



Effect of freezing and frozen storage on microstructure of Mozzarella and pizza cheeses

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ABSTRACT

Scanning electron microscopy was used to assess the effect of aging before (2, 7, and 14 days at 7 °C) or tempering after (1, 7, and 14 days at 7 °C) freezing, and frozen storage (1 and 4 weeks at −20 °C) on protein matrix of pasta filata Mozzarella and non-pasta filata pizza cheeses using unfrozen samples as controls. Pores and ruptures of reticular structure were observed in frozen-stored pasta filata Mozzarella cheese protein matrix, but cracks and clumps of bacteria were found in frozen-stored non-pasta filata pizza cheese. No obvious differences were discernable between the microstructures of pasta filata Mozzarella cheeses frozen stored 1 and 4 weeks. Formation of the reticular structure in frozen-stored pasta filata Mozzarella cheese progressed during tempering. Microstructure of non-pasta filata pizza cheese frozen stored for 4 weeks contained more extensive cracking and more areas of clumps of bacteria than that was frozen stored for 1 week. Aging of cheese before frozen storage was considered responsible for microstructural cracking; fewer cracks were found in the frozen-stored cheese tempered 1 and 2 weeks, but the clumps of bacteria were still observed.

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1. Introduction

Frozen storage of Mozzarella cheese just after its manufacture is of significant commercial interest as a means of arresting physicochemical changes in cheese during ripening and to extend its shelf-life. However, past research indicates some adverse effect on Mozzarella cheese texture, rheological and functional properties, and proteolysis due to freezing (Bertola, Califano, Bevilacqua, & Zaritzky, 1996a, 1996b; Cervantes, Lund, & Olson, 1983; Dahlstrom, 1978; Diefes, Rizvi, & Bartsch, 1993; Kuo & Gunasekaran, 2003; Oberg, Merrill, Brown, & Richardson, 1992). The textural changes during freezing could lower the end product quality.

It is well known that cheese texture, determined by chemical composition and physical properties, is largely a function of cheese microstructure (Emmons, Kalab, Larmond, & Lowrie, 1980). The microstructure of Mozzarella cheese has been studied by transmission electron microscopy (TEM), confocal scanning laser microscopy (CSLM), and scanning electron microscopy (SEM) to determine the changes occurring during cheese making (Auty, Twomey, Guinee, & Mulvihill, 2001; Kalab, 1977; Kiely et al., 1992, 1993; Masi & Addeo, 1986; Paquet & Kalab, 1988; Taranto, Wan, Chen, & Rhee, 1979). However, there have been few investigations

reporting the changes in the microstructure of cheeses during frozen storage (Fontecha, Kalab, Medina, Pelaez, & Juarez, 1996; Perez-Munuera, Estevez, & Lluch, 1999), and only one pertinent to Mozzarella cheese (Reid & Yan, 2004). The objective of this research was to investigate the effect of freezing on the microstructure of pasta filata Mozzarella and non-pasta filata pizza cheeses.

2. Materials and methods

2.1. Cheese manufacture

Low moisture, part-skim (LMPS) pasta filata Mozzarella and non-pasta filata pizza cheeses (Chen & Johnson, 1999) that have characteristics similar to the traditional LMPS Mozzarella cheese were manufactured in the Wisconsin Center for Dairy Research pilot plant at University of Wisconsin-Madison.

2.1.1. Pasta filata Mozzarella cheese

Milk was standardized to 2.5 g/100 g milk fat, pasteurized at 72.2 °C for 17 s, and held at 34.4 °C. Milk was then inoculated by adding the starter culture, one-to-one ratio of *Streptococcus thermophilus* C 90 to *Lactobacillus bugarius* R 160 (Rhodia, Madison, WI). Chymosin (Chymostar, Rhodia, Madison, WI) of 20 mL was added when the pH of milk decreased by 0.1. After 30 min, the curd was cut with 0.95-cm knives and allowed to heal for 15 min. The curd and whey were then stirred at 41.1 °C for 30 min, and the whey was

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drained. After the whey was removed, the curd was ditched and cut into slabs. The slabs were turned and piled double immediately and held until pH dropped to 5.2–5.3. The curd was then milled and dry-salted. The salted curds were mechanically heated, stretched, and molded under 76.7 °C hot water and then formed into 2.2-kg loaves. The loaves were immersed in cold water for 1 h, then in 23 g/L brine for 90 min, and stored in vacuum-sealed barrier bags (VF-400, Vilutis & Co. Inc., Frankfort, IL) at 6–8 °C.

2.1.2. Non-pasta filata pizza cheese

Milk was standardized to 2.5 g/100 g milk fat, pasteurized, and pre-acidified with acetic acid to pH 6.3. The cheese milk was heated to 34.4 °C and inoculated with a mesophilic starter culture (DVS 970, Chris Hansen, Inc., Milwaukee, WI), which has a slower rate of acid production than the mixed culture used for manufacturing pasta filata Mozzarella cheese. Cheese milk was then ripened for 1 h until pH dropped by at least 0.5 and 8 mL of chymosin (Chymostar, Rhodia, Madison, WI) was added. The curd was cut with 0.95-cm knives 20 min after coagulant addition. Curds were heated for 10 min and then stirred at 36.7 °C for 20 min. After the whey was removed, the curd was rinsed with 12.8 °C water for 20 min. The water was then drained. The curd was salted, ladled into sanitized hoops, and pressed for 4 h. The cheese was stored in vacuum-sealed barrier bags (VF-400, Vilutis & Co. Inc., Frankfort, IL) at 6–8 °C.

