Nonfat Mozzarella cheese curd was manufactured in 227-kg batches on 3 separate days using direct acidification. Cheeses with differing NaCl concentrations were obtained by dividing curd into separate lots that received various applications of dry NaCl (0, 0.5, and 1.0% NaCl, wt/wt) and hot brine (0, 5, and 10% NaCl, wt/vol) stretching treatments. The NaCl, Ca, ash, fat, moisture, and protein contents as well as cheese meltability and expressible serum of each cheese were determined. In addition, observations were made on cheese color and functionality over 24 days of storage at 4°C. Transmission and scanning electron micrographs of unsalted and salted cheeses were evaluated to determine the differences in the protein matrix. The type of NaCl application and the NaCl content of the cheeses influenced the cheese moisture, meltability, expressible serum, microstructure, and ultrastructure. The moisture content was highest in cheeses in which the curd was salted before stretching. The melt was the lowest in cheeses that were unsalted. Cheeses that were stretched in either 5 or 10% brine had <1% of the amount of expressible serum observed in unsalted cheese. Unsalted cheeses had a more open structure than did salted cheeses. Pockets of free serum were distributed throughout the protein matrix of the unsalted cheese, thus producing light-scattering surfaces and making the cheese opaque. In contrast, the salted cheeses had a more homogeneous protein matrix that lacked light-scattering surfaces, resulting in a translucent cheese. Neither NaCl concentration nor method of salting affected the Ca content of the cheeses.

INTRODUCTION

When fat is removed from Mozzarella cheese, several undesirable characteristics develop during cooking, including poor melt, a tough and rubbery texture, translucent color, and rapid skin formation. These changes occur because fat globules normally act as a filler between the protein fibers that are formed during hot stretching of the cheese curd, thus reducing the interactions among proteins within the protein matrix. Consequently, nonfat cheese, because of the increased protein concentration and increased interactions between proteins within the cheese matrix, would require more energy to melt when heated than full fat cheese would. It has been claimed that melt can be greatly improved when the combined fat and moisture content of Mozzarella cheese is ~70%. Applied to nonfat cheese, this principle suggests that the optimum melting of nonfat cheese could be obtained when the moisture content is increased because of the decrease in interactions between proteins as the protein concentration within the cheese is decreased.

When the fat content of a cheese is reduced, the moisture is usually increased to compensate for the lower fat content. When considered on a fat-free basis, however, the moisture content often does not increase sufficiently, and the ratio of moisture to protein remains comparable with that of the full fat cheese. Increased moisture content can help soften the cheese to avoid a rubbery texture; however, at high moisture contents, the cheese may become so soft that it cannot be shredded or sliced. Another pitfall is that, if the moisture content is increased without consideration of other parameters, such as the water-holding capacity of the proteins, continued syneresis can occur after packaging, resulting in a whey expulsion in the package. Thus, manufacturing protocols have to be chosen carefully so that the moisture content can be elevated, but the moisture in the cheese must be stabilized and held within the protein matrix.
When Mozzarella cheese is heated, the fat melts and becomes fluid, which helps the molten cheese to flow. Concomitantly, the protein matrix absorbs energy, which affects the interactions that are maintaining the protein structure (29). Interactions under entropic control (e.g., hydrophobic interactions) become stronger (up to 60 to 80°C) while those under enthalpic control (i.e., electrostatic and van der Waals' interactions, and hydrogen bonds) become weaker. Because of these opposing temperature dependencies, many proteins unfold in the temperature range of 60 to 80°C (the range to which Mozzarella cheese is typically heated to bring about melting). The same temperature dependence would also exist for interactions among protein molecules. Therefore, as the cheese is heated, the enthalpically controlled interactions between proteins are disrupted, allowing the cheese to deform and flow as the proteins move past one another.

At temperatures achieved during the initial stages of cooking a pizza, fat is stable and remains in the cheese or on the cheese surface as moisture is lost to evaporation. Fat protects the protein fibers from dehydration during cooking by adsorbing heat energy (3) and retarding moisture evaporation. When low or nonfat cheese is used on a pizza that is cooked in a forced-air convection oven, the cheese rapidly chars unless oil or water is placed on the surface of the moisture (personal observation) because the surface moisture is cooked away (35). Because there is little or no fat to protect the proteins once the moisture is cooked away, the proteins dry and form a skin on the surface of the cheese. As the pizza continues to cook, the dehydrated proteins rapidly char. Consequently, nonfat cheese that has a high moisture content and can retain its moisture will have better melt characteristics.

