). However, it is necessary to ripen the cheese for a longer time period because of the development of other organoleptic properties. These results are similar to those reported by Guinee and Fox (16) and Geuerts et al. (14). Salt uptake decreased slightly with time. These observations are different from the results of Robinson and Tamime (30). The percentage of NaCl increased during ripening in that study. There is no good explanation for this disagreement except probable limitations of the sampling technique. The outside discarded layer may have contributed to a nonrepresentative sample at the early ripening stage when there was a large gradient of salt concentration in the cheese samples. A wide variation in NaCl concentration as a function of the distance from the cheese-brine interface was demonstrated in brine-salted cheese (16). The final salt-in-moisture content of Feta cheese is targeted around 3 to 4.5% in the matured product (23, 26) and both the 15% and 18% brine produced cheese with concentrations in this predicted range (Figure 2).

The concentration of rennet affected ($P \leq 0.05$) salt concentration during ripening. A decrease in salt content was observed after d 7 through the end of ripening. Salt content decreased from 4.25 to 4.03%, 4.36 to 3.6%, and 3.81 to 3.58% in cheese samples prepared

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**TABLE 2. Probability values from ANOVA of the effects of different brine concentrations and rennet level on moisture, salt, and protein contents and pH and hardness of Feta cheese.**

<table>
<thead>
<tr>
<th>Source</th>
<th>NDF</th>
<th>DDF</th>
<th>Moisture</th>
<th>Salt</th>
<th>pH</th>
<th>Hardness</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brine</td>
<td>2</td>
<td>234</td>
<td>0.001*</td>
<td>0.0001*</td>
<td>0.0001*</td>
<td>0.0001*</td>
<td>0.0976</td>
</tr>
<tr>
<td>Rennet</td>
<td>2</td>
<td>234</td>
<td>0.9198</td>
<td>0.0001*</td>
<td>0.2735</td>
<td>0.0471*</td>
<td>0.8244</td>
</tr>
<tr>
<td>Time</td>
<td>8</td>
<td>234</td>
<td>0.0001*</td>
<td>0.0005*</td>
<td>0.0001*</td>
<td>0.0001*</td>
<td>0.0001*</td>
</tr>
<tr>
<td>Brine × Time</td>
<td>16</td>
<td>234</td>
<td>0.2128</td>
<td>0.0001*</td>
<td>0.0002*</td>
<td>0.0001*</td>
<td>0.9918</td>
</tr>
<tr>
<td>Rennet × Time</td>
<td>16</td>
<td>234</td>
<td>0.2646</td>
<td>0.0001*</td>
<td>0.0001*</td>
<td>0.0157*</td>
<td>0.8395</td>
</tr>
<tr>
<td>Brine × Rennet</td>
<td>4</td>
<td>234</td>
<td>0.7733</td>
<td>0.0001*</td>
<td>0.6091</td>
<td>0.4643</td>
<td>0.6506</td>
</tr>
<tr>
<td>Brine × Rennet × Time</td>
<td>32</td>
<td>234</td>
<td>0.0256*</td>
<td>0.0001*</td>
<td>0.1777</td>
<td>0.0001*</td>
<td>0.9559</td>
</tr>
</tbody>
</table>

1 NDF and DDF are the numerator and denominator degrees of freedom of the approximate $F$ tests, the type III Wald $F$ tests. Type III Wald $F$ test was significant at the 0.05 level.

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Figure 2. Effect of different concentrations of salt on percentage of salt in moisture phase of Feta cheese during ripening. Each data point is a mean of treatment combinations replicated in triplicate. Significance was established at $P < 0.05$. 

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Several factors responsible for salt diffusion in cheese were postulated by Guerts et al. (14). They reported that the principal factors are the effect of the protein matrix on the mass ratios of salt and water migrating in opposite directions, fractional effects of protein-bound water, the high relative viscosity of water in the cheese, and interference of fat globules and globular protein particles. A partial exchange of water-soluble nitrogen compounds between the Feta cheese and the brine in which it is ripened was explained as a result of proteolysis caused by residual rennet during ripening (1). However, the differences in salt concentrations in cheese made with different levels of rennet were small in magnitude. Other factors influencing salt absorption and diffusion in cheese are the existence of a salt-in-moisture gradient between the cheese and the salting medium, surface area to volume ratio of the cheese, cheese shape, and salting time (16). The decrease of salt observed in our results may not be associated with rennet effect only. Table 2 shows that salt content was also affected by brine concentration and the interaction between brine concentration and rennet as a function of the time. A possible explanation might be changes in the protein matrix caused by residual rennet and the lower salt concentration in the brine at the end of ripening.