The composition of the cheeses made was analyzed as described in Kuo, Gunasekaran, Johnson, and Chen (2001).

2.2. Experimental design

A single vat of each cheese was produced for this study. Cheese loaves were cut into 5 × 10 × 7-cm blocks. The cut cheese blocks were vacuum sealed in plastic cheese packing bags, divided into four equal groups, and stored in refrigerator (Model 326R-2, Frigidaire Commercial Products Co., Convey, AR) at 7 °C until the freezing tests. One group was used as unfrozen control and the other three were frozen and stored at –20 °C in a conventional freezer (Kenmore, Model 253.918321, Sears, Roebuck and Co., Hoffman Estates, IL) by taking each group at 2, 7, and 14 days after manufacture. Cheese blocks were removed from freezer after 1 and 4 weeks and then thawed at 7 °C. Thawed cheese blocks were tempered in the refrigerator for 1, 7 and 14 days. Unfrozen cheese samples were stored at 7 °C for 2, 7, and 14 days.

2.3. Scanning electron microscopy

Scanning electron microscopy (SEM) was used to examine the changes in cheese microstructure. Cheese samples approximately 1 × 1 × 10-mm, were cut using razor blade from the interior of the cheese block and immediately fixed in 2.8 g/100 g glutaraldehyde in a 0.05 mol/L sodium phosphate buffer (pH 6) for 48 h at 7 °C. The fixed cheese samples were dehydrated in a graded ethanol series. This consisted of 15 min in each of a 25, 50, 70, 80, 95, 100, 100, and 100 mL/100 mL ethanol solution. The samples were then defatted three times with chloroform 15 min each. The defatted samples were dehydrated three times with absolute ethanol 15 min each. Samples were then frozen in liquid nitrogen and fractured longitudinally (i.e., along the axis fiber orientation in Mozzarella cheese) and cross-sectionally (i.e., across the fiber orientation). Frozen fractured samples were thawed in 100 mL/100 mL ethanol and critical point dried with liquid carbon dioxide using a Samdri 780A critical point drier (Tousimis Research Co., Rockville, MD). The dried, fractured cheese samples were mounted on aluminum SEM stubs using a carbon-based tape and coated with gold in a DC sputter coater (Seevac Auto Conductavac IV, Seevac Inc., Pittsburgh, PA). The samples were examined in a Hitachi S-570 LaB₆ scanning

electron microscope operated at an accelerating voltage of 10 kV (Biological and Biomaterials Preparation, Imaging, and Characterization Laboratory at Department of Animal Sciences in University of Wisconsin-Madison).

3. Results and discussion

3.1. Pasta filata Mozzarella cheese

The composition of pasta filata Mozzarella was: moisture 46.95 g/100 g, fat 21.75 g/100 g, salt 1.32 g/100 g, protein 24.87 g/100 g, with a pH of 5.16. The longitudinally cryofractured pasta filata Mozzarella 2 days post-manufacture revealed an oriented structure under the SEM (Fig. 1). During the hot water stretching step of pasta filata Mozzarella manufacture, the proteins form into fibers and orient in a roughly parallel manner as shown in Fig. 1. Consequently, fat and water accumulate between the long strands of protein. The microstructure of cross-sectionally cryofractured samples were very different (Fig. 2A). Only the micrographs of cross-sectionally cryofractured samples were considered for discussion presented herein.

Fig. 2 shows the microstructure of unfrozen pasta filata Mozzarella 2, 7, and 14 days post-manufacture. At 2 days post-manufacture, there were a large number of irregular cavities dispersed randomly throughout the protein matrix (Fig. 2A). Some of these cavities may represent the sites originally occupied by the extracted fat, and some were originally occupied by serum. When viewed at higher magnifications (Fig. 2B), the cavities show many chains and rods of bacteria and the cavity walls are textured by numerous indentations, which correspond to fat globules or the cocci starter culture. The filamentous material clumps associated with the coccal bacteria (Schellhaass & Morris, 1985) might be exocellular polysaccharide, although the cocci used were not a typical ropy strain, or residual fat membranes (Ober, McManus, & McMahon, 1993). Very few bacteria were embedded in the protein matrix; the majority was at the interface of fat/protein. The tendency of the bacteria to congregate at the surface of the fat droplets was reported by Dean, Berridge, and Mabbitt (1959) and Tunick (1993).