It has been reported (6, 38) that NaCl alters the water-binding properties of casein within the cheese matrix and, thus, influences the physical properties of the cheese. Guo and Kindstedt (15, 16) showed that unsalted blocks of part-skim Mozzarella cheese had higher levels of expressible serum than did brined part-skim Mozzarella cheese, which suggests that salting of the cheese increases the water-holding capacity of the cheese matrix by increasing the hydration of the casein molecules that make up the cheese matrix. At the same time, the amount of soluble proteins (e.g., \(\beta\)-CN) also increases (16).

The objective of this study was to determine whether variation in the amount of NaCl, applied using either dry-salting, by stretching the curd in hot brine or a combination of both methods, could increase the moisture retention and alter the structure of the cheese matrix. Such changes could lead to improved melt and functionality of nonfat Mozzarella cheese. Directly acidified nonfat Mozzarella cheese curd at pH 5.4 was used as a model to reduce the secondary proteolytic changes to the cheese matrix during storage and to minimize other confounding effects from the starter cultures. The curd manufacture was based on the methods of Breene et al. (5); hot brine stretching (10) being used to evenly distribute the NaCl and to eliminate brining of the cheese after stretching the curd.

### MATERIALS AND METHODS

#### Cheese Manufacture

Skim milk (227 kg) fortified with 1.0% NDM was pasteurized at 80°C for 29 s (18) and then cooled to 4°C overnight. The milk was placed in an open, rectangular vat and acidified to pH 5.40 ± 0.03 using glacial acetic acid diluted 1:10 with distilled water. The milk was heated to 35°C, and 16 ml of single-strength calf rennet (Rhône-Poulenc, Madison WI) was added. After 15 min, the curd was cut with 1.9-cm knives and allowed to heal for 15 min. The curd was then stirred at 35°C for 45 min, and the whey was drained. The curd was divided into three equal portions, which were dry-salted with 0, 0.5, or 1.0% (wt/wt) NaCl in a random order. The NaCl was sprinkled over the curd, and the curd stirred to distribute the salt evenly. The curd was then allowed to stand for 5 min (at 35°C) and occasionally stirred to prevent matting. Each portion of curd was then subdivided into three equal lots, which were stretched in either hot (82°C) water, 5% (wt/wt) brine, or 10% (wt/wt) brine in a random order. The cheeses were stretched by hand until smooth and assessed for ease of stretching. The cheeses were placed in stainless steel molds (9 × 9 × 9 cm), cooled in ice water for 1 h, and then vacuum-packaged and stored at 4°C.

#### Cheese Analysis

The cheese was shredded in a hand-held electric shredder (Presto Professional SaladShooter, National Presto Industries, Inc., Eau Claire, WI) prior to analyses. The cheese moisture was determined on d 1 and 24 using a vacuum oven (1). Expressible serum was measured using the centrifugation method of Guo and Kindstedt et al. (15); sample size was 125 g. Protein was determined using the Kjeldahl method (1). Fat was determined using the modified Babcock test (40). Melt was determined at d 1, 8, 16, and 24 using the horizontal melt tube method and a cooking
TABLE 1. Means (±SEM) for d-1 concentrations of moisture, protein, ash, fat, NaCl, and Ca of directly acidified nonfat Mozzarella cheese manufactured with varying levels of application of dry salt to the curd and stretched in hot water or brine solutions.

