Water Partitioning in Mozzarella Cheese and Its Relationship to Cheese Meltability[1]

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ABSTRACT

The aim of this study was to determine what happens to water in Mozzarella cheeses during storage and to relate those changes to cheese microstructure and functionality. A reduced fat (8% fat) Mozzarella cheese and a control cheese with 19% fat were made and evaluated over 21 d of refrigerated storage at 4°C. Fat, protein, ash, salt, and water were measured on d 1. Meltability, total water, freezable water, and expressible water were measured on d 1, 7, 14, and 21. Even though the reduced fat cheese had a higher total water content than did the control cheese, the reduced fat cheese contained less water on a fat-free basis. The amount of water expressible at 25°C was higher in the control cheese than in the reduced fat cheese and was proportional to the fat content of the cheese. During storage, the expressed serum for both cheeses decreased to zero by d 21. Based on changes observed in microstructure of a commercial Mozzarella cheese (19% fat) during storage, we concluded that the expressed water was derived from water contained in the fat-serum channels that were interspersed throughout the protein matrix. The amount of bound water was lower in the control cheese than in the reduced fat cheese and was proportional to the protein content of the cheese. Bound water levels remained constant throughout storage. During storage of the commercial Mozzarella cheese, the fat-serum channels became smaller with the protein matrix expanding into the areas between the fat globules. By d 21, the fat globules were completely encased by the protein matrix. This expansion of the protein matrix in the commercial cheese occurred over the same time span as the decrease in expressible water of the experimental cheese and indicated that the protein matrix was absorbing the water originally located in the fat-serum channels. Because no change in bound water was observed, the water that had been expressible at d 1 was being absorbed into the protein matrix as entrapped water. The meltability of both cheeses increased during storage while the percentage of entrapped water increased.
(**Key words**: Mozzarella cheese, bound water, microstructure, fat)

Abbreviation key: **DSC** = differential scanning calorimeter.

INTRODUCTION

When milk is renneted, casein micelles are converted from a stable colloid into a protein gel with individual casein micelles joined together into chains. As cheese making proceeds, and whey is expelled from the curd, the protein chains become thicker through continued association with one another until they form a protein matrix in which water and fat are distributed (19, 24). For Mozzarella cheese, this process is the same as for Cheddar cheese up until the time the Mozzarella cheese curds are transferred to the cooker-stretcher to produce a pasta filata cheese (19) rather than being cheddared (11).

In pasta filata cheese, the proteins in the cheese curds coalesce into larger strands that are oriented in the direction of stretching. This coalescence results in a redistribution of the water and fat during stretching and molding with the larger strands (or fibers) of protein being separated by channels containing water, water-soluble cheese components, bacteria, and fat globules (19, 22). Changes in the microstructure and functionality of Mozzarella cheese during storage (4, 13, 21) suggest that the proteins are not in a quiescent state immediately after stretching and molding but undergo further structural rearrangement.

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The improved meltability of Mozzarella cheese observed during storage is generally associated with the proteolytic hydrolysis of α_{s1}-CN by chymosin, plasmin, and proteolytic enzymes originating from the starters (9, 13, 14, 16). Hydrolysis of β-CN in cheese made using chymosin occurs at a much slower rate and is not usually associated with improved meltability. However, Bogenrief and Olson (3) demonstrated that meltability correlated more with β-CN hydrolysis than with α_{s1}-CN hydrolysis within a group of experimental Cheddar cheeses at 15 and 45 d of aging. In cheese made using *Cryphonectria parasitica*, the meltability was greater compared with cheeses made with chymosin. They attributed the improved meltability to increased β-CN hydrolysis.

It has also been shown (27) that increased β-CN hydrolysis improves cheese meltability, increases free oil, and decreases apparent viscosity in Mozzarella cheese. However, we have observed (unpublished data) that changes in cheese meltability can occur during storage without any significant decrease in intact β-CN levels. This observation suggests that proteolysis may not be the only causal effect for changes in the meltability of Mozzarella cheese during storage, and that other changes occurring concomitantly in the cheese during storage should be investigated. One such change that has been observed, as shown by a decrease in expressible serum, is a redistribution of water in cheese during storage (12). However, it is not known what causes this change in water or what subsequent role water plays in cheese functionality.

The various states in which water may exist in a material such as cheese can be described in terms of the spatial relationship between water and the solid constituents of the food (in cheese, these solids are predominantly protein although water interactions also exist between other cheese constituents). Bound water has been characterized as nonsolvent water or chemisorbed water (10), sorbent- or solute-associated water and unfreezable water (2), constitutional water (i.e., water that occupies interstitial protein spaces), and vicinal water (i.e., water that covers a protein as a single layer) (7). Bound water also has been characterized in terms of hydrodynamically influenced layers of water that surround a protein (6). Such isotropically bound water is intimately associated with (or is in close proximity to) the protein surface. This water exhibits slower rotational and translational speeds than pure water, is not available as a solvent, and cannot be frozen at –40°C (6).

