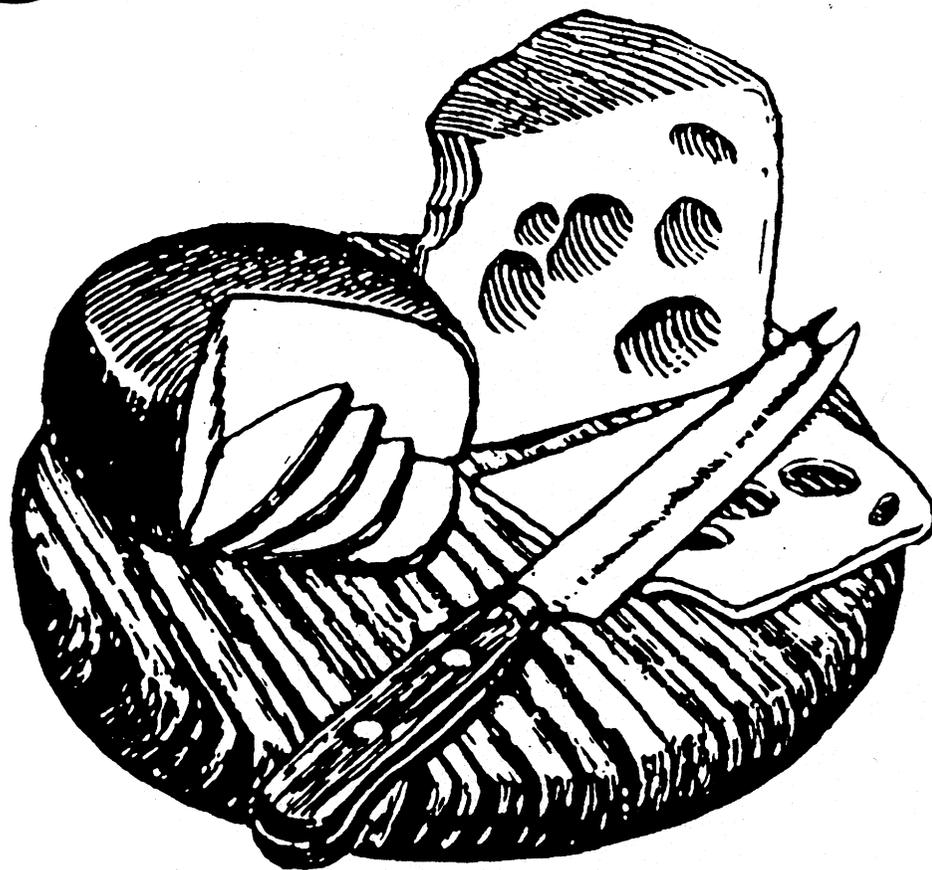


6096

CHEESE SYMPOSIUM



and

**California Dairy Foods
Research Center
Annual Conference**

February 13-14, 1995

Rheology, Proteolysis, and Microstructure of

Low-Fat Mozzarella Cheese

Michael H. Tunick, Edyth L. Malin, Philip W. Smith,

Peter H. Cooke, and V. H. Holsinger

United States Department of Agriculture

Agricultural Research Service

Eastern Regional Research Center

Philadelphia, PA

INTRODUCTION

The 1990 Dietary Guidelines for Americans call for a maximum of 30% of calories from fat in the diet. Compliance with this guideline is expected to result in a decline in nutrition-related diseases such as coronary heart disease and cancer. Many diet-conscious consumers are therefore turning away from cheese and other dairy products because of the fat they contain. The development of acceptable reduced-fat cheeses has thus become a matter of economic necessity for cheesemakers. In addition, low-fat cheese will soon become a regulatory necessity for companies that sell cheese to the National School Lunch Program: by 1998, school lunches must conform with the 1990 Dietary Guidelines.

The Agricultural Research Service is committed to improving the utilization of farm products. At the Eastern Regional Research Center, the Dairy Products Research Unit is

involved in several projects which address this goal, including the development of reduced-fat cheese products. This research will eventually enable consumers and schools to purchase cheeses that contain less fat than the full-fat products now available. Successful development of such cheeses requires a full investigation into proteolysis, microstructure, and rheology.

We decided to conduct research into fat reduction of Mozzarella cheese, which in the United States is second in popularity only to Cheddar. Various manufacturing parameters were adjusted so that breakdown of the protein matrix in the cheese would be accelerated. The resulting decrease in rigidity of the cheese structure would then compensate for the loss of fat. Cheeses were made at normal and reduced cooking temperatures, with the lower temperature resulting in greater moisture retention and increased proteolysis. The stretching temperature was kept below 80°C to minimize inactivation of chymosin and starter culture enzymes. The period of refrigerated storage was extended to allow proteolysis to continue. Experiments involving homogenization of cheese milk with and without fat were also performed.