<table>
<thead>
<tr>
<th>Salt treatment</th>
<th>Lot</th>
<th>Dry</th>
<th>Brine</th>
<th>Moisture (X ± SEM)</th>
<th>Protein (X ± SEM)</th>
<th>Ash (X ± SEM)</th>
<th>Fat (X ± SEM)</th>
<th>NaCl (X ± SEM)</th>
<th>Ca (X ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>0.0</td>
<td>0</td>
<td></td>
<td>61.2 ± 0.29</td>
<td>32.3 ± 0.46</td>
<td>1.94 ± 0.04</td>
<td>0.22 ± 0.03</td>
<td>0.14 ± 0.02</td>
<td>0.42 ± 0.03</td>
</tr>
<tr>
<td>A2</td>
<td>0.5</td>
<td>0</td>
<td></td>
<td>63.2 ± 0.28</td>
<td>29.8 ± 0.46</td>
<td>1.83 ± 0.10</td>
<td>0.27 ± 0.02</td>
<td>0.40 ± 0.07</td>
<td>0.39 ± 0.04</td>
</tr>
<tr>
<td>A3</td>
<td>1.0</td>
<td>0</td>
<td></td>
<td>60.9 ± 0.20</td>
<td>30.3 ± 0.31</td>
<td>2.05 ± 0.04</td>
<td>0.28 ± 0.03</td>
<td>0.56 ± 0.07</td>
<td>0.37 ± 0.09</td>
</tr>
<tr>
<td>B1</td>
<td>0.0</td>
<td>5</td>
<td></td>
<td>60.7 ± 0.29</td>
<td>31.8 ± 0.51</td>
<td>2.32 ± 0.21</td>
<td>0.23 ± 0.03</td>
<td>0.86 ± 0.13</td>
<td>0.59 ± 0.03</td>
</tr>
<tr>
<td>B2</td>
<td>0.5</td>
<td>5</td>
<td></td>
<td>61.9 ± 0.60</td>
<td>29.0 ± 0.79</td>
<td>2.43 ± 0.17</td>
<td>0.25 ± 0.03</td>
<td>1.01 ± 0.12</td>
<td>0.55 ± 0.01</td>
</tr>
<tr>
<td>B3</td>
<td>1.0</td>
<td>5</td>
<td></td>
<td>63.4 ± 0.31</td>
<td>30.1 ± 0.54</td>
<td>2.86 ± 0.17</td>
<td>0.28 ± 0.02</td>
<td>1.47 ± 0.14</td>
<td>0.36 ± 0.01</td>
</tr>
<tr>
<td>C1</td>
<td>0.0</td>
<td>10</td>
<td></td>
<td>60.9 ± 0.50</td>
<td>31.3 ± 0.46</td>
<td>2.88 ± 0.28</td>
<td>0.30 ± 0.03</td>
<td>1.36 ± 0.19</td>
<td>0.37 ± 0.05</td>
</tr>
<tr>
<td>C2</td>
<td>0.5</td>
<td>10</td>
<td></td>
<td>62.1 ± 0.47</td>
<td>29.9 ± 0.79</td>
<td>3.00 ± 0.21</td>
<td>0.27 ± 0.04</td>
<td>1.72 ± 0.13</td>
<td>0.38 ± 0.03</td>
</tr>
<tr>
<td>C3</td>
<td>1.0</td>
<td>10</td>
<td></td>
<td>62.3 ± 0.36</td>
<td>29.9 ± 0.58</td>
<td>3.51 ± 0.27</td>
<td>0.28 ± 0.02</td>
<td>2.18 ± 0.14</td>
<td>0.32 ± 0.05</td>
</tr>
</tbody>
</table>

1Percentage of NaCl added to curd before stretching.
2Salt concentration added to water used for stretching the curd.

...continued from the previous text...

...continued from the previous text...
hand-stretching. After the curd was worked slightly, the adhesiveness lessened, and the cheese stretched well. All cheeses that were stretched in 5 or 10% hot brine solutions showed good elasticity during stretching without adhering to the rubber gloves but did exhibit a less elastic texture.

The color and appearance of the cheese were also affected by the medium in which the cheeses were stretched. All cheeses were opaque while being stretched. After the cheeses were cooled to room temperature (ca. 22°C), the cheeses stretched in hot water were white and opaque, although their opacity decreased with increased dry-salt treatments. When cooled to 4°C, these cheeses became translucent, as is typical for nonfat cheeses. In contrast, the cheeses that had been stretched in brine lost opaqueness more quickly and were translucent by the time they were, cooled to room temperature (ca. 22°C). During the melt test, when the cheeses were heated to ca. 90°C, all cheeses became opaque and white. Then, after subsequent cooling, all of the cheeses became translucent again.

Cheeses with low NaCl concentrations shredded better than did cheeses with high NaCl concentrations throughout the 24 d of storage and analysis, regardless of moisture content. At approximately 0.8% NaCl content, cheese shredding became difficult, and cheese would adhere to the knives and crumble in the shredder.