In contrast, bulk water is water that is more loosely associated with the proteins even though it retains a large solvent capacity and is freezable at –40°C. Farrell et al. (6) described bulk water as being anisotropic, because the rotational and translational speeds of such water molecules are not significantly effected by their interaction with the proteins. Bulk water may be further divided into entrapped or free water depending upon its mobility within the protein matrix. Water that is impeded by the macrostructure of the protein matrix, such that it cannot be expressed by centrifugation, is considered to be entrapped. Water that is not impeded by the protein matrix and may be expressed by centrifugation is known as expressible water (7). Thus, the distribution and movement of water in cheese may be characterized as being either bound to proteins, entrapped by those proteins, or expressible by centrifugation.

The objective of this research was to explain changes observed in Mozzarella cheese microstructure and functionality over time in terms of changes in water distribution in the cheese and to determine whether those changes are the same for cheeses with high or low fat levels.

MATERIALS AND METHODS

Milk, Cultures, and Rennet

Skim milk and nonhomogenized 2% milk from the Gary H. Richardson Dairy Products Laboratory at Utah State University were pasteurized at 80°C for 29 s, cooled to 4°C, and used to standardize cheese milks to a casein to fat ratio of 1.2 or 2.4. Lyophilized direct-set cultures of *Lactobacillus helveticus* LH 100 and *Streptococcus thermophilus* TA 061 were obtained from Rhodia (Madison, WI), and double-strength Chymax® rennet was from Chris Hansen, Inc. (Milwaukee, WI).

Mozzarella Manufacturing Procedure

Double O vats, with capacity to hold 454 kg of milk per vat (DEC International, Damrow, Fond DuLac, WI) and equipped with vertical agitator and knives and Para Just AC motor speed controls (Parametrics, Orange, CT), were used to manufacture cheese curds. Curds were mechanically stretched in a cooker-stretcher (model ALIS LAB; Alfa-Laval Cheese Systems Ltd., Tetrapak, Inc., Chicago, IL), and the melted cheese was formed into rectangular loaves (Alfa-Laval Alform; Tetrapak, Inc., Chicago, IL).

The control (i.e., low moisture part-skim Mozzarella cheese) and reduced fat Mozzarella cheeses were made by modifying the cheese-making procedure for reduced fat Mozzarella described by Merrill et al. (17). After preacidification to pH 6.1 and adjustment of the milk temperature to 34°C, 20 U of each culture were added, and the milk was ripened for 45 min. Thirty-five milliliters of rennet (diluted in 350 ml

distilled water) were added, and the coagulated milk was cut 15 min after rennet addition. Curds were healed for 5 min and then agitated for the remainder of the cheese-making procedure. After the curds healed, the temperature was raised to 39°C over 30 min, then approximately half of the whey was removed. When the curd pH reached 5.2, the remaining whey was drained, and the curds were salted (1 kg of salt/1000 kg of milk) in three increments, 10-min apart. The salted curds were cooked and stretched at 80°C in 5% (wt/vol) brine solution and then formed into 2.2-kg rectangular loaves. The loaves were immersed in cold water for 1 h, cut longitudinally in half, individually vacuum-packaged, and stored at 4°C.

Cheese Composition

Fat was measured in duplicate using a modified Babcock method (23). Cheese moisture was measured in triplicate by vacuum-oven AOAC method 926.08 (1). Ash was measured in duplicate by gravimetric method AOAC 935.42, protein in duplicate by AOAC method 920.123, and salt in duplicate by chloride analysis by AOAC method 971.19 (model 926 salt analyzer; Corning, Medfield, MA) (1). Moisture content as measured by vacuum oven was used as total moisture for all calculations of water partitioning in the cheese.

Melt

Melt was measured by modifying the method of Bogenrief and Olson (3). Cheese plugs weighing approximately 15 g were placed into glass tubes, which were sealed with rubber stoppers. Sample tubes were immersed in hot (95°C) mineral oil, and the distance (millimeters) the cheese melted was measured at 0, 4, 8, 12, and 16 min. Meltability measurements at 12 min were used to compare control and reduced fat cheeses.