CHEESEMAKING

Milk Preparation

Low-fat and full-fat Mozzarella cheeses were made from three kinds of milk: nonhomogenized, homogenized, and homogenized skim to which nonhomogenized cream was added. Each type of cheese was prepared from 22.7 kg of milk and one batch was prepared

on a given day. The full-fat cheese milk was standardized with cream or skim milk to 3.5% milk fat and the low-fat cheese milk to 1.0% milk fat. Milk intended for nonhomogenized and homogenized milk cheeses was standardized prior to pasteurization at 63°C for 30 min. The milk for homogenized milk cheese next underwent two-stage homogenization at 63°C with pressures totaling 10.3 or 17.2 MPa. The second stage pressure was 3.43 MPa. The milk intended for homogenized skim milk cheese was separated before the cream and skim milk were each pasteurized at 63°C for 30 min. The skim was homogenized at 10.3 MPa and the cream was then added back to the skim milk until the desired fat content was achieved.

Cheese Preparation

Cheese milk was held at 32.4°C and inoculated by direct addition of 125 ml of CR7 starter culture from Marschall-Rhône Poulenc*, which contains 50% *Streptococcus thermophilus* and 50% *Lactobacillus bulgaricus*. After the pH decreased 0.1 unit, 4.4 g of #01034 single strength calf rennet (chymosin) from Chr. Hansen's Laboratory were added. The milk was held for 35 min, cut, and held another 15 min. Low temperature cheeses were stirred at 32.4°C for 15 min, and held at that temperature for 45-60 min. High temperature cheeses were gradually heated over a 60-min period to 45.9°C and held there for 50 min.

*Use of brand names or firm names do not constitute endorsement by the USDA over others of a similar nature not mentioned.

After the holding period, half of the whey (pH 6.3-6.4) was drained and the curd was held an additional 30-45 min before the rest of the whey was drained. The low temperature cheeses were rinsed twice with water at 33°C, and the high temperature cheeses were rinsed once with 40°C water, covered with 40° water for 30 min, and drained. The curd was cut into slabs and cheddared until the pH decreased to 5.2-5.3. The slabs were covered and iced overnight since time constraints did not allow the entire procedure to be completed in one day. 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. The samples were pressed into 224-ml polyethylene cups measuring approximately 80 mm in diameter and 55 mm high, cooled, removed from the cups, brined for 2 h in 23% salt solution, blotted dry with clean paper towels, and stored in vacuum-sealed pouches at 4°C for up to 6 wk.

Composition

Average compositions of the various Mozzarella cheeses studied are shown in Table 1. Moisture was measured by the forced-draft oven method and the fat content was determined by the modified Babcock method. Moisture in nonfat substance (MNFS) was calculated by subtracting percentage of fat from 100 and dividing into percentage of moisture. Fat in dry matter (FDM) was calculated by subtracting percentage of moisture from 100 and dividing into percentage of fat.

EFFECTS OF FAT, MOISTURE, AND STORAGE TIME

Proteolysis

Proteolysis was examined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Cheese samples were grated, placed in Tris-EDTA buffer, and processed in a Virtis Model 23 homogenizer at approximately 10000 rpm to extract proteins and peptides. After the addition of dithiothreitol, the samples were centrifuged at 40000 x g at 2°C and the supernate filtered and lyophilized. These extracts were stored at -20°C prior to SDS-PAGE, which was performed with Pharmacia's PhastSystem. Gels were stained with Coomassie blue and scanned with a Bio-Rad Model 620 Video Densitometer. Bio-Rad 1D Analyst II software was used to scan the gels and determine percentages of individual caseins, which were expressed as percent of total α_s - and β -casein.

Significant proteolysis of α_{s1} -casein occurred during refrigerated storage of high moisture Mozzarella cheeses. Chymosin, which degrades α_{s1} -casein, retains activity in Mozzarella cheese. Initial cleavage of α_{s1} -casein by chymosin results in the formation of α_{s1} -I-casein, the large peptide remaining after the loss of residues 1 to 23, and both chymosin and plasmin continue to break down α_{s1} -I-casein into smaller fragments, including γ -caseins. A typical SDS-PAGE gel of caseins present in low- and full-fat Mozzarella after 1, 3, and 6 wk storage is shown in Figure 1. Table 2 shows the percentages of α_{s1} - and α_{s1} -I-casein during storage.