Cheese Composition

The composition of the cheese is shown in Table 1. Fat contents for all cheeses were ≤ 0.30%. When the curd was stretched in hot water, only about half of the NaCl that was applied to the curd was retained in the cheese. The addition of 1% (wt/wt) of NaCl to the curd produced a cheese with a total NaCl content of 0.68%; the unsalted cheese had a baseline level of 0.14% NaCl. The remainder of the NaCl was lost in the water used for stretching the cheese. As NaCl content of the stretching water was increased, the final NaCl content of the cheese also increased. Stretching of the unsalted curd in 5% brine yielded a cheese with an NaCl content of 0.85%; stretching of the curd that had received 1.0% NaCl prior to stretching in 5% brine produced a cheese with 1.47% NaCl. This result is similar to that obtained by Barbano et al. (2) who added 2.2% NaCl to curd and then stretched the curd in a 6% brine solution to produce a cheese with 1.76% NaCl.

Cheese moisture contents were in the range of 62.1 ± 1.4 and were influenced by dry-salting of the curd. The addition of 1% NaCl to the cheese curd before stretching increased the cheese moisture by 2%; stretching in brine did not effect the cheese moisture. Consequently, cheeses that were not dry-salted before stretching had the lowest total moisture contents, regardless of the stretching conditions. They also had lower ratios of moisture to protein content.

The mean Ca content for the directly acidified cheeses was 0.37%, which was considerably lower than the 0.7% Ca typically found in Mozzarella cheese (2, 15, 44). Although the dry-salted cheeses tended to have lower Ca contents than cheese that was not dry-salted (Table 1), these differences were not significant (P = 0.30). The effect of brine concentration at stretching on Ca content was also not significant (P = 0.17).

Expressed Serum

Expressible serum was affected (P < 0.0001) by the hot brine stretching treatment. Cheeses stretched in 5 and 10% hot brine solutions had no expressible serum on d 1 or subsequently. These cheeses had salt contents ranging from 0.85% to 2.18%. Ramkumar et al. (38) reported a similar occurrence in directly acidified curd; no expressible serum was obtained when the milk was coagulated at pH 5.4 or 5.1. Cheeses stretched in hot water had expressible serum, the quantity of which decreased as concentrations of dry-salt treatment and age increased (Figure 1). By d 24, some of the serum could still be expressed from these cheeses. These cheeses also suffered from syneresis when held at room temperature (ca. 22°C) and had some whey in the packages during storage at 4°C. Unsalted cheese had the highest level of expressible serum even though it had the lowest moisture content. Those cheeses that contained expressible serum also exhibited syneresis when the cheese was heated, as was evident from the free serum that separated from the molten cheese during the melt test.

Cheese Melt

Salting of the cheese influenced melt: unsalted cheese had 1-cm lower melt on d 1 than did all other cheeses (P = 0.04). Cheeses that had received any of the salt treatments (and had NaCl contents of 0.4 to 2.2%) all had similar melt and did not change significantly during storage. Similarly, no change occurred in the functional properties of directly acidified part-skim Mozzarella cheese during storage (32). Meltability of the unsalted cheeses increased by 0.5 cm during 24 d of storage, and no significant differences were observed between cheese melt for any of the cheeses by the end of the experiment (Figure 2).
DIRECTLY ACIDIFIED NONFAT MOZZARELLA CHEESE

Cheese Microstructure and Ultrastructure

The addition of NaCl to cheese had an impact on both the microstructure and ultrastructure of nonfat Mozzarella cheese. When the microstructure of the cheeses was examined at low magnification, differences were observed between salted and unsalted Mozzarella cheeses (Figure 3). When the fractured surface of the unsalted cheeses was examined using the scanning electron microscope, many fissures and folds (1 to 25 μm in size) were found to be distributed throughout the protein matrix (Figure 3A). These structures were pockets of whey that remained trapped within the cheese curd after stretching. The larger folds were similar to the fat-serum channels formed during hand-stretching of higher fat Mozzarella cheese (27), although these folds were smaller and less numerous in the nonfat cheese. In contrast, the salted cheeses had a more homogeneous microstructure that lacked areas of entrapped whey (Figure 3B).

This difference among cheeses was also observed within the cheese matrix at higher magnification using transmission electron microscopy (Figure 4). Areas of lower electron density (20 to 200 nm in size) were present in the unsalted cheese (Figure 4A). Such regions were fewer and smaller in the salted cheese, giving them a more homogeneous protein matrix (Figure 4B).