Water

Freezable and bound water. Freezable water, defined as water freezable at –40°C, was measured using a differential scanning calorimeter (**DSC**) (model 2910; TA Instruments Inc., New Castle, DE) equipped with a refrigerated cooling system. Nitrogen was used to purge the DSC cell (50 ml/min) and refrigerated cooling system (150 ml/min) prior to and during sample analysis. Cheese samples, approximately 10 to 20 mg, were accurately weighed, then sealed in aluminum pans (TA Instruments Inc.), and placed inside the DSC cell. A pan containing indium, which served as the reference, was also placed inside the DSC cell. Both pans were simultaneously equilibrated to 25°C, cooled at the rate of 10°C/min to –40°C, and then heated at a rate of 10°C/min to 40°C. Universal Analysis 1.5B software (TA Instruments Inc.) was used to measure the area under the ice melting peak, and the enthalpy (Joules per gram) of the transition was determined. The percentage of freezable moisture in the sample was then calculated using the latent heat of fusion of water (334.4 J/g) (20). Bound water was defined as the water that did not freeze at –40°C (2).

Expressible water. Samples were centrifuged at 12,500 × *g* for 75 min (25°C) (12). The expressed fluid was decanted into a weighing pan, and the fat was removed using a micropipette. The expressible water was calculated as the expressed fluid minus fat.

Entrapped water. Entrapped water was calculated as the difference between the freezable water and the expressible water.

Statistical Analyses

Cheese was made in duplicate on 2 different d with two vats of cheese (a control cheese and a reduced fat cheese) being manufactured on the same day. Differences between means were tested using Student's *t* test. Significance was declared at $P < 0.05$ unless otherwise stated.

Cheese Microstructure

In a separate experiment, scanning electron microscopy was used to examine changes in the microstructure of a commercially prepared low moisture part-skim Mozzarella cheese (19% fat, 48.8% moisture, 1.3% salt, pH 5.2) during storage. Samples were obtained at 1, 7, 14, and 21 d and were prepared for electron microscopy using the method described by Oberg et al. (19).

RESULTS AND DISCUSSION

Cheese Microstructure

Initial structure. As the commercial Mozzarella cheese curd was heated, stretched, and molded, the proteins formed into fibers that were oriented in a roughly parallel manner as shown in Figure 1 and as previously reported (19). While the cheese was still hot (ca. 54°C), the proteins had the appearance of continuous, interconnected, smooth-walled fibers separated by channels that contained molten fat globules, serum, bacteria, and water-soluble cheese components. Although most of the fat and bacteria were

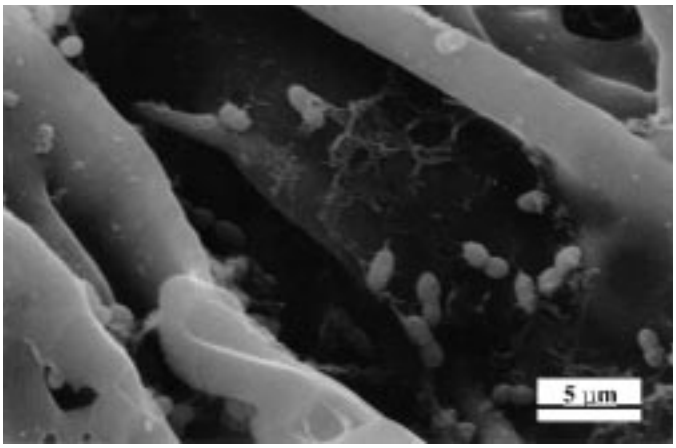

Figure 1. Scanning electron micrograph of commercial part-skim Mozzarella cheese taken immediately after hot-water stretching. Cheese matrix proteins appear as smooth, elongated fibers separated by serum channels, which once contained fat, water, and bacteria. Bacterial cells and residual fat globule membrane material adhere to the fat-serum channel walls.

removed during sample preparation for electron microscopy, some bacteria (along with residual fat globule membrane material) could still be found within the fat-serum channels between the protein fibers of the cheese.

d 1. After the commercial cheese had been brined, the appearance of the protein matrix surface surrounding the fat-serum channels changed (Figure 2). The original smooth appearance of the fat-serum channel walls, present when the cheese was hot, changed to a rough-textured appearance. These channel walls were textured by numerous circular indentations that ranged in size from approximately 1 to 10 μm in diameter. In addition, there were also many smaller circular and elliptical indentations approximately 0.5 to 1 μm in diameter. The larger indentations corresponded to the size of fat globules, and the smaller indentations corresponded to that of the cocci starter culture. Some cells (often as diplococci) were observed to still occupy some of these indentations.

Based on these observations, it appeared that the protein matrix pressed upon the rigid components of the fat-serum channels and molded around any solid object adjacent to the fat-serum channel walls. While the cheese was still warm, the fat globules provided no resistance, because the fat was still molten, and left no indentations in the fat-serum channel walls. However, as the cheese cooled, the fat globules solidified and acted as a template around which the pliable protein matrix molded.

It was also concluded from the formation of these indentations that the fat globules were closely packed in the fat-serum channels. If not, they would have provided little resistance to the protein matrix, and very shallow, if any, indentations would have been formed. Therefore, fat appears to have the important function during the cooking and stretching steps of the cheese-making process of interrupting the fusion of the protein matrix and providing space in which excess serum can be retained.

