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MICROSTRUCTURE OF MOZZARELLA CHEESE DURING MANUFACTURE

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Abstract

Scanning electron microscopy was used to examine structural developments during the manufacture of low-moisture, part-skim Mozzarella cheese made by a stirred curd procedure. The micrographs showed changes in the protein matrix, dispersion of fat globules, and bacteria during processing. Most curd knitting occurred during the curd stirring step, particularly dry stirring. A thin (5 μm) curd skin was observed on curd particles at the end of dry stirring. Dry salting prior to stretching resulted in the rapid loss of whey from the curd particle. Protein fibers were aligned and longitudinal columns of whey and fat were formed when the cheese was stretched and molded. Bacteria were initially dispersed throughout the protein matrix, but after stretching, most of the bacteria were located at the fat-whey/protein interface. Brining cooled the cheese causing the fat globules to solidify and additional whey to be expelled, resulting in fat globule indentations in the protein matrix. These micrographs can be used to monitor changes in curd structure when Mozzarella manufacturing procedures are modified. An understanding of the protein/fat interactions and curd structure development during manufacture will help optimize the physical properties of reduced-fat, low-fat, and non-fat Mozzarella cheese.

Introduction

Quality of Mozzarella cheese depends on its physical properties, particularly stretch, melt, cook color, and oiling off during baking (Alvarez, 1986). These properties are derived primarily from the interaction of casein and fat in the cheese matrix. The composition of the cheese, degree of proteolysis, storage time, and storage temperature also influence these physical properties (Parkye et al., 1991; Masei and Addeo, 1986; Oberg et al., 1991, 1992a, b; Tunick et al., 1991). Surveys concerning Mozzarella cheese quality indicate that improvement in manufacturing, resulting in a more consistent product with predictable physical properties, is needed (Nilson and LaClair, 1976; Pilcher and Kindstedt, 1991).

Kalab (1977) examined the microstructure of several brands of commercially manufactured Mozzarella cheese. He found differences in the microstructure of stirred curd Mozzarella and conventionally stretched Mozzarella, particularly in the shape of the curd granules. Stretched Mozzarella had no curd junctions and a definite curd orientation. Paquet and Kalab (1988) also studied the structure of stirred curd and stretched Mozzarella noting a parallel orientation of protein fibers in stretched Mozzarella that was lacking in stirred curd Mozzarella. Fat globules in stirred curd Mozzarella appeared to be evenly distributed, but not in stretched Mozzarella. Taranto et al. (1979) also found similar structures in their study of Mozzarella cheese. While studying the effects of draw pH on the microstructure of Mozzarella cheese, Kiely et al. (1992) showed that significant changes in curd structure occur during manufacture, especially during stretching. Taneya et al. (1992) attributed stringiness in string cheese to the uniform longitudinal orientation of the protein matrix. Only Kiely et al. (1992) followed curd structure development through the manufacturing process.

Understanding the microstructure of Mozzarella cheese, particularly how protein and fat interact during and after manufacture, can provide information useful in determining what constitutes a quality product. In addition, this understanding can indicate how to process reduced-fat, low-fat, and non-fat Mozzarella cheeses,
since changes in the fat content of Mozzarella cheese significantly affects textural and melt properties (Tuckey, 1974; Tunick et al., 1991). This study examined the development of curd structure through the manufacture of low-moisture, part-skim Mozzarella cheese made with a stirred curd procedure. It also documented the effects of dry curd stirring and dry salting prior to stretching, and changes in curd structure following stretching, molding, and brining.