Although the optimal temperature for chymosin activity is about 45°C, cooking the

curd at this temperature resulted in decreased α_{11} -casein degradation during storage compared with that of cheeses prepared at 32.4°C. The difference in proteolysis at the two temperatures was evidently due to the higher MNFS in the cheeses prepared at 32.4°, as increased MNFS accelerates the rate of proteolysis in cheese. Moisture requirements for short-term proteolysis are high since a molecule of water is needed for every peptide bond broken.

The average α_{22} -casein content in all cheeses was 12.6 to 16.0% at 1 wk and 11.2 to 14.7% at 6 wk. The average β -casein content in all samples was 36.4 to 42.6% at 1 wk and 34.1 to 39.7% at 6 wk. No significant variations were attributable to processing parameters. Although chymosin is active on α_{22} - and β -caseins individually in solution, relatively little research has dealt with these caseins in cheese, and there have been no confirmed reports of chymosin attacking α_{22} - or β -casein in cheese. The small amount of degradation observed may have been due to plasmin, a heat-stable protease in milk. Starter bacteria enzymes may also account for some of the casein degradation observed during the 6-wk period.

Hardness and Springiness

Texture profile analyses were performed with an Instron Universal Testing Machine Model 4200. After samples were tempered at room temperature for 1 h, six cylindrical specimens measuring approximately 14 mm high and 14 mm in diameter were removed from the interior of the cheese. Specimens were placed on parallel plates and twice compressed by 75% using a crosshead speed of 100 mm/min with no dwell time. The force required for

the first compression was designated as hardness, and the height of recovery before the second compression was the springiness.

Hardness increased with cooking temperature. MNFS drops when the cooking temperature is raised, leading to less hydration of protein, to less freedom of movement for the protein molecules, to larger amounts of intact caseins, and to a firmer casein matrix. Figure 2 shows that high percentages of MNFS are needed to produce cheese with relatively low hardness. Hardness also increased when the fat content decreased, since the loss of fat increases the percentage of casein, resulting in a denser protein network. The effect was more pronounced at the higher cooking temperature because of the lower degree of proteolysis.

The values for hardness decreased with storage time. The casein matrix in cheese becomes softer during storage because of the breakdown of α_{s1} -casein, which provides the major contribution to the structure of casein in the curd. When α_{s1} -casein is degraded into peptides by proteolytic cleavage, it loses the ability to interact with other caseins, causing the protein matrix to lose strength. Cheese can be described as a filled gel in which fat globules and other components are dispersed within the casein network. Peptides are more compact than intact casein and, in low-fat Mozzarella, take the place of fat as filler in the gel.

Springiness increased as fat content decreased. Fat globules are physically entrapped in the protein matrix of cheese and limit its deformation; the absence of fat in low-fat cheese thus causes an increase in elasticity. Figure 3 shows that the springiness decreased during storage, indicating structural breakdown in the cheese. This decrease was greater at low cooking temperatures and at high fat percentages.

Storage and Loss Moduli

The storage modulus G' and the loss modulus G'' were measured at room temperature with a Rheometrics Dynamic Analyzer RDA-700 using a frequency of 100 rad/s at 0.8% strain. Three disks, each 25 mm in 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.

G' and G'' decreased with MNFS, again showing the effect of cooking temperature (Figure 4). Proteolytic breakdown caused both to decrease with storage time. The loss tangent, also known as $\tan \delta$, is obtained by dividing G'' by G' . The values, 0.32-0.39, indicate strong structure and predominately solid and elastic behavior.

Meltability

Meltability, which is unitless, was determined by the Schreiber test (Figure 5). Three disks, each 37 mm in diameter and 5 mm thick, 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 amount of spread of each disk 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. Meltability is particularly important in Mozzarella cheese since about 75% of the Mozzarella manufactured in the U.S. is used to make pizza.

As seen in Figure 6, meltability increased with fat content, MNFS, and storage time. Mozzarella spreads when it melts because the base of the cheese disk flows under the weight of the upper layer. This spread increased with fat content and also increased with proteolysis during storage. The peptide fragments resulting from the breakdown of α_{s1} -casein are smaller and more compact, allowing fat to flow more freely. Lower levels of MNFS did not result in the level of breakdown observed in high-moisture Mozzarella and thus the protein matrix was firmer and more likely to support its own weight when heated.

Microstructure

Samples for scanning electron microscopy were cut with a razor blade from the interior of the cheese and diced into blocks approximately 5 x 2 x 2 mm. These were fixed in a solution of 1% glutaraldehyde in 0.1 M sodium cacodylate, dehydrated in a graded series of ethanol solutions, and extracted with three changes of chloroform to remove lipids. The samples were then transferred into ethanol, freeze-fractured in liquid nitrogen, thawed in ethanol, and