At high magnification (140,000x), differences in the ultrastructure were also observed between the unsalted and salted cheeses (Figure 5). The unsalted cheese had larger and more distinct protein aggregates, and the micrographs of those cheeses had a grainy appearance (Figure 5A). In contrast, the salted cheese had a more evenly dispersed protein arrangement (Figure 5B), and large protein clusters were less obvious.

Image Analysis

Processed binary images of the high magnification micrographs used for the Fourier transform calculations are shown in Figure 6. The black regions

Figure 1. Decline in expressed serum (ES; weight of expressed serum divided by the total moisture of the cheese) over 24 d of storage at 4°C for cheeses A1 (○), A2 (□), A3 (△), and B1 (●) from Table 1.

Figure 2. Mean (±SEM) measurements of melt for Mozzarella cheeses manufactured using hot water stretching (A), 5% hot brine stretching (B), and 10% hot brine stretching (C). Within each stretching treatment, 0.0% (open), 0.5% (closed), and 1.0% (shaded) dry NaCl was applied to cheese curd. Error bars represent standard errors of the mean.

represent protein aggregates, and the white regions represent the spacing between aggregates. Unsalted cheese had larger protein aggregates and greater space between aggregates (Figure 6A). In contrast, salted cheeses had smaller protein aggregates and smaller spacing between them (Figure 6B). The spacing between bars on the grid along the top of these two images represents 15.9 nm.

The average distance between protein aggregates in both the salted and unsalted cheeses were calculated using Fourier transforms of the binary images (Figure 6, C and D), which were generated from Figure 6, A and B, respectively. The cluster of pixels around the origin in Figure 6, C and D, represent the inverse spacing of the electron-dense regions calculated from the processed images. The lines along the equator represent inverse spacings of 1/15.9 nm. The average spacing of the electron-dense regions in the micrographs were calculated to be 3.5 nm in the unsalted cheese and 2.4 nm in the salted cheese.

The actual distance between protein aggregates would be an order of magnitude greater than that calculated for the micrographs because of the manner in which the micrographs were obtained. For transmission electron microscopy, an ultra-thin section of plastic-embedded cheese is the object viewed in the microscope. Although these sections are only 70 nm thick, this thickness is significant on a molecular
basis, so the conversion from a three-dimensional section to a two-dimensional micrograph must be considered when measuring the objects, and, more importantly, the distances between objects must be considered when those distances are much less than the thickness of the section.

Thus, the number of electron-dense regions shown in Figure 6, A and B, represent not only the number of protein aggregates distributed in a $184 \times 184$-nm square but rather the total number of protein aggregates distributed in a $184 \times 184 \times 70$-nm block (Figure 7). Consequently, the average spacing between aggregates in the cheeses would be of the order of 20 to 30 nm.

![Figure 5: Transmission electron micrographs, at identical magnification, of d-7 nonfat Mozzarella cheese at high magnification, showing distinct protein aggregates in unsalted cheese (A) and a more diffuse appearance of proteins in salted cheese (B).]

![Figure 6: Binary images (from Figure 5) of the unsalted cheese matrix (A) and the salted cheese matrix (B) and the respective Fourier transforms for unsalted cheese (C) and salted cheese (D) that were used to calculate the average spacing between the protein aggregates. Spacing of lines in grating (A and B) are 15.9 nm. The spacing of lines on the equator (C and D) represents $1/15.9$ nm.]

The same dimensional compression must also be considered when the size of the protein aggregates is calculated. In this case, however, the actual size of the protein aggregates may be slightly smaller than that observed in the micrographs because of the way in which objects are viewed in the transmission electron micrograph. As the electron beam passes through the sample section, electrons scatter when they interact with electron-dense regions (proteins that have been stained with Os, Ur, and Pb) of the section. Then, as the electrons that passed through the section without being scattered are collected, a gray-scale image is formed that represents the proportion of electrons passing through the sample. Consequently, the shadows formed by two electron-dense particles that may be separated by up to 70 nm can appear to be linked together or as a single larger shadow (Figure 7).

**DISCUSSION**

Differences in curd behavior during manufacture, cheese appearance, moisture content, and meltability can all be related to how NaCl affected the organization of the proteins in the cheese matrix.