Prior to heat treatment, the cheese curd consists of a protein matrix that contains randomly distributed pockets of serum and fat (19). When the curd is heated and stretched, the protein matrix becomes molten and starts to fuse together, most likely through hydrophobic interactions. This fusion continues, unless it is interrupted by any material that is incompatible with the protein matrix, such as fat globules, bacteria, or microparticulated fat replacers (15). The mixing that occurs during stretching then causes the fat globules and much of the bacteria to be pushed together into increasingly smaller spaces until the fat globules are sufficiently packed to resist the pressure from the protein matrix. Thus, closely packed pockets of fat globules and bacteria are formed that are then oriented into channels between the protein fibers as the molten cheese is extruded and molded into its final shape. Consequently, it would be expected that the size and amount of fat-serum channels that are present in Mozzarella cheese would be a function of fat content of the cheese rather than the moisture content. Any excess moisture would be pushed out of the cheese until the fat globules are closely packed, which is one reason why increasing

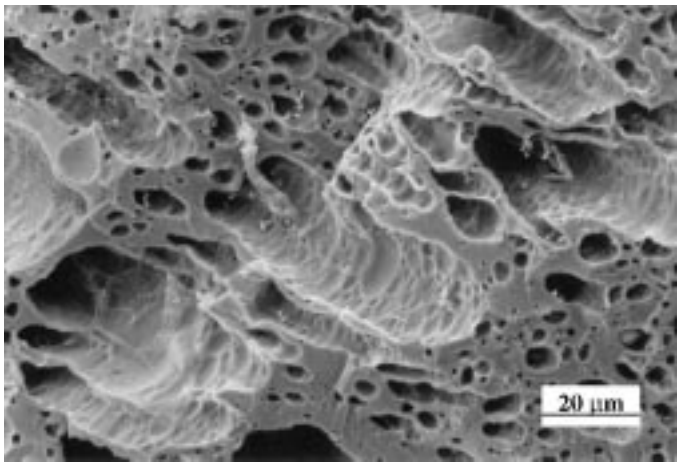

Figure 2. Scanning electron micrograph of commercial part-skim Mozzarella cheese 1 d after cooling and brining. Fat-serum channel walls show indentations formed by solidified fat globules of varying size and starter bacteria. Bacterial cells and residual fat globule membrane material adhere to the fat-serum channel walls.

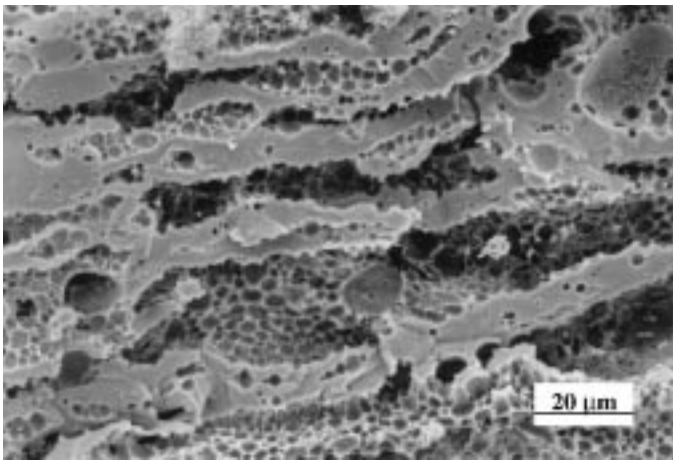

Figure 3. Scanning electron micrograph of commercial part-skim Mozzarella cheese after 7 d of storage at 4°C. Fat globule indentations are more pronounced than at d 1, indicating that the cheese protein matrix has expanded into the fat-serum channels.

the moisture content of lower fat Mozzarella cheese is difficult without also having continued syneresis of serum from the cheese after packaging.

d 7 through 21. When the microstructure of the commercial cheese was examined during refrigerated (4°C) storage, the appearance of the fat-serum channels continued to change (Figures 3 to 5). The fat globule impressions in the protein matrix surface of the channel walls became more pronounced and changed from small indentations to large depressions. By d 7 of storage, the protein matrix extended 0.5 to 1.0 μm into the fat-serum channels, so that the spherical shape of the fat globules were distinctly defined. After 14 d, the protein matrix had loosely surrounded the fat globules. Thin strands of protein material, encroaching from all sides, connected the fat-serum channel walls and partially occupied the space previously filled by the interstitial serum between the closely packed fat globules. In the micrographs of d-14-old cheese, the fat-serum channels had a honeycomb appearance.