Materials and Methods

Mozzarella Manufacturing Procedure

Mozzarella cheese was manufactured in double O vats, 25,000 kg of milk per vat, in a commercial cheese plant. One hundred and seventy liters of starter culture produced by external pH control in a milk-based medium were used, with a four to one ratio of cocci (Streptococcus salivarius ssp. thermophilus) to rods (Lacticoccus helveticus) by weight. Cultures were grown separately and combined prior to inoculating the cheese vat. Following starter addition, the milk was set with fermentation-produced chymosin at 34.4°C. After a 30 minute set, curd was cut in one direction only and allowed to heat for 10 minutes. The curd and whey were then heated to 40.5°C over 30 minutes. A portion of the whey was drained and the curd was stirred at 40.5°C for another 45 minutes. Curd and whey were then pumped to a finishing vat where the whey was drained at a pH of between 6.2 and 6.3, and the curd was dry-stirred until a pH of 5.3 was reached. Curd was then dry-salted and sent to the stretcher. Pre-salting resulted in a salt level of 0.7% to 0.8% in the curd when it came out of the stretcher. Water temperature in the stretcher was 61.7°C and curd temperature increased to 50 to 54°C during its 2.5 to 3 minute exposure. Melted curd was packed into 2.3 kg molds and cooled in a 6.7°C water bath for 1 hour. Cheese loaves were then removed from the molds and soaked in saturated brine solution at 15.6°C for 3 hours before storage at 5°C.

Curd Sample Preparation

Samples were taken at various stages of manufacture following two vats through the entire process. Micrographs showed little difference between the vats so only one vat was chosen for this study. Curd particles were removed during cooking, before and after dry-stirring, dry-salting, stretching, and brining. Curd particles were either removed with a small stainless steel collection vial or sliced off with a sterile blade. The samples were cut into 3 mm x 3 mm x 10 mm pieces and immersed in 2% glutaraldehyde in a 0.001 M sodium phosphate buffer for 2 hours. Samples were then placed in small glass vials, filled with a fresh 2% glutaraldehyde solution in the same buffer, and stored at 5°C.

Scanning electron microscopy

Freon 22 was solidified by cooling in a double walled container, jacketed with liquid nitrogen. Pressing a metal rod into the frozen Freon 22 formed a pool of liquid, in which the glutaraldehyde-fixed Mozzarella curd samples were immersed and rapidly frozen. Each sample was transferred into liquid nitrogen and was fractured perpendicular to its long axis. The fragments were then thawed in the glutaraldehyde solution. They were subsequently rinsed with water for 5 minutes, dehydrated in an ethanol series to 100% ethanol (30% - 50 - 70 - 95 - 100 - 100% - 100%, 10 minutes in each solution), transitioned into Freon 113 (1:3, 1:1, 3:1, pure, 10 minutes in each solution) and stored. During this extraction step, milk fat was extracted from the samples. Unless specified, these procedures were conducted at room temperature. The following morning, the fragments were rehydrated with distilled water, step-wise, using an inverse set of times and concentrations of the previous dehydration and transition, followed by buffer rinse in 0.1 M sodium cacodylate buffer, pH 7.2 (1 x 5 minutes), and postfixation in 1% OsO₄, 1.5% K₃[Fe(CN)]₆ (O-F solution) in the same 0.1 M sodium cacodylate buffer for 2 hours. The O-F solution was replaced with 2% tannic acid (w/v) in 0.1 M sodium cacodylate buffer and the samples were stored overnight. The following morning, the tannic acid was replaced with O-F solution for 4 to 8 hours. This solution was then replaced with a 1% aqueous hydroquinone solution and stored overnight.

The following morning, the samples were washed in distilled water (4 x 5 minutes), dehydrated with ethanol, and transitioned to Freon 113 as before, followed by critical point drying in Freon 13. Specimens were mounted with a clear fingernail polish, so that the fractured surfaces faced upward. Specimens were coated with 2 nm of iridium using an ion beam sputtering system (VCR Group, San Francisco, CA) at a 90° tilt, 75% maximum speed, and 75% maximum rotation.

The specimens were viewed in a Hitachi S-4000 field emission scanning electron microscope operated at 10 kV, 0.01 nA at sample, a condenser setting of 10, the smallest objective aperture, and a working distance of 15 mm. Images were recorded on Polaroid P/N 55 film at a photo speed of 80 seconds.