Cheese Manufacture

During the preliminary trials, we determined that two modifications to the Breene et al. (5) method were necessary when direct acidification was used to manufacture a nonfat cheese at pH 5.4. Breene et al. (4, 5) found that curd from milk acidified to pH 5.6 had better characteristics than curd formed at pH 5.3 to 5.4. However, we wanted to have the curd at a lower pH so that our model would be close to actual Mozzarella cheese-making practices. Skim milk only produced a weak curd when it was renneted and was too weak to cut satisfactorily. The addition of 1% NDM before acidification increased the milk protein concentration sufficiently to produce a firm curd that did not fragment when cut. Also, heat treatment of the curd and whey to 49°C after cutting caused the nonfat curd to melt and stick to the vat, so this heating step was eliminated, and the set temperature of 35°C was maintained throughout curd manufacture.

The tackiness of directly acidified cheese curd during stretching (especially curd with pH <5.6) has been reported by other researchers (17, 20), although no explanation has been given for such tackiness. This problem did not occur when the curd was stretched in hot brine. A possible explanation is the tendency for para-casein in the curd to associate with hydrophobic materials (such as rubber gloves) rather than with the solvent water. The addition of NaCl to the stretching water apparently increased the protein hydration in the nonfat curd, and, by implication, reduced the surface hydrophobicity of the proteins in the curd. Whether an exchange of Na+ for Ca2+ occurred was not determined. The cheeses stretched in brine tended to have lower Ca contents (Table 1), but additional experiments would be required to show a statistically significant difference.

Protein Interactions

Before the cheese curd is salted, the proteins in the cheese at pH 5.4 can be considered still to be undergoing aggregation. This drive toward aggregation (initiated by the renneting of the milk) occurs because the free energy of the cheese curd can be lowered as the hydrophobic regions of the proteins are shielded from water as the proteins aggregate. Such hydrophobic interactions increase in strength as the temperature increases (29), and any functional characteristics of the curd that are based upon hydrophobic interactions become more apparent when the curd is heated during the stretching process.

The addition of NaCl to the nonfat cheese apparently increased the interactions between proteins and the surrounding water, thus reducing the hydrophobic interactions between protein molecules. Consequently, less aggregation of the protein into protein-dense groupings was observed. Although the actual distance between protein aggregates is somewhat uncertain because of dimensional compression in the micrograph images, the differences observed among the cheeses provides an indication of the change in the ultrastructure caused by salting. In the salted cheeses, the spacing between the aggregates decreased 31% compared to that in the unsalted cheese. Such increased hydration of proteins, as shown at the ultrastructural level, demonstrates why cheese curd in the pH range of 5.6 to 5.2 is soluble in warm NaCl solutions (19).

As the protein hydration increases, voluminosity of the cheese matrix also increases, causing a migration of water from the large and small voids into the protein matrix. The increased water-holding capacity of the proteins with increased NaCl concentration is further demonstrated when the ratios of moisture to protein of the cheeses are compared. The unsalted cheese contained only 1.9 g of water/g of protein, and, if the quantity of expressible serum was used as a first approximation of the amount of water in the serum pockets, then the hydration of the cheese matrix in the unsalted cheese would be even lower (<1.8 g of water/g of protein). In contrast, the salted cheese contained 2.1 g of water/g of protein, which, although greater than the unsalted cheese, is still considerably less than the hydration of native casein micelles, which is 3.7 g of water/g of protein (23).
When milk is renneted, a series of changes in how the proteins interact with each other is initiated. The subsequent coagulation and syneresis that occur after renneting reflects the sum of all thermodynamic forces acting on the casein proteins, including non-specific hydrophobic and electrostatic interactions as well as specific interactions (such as Ca bridging) between the renneted para-casein micelles (19). Hydrophobic interactions are important in the destabilization and aggregation of the casein micelles by rennet and continue to be important in the syneresis of the resultant coagulum (39). This syneresis, or whey expulsion, is enhanced 1) by heating the curd, which increases the strength of hydrophobic interactions and, therefore, increases interactions among proteins so that more water is excluded from the cheese curd, and 2) by lowering curd pH, which reduces the net charge of the caseins as they approach their isoelectric point so that the proteins become less soluble. By the time the cheese curd is ready to be pressed (or stretched in the case of Mozzarella cheese), the protein matrix usually has reached a point at which little moisture can be removed from the curd without mechanical force, and cheese curd (which is essentially hydrated para-casein) contains less than half of the hydration of native casein micelles (8). Then, when NaCl is added, the ionic environment around the proteins changes such that the various components of the cheese curd system may be redistributed to obtain an equilibrium state of minimum energy (38). For the directly acidified nonfat cheese curd, salt addition results in an increase in protein hydration.