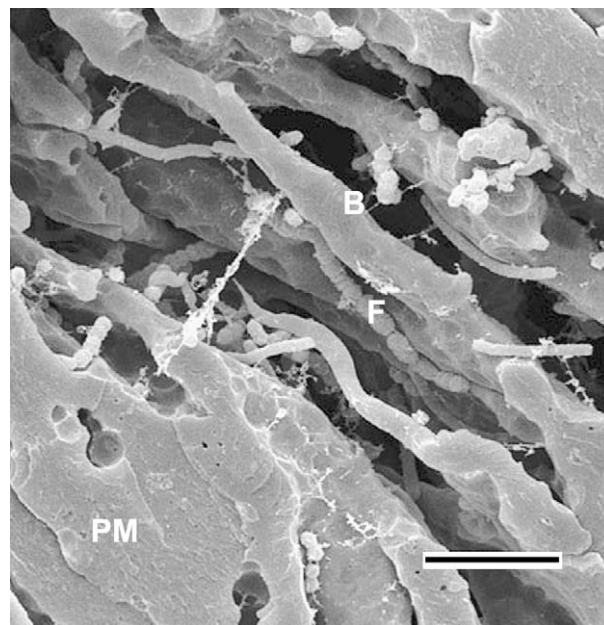


Fig. 1. Scanning electron micrograph of unfrozen pasta filata Mozzarella with longitudinal view (along the axis of protein fibers), taken at 2 days post-manufacture. (PM = protein matrix, F = protein fiber, B = bacteria). Scale bar represents 8 μm.

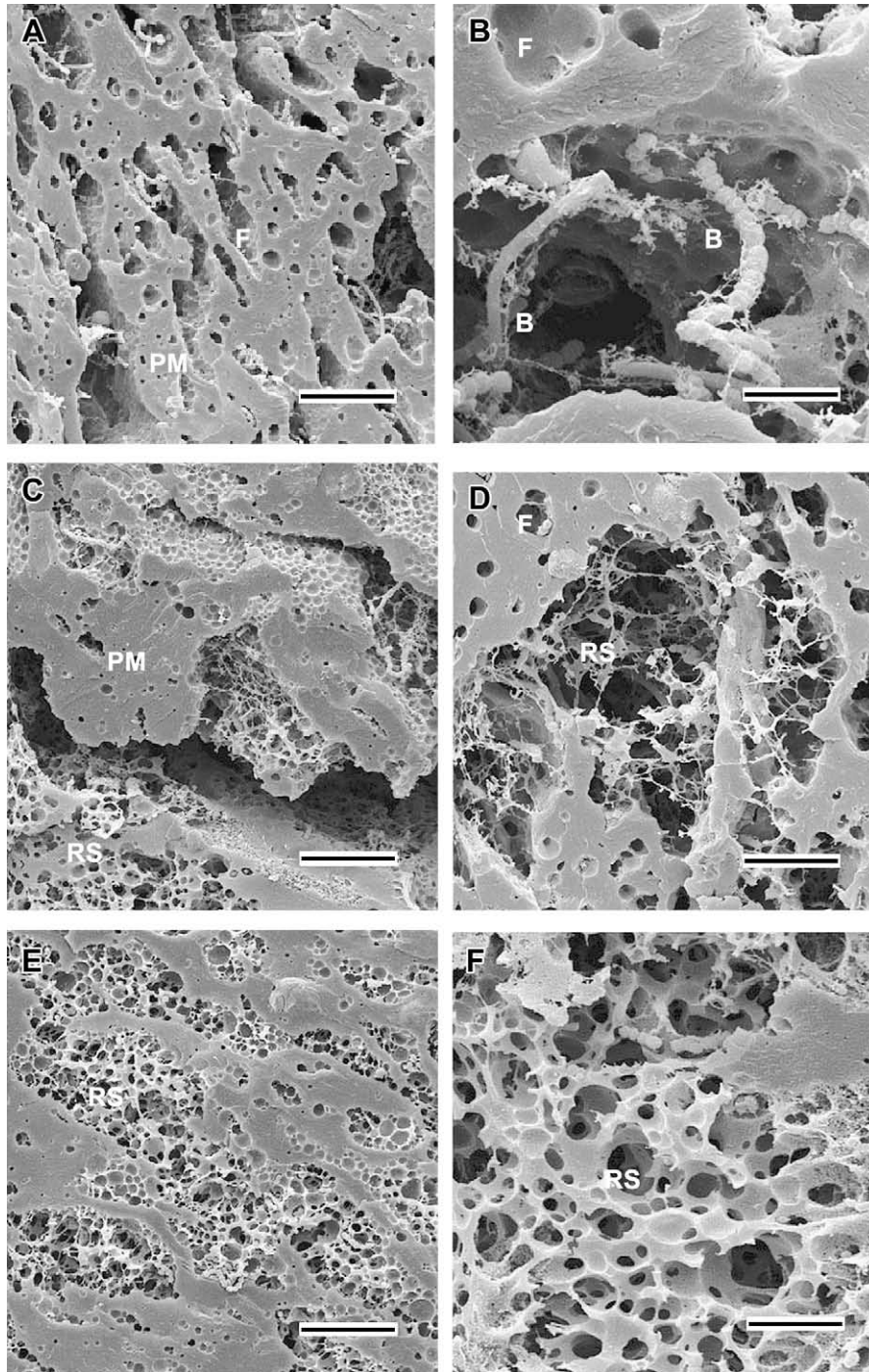


Fig. 2. Scanning electron micrographs of unfrozen pasta filata Mozzarella with cross-section view during ripening at two (A, C, and E at low; and B, D, and F at high) magnifications. A and B, C and D, and E and F are representative of sample structure after 2, 7, and 14 days, respectively. (PM = protein matrix, F = fat globule, RS = reticular structure, B = bacteria). Scale bars represent: A: 20 μ m, B: 5 μ m, C: 20 μ m, D: 10 μ m, E: 20 μ m, F: 8 μ m.