Results and Discussion

Mozzarella cheese examined in this study was a commercially manufactured low-moisture, part-skim Mozzarella cheese in which a stirred-curd step was used during acid production. In addition, the curd was dry-salted prior to stretching. The cheese had a moisture of 48.8%, pH of 5.16, fat on dry basis (FDB) of 37.1%, and a salt level of 1.3%.

Scanning electron microscopy (SEM) was used to study curd structure at different steps in the manufacturing procedure. The fat and water (whey) were removed during sample preparation, leaving only the protein matrix and bacteria to be visualized. Therefore, the vacuoles in the curd structure were sites where either fat or whey had been located.

Figure 1 shows the curd structures at 10, 20, and 40 minutes after the curd was initially heated to 40.5°C. Curd particles were suspended in the whey and were
continually stirred during this period. Aggregation of casein micelles during cooking was evident, and fat globules and whey pockets were uniformly dispersed. A significant reduction in porous structure was seen over the 30 minute time span at which the curd was sampled.

Figure 1. Scanning electron micrographs of Mozzarella curd sampled at 10 (a, b), 20 (c, d), and 40 (e, f) minutes after curd was heated to 40.5°C. In Figures 1a and 1b, arrows point to micelle structure in the protein matrix. In Figure 1f, the short arrow indicates fused protein matrix and the long arrows point to dividing bacterial cells.
Figure 2. Scanning electron micrographs of Mozzarella curd taken just before and after the dry stirring step. In Figure 2a, the arrow points to a fat globule/whey pocket. In Figure 2b, the long arrow indicates a fat globule indentation and the short arrow a micro-colony of bacteria.

Individual micelles were visible when the curd was first heated (Figures 1a,b). Micelles were fused into chains, but the micelle-based structure was still visible. As the curd was stirred during cooking, continued micelle aggregation and protein knitting occurred (Figures 1c,d) until the curd had lost all of the globular micelle structure (Figures 1e,f). Bacteria were difficult to distinguish in the early curd samples (Figures 1a,b), probably because they were not evenly distributed in the curd at this stage in manufacture or because cell numbers were too low at this point in the manufacturing process. After curd was held at 40.5°C for 40 minutes, bacteria were seen entrapped in small whey pockets throughout the protein matrix of the curd.

The effect of syneresis of whey from curd particles could be seen as the protein structure became more compact during heating (Figures 1b,d,f). Micelles were compressed into a uniform protein matrix at this stage.

Figure 3. Scanning electron micrographs of Mozzarella curd taken prior to dry salting, following dry salting, and following brining. In all three figures, the short arrows indicate the surface of the curd particle, while the long arrows point to vacuoles in the curd matrix where fat and whey are found.
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Figure 4. Scanning electron micrographs of Mozzarella curd taken after it has been through the stretcher. (a) Longitudinal view (long arrows point to micro-colonies of bacteria, small arrow indicates fibrous material, and the medium arrow points to the smooth protein surface); and (b) cross-section view (arrows indicate fat/whey columns).

Figure 5. Scanning electron micrographs of Mozzarella cheese curd immediately after stretching (a) and following one day of storage (b). In Figure 5a, the long arrow points to a fat/whey column and the short arrow indicates the smooth protein surface. In Figure 5b, arrows indicate indentations of fat globules in the protein matrix.

Dividing bacteria were also visible in the sample taken 40 minutes after the curd was heated (Figure 1f). At this point in the manufacturing, temperature and growth conditions were optimal for bacterial growth, explaining the increase in bacteria seen during heating.

After heating the curd and whey to 40.5°C, one third of the whey was removed. As shown in Figure 2, considerable curd knitting occurred as the curd was stirred for 1.5 hours (Figure 2a compared to Figure 2b). The curd was dry-stirred until the pH reached 5.3. In both curd samples, bacteria were distributed throughout the curd matrix. The majority of bacteria seen are cocci. Curd compression and knitting continued during dry stirring, and the size of whey/fat pockets were markedly reduced due to syneresis. Vacuoles were round and evenly dispersed. As syneresis continued, the shapes of the fat globules, imprinted in the protein matrix, became clearer (Figure 2b). Micelle structure was no longer visible at the finish of dry stirring and the protein matrix became very dense. Fat globules coalesced as the curd shrank and became compressed around the fat globules.