Cheese Composition

The higher moisture of the nonfat cheeses that were dry-salted before stretching suggests a behavior for curd made by direct acidification that is different from that typically observed. It is generally accepted that the cheese moisture content is inversely proportional to the NaCl content (14) because of the increase in whey release that takes place when Cheddar cheese curd (43) or Mozzarella cheese curd (2) is salted. One of the differences between the directly acidified cheese curd and curd made using starter cultures is that there is no internal pH gradient within directly acidified curd. The drop in the pH of the cheese curd induces more syneresis than that occurring if milk has previously been brought to the same pH (47). So, instead of a continual acidification that affect the curd proteins, the protein conformations within the directly acidified curd are set by the initial acidification of the milk before the curd is cut. The subsequent whey drainage from the curd above that pH resulting from renneting and cutting the curd can only be induced by mechanical agitation or heating.

Another difference is that the directly acidified cheese had a much lower Ca content because of the acidification of milk to pH 5.4 before renneting. At this pH, all of the inorganic phosphate and 75% of the Ca have been solubilized from the casein micelles (46). Consequently, when the whey is drained from the directly acidified curd, loss of Ca and P from the curd is greater. The resultant cheese contained only 0.37% Ca (i.e., 1.2 g of Ca/g of protein). In comparison, Mozzarella and Cheddar cheeses made using starter cultures typically contain 2.7 and 2.9 g of Ca/g of protein, respectively (44). Directly acidified curd would, therefore, be more susceptible to the peptizing action of NaCl, which has been shown to cause cheese curd to absorb water if the Ca concentration of the NaCl solution is low (ca. 0 to 0.5%) (12). Such peptizing action causes the cheese curd to absorb water so that the casein matrix swells to form a hydrated gel.

In a study (34) of syneresis of milk gels, syneresis was inhibited when NaCl was added to whey surrounding the curd, which was attributed to an increase in hydrophilic capacity of the proteins because of their interaction with Na+, which resulted in an increase in water-holding capacity of the proteins. Creamer (8) also observed that the addition of NaCl increased the water retention of renneted milk. He suggested this increase was a result of a displacement of Ca from the protein matrix, leading to an increase in the number of ionic groups in the matrix and a consequent increase in volume of the matrix. Robertson et al. (41) observed that NaCl addition to whey before draining the curd resulted in an increase in the moisture content of the drained curd. Other researchers (13, 36, 47), however, have reported less influence of salt on curd syneresis when NaCl is added to the milk prior to rennet coagulation.

Normally, when the curd is salted, some of the salt dissolves on the curd surface, which induces a counterflow of water from the curd onto the surface (because of osmotic pressure) and produces a concentrated brine layer on the surface. This expulsion of whey from the curd is usually further enhanced by a localized contraction of proteins on the curd surface as they are salted-out. Evidently, for the directly acidified cheese curd, there must be an induced expansion of the protein network sufficient to counteract these osmotic forces so that this release of whey does not
occur. The pore width of the protein matrix in nonfat curd has been observed (14) to be smaller than for a cheese curd with higher fat content (ca. 20% fat), which reduces the diffusion coefficient by 30% and would also affect how the curd reacts to salt addition.

Apparently, whether salting of the cheese curd promotes or inhibits syneresis is determined by the physico-chemical environment of the proteins that constitute the gel matrix of the curd particles, including pH (and pH gradient within the curd particles), Ca (and PO₄) concentrations, moisture content, and temperature. As stated by Guinee and Fox (14), although a considerable amount of information is available on the significance of NaCl in cheese, many gaps in the knowledge remain. Whatever the reason, in our experiments with directly acidified curd, the addition of NaCl to the curd resulted in a salting-in of the proteins rather than the salting-out observed (14) for proteins at milled-curd surfaces during Cheddar cheese manufacture.

Expressible Serum

The water in the large serum pockets, observed in unsalted cheese, would correspond to the water that can be removed by centrifugation as expressible serum. These pockets, formed by the entrapment of water between the protein strands during stretching, must be interconnected to some extent or the serum would not be able to diffuse as readily from the cheese. The pockets can be considered analogous to an open cell foam with water contained in open cells within the relatively solid protein matrix. The smaller pockets (observed as areas of lower electron density) appear as closed cells and probably do not contribute to expressible serum.

