

Rheology and Microstructure of Low-Fat Mozzarella Cheese

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ABSTRACT

The contributions of fat and moisture content to Mozzarella cheese texture were investigated to provide a basis for developing low-fat cheese with consumer acceptability. The characteristics of low-fat high-moisture (LFHM) experimental Mozzarella cheeses before and after 6 weeks of refrigerated storage were compared with those of high-fat low-moisture controls. High levels of either moisture in nonfat substance or fat in dry matter (FDM) were accompanied by decreases in hardness, complex viscosity, and elastic modulus and increases in meltability during the storage time. Starter culture bacteria were observed at the surface of the fat droplets, the latter having a tendency to coalesce during storage. Development of texture and meltability in LFHM Mozzarella appeared to be directly related to increased proteolysis of α_{s1} -casein observed during storage. These results show the feasibility of making Mozzarella cheese containing < 25% FDM with textural properties similar to those of a full-fat cheese if the product contains enough moisture and is stored under refrigeration for several weeks.

INTRODUCTION

Health concerns linked to dietary fat are motivating American consumers to adjust their eating habits. In a recent Gallup poll, 23% of the

respondents claimed that they had stopped eating dairy products (Barr, 1990). As a result, low-fat cheeses are becoming more desirable to both consumers and the dairy industry. Consumption of Mozzarella cheese in the US has been growing steadily in the past 10 years, with a per capita average of 3.2 kg (USDA, 1991). Low-moisture, part-skim Mozzarella, which has been available for many years, can contain as little as 30% fat in dry matter (FDM), the minimum specified in the 'US Federal Standard of Identity for Mozzarella' (FDA, 1989). An acceptable Mozzarella with a fat content lower than that of the part skim cheese has the potential of finding an important market among light dairy products.

A previous report compared Mozzarella cheeses of various ages and compositions (Tunick *et al.*, 1991). In that study, reduced moisture levels in Mozzarella resulted in higher values for hardness and springiness and lower values for cohesiveness and meltability. Decreasing the fat levels to less than 30% FDM increased the values of each texture profile analysis (TPA) parameter and decreased meltability values. In fresh samples, hardness and springiness were higher and meltability lower than in samples stored for 6 weeks at 4°C. The influence of protein breakdown and cheese microstructure were not investigated at that time. It is important to determine the relationships among composition, textural parameters, protein breakdown and microstructure in cheese in order to develop a low-fat cheese with acceptable texture. In this expanded study, the effects of moisture, fat, and storage time on rheological and textural properties of Mozzarella cheese were related to casein proteolysis and changes in microstructure.

MATERIALS AND METHODS

Cheese preparation

Low-fat (LF) and high-fat (HF) Mozzarella cheeses were prepared as described by Tunick *et al.* (1990). As before, 11.0-kg batches of milk were standardized with cream or skim milk to the desired fat level prior to pasteurization at 63°C for 30 min. LF cheese milk was standardized to 1.0% butterfat, and HF cheese milk to 3.2–3.7% butterfat. The milk was inoculated with CR5 starter culture[†] (Marschall-Rhone Poulenc, Madison, WI, USA) which is described by the manufacturer as consisting of 50% *Streptococcus thermophilus* and 50% *Lactobacillus bulgaricus*. After the pH dropped 0.1 unit, #01034 single strength rennet (Chr. Hansen's Labora-

[†]Use of brand or firm name does not imply endorsement by the USDA over others of a similar nature not mentioned.

tory, Milwaukee, WI, USA) was added. Low moisture (LM) cheeses were cooked at 45.9°C and contained less than 52.0% moisture. High moisture (HM) cheeses were prepared at 32.4°C and contained between 52.0 and 60.0% moisture. After whey drainage, the curd was rinsed and cut into slabs. When the pH dropped to 5.2–5.3, the slabs were covered and iced overnight. The next day, the curd was divided into eight parts and stretched and kneaded multidirectionally by hand for 7 min in 70–80°C water. This type of stretching reduced orientation of the curd. The samples were shaped into 224-ml polyethylene cups (approx. 80 mm diameter, 55 mm height), cooled, removed from the cups, brined for 2 h in a 23% salt solution, wiped dry with clean paper towels, and stored in vacuum-sealed pouches at 4°C. Samples were prepared according to a 2 × 2 factorial arrangement and randomly assigned in time, assuring sufficient replicates of each type of cheese. Cheeses containing less than 25% FDM were classified as LF Mozzarella; cheeses with a higher FDM level were designated HF cheeses.