The structure of cavities and protein matrix continued to change through 7 and 14 days post-manufacture. By 7 days aging, thin strands of protein material encroached from all sides, connected the cavity walls such that the spherical shape of fat globules were distinctly defined (Fig. 2C and D). After 14 days aging, the cavities typically found in younger cheese samples were absent. Instead, those cavities had a reticular appearance (Fig. 2E & F). The fat globules were completely encased within the protein matrix. There was an apparent increase in porosity of the protein matrix in 14 days cheese samples (Fig. 2E) with respect to 2 days samples

(Fig. 2A). Kiely et al. (1993) also reported an increase in the porosity of the Mozzarella cheese protein matrix during 50 days of aging.

McMahon, Fife, and Oberg (1999) suggested that changes in the microstructure of Mozzarella cheese during storage were a result of the redistribution of protein and water. The position of the fat globules does not change during aging. Guinee, Feeney, Auty, and Fox (2002) confirmed this conclusion using CLSM. According to Guo and Kindstedt (1995) and Kuo et al. (2001), most of the expressible water, the water originally at the irregular cavities, in 2 days pasta filata Mozzarella appears to become absorbed into the protein

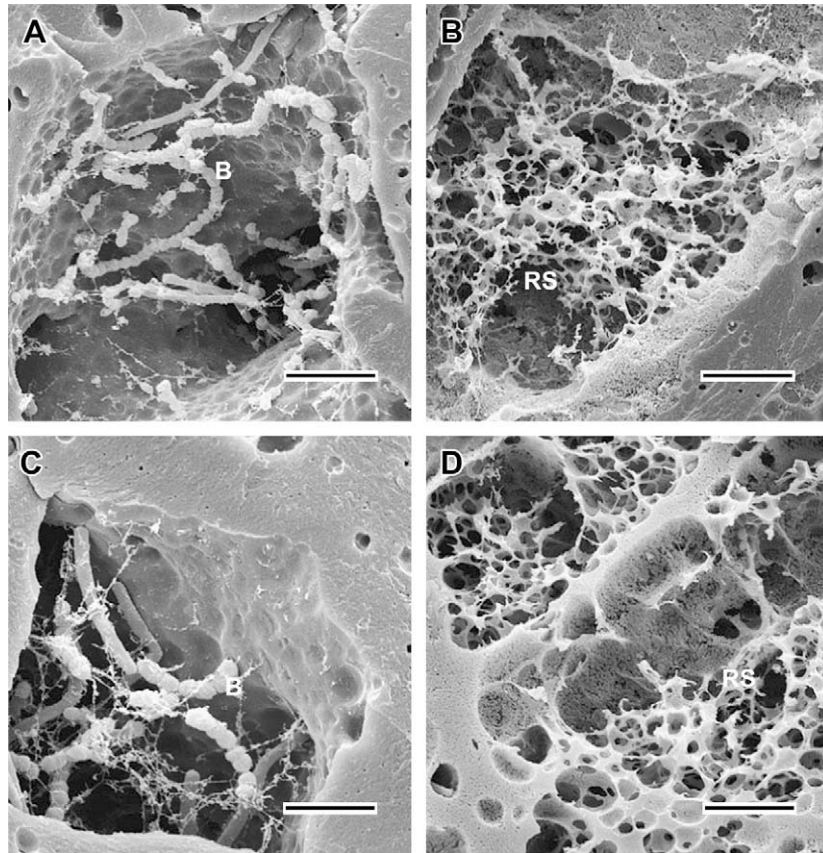


Fig. 3. Scanning electron micrographs of pasta filata Mozzarella frozen 2 days post-manufacture, thawed after 1 week of frozen storage, tempered for 1 day (A) and 7 days (B); and thawed after 4 weeks of frozen storage, tempered for 1 day (C) and 7 days (D). (RS = reticular structure, B = bacteria). Scale bars represent: A: 8 μ m, B: 8 μ m, C: 5 μ m, D: 8 μ m.

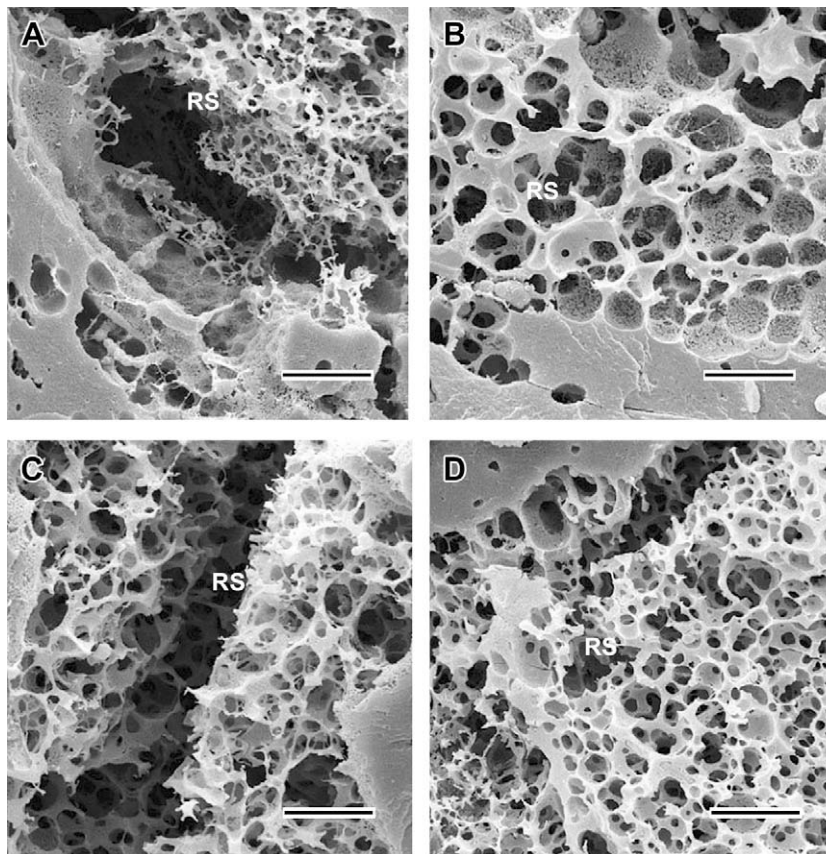


Fig. 4. Scanning electron micrographs of pasta filata Mozzarella frozen 7 days post-manufacture, thawed after 1 week of frozen storage, tempered for 1 day (A) and 7 days (B); and thawed after 4 weeks of frozen storage, tempered for 1 day (C) and 7 days (D). (RS = reticular structure). Scale bars represent: A: 8 μ m, B: 5 μ m, C: 5 μ m, D: 8 μ m.

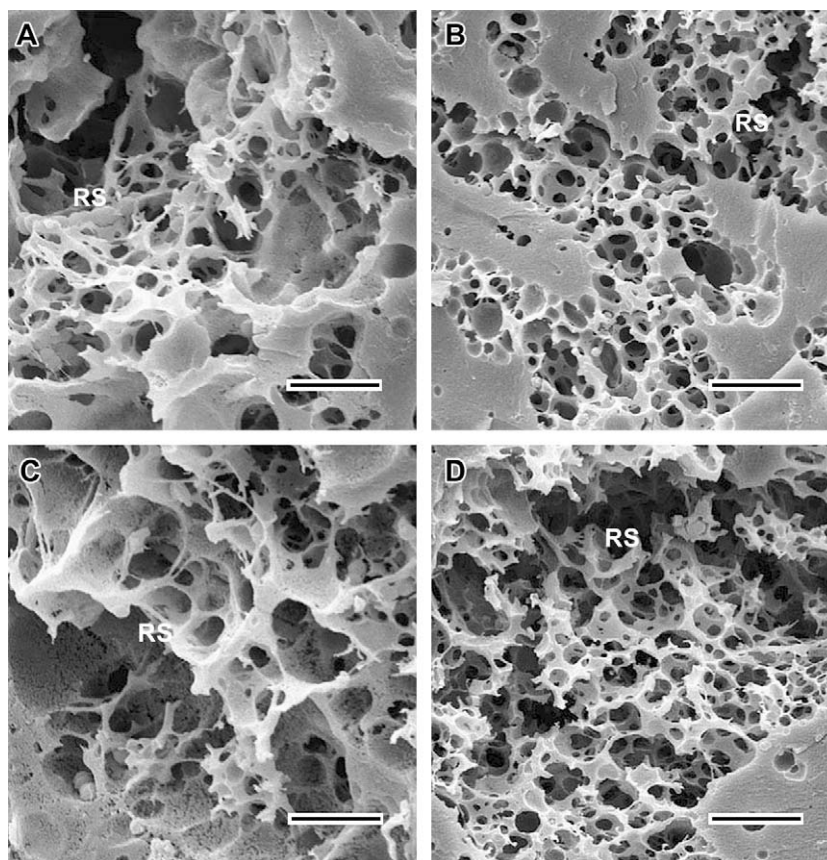


Fig. 5. Scanning electron micrographs of pasta filata Mozzarella frozen 14 days post-manufacture, thawed after 1 week of frozen storage, tempered for 1 day (A) and 7 days (B); and thawed after 4 weeks of frozen storage, tempered for 1 day (C) and 7 days (D). (RS = reticular structure). Scale bars represent: A: 5 μ m, B: 8 μ m, C: 5 μ m, D: 8 μ m.

matrix during the early stage of maturation. This was accompanied by a swelling of the protein matrix that continued until the spaces between the fat globules were completely filled with protein matrix as evidenced by the formation of reticular structures observed.

Effects of frozen storage and tempering on the microstructure of pasta filata Mozzarella frozen 2 days post-manufacture are shown in Fig. 3. There were no obvious differences in the microstructure of cheese sample frozen stored 1 and 4 weeks then tempered for 1 days (Fig. 3A and C) with respect to unfrozen sample aged 2 days post-manufacture (Fig. 2B). During 7 days tempering, the formation of reticular structure in frozen-stored cheese sample is in progress (Fig. 3B and D), indicating that the water in cavities became absorbed into the protein matrix. The microstructure of frozen-stored cheese sample did not change significantly when further tempered for 14 days. Compared with unfrozen cheese sample aged 2 days (Fig. 2B), the frozen-stored cheese sample showed a porous protein matrix (Fig. 3B and D). No obvious differences were found between cheese samples frozen stored for 1 and 4 weeks.

Fig. 4 shows the effect of frozen storage and tempering on the microstructure of pasta filata Mozzarella frozen 7 days post-manufacture. Partial breakdown of the reticular structure and increase of the porosity of the protein matrix were found in cheese sample frozen stored for 1 and 4 weeks then tempered for 1 days (Fig. 4A and C). During 7 days tempering, water in frozen-stored cheese sample had been absorbed into the protein matrix, as evidenced by the formation of reticular structures observed in Fig. 4B and D. The microstructure of frozen-stored cheese sample did not change significantly when further tempered for 14 days.

The effects of frozen storage and tempering on the microstructure of pasta filata Mozzarella frozen 14 days post-manufacture are shown in Fig. 5. A large portion of the reticular structure was lost and the protein matrix was porous in the frozen-stored cheese sample tempered for 1 days (Fig. 5A and C). Upon further tempering the frozen-stored cheese sample up to 7 days, no obvious changes were observed (Fig. 5B and D) in contrast to those in samples tempered for 1 days (Fig. 5A and C).

Formation of ice crystals in cheese during freezing might cause local dehydration of proteins, resulting in breaks in the protein structure. Recrystallization of ice crystals during frozen storage might lead to a more extensive breakdown of the cheese structure. Upon tempering, water clusters are unable to fully rebind into proteins (Diefes et al., 1993). Thus, this might lead to an increase in porosity of the protein matrix in frozen-stored cheese sample. Kuo & Gunasekaran (2003) suggested that structural changes in cheese during freezing and frozen storage could increase the meltability and decrease the stretchability of pasta filata Mozzarella cheese.

3.2. Non-pasta filata pizza cheese

The composition of non-pasta filata pizza was: moisture 46.74 g/100 g, fat 22.91 g/100 g, salt 1.88 g/100 g, protein 24.56 g/100 g, with a pH of 5.24. The microstructures of unfrozen non-pasta filata pizza cheese aged 2, 7, and 14 days post-manufacture are shown in Fig. 6. Considerable differences were observed. Non-pasta filata pizza cheese showed no orientation of protein strands. Instead, an open irregular protein matrix was observed in which the serum and fat globules (~1–10 μ m in diameter) were relatively evenly distributed (Fig. 6A).

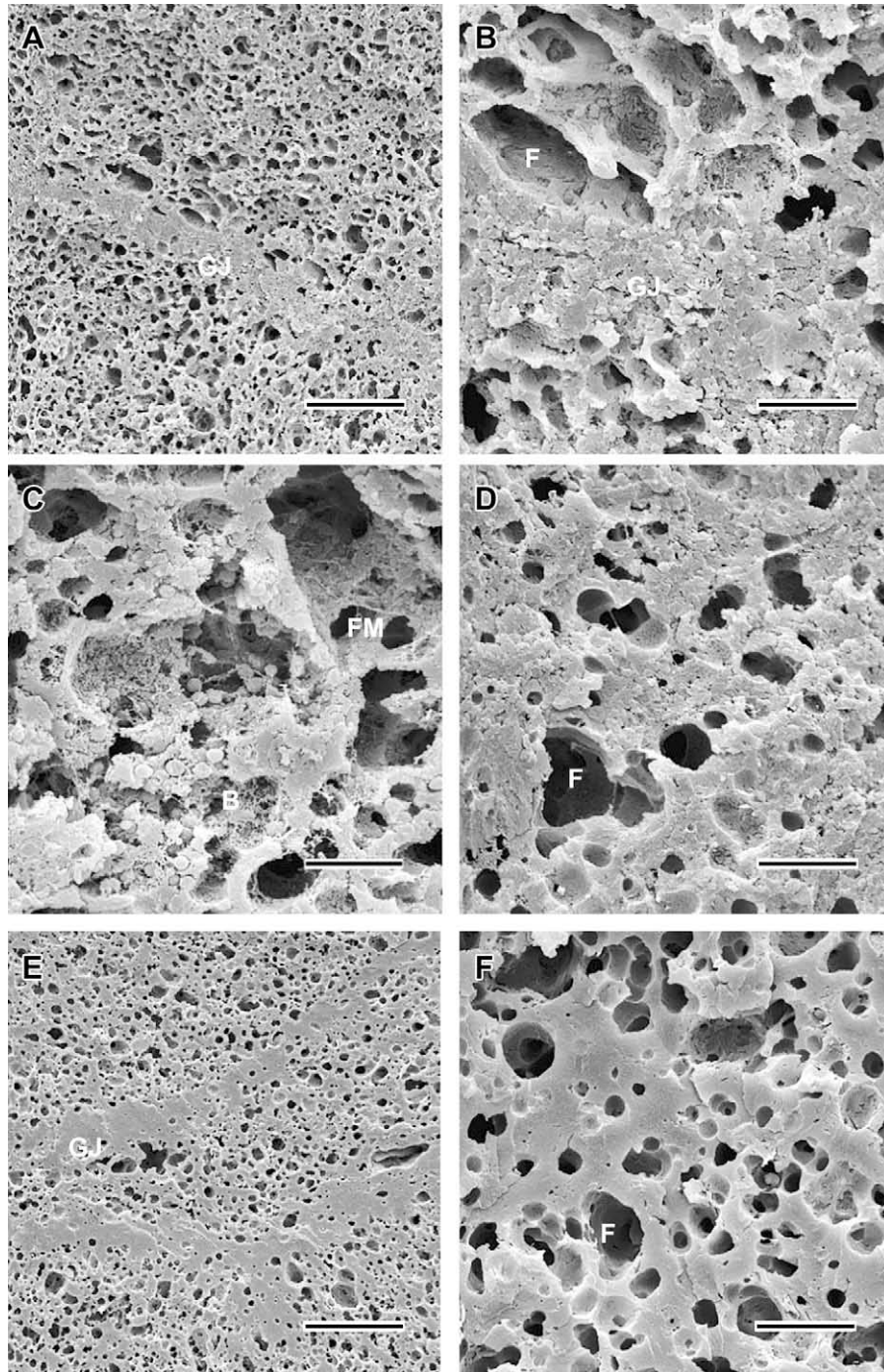


Fig. 6. Scanning electron micrographs of unfrozen non-pasta filata pizza cheese sampled at 2 (A, B, and C), 7 (D), and 14 (E and F) days post-manufacture. (GJ = granule junction, F = fat globule, FM = fat membrane, B = bacteria). Scale bars represent: A: 40 μm , B: 8 μm , C: 5 μm , D: 8 μm , E: 40 μm , F: 8 μm .

In the microstructure of 2-days aged unfrozen cheese sample, some coarse particles were noticeable (Fig. 6B), which may be the proteins that are loosely distributed more or less as micelle clusters. Bacteria were associated with the interior areas of the protein matrix (Fig. 6C). The curd granules junctions, consisting of compacted protein zones containing less fat than the interior of the protein matrix, were observed (Fig. 6A and B). At 7-days post-manufacture, the particles have not completely fused (Fig. 6D). However, a more uniform protein matrix was found in 14-days aged unfrozen cheese sample (Fig. 6E and F), since the particles have completely fused.

Effects of frozen storage and tempering on the microstructure of non-pasta filata pizza cheese sample aged 2 days before frozen storage are shown in Fig. 7. Cracks (Fig. 7A and B) and noticeable clumps of bacteria, about 0.8 μm in diameter (Fig. 7C and D) were found in the cheese sample frozen stored 1 week, and tempered for 1 day, which were not observed in the unfrozen sample. There were no obvious differences in the size distribution and morphology of the fat globules that could be attributed to freezing. Except for the cracks, the frozen cheese sample showed a more uniform and compact protein matrix (Fig. 7B) than the unfrozen sample (Fig. 6B). Fewer cracks were found in the frozen-stored cheese sample

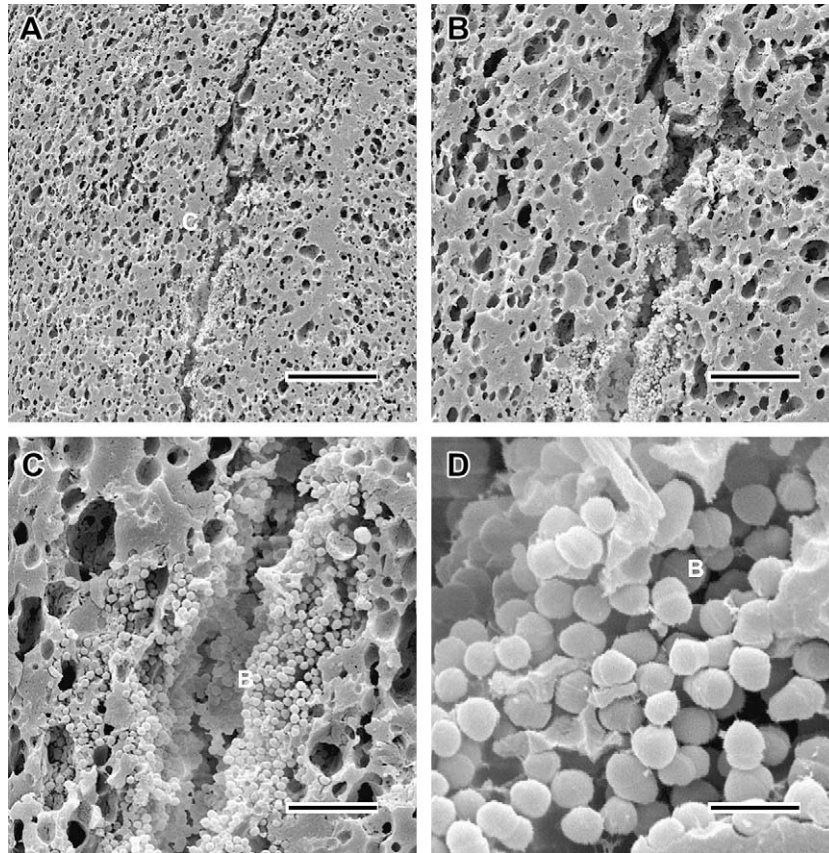


Fig. 7. Scanning electron micrographs of non-pasta filata pizza cheese frozen 2 days post-manufacture, thawed after 1 week of frozen storage, and tempered for 1 day at four magnifications. (C = crack, B = bacteria). Scale bars represent: A: 40 μm , B: 20 μm , C: 8 μm , D: 1.7 μm .

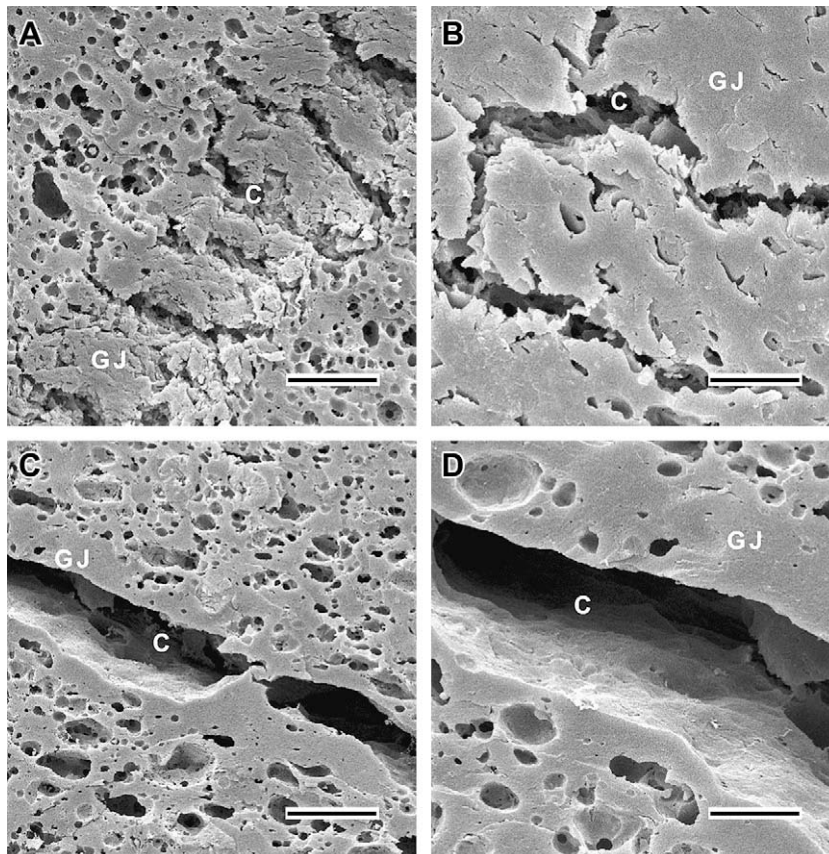


Fig. 8. Scanning electron micrographs of non-pasta filata pizza cheese frozen 7 days post-manufacture, thawed after 4 weeks of frozen storage, and tempered for 7 days at two magnifications (A at low, and B at high); and frozen 14 days post-manufacture, thawed after 1 week of frozen storage, and tempered for 7 days at two magnifications (C at low, and D at high). (C = crack, GJ = granule junction). Scale bars represent: A: 40 μm , B: 20 μm , C: 8 μm , D: 1.7 μm .

tempered 7 and 14 days. However, the clumps of bacteria were still observed in the tempered sample (data not shown).

Similar results were found in the frozen-stored cheese sample aged 7 and 14 days before frozen storage. However, sample aged 7 and 14 days before frozen storage was more susceptible to cracking (Fig. 8A and B) than the frozen-stored sample aged 2 days before frozen storage. Ruptures in cheese might be attributed to ice crystals formation which results in cracks and clumps of bacteria. The granule junctions were more susceptible to cracking than the interior of the matrix (Fig. 8C and D), probably because the fat globules in the interior matrix absorbed the stress developed during freezing and/or the junctions had a higher moisture and protein content than the interior matrix (Fontecha et al., 1996). The cheese frozen-stored for 4 weeks showed more extensive cracking and more areas of bacterial clumps as expected than cheese frozen-stored for 1 weeks due to recrystallization of ice crystals. However, the cracks and clumps of bacteria were still observed in the tempered sample (data not shown). Changes in cheese microstructure during freezing and frozen storage may be related to increase meltability and decrease stretchability of non-pasta filata pizza cheese sample (Kuo & Gunasekaran, 2003).

4. Conclusions

Significant differences in the microstructure were observed between pasta filata Mozzarella and non-pasta filata pizza cheeses. The effect of freezing on the protein matrix of pasta filata Mozzarella and non-pasta filata pizza cheeses were different. Pores and ruptures of reticular nature were found in frozen-stored pasta filata Mozzarella cheese protein matrix, but cracks and clumps of bacteria were shown in frozen-stored non-pasta filata pizza cheese. The protein matrix of frozen stored non-pasta filata pizza cheese was more prone to breakdown than that of frozen-stored pasta filata Mozzarella cheese when the aging and frozen storing duration was 14 days and 4 weeks, respectively. Tempering could modify the effect of freezing on the protein matrix of Mozzarella cheeses. Thus, the effect of freezing and frozen storage on the protein matrix of Mozzarella cheeses might be controlled to a certain extent by a suitable combination of aging and tempering.

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