Changes on the surface of curd particles before and after dry-salting, and after brining are shown in Figure 3. Cross-sections through the edges of individual curd particles were scanned at a lower magnification to show changes on the surface. The curd skin was also clearly visible and was only 10 μm thick (Figure 3a). There was continued loss of open structure, associated with continued expulsion of whey. Since dry-salting took only a few minutes, the loss of water/whey from the curd particle surface was very rapid (Figure 3b). The body of the curd was slightly more compact after the 3 hour brining step (Figure 3c). Some of the textural changes in the brined sample were also due to stretching. These micrographs show the dramatic changes in curd structure during manufacture.
Lateral (Figure 4a) and cross-section (Figure 4b) views of Mozzarella cheese curd after it passed through the stretcher clearly show the formation of a fibrous structure in the cheese. During stretching, curd was placed in 61.7°C water for 2.5 to 3 minutes, and curd temperature increased to 49 to 54°C. The molten curd was then pressed into 2.3 kg loaf molds and placed in a cold water bath (6 to 7°C) for 1 hour. Long strands of protein were visible in the lateral section (Figure 4a) with large areas of fat/whey accumulation between the strands. The fat and whey apparently were squeezed into long column-like structures as the protein fibers were compressed and elongated in the stretcher. Milk-fat would be molten at stretcher temperatures and coalesce where possible into these column-like structures. A cross-section (Figure 4b) shows that the diameter of the columns of fat/whey in the stretched curd were irregular.

There were large numbers of bacteria on the surface of the protein strands (Figure 4a). The bacteria were intact and showed the characteristic diplococcus arrangement. At higher magnification, the protein surface appeared very smooth, suggesting the fat was in the liquid state. Clumps of fibrous material that were associated with the coccoid bacteria (Schellhaass and Morris, 1985), might be exocellular polysaccharide, although the cocci used are not a typical ropy strain, or residual fat globule membranes.

Bacteria are now seen at the fat/protein interface instead of evenly dispersed throughout the curd matrix. Bacteria appeared to be concentrated along the outside surface of the protein, as if they were entrapped or suspended in whey at this interface, or were pressed between the fat and protein layers in the cheese. Very few bacteria were seen entrapped in the protein matrix. Figure 5 shows the structure of Mozzarella cheese immediately after removal from the stretcher (Figure 5a) and after 1 day of storage (Figure 5b). Cheese aged 1 day had more matted protein fibers, which contained columns of fat and whey. Very few bacteria were embedded in the protein matrix; the majority were at the fat/protein interface.

Longitudinal sections of the interior of 1-day-old Mozzarella cheese contained fibers of protein with numerous surface indentations (Figure 5b). The space between these protein fibers result from the extraction of compressed fat globules and a small amount of whey during sample preparation. These indentations would have been formed as cooling protein fibers pressed against solidifying fat globules during brining and cold storage. Some additional whey expulsion may also have played a role. Since proteins were fused with glutaraldehyde before the fat was extracted, the imprint of the fat globules remained on the protein surface. It appeared that liquified fat globules, suspended in the whey, retained some structural integrity. Upon cooling the fat globule returned to its defined spherical shape, which imprinted on the protein fibers.

The new sample preparation techniques provided exceptionally clear electron micrographs of Mozzarella curd development during manufacture. Due to increased resolution, more detail can be seen than in previously published micrographs of Mozzarella cheese (Kalab, 1977; Kiely et al., 1992; Taneya et al., 1992). Knitting of casein micelles during and after set, and during the cooking steps were clearly visible. In addition, bacteria and their distribution could be easily followed. The casein micelle structure had entirely disappeared in the protein matrix when the curd was stirred in the vat. Dry stirring did not have a significant impact on distribution of fat and protein in the curd. Fat and whey pockets were evenly distributed in the protein matrix and there was a very thin curd skin formed during dry stirring. Starter culture bacteria had also multiplied and microcolonies had formed throughout the curd matrix. Dry salting prior to stretching rapidly expelled whey from the surface of the curd particles and increased the density of the protein matrix.