Electrophoresis

Cheese samples were extracted for protein analysis with 0.166 M Tris/1 mM EDTA, pH 8.0, containing 2.9% sodium dodecyl sulfate (SDS) and 1.7 mM dithiothreitol, as described by Basch *et al.* (1989), except that a Potter–Elvehjem tissue grinder was used for all homogenization steps. Lipid plugs were discarded after centrifugation, and supernatants were filtered through laboratory wipe tissues before lyophilization. Lyophilized extracts were stored at –20°C. Polyacrylamide gel electrophoresis with SDS (SDS-PAGE) of extracts was performed in duplicate with the PhastSystem (Pharmacia, Piscataway, NJ, USA) using 20% gels. Gels were stained with Coomassie blue R250, destained and dried. A Bio-Rad (Richmond, CA, USA) Model 620 Video Densitometer interfaced with a computer and 1D Analyst II (Version 3.10) software (Bio-Rad) was used to scan the gels and integrate peak areas.

Microscopy

Samples for scanning electron microscopy (SEM) were cut with a razor from the interior of the cheese and then diced into rectangular blocks approximately 5 × 2 × 2 mm. These were immersed in a solution of 1% glutaraldehyde in 0.1 M sodium cacodylate (pH 7.2) at room temperature for 1–2 h and then stored at 4°C. Groups of samples were subsequently washed in cacodylate buffer, dehydrated in a graded series of ethanol solutions, extracted with three changes of chloroform, transferred into

ethanol, freeze-fractured in liquid nitrogen, thawed into ethanol, and finally dried at the critical point in carbon dioxide. The dried blocks were mounted on aluminum stubs, coated with a thin layer of gold in a DSM-5 Cold Sputtering Module (Denton Vacuum, Inc., Cherry Hill, NJ, USA) and examined by secondary electron imaging in a JEOL 840A (JEOL USA, Peabody, MA, USA) scanning electron microscope.

Frozen sections (10 μm thick) of samples for optical microscopy were cut with a cryostat microtome, mounted on cover slips coated with Mayer's albumen, thawed at room temperature, and exposed to the vapor of a 4% solution of osmium tetroxide. Darkened sections were mounted in glycerol and examined with Koehler optics for brightfield. Separate experiments were carried out to demonstrate the absence of anisotropy in the cheese samples.

Textural and rheological analyses

TPA was performed as previously described (Tunick *et al.*, 1990), with hardness, springiness, and cohesiveness being determined at 25°C using an Instron Universal Testing Machine Model 4201 (Instron, Inc., Canton, MA, USA). Four to six cylindrical specimens approx. 14.5 mm in diameter and 15 mm high were removed from the interior of the cheese sample with a cork borer. Specimens were removed at different angles relative to the axis of the cheese cylinder to avoid effects due to orientation of the curd. The elastic modulus (G'), viscous modulus (G''), and complex viscosity (η^*) were determined with a Rheometrics Dynamic Analyzer RDA-700 (Rheometrics, Inc., Piscataway, NJ, USA) at 25°C at frequencies of 1–100 rad/s (Tunick *et al.*, 1990). Three disks, 25.4 mm diameter and 4–5 mm thick, were removed from the interior of the cheese and glued with cyanoacrylate bonding agent to pairs of parallel aluminum plates for the analyses. Note that G' , which is measured at small amplitudes, is not directly related to springiness; the latter is measured after the sample has been compressed 75% of its original height.