By d 21, the interstitial spaces between the fat globules of the commercial cheese appeared to be completely filled by the protein matrix. Rather than being contained within fat-serum channels, the columns of fat globules were completely encased within the protein matrix. From the micrographs, it could be concluded that there had been no change in

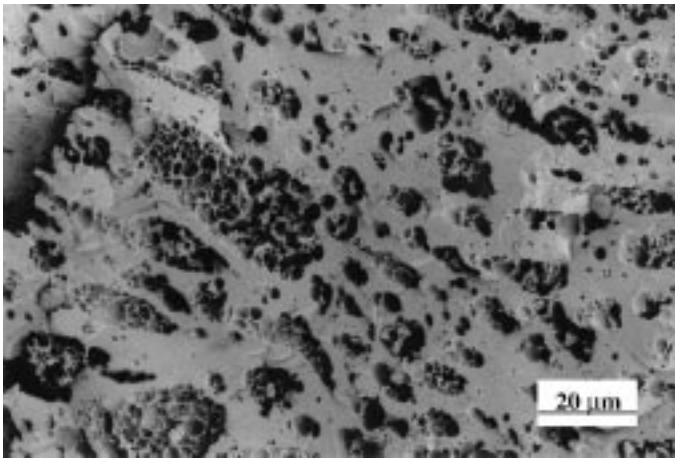

Figure 4. Scanning electron micrograph of commercial part-skim Mozzarella cheese after 14 d storage at 4°C. Thin fibers of cheese protein matrix surround many fat globules and partially occupy the inter fat globule spaces once filled by serum.

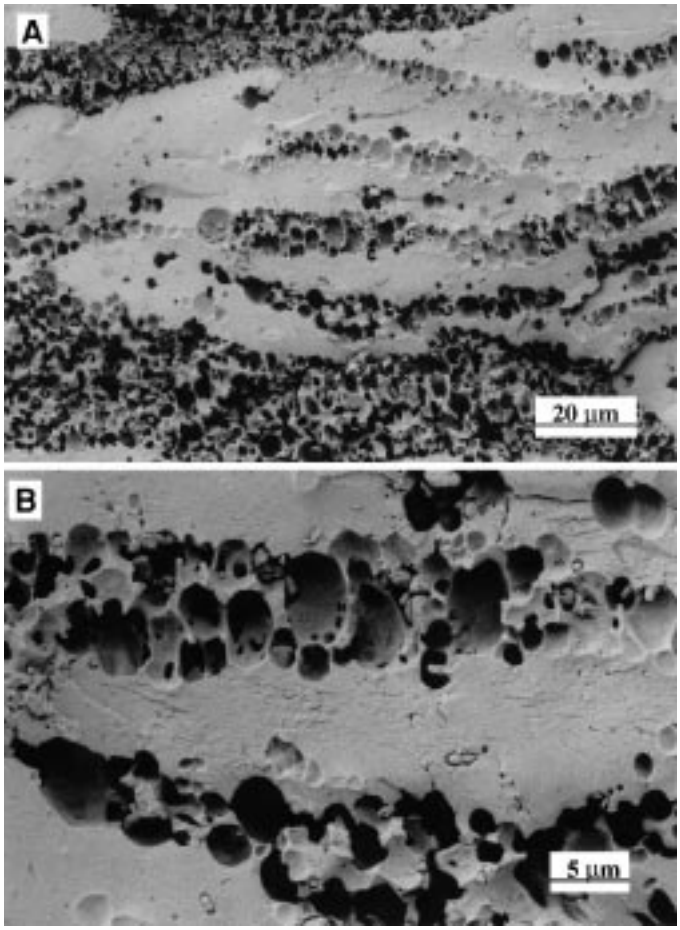

Figure 5. Scanning electron micrographs at low (A) and high (B) magnification of commercial part-skim Mozzarella cheese after 21 d of storage at 4°C. The hydrated cheese protein matrix fills the spaces between the solidified fat globules. Impressions of discrete fat globules attest to the completeness of the cheese protein matrix hydration and subsequent expansion into the fat-serum channels. Starter bacterial cells embedded in the matrix are evident.

TABLE 1. Mean (± SEM) percentages of fat, protein, ash, salt, moisture, and moisture in fat-free component (MFFC) and pH in part-skim and reduced fat Mozzarella cheese after 1 d of refrigerated (4°C) storage.

	Fat		Protein		Ash		Salt		Moisture		MFFC		pH	
	(%)													
	$\bar{X}$	SEM	$\bar{X}$	SEM	$\bar{X}$	SEM	$\bar{X}$	SEM	$\bar{X}$	SEM	$\bar{X}$	SEM	$\bar{X}$	SEM
Part skim	19.2	0.9	26.4	0.7	2.6	0.1	1.4	0.3	49.7	0.6	61.4	0.1	5.4	0.1
Reduced fat	8.0	0.9	32.7	0.1	3.2	0.0	1.4	0.1	53.3	0.2	58.0	0.2	5.3	0.1

the position of the fat globules during storage. Rather, any changes in cheese microstructure during storage were a result of the redistribution of protein and water.