Effect on pH

A slight increase in pH from 5.09 to 5.18 followed by a slight decrease to 5.0 was observed in the 18% NaCl cheese during the first 2 wk of ripening. The pH of cheeses containing 8% and 15% NaCl decreased slightly from 5.09 to 4.9 in the same period. After 2 wk, the pH in all cheeses remained constant. The decrease in pH may have resulted from acid production by halotolerant bacteria (36). Brine-ripened cheeses that are similar to Feta cheese have localized changes in levels of carbohydrates, lactose, and pH in response to variations in concentrations of salt in the moisture phase (27, 36). Hence, some of the microorganisms may possibly survive for a few weeks in the center of the cheese and cause a slight variation of pH over the first 2 wk of ripening. A comparison of treatments of 18, 15, and 8% salt revealed that higher salt concentrations produced consistently higher pH of 5.07, 5.03, and 4.89, respectively (Table 1). Thus, the higher salt concentration tends to produce a cheese with a higher (P ≤ 0.05) pH. O’Connor (28) reported that at concentrations of salt-in-moisture phase below 5%, the pH is likely to decrease after salting, presumably because of the action of starter, but, at higher salt-moisture ratios, starter activities decrease abruptly, and the pH remains high. The lower salt cheese also had substantially more moisture (Figure 1) and, therefore, presumably more residual lactose, the fermentation of which could have contributed to the lower pH values. Rennet was found to have no effect (P ≤ 0.05) on the pH of Feta cheese during ripening (Table 2).

Effect on Hardness

An initial increase in hardness of the cheese followed by a weakening of the cheese structure was observed (Figure 3). The initial increase in hardness was greater as the concentration of salt in brine increased. This relationship may be due to the relatively high loss of water because of the increased salt content of the cheese in wk 1. Salt was important in regulating cheese texture because of the interactions of NaCl with paracasein, which reduced the moisture content. The increase of firmness and reduction in elasticity and plasticity of cheese was related to weakly bound moisture (1).

A gradual weakening of the cheese was measured in cheese samples, prepared with 2.5 ml of rennet/10 L of milk, after 35 d of ripening. Hardness decreased from 22.58 N to 14.91 N. This reduction in hardness was not observed in cheeses made with 0.5 or 1.0 ml of rennet/10 L. Cheese structure was weakened probably because of the hydrolysis of the protein matrix with ripening. Prentice (29) has attributed the rheological role of the casein matrix hydrolysis in the cheese to a softer and more crumbly cheese. The effect of rennet retention on hardness has also been
reported by De Jong (7). That author reported a linear relationship between the protein content of the cheese and firmness. A decrease in total protein content of the cheese over time due to proteolysis and diffusion of smaller peptides could also contribute to the decrease in the hardness of the cheese.

**Effect on Protein Content**

The total protein content of cheese decreased \( P \leq 0.05 \) over time during the ripening period of Feta cheese (Table 1) regardless of the concentration of salt and rennet. The decrease of protein content in 8 and 15% salt cheeses was greater than that in 18% salt cheese. Protein content decreased approximately 1.75% in 8 and 15% salt cheeses and 0.91% in 18% salt cheese after 63 d of ripening. The decrease of protein content has been well documented (1, 2, 11, 30). Some proteins are converted to water-soluble nitrogen compounds (31). The decrease of total nitrogen of Feta during the first stages of ripening is related to salting and drainage of the cheese. Further losses are attributed to proteolysis and diffusion of water-soluble nitrogen into the brine (1). In several studies, researchers concluded that concentrations of up to 5% NaCl in the moisture phase of cheese significantly reduced the hydrolysis of \( \beta-CN \) by rennet. At higher NaCl concentrations, the hydrolysis of \( \beta-CN \) was completely inhibited (2, 12, 13). The reasons just mentioned and the probable inhibition of rennet activity may explain the decrease of only 0.91% in protein content in 18% salt cheese.

**CONCLUSIONS**

Different salt percentages in brine were found to have a significant effect on moisture content, uptake of salt, pH, and hardness. Results indicated that increasing the percentage of salt in brine produced a harder cheese with higher salt content and pH but lower moisture content. Salt had no significant effect on the protein content of the cheese. This study demonstrated that both salt and rennet were important in the manufacture of Feta cheese. High rennet concentrations had a significant effect on uptake of salt. Increased concentration of rennet gave a softer cheese with higher salt content. Rennet had no effect on the moisture and protein contents and pH of the cheese. The results show that the protein matrix is important in its effects on physico-chemical properties of Feta cheese. Special consideration should be given to sampling technique because it may affect the results. A further study on the hydrolysis of the protein matrix and salt gradients within the cheese would give a better insight into the changes in physico-chemical properties of Feta cheese made from bovine milk.

**REFERENCES**