Other analyses

Meltability was determined by the Schreiber test (Kosikowski, 1982; Park *et al.*, 1984). Three 5 mm thick disks of 37 mm diameter were cut from the inside of a cheese sample, placed on glass Petri dishes, and heated in a 232°C oven for 5 min. The dishes containing the melted disks were then cooled on a flat surface for 30 min. The amount of spread was measured on a target graph containing numbered concentric circles starting at a diameter of 37 mm (labeled 1) and increasing by 5 mm (labeled 2, 3, 4,

etc.). The outer edge of each melted sample was measured in six places and averaged.

The moisture content of the samples was measured by the forced-draft oven method (AOAC, 1990) and fat content by the modified Babcock test (Kosikowski, 1982). The data were analyzed by the Statistical Analysis System—General Linear Models procedure (SAS, 1987). Differences are described as significant only when $P < 0.001$.

For SEM analyses, 0, 1, 3, and 6-week-old samples were used, while for SDS-PAGE samples were extracted at 1, 3, and 6 weeks. TPA and meltability analyses took place at 1 and 6 weeks. The other analyses were with 1-week-old samples only.

RESULTS AND DISCUSSION

Proteolysis

The α_{s1} -casein in the fresh cheeses was degraded into α_{s1} -I casein and other peptides during the storage period (Fig. 1, Table 1). The band between α_{s1} - and β -casein, presumed to be α_{s1} -I casein, can be readily observed in Fig. 1. A target for proteolysis in cheeses made with rennet is α_{s1} -casein (Fox,

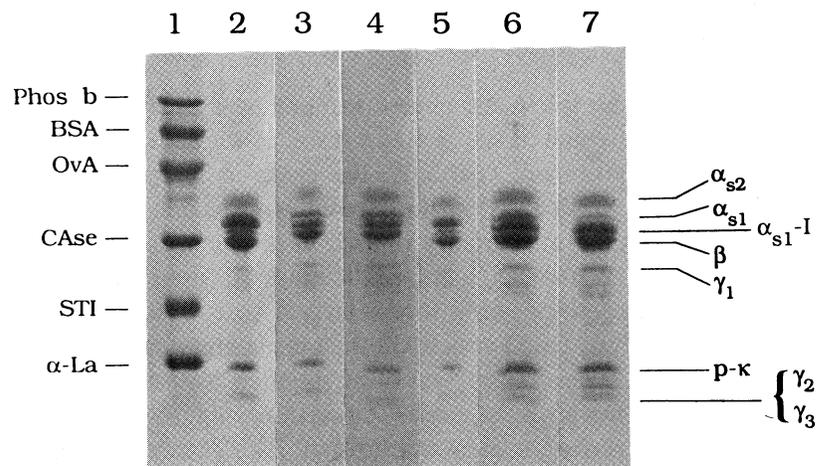


Fig. 1. Electrophoresis gels of Mozzarella illustrating the caseins present. Lane 1 — molecular weight standard (Phos b — phosphorylase B; BSA — bovine serum albumin; OvA — ovalbumin; CAse — carbonic anhydrase; STI — soybean trypsin inhibitor; α -La — α -lactalbumin); lanes 2–4 — LFHM cheese after 1, 3, and 6 weeks storage; lanes 5–7 — HFLM cheese after 1, 3, and 6 weeks storage. The band between α_{s1} - and β -casein is tentatively identified as α_{s1} -I casein.

TABLE 1
 Proteolysis of Caseins During Storage of LF Mozzarella.
 Determined by SDS-PAGE

Week	α_{s1}	α_{s1-I}	α_{s2}	β	Peptides
(% of total area)					
1	42.6	4.6	12.5	37.0	3.2
3	14.4	27.5	11.1	25.5	21.6
6	6.5	30.7	8.6	30.9	23.4

1989), which suggests that the rennet retained some activity after our preparation process. This is in agreement with previous work on Mozzarella (DiMatteo *et al.*, 1982; Farkye *et al.*, 1991). Plasmin, a heat-stable proteinase found in milk, apparently causes hydrolysis of β -casein in Mozzarella (Farkye *et al.*, 1991), and evidence of this breakdown was also indicated by the results. Table 1 also suggests that proteolysis of α_{s2} -casein may have occurred, but studies of the hydrolysis of α_{s2} -casein by plasmin (Visser *et al.*, 1989) show that the largest peptide formed could have a mobility similar to that of α_{s1-I} , α_{s1-} , or β -casein. Large peptides of α_{s2} -casein therefore would not be detected. Plasmin hydrolysis of α_{s2} -casein in cheese has been postulated (Farkye & Fox, 1992), but not yet verified.