Curd structure was affected most by the hot water bath and mechanical stretcher (Kiely et al., 1992). The protein matrix was extensively modified to form a bundle of long strands that contained columns of melted fat suspended in whey. Fat and bacteria in these columns were forced out of the protein matrix and into these fat/whey columns. Paquet and Kalab (1988) also observed an uneven distribution of fat in the curd of stretched Mozzarella cheese. After curd had been through the stretcher, bacteria were found primarily in the interface between the fat and protein layers. Bacteria appeared to be alive and very few lysed cells were observed. After a 3 hour brining step and 1 day of refrigerated storage, the fat solidified and additional whey was lost from the curd. Imprints of fat globules were visible in the protein matrix and fat and protein retained a columnar structure.

A reduction in milk fat could have an effect at several points in the manufacture of Mozzarella cheese. Yang and Taranto (1982), Tuckey (1974), and Tunick et al. (1991) found that changes in the fat content of Mozzarella cheese can change the physical properties of cheese, particularly melting properties. Changes in fat content will affect protein aggregation as soon as the curd is cut and becomes increasingly significant as the protein structure develops. In addition, decreased fat levels may affect the location of bacteria in the curd, which are now found at the fat/protein interface after stretching. Further SEM examinations of reduced-fat Mozzarella will identify ways in which the manufacturing process can be modified to enhance the physical properties of reduced-fat, low-fat, and non-fat Mozzarella cheese.

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References


Discussion with Reviewers

R.K. Thunell: It was suggested in the conclusion of the article that further SEM examinations will identify ways in which the manufacturing process can be modified to enhance the physical properties of Mozzarella cheese. What improvements to manufacturing could be suggested, based on these first micrographs?

Authors: These initial micrographs provide a baseline for further studies. As changes are incorporated in the manufacturing procedure and fat levels are reduced in the cheese, then improvements should become evident.

R.K. Thunell: Does cheese microstructure change significantly from starter culture to starter culture, or is microstructure more a function of cooking/stretching?

Authors: Previous research has shown that when different strains of starter culture are used, particularly when the strains differ in the degree of proteolysis of the rod component, changes the physical properties of the cheese during aging. In this study we did not examine any aged samples. Most observed changes in the microstructure during manufacture can be directly attributed to the various steps in manufacture, i.e., compaction and loss of micelle structure during syneresis and the elongation of protein fibers during the stretching step.

R.K. Thunell: How would the cheese microstructure be altered by higher rod numbers, and greater proteolysis?

Authors: Previous studies have shown that increased proteolysis by the starter cultures does affect the physical properties of Mozzarella cheese as it ages. We do not think that much difference in the structure could be observed during the manufacturing process as the time is too short for extensive proteolysis to occur. Often, highly proteolytic cultures are also very active acid producers, and if this is the case, then accelerated acid production might affect the way in which the protein knits during manufacture.

R.K. Thunell: How would cheese microstructure be altered by higher fat concentrations?

Authors: Increased fat concentrations in the cheese would increase the size and number of fat columns seen in the stretched cheese and probably weaken the protein matrix making it more difficult for the protein to retain fat. In addition, increased oiling-off would occur during cooking. We are most concerned about the effects of

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decreased fat concentrations in the cheese on the microstructure and physical properties. A denser protein matrix with fewer fat columns should change the melting and stretching characteristics, probably in an adverse way.

D.G. Pechak: The presence of fat globule membranes in cheeses and their relationship to the original milk fat globule membrane has remained an unresolved issue. Using your techniques and higher resolution SEM, can you comment further on the detailed structure or origin of these structures in this cheese?

Authors: During sample preparation, the fat is extracted from them, so it is difficult to make specific conclusions related to location or nature of the fat globule membrane. Not only is there collapse of the membrane structure, but depending on the location and nature of the fracture surface of the sample examined, the membrane remnant can either remain entrapped in the sample matrix or be washed away. This can be observed in Fig. 5b.