Cheese Composition

Composition of the cheeses made for the study of moisture status in cheese is shown in Table 1. Actual fat contents for the cheeses were within the range expected for low moisture part skim Mozzarella cheese (i.e., 30 to 45% fat dry weight) and its 50% fat reduced equivalent. This fat reduction corresponded to increases in the protein, ash, and moisture contents that were similar to increases in other reduced fat cheese made using this method (8).

Interestingly, despite the higher moisture content in the reduced fat cheese, the moisture was 3.4% lower when expressed on a fat-free basis. When compared on a protein basis, the amount of water in the cheese decreased from 1.9 g of water/g of protein to 1.6 g of water/g of protein, which would probably have an influence on meltability of the reduced fat cheese. The increase in the ash content is probably a result of increased protein content, which would increase protein-bound minerals such as colloidal calcium phosphate, and of increased moisture (replacing fat), which would increase the total amount of soluble minerals such as ionic Ca, Na, and K. Because of its higher moisture content, the reduced fat cheese had a slightly lower salt-in-moisture content (2.6%) compared with the part-skim cheese (2.8%).

Water Partitioning

Expressed serum. When expressed on the basis of cheese weight or total moisture content (Figure 6), the control cheese contained twice as much expressible serum as the reduced fat cheese. The control cheese contained 13.3 g of expressed serum/g of cheese compared with 6.2 g of expressed serum/g of cheese in the reduced fat cheese. This serum weight represented 54% of the water in the control cheese but only 22% of the water in the reduced fat cheese. When the fat content of the cheeses was taken into account, no significant difference ($P > 0.40$) was found between the cheeses. On average, the cheeses contained 0.75 g of expressed serum/g of fat. This result further implies that the serum that can be expressed from cheese by centrifugation is associated in some manner with the fat globules.

During storage, the amount of expressible serum decreased so that by d 14, very little serum was expressed, and by 21, there was no expressible serum (Figure 6). This same trend has been previously reported by Guo and Kindstedt (12). They reported that 30 to 40% of the water in low moisture part-skim cheese was expressible after 2 d of storage. Reduction of the expressible serum to 0% occurred by d 8, 12, and 16 in the three commercially prepared cheeses that they analyzed. Based on our observations and those reported by Guo and Kindstedt (12), the majority of the expressible water initially in Mozzarella cheese appears to become impeded by the macrostructure of the protein matrix during the first 2 wk of storage at 4°C.

Guo and Kindstedt (12) concluded from increases in protein concentration in the expressed serum that a continual solubilization of casein (mainly β-CN) from the protein matrix into the expressed serum had occurred. Subsequently, they proposed that the solubilization of casein resulted from changes to the protein brought about by addition of salt to the cheese, and that the serum in the fat-serum channels became converted into a hydrated paracasein gel (14). From our observations of changes in microstructure during storage of commercial Mozzarella cheese, a different conclusion was made. It appears that serum, along with the protein contained therein, is absorbed into the protein matrix and becomes an integral part of the protein matrix by d 21. There was no observable discontinuity between the material that filled the serum channels and that of the protein fibers (Figure 5B).

In an effort to understand the processes occurring during cheese storage, we reexamined the data reported by Guo and Kindstedt (12). When expressed on a mass basis rather than a concentration basis, it became apparent that there was an initial increase in amount of caseins. The increase was then followed by

a gradual decline in protein as the volume of expressed serum decreased. The solubilized protein was predominantly β-CN, which has increased solubility at cold temperatures (5) as does α_{s1}-CN to a lesser extent. This solubilization is probably due to changes to the protein brought about by addition of salt to the cheese as suggested by Kindstedt and Guo (14).