Microstructure

The distribution of the fat in the cheese samples was evaluated from the void spaces (cavities) which developed as a result of the sample preparation technique. These cavities are visible in the electron micrographs of the samples (Figs 2 and 3). Proof that these cavities were due to fat globules was provided by optical microscopy of frozen sections that were treated with osmium vapor. At 0 weeks, osmiophilic material (lipid) partially filled many large cavities corresponding to the largest sizes observed in freeze-fractured samples. Many small vesicles in the matrix were osmiophilic. At 6 weeks, large fusiform areas comprised of aggregated osmiophilic spherical particles matched the sizes of areas containing aggregated spherical particles in the freeze-fractured preparations. The intervening areas of matrix contained many dispersed vesicles of osmiophilic material with a broad size distribution. Aggregation of lipid was observed in all samples at 6 weeks and none at 0 weeks. This effect has been observed in Mozzarella by Kiely *et al.* (1992) and may be due to breakdown of the casein network which holds the fat globules in place.

At 0 weeks, HF and LF cheese samples contained irregular, smooth-

surfaced cavities separated by thick and thin fractured faces of matrix (Figs 2(a) and (c)). The cavities contained many chains of *S. thermophilus* and comparatively few rods of *L. bulgaricus*. The tendency of the bacteria to congregate at the surface of the fat droplets was first observed by Dean *et al.* (1959). The LFHM cheese, which was prepared at a temperature more conducive to bacterial survival, contained about 50% more bacterial colonies than the HFLM cheese. Fractured faces of matrix, which comprised about two-thirds of the projected surface area in LF samples and about one-half of the surface area in HF samples, also contained many circular profiles of small vesicles ranging from about 5 μm to less than 50 nm in diameter.

At 1 week, the spaces occupied by the large, smooth-surfaced cavities were reduced, increasing the area occupied by fractured faces of matrix. The shapes of the cavities changed from irregular to flat and elongated in the LF cheese samples, and to open, circular areas in the HF cheese samples.

At 3 and 6 weeks, the cavities typically found in younger cheese samples were absent from the LF cheese (Fig. 2(b)). Instead, the areas between the fractured faces of matrix contained profiles of aggregated spherical spaces, often containing chains of *S. thermophilus*, embedded in the matrix at the surfaces. HF cheese samples at 3 and 6 weeks contained very large fusiform areas of aggregated spherical spaces in addition to the large, open, smooth-surfaced cavities that were typically observed at 1 wk (Fig. 2(d)). At 6 weeks, the LFHM cheese contained 25% fewer bacterial colonies than at 0 weeks, whereas the HF samples showed a 47% decrease. The release of proteolytic enzymes from bacteria apparently contributed to the observed breakdown of casein.

Effects of moisture

Cheeses are frequently classified according to their moisture in the nonfat substance (MNFS), which is equal to the percentage of moisture divided by (100 – percentage of fat) (Olson & Johnson, 1990). Variations in MNFS, which is basically a ratio of water to protein, can lead to differences in textural quality (Olson & Johnson, 1990). In both HF and LF cheese, hardness, springiness, G' , G'' , and η^* decreased significantly with increasing MNFS, whereas meltability increased significantly. Linear regressions of these relationships are shown in Figs 3–6. Elevating the water content in cheese results in greater hydration of the casein network which has been found by Taranto *et al.* (1979) to cause a decrease in hardness, and by Fukushima *et al.* (1965) to cause a decrease in G' . The increase in meltability with increasing MNFS can be related to decreases in η^* .