The rate of decline of expressible serum during storage of those nonfat cheeses that had any expressible serum was much slower than that typically observed for cultured part-skim Mozzarella cheese. In Mozzarella cheese made with starter culture, expressed serum decreases to 0% within 10 to 20 d (15), although unbrined Mozzarella continues to express serum longer (16). In the directly acidified low NaCl, nonfat Mozzarella cheeses, the expressed serum only decreased by 40% after 24 d. This result suggests that, without added Na⁺, there is a delay in the changes occurring within the protein matrix that increases the water-holding capacity of the matrix.

Cheese Appearance

The change in opacity of the cheeses as a result of salting can be attributed to changes in the protein matrix. In unsalted curd, the proteins are more aggregated, and the protein matrix has numerous pockets of free serum throughout. The edges of these pockets would provide a surface at which light can be scattered, giving the cheese an opaque appearance. In addition, the aggregation of the protein may also contribute somewhat to light scattering, although the small size of the aggregates compared with the size of the serum pockets suggests that most of the light scattering occurs at the serum-matrix interface. Salting the cheese results in the absorption of free serum into the matrix, giving a homogeneous matrix with few discontinuities or surfaces to cause light scattering. Thus, the salted cheese becomes translucent.

The cheeses stretched in brine became translucent by the time the cheese was cooled to room temperature, which suggests a change in the protein structure upon addition of NaCl. This result would be expected as the cheese curd and brine are intimately mixed during stretching. In contrast, cheeses that were stretched in hot water had lower NaCl contents, and, consequently, absorption of serum into the matrix would take longer. In this case, not until the cheeses were cooled to 4°C did they lose their opaqueness. This temperature dependence of opaqueness is a strong indication that hydrophobic interactions are involved.

The effect of temperature on the association and disassociation of caseins in milk is well known (23) and has also been used to explain changes in protein content of expressed serum of Mozzarella cheese (15). At pH 5.4 (the pH of the cheeses in this study), the dissociation of casein from micelles has also been shown to be much greater at 4°C than at 20°C (9). The importance of the hydrophobic interactions was also evident in the cycling from translucency to opaqueness and back to translucency that occurred as the cold cheese was heated during stretching (or during the melt test) and subsequently cooled. Whether serum pockets were reformed during heating or whether the light scattering was solely a function of change in protein aggregation size was not determined as samples for electron microscopy were only prepared from cheese on d 1.

Cheese Melt

The lower meltability of unsalted cheese can be explained in terms of the energy required to disrupt the matrix network and to allow the proteins to flow. Proteins that are more highly aggregated would require more thermal energy to disrupt the aggregates and disassociate the proteins. In contrast, salted
cheeses with smaller protein aggregates and a more hydrated protein matrix had better melt. The slight increase in the melt of the unsalted cheeses during storage corresponds with the decrease in the expressible serum over the same time and implies that the increased meltability observed with cheese of greater moisture content (2, 37) is a function of the moisture being held within the cheese matrix and not the total cheese moisture. As water migrates into the protein matrix, the interactions of protein to water increase, and the hydration sphere of the proteins increases to accommodate the extra water molecules. Concomitantly, the volume of the protein matrix increases, resulting in the protein matrix filling the spaces previously occupied by the serum pockets and voids.

CONCLUSIONS

The influence of salting on the color, expressible serum, and melting of nonfat Mozzarella cheese can be explained by the changes in the microstructure and ultrastructure of the cheese. Unsalted cheese had larger protein aggregates with free serum existing in small and large pockets within the protein matrix, and salted cheese had a more homogeneous structure with more hydrated proteins. There was no expressible serum in the cheeses with an NaCl content ≥0.85%. These cheeses were also relatively translucent in appearance. Salted cheeses also had a slightly improved meltability; however, the meltability did not change during 24 d of storage.

When all factors were considered, the nonfat cheese made from curd that had 1.0% NaCl that was added before stretching and was stretched subsequently in hot water produced the best cheese. This cheese had only 0.4% NaCl content but similar melt characteristics to cheeses manufactured with a higher NaCl content; this cheese was also more opaque at room temperature and shedded better.

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