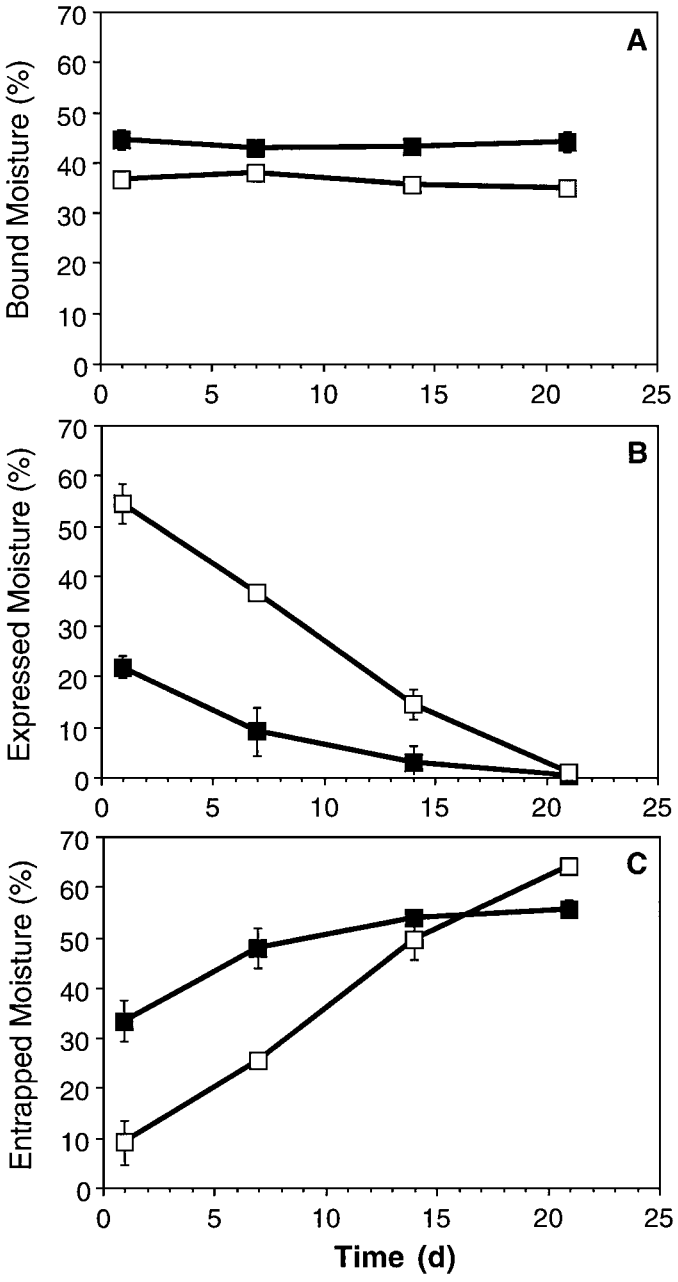

Figure 6. Percentage of moisture in control (open square) and reduced fat (closed square) Mozzarella cheese that was bound (A), expressible (B), or entrapped (C) during 21 d storage at 4°C.

Bound water. On d 1, the control cheese contained 18.1 g of bound water/g of cheese, and the reduced fat cheese contained 23.8 g of bound water/g of cheese. A similar increase in bound water content with a decrease in fat content was shown when the bound water was calculated as a percentage of the total moisture in the cheese (Figure 6). However, when the protein content of the cheeses was taken into account, no significant difference ($P > 0.50$) was found between the cheeses, and that on average, they contained 0.71 g of bound water/g of protein.

During the 21-d storage, the level of bound water in cheese remained constant. Therefore, the process of transfer of water from the fat-serum channels into the protein matrix was not being driven by an increase in the amount of water that was chemically bound to the proteins. Instead, some other driving force must be causing the transfer of water into the protein matrix.

Entrapped water. At d 1 of storage, the reduced fat cheese contained a greater proportion of its water as entrapped water than did the control cheese (Figure 6). This result was expected because 1) the higher protein content of the reduced fat cheese means there is more protein matrix per unit weight of cheese, and 2) the higher fat content of the control cheese results in the presence of more fat-serum channels interspersed throughout the protein matrix of the control cheese than occurs in a reduced fat cheese (18). During storage, the amount of entrapped water increased for both cheeses and indicated that the expressible water was being absorbed into the protein matrix.

Recently, Cooke et al. (4) demonstrated that the spaces between electron-dense centers (i.e., the protein subaggregates that compose the backbone of the protein matrix) shown in transmission electron micrographs of low fat Mozzarella cheese increased with storage time (6 wk). They postulated that the increase in spacing, as well as an increase in the size of the electron-dense center, was a result of a reorganization of the protein matrix in response to proteolysis. More recently, Paulson et al. (21) showed that the spaces between electron-dense centers (protein subaggregates) of nonfat Mozzarella cheese changed in response to salt concentration. It is our hypothesis that the increase in spacing between protein subaggregates results from a reorganization of the protein matrix brought about by salting of the cheese and subsequent absorption of water from the fat-serum channels into the protein matrix during refrigerated storage. The spaces between the protein subaggregates would represent regions of entrapped water within the protein matrix.

Because of the larger reservoir of water contained in the fat-serum channels of the control cheese, there was more water that could be absorbed into the protein matrix in the control cheese than in the reduced fat cheese. Although physically impeded by the macrostructure of the protein matrix, entrapped water is available to participate in both protein hydration and to act as a solvent. Such entrapped water is considered to be part of the bulk water phase in cheese because the water molecules retain the anisotropic properties of free water, even though the water molecules cannot be expressed by centrifugation (i.e., their rotational and translational speeds are not significantly affected by their interactions with the proteins). If it is assumed that there is no difference in the proteins between the control and reduced fat cheeses, the challenge for the cheesemaker is to produce reduced fat cheeses with the same water to protein ratio as the reference cheese. To do this would require that a similar reservoir of water was initially present in the reduced fat cheese even though the volume occupied by fat-serum channels decreases as fat content is lowered.