D.G. Pechak: When did you first see an indication of curd boundaries forming? The structure is clearly visible in Fig. 3a, but at what point in the process of curd formation was it first observed? The formation of this boundary may play an important role in retaining unbound fat and/or whey proteins within the curd unit.

Authors: Strictly speaking, the structure shown in Fig. 3a is not a curd boundary but rather a cross-section through the edge of a curd particle. In pressed cheese (such as Cheddar), it would result in the formation of a curd boundary. Curd boundaries were observed during sample preparation after glutaraldehyde fixation, but those specific regions were not examined in this study.

P.S. Kindstedt: Progressive aggregation and fusion of paracasein micelles during cooking (Fig. 1a-f) may be related to loss of calcium from the curd as pH decreased. Kiely et al. (1992) reported a similar loss of micellar identity during cooking with decreasing pH and an increasing calcium loss. Would you comment on the factors that you believe might be responsible for the observed changes in micellar aggregation during cooking and specifically the role of acidification?

Authors: The loss of micellar identity during cheesemaking has long been observed (Green et al., 1981). When considering the effect of acidification on the changes in microstructure of cheese curd, we have observed that when rennet and acid coagulation are combined, their effects on aggregation are additive (McMahon, 1989). Thus, dissociation of calcium, phosphate, and protein from the casein micelles would follow the same pH profile in renneted milk as it does in acid-coagulated milk.

P.S. Kindstedt: Your micrographs suggest that during stretching, fat and whey were squeezed into long column-like pools containing water and liquid fat. The behavior of the fat upon cooling is intriguing. Instead of separating into water and oil phases, the fat apparently was emulsified as relatively small droplets or globules which, upon cooling and solidifying, left imprints on the protein matrix (Fig. 5b). We have observed similar imprints of fat globules within the columns of bulk phase water in stretched, cooled LMPS Mozzarella. Would you care to speculate on whether the original milkfat globule membrane remained intact during cheesemaking and subsequently maintained the emulsion stability during stretching and cooling, or whether other factors may have influenced the emulsion? What are the implications for the oiling off characteristics of the final cheese, and how might this be related to the increase in free oil formation that characteristically occurs in Mozzarella cheese during the first weeks of aging?

Authors: As a rough approximation obtained by measuring the size of the indentations in Fig. 5b, the fat droplets have sizes of 1-5 μm. This would suggest that some reduction in fat droplet size has occurred during cooking and stretching of the cheese curd. This would result in an increase in fat droplet surface areas above which is covered by the native milkfat globule membrane. For the fat droplets to remain stable, it would be essential for casein molecules to be adsorbed at these new fat/water interfacial surfaces. It is thus possible that as proteolysis occurs during storage, this casein could be hydrolyzed and thus allow a breakdown of emulsion stability.

P.S. Kindstedt: Fresh melted Mozzarella can literally be wrung out like a sponge because a significant portion of the total moisture content is held very loosely within the curd. It is likely that the poor water-binding characteristics of fresh Mozzarella are related to the pooling of whey into large columns between the elongated protein fibers during stretching, as shown in Figures 4a and 5a. However, water-binding increases dramatically during the first week of aging. Could you speculate on what happens to the columns of pooled bulk phase water during short-term aging that leads to increased water-binding?

Authors: The water is slowly absorbed back into the protein matrix and bound by the proteins. Stretching is a harsh treatment that forces the water out of the protein matrix and into the fat columns. As the fat solidifies, the water is forced away from it and back into the protein fibers.

M. Tunick: Which aspects of your sample preparation technique were responsible for the enhanced clarity of your micrographs?

Authors: We used a new metal impregnation technique, the tannin-ferrocyanide-osmium procedure, in place of conventional procedures. In addition, we examined the samples in a high resolution cold field emission scanning electron microscope so we could use a much thinner metal coat, thus seeing fine structural detail. The protein matrix must resist electron beam damage. The entire procedure has been described in greater detail in a separate manuscript now undergoing reviewing for publication in Food Structure by W. R. McManus et al.