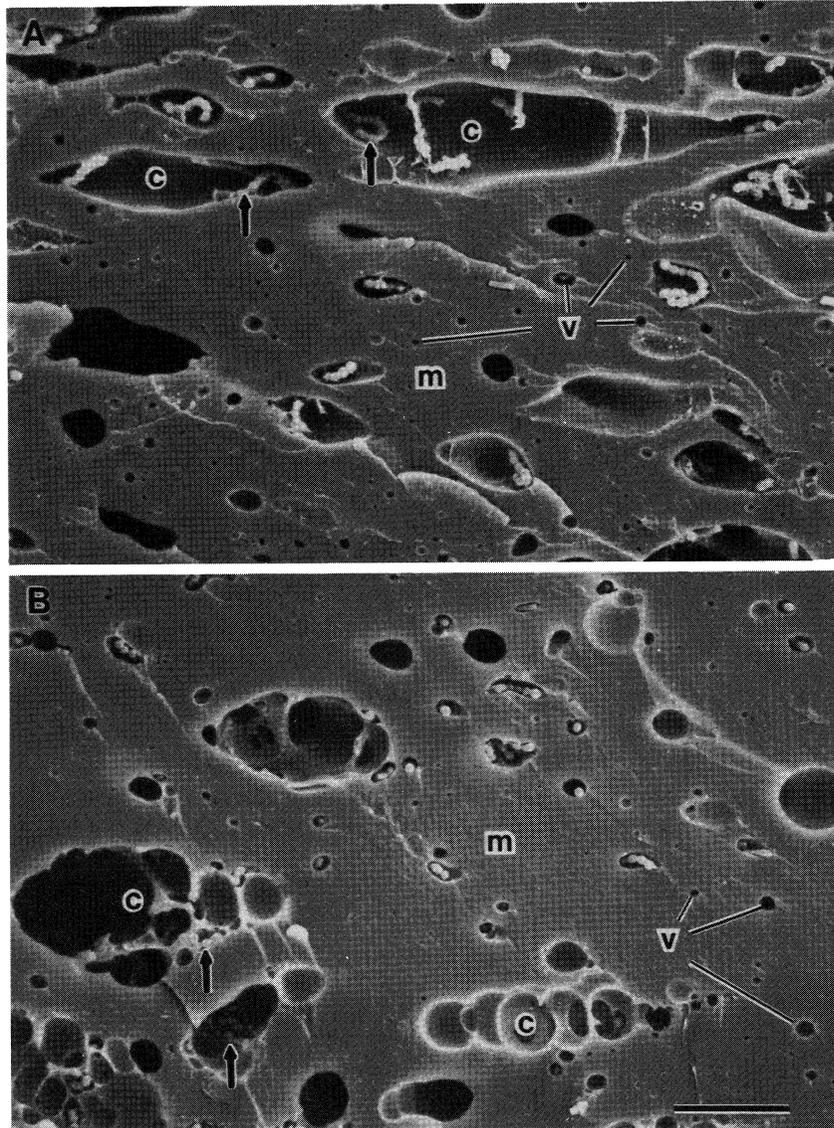


Fig. 2. Secondary electron images at 1600 \times (original magnification) of (A and B) LFHM Mozzarella, and (C and D) HFLM Mozzarella. (A) 0 weeks sample. Large, smooth-surfaced cavities (c) containing *Streptococcus thermophilus* (arrows) are separated by fractured faces of matrix (m) containing profiles of numerous small vesicles (v). (B) 6 weeks sample. Cavities containing *S. thermophilus* are composed of aggregated spherical areas and separated by expanses of fractured faces of matrix. Small vesicles also contain *S. thermophilus*. Bar indicates 10 μm .

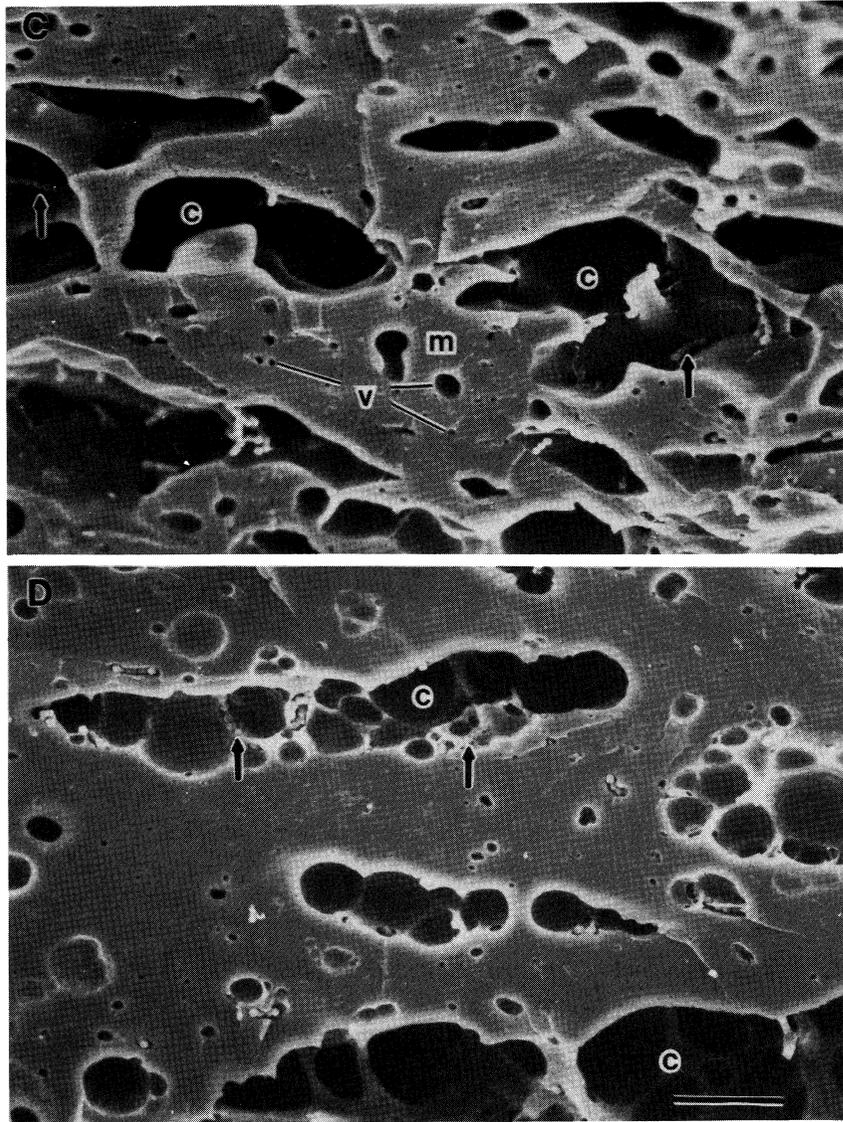


Fig. 2—contd. (C) 0 weeks sample. Large, irregular, smooth-surfaced cavities containing *S. thermophilus* are separated by irregular bands of fractured matrix. The matrix contains profiles of vesicles which are comparable in size and distribution to those in (A). (D) 6 weeks sample. Large cavities are shaped like aggregates of spherical areas with *S. thermophilus* embedded at the matrix interface. Bar indicates 10 μm .

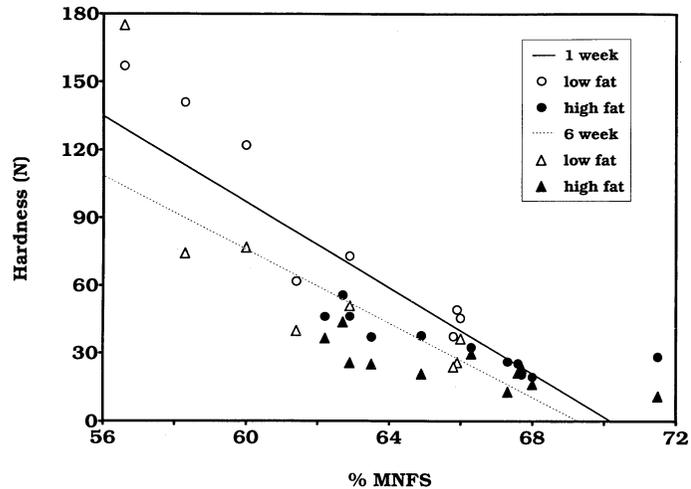


Fig. 3. Variation with moisture in non-fat substance (MNFS) of Mozzarella hardness after 1 weeks storage ($R^2 = 0.760$) and 6 weeks storage ($R^2 = 0.636$).

Because the meltability test used measures the increase in diameter of a melting sample, a decrease in viscosity would allow the sample to flow more readily.

Effects of fat

LF cheese exhibited higher hardness, G' , G'' , and η^* values than HF cheese of comparable age and MNFS (Figs 3 and 5). Fat globules are physically entrapped in the protein matrix of cheese and limit its deformation (Jameson, 1990); the absence of fat in LF cheese thus causes an increase in elasticity. LF Mozzarella is harder, more viscous and more elastic than HF Mozzarella. The meltability in the LF samples was lower than in the HF samples (Fig. 6), which is again related to the higher values of η^* . The loss tangent, which is G''/G' , was 0.37 ± 0.02 in the LF samples, and 0.39 ± 0.03 in the HF samples. The fact that these values are less than 1.0 indicates that both types of cheese exhibit predominantly solid and elastic behavior.

Effects of age

The values for hardness and springiness decreased significantly with time (Figs 3 and 4). The casein matrix in cheese became softer and less elastic during storage because of the breakdown of α_{s1} -casein, which provides the major contribution to the structure of casein in the curd. If the α_{s1} -casein in Mozzarella is degraded by proteolytic cleavage, it loses the ability to link

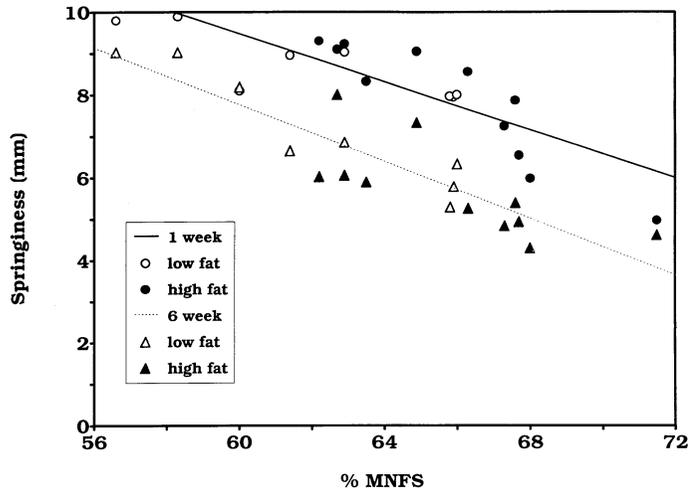


Fig. 4. Variation with MNFS of Mozzarella springiness after 1 weeks storage ($R^2 = 0.704$) and 6 weeks storage ($R^2 = 0.782$).

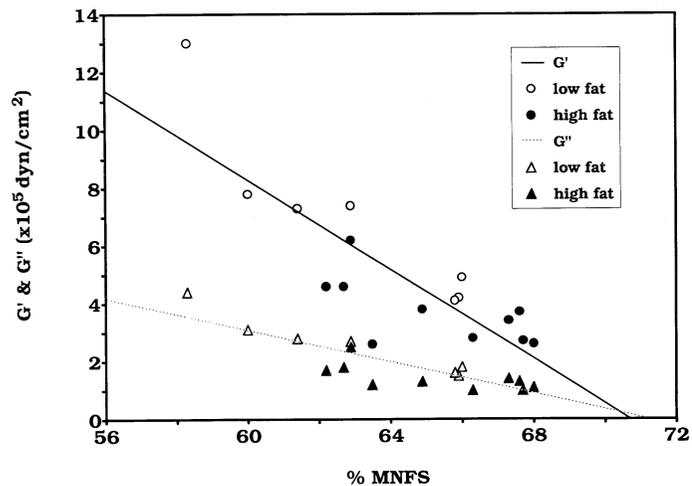


Fig. 5. Variation with MNFS of elastic modulus G' ($R^2 = 0.698$), viscous modulus G'' ($R^2 = 0.748$), and complex viscosity η^* ($R^2 = 0.711$) of Mozzarella after 1 weeks storage.

with other caseins, causing the protein matrix to lose strength and elasticity (Lawrence *et al.*, 1987). Meltability showed the expected increase with age (Fig. 6), although the HF results at 6 weeks were quite variable. The cohesiveness of HF Mozzarella increased marginally with storage, from 0.39 ± 0.04 at 1 week to 0.43 ± 0.06 at 6 weeks. The cohesiveness of LF

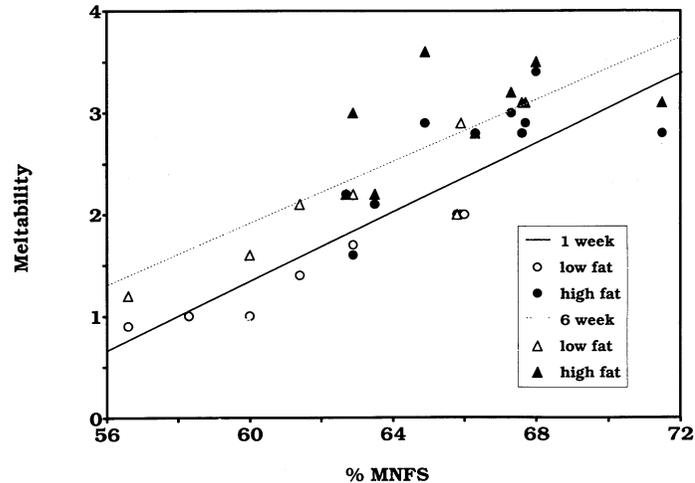


Fig. 6. Variation with MNFS of meltability of Mozzarella after 1 weeks storage ($R^2 = 0.761$) and 6 weeks storage ($R^2 = 0.632$).

Mozzarella also showed a slight increase, from 0.43 ± 0.04 at 1 week to 0.47 ± 0.09 at 6 weeks.

Allowing the LF Mozzarella to be stored for 6 weeks produces textural properties similar to those of HF Mozzarella at 1 week, as long as the MNFS levels are similar. Table 2 shows a comparison of 6-week-old LFHM cheeses with 61.4–66.0% MNFS, and 1-week-old HFLM cheeses with 62.2–68.0% MNFS. Although the FDM values of the HF cheese are about twice those of the LF cheese, the hardness and meltability values of the two types are not significantly different.

CONCLUSIONS

Textural properties of the experimental Mozzarella cheeses were affected by moisture and fat content and by age. Hardness, springiness, complex viscosity, and elastic and storage moduli all decreased with increasing MNFS, and all but springiness decreased with increasing fat. Refrigerating Mozzarella at 4°C for 6 weeks produced lower values for hardness and springiness. Meltability showed increases with increasing moisture, fat, and age. Fresh LF cheese was harder, more viscous, more elastic, and less meltable than fresh HF cheese. Extending the refrigerated storage period and controlling the MNFS may allow for the production of a Mozzarella containing approximately 25% less FDM than the minimum listed in the Standard of Identity (FDA, 1989). The relatively low cooking temperature

TABLE 2Comparison of LFHM Mozzarella Stored for 6 Weeks and HFLM Mozzarella Stored for 1 Week^a

	<i>LFHM</i>	<i>HFLM</i>
MNFS (%)	64.4 ± 2.1	63.8 ± 1.7
FDM (%)	22.9 ± 1.0	45.4 ± 3.6
Hardness (N)	35.5 ± 11.0	43.6 ± 8.9
Springiness (mm)	6.2 ± 0.6	9.0 ± 0.3
Meltability	2.2 ± 0.4	2.4 ± 0.5

^aAverage of five samples ± standard deviations.

of the LFHM cheese may permit increased survival of starter culture bacteria, their proteases, and rennet, all of which can contribute to degradation of α_{s1} -casein. Proteolysis of α_{s1} -casein appeared to cause LF Mozzarella of sufficiently high MNFS to have textural properties similar to those of the HF type.

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