Melt

The meltability of the control and reduced fat cheese during 21 d storage is shown in Figure 7. The control cheese melted to a greater extent than did the reduced fat cheese, although the difference between the cheeses diminished as storage time increased. These observations are similar to previously reported trends for both low moisture part-skim, reduced fat, and low fat Mozzarella cheese (8, 12, 15, 17, 26).

The largest increase in meltability of the control cheese was from d 1 to 7, followed by minimal increase from d 7 to 21. The meltability of the reduced fat cheese also increased during storage with the largest increase occurring from d 1 to 14. There was only a slight change in meltability of the reduced fat cheese from d 14 to 21. This change in meltability corresponds to the transfer of water from the fat-serum channels to the protein matrix of the reduced fat cheese being virtually complete by d 14.

The improvement in cheese meltability during the first 21 d of storage can also be explained in terms of changes in water and protein states in the cheese. At the beginning of storage, the cheeses had the least meltability. This phenomenon suggests that, when heated, relatively strong interactions are maintained between protein molecules within the protein matrix. The cheeses resist the tendency to flow even though expressible water and fat are present within the fat-serum channels. Thus, the presence of considerable free water and fat in the fat-serum channels is not

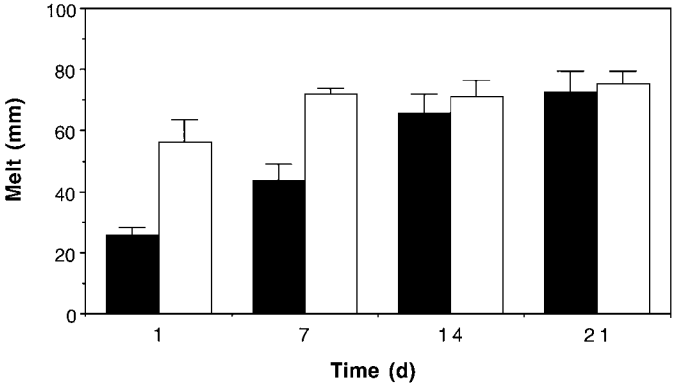

Figure 7. Meltability after 12 min at 95°C of control (open bar) and reduced fat (solid bar) Mozzarella cheese that was stored for 21 d at 4°C.

enough to ensure good meltability. During the initial weeks of storage, water is transferred from the fat-serum channels into the protein matrix as the proteins become more hydrated, and some interactions between proteins are replaced with interactions of proteins with the bulk phase water molecules. This increased hydration of proteins has been attributed to salting-in of proteins in the cheese (21). During most of the cheese-making process, the protein matrix of the renneted curd is dehydrated through the action of cutting, agitation, heating, and acidification of the curd. It appears (21) that the presence of added salt results in the protein becoming more hydrated and less tightly aggregated. As the proteins that constitute the matrix became more hydrated, their hydrodynamic volume increases, and the matrix begins to extend into the spaces between fat globules in the fat-serum channels. In regard to the functionality of the cheese, a more hydrated protein structure would allow the proteins to slip past one another more easily and, when combined with the lubricating properties of the fat (25, 26), result in improved meltability. Thus, the overall meltability of Mozzarella cheese can be considered as the combined effects of 1) fat content and 2) the balance between protein-to-protein interactions and interactions between proteins and the bulk water entrapped within the protein matrix.

An initially high level of interactions between proteins would restrict the ability of the proteins to flow when heated. Then, as expressible water is absorbed into the protein matrix, increased protein hydration allows the proteins to flow more easily when heated and results in improved meltability. Further improvements in meltability could therefore be obtained by allowing the protein matrix to become fully hydrated. It would appear that this state of full hydration was

not reached in the reduced fat cheese because the initial reservoir of water contained in the fat-serum channels was insufficient for the quantity of protein present in the cheese.

CONCLUSIONS

During the first 3 wk of storage, the expressible water contained within the fat-serum channels of Mozzarella cheese was absorbed into the cheese protein matrix where it became entrapped water. Presumably, this change was caused by a rearrangement of the protein molecules contained within the protein matrix that was induced by salting and refrigeration of the cheese. The water absorption was accompanied by a swelling of the protein matrix, which continued until the spaces between the fat globules were completely filled with matrix proteins. The increase in entrapped water during storage was limited in the reduced fat cheese because of the lower volume of expressible water that was initially present in the fat-serum channels. Improvements in the meltability of the cheese occurred concomitantly with the matrix proteins becoming more hydrated. This phenomenon implies that a reduction in the extent of protein-to-protein interaction within the protein matrix is a principal reason for increased meltability of Mozzarella cheese during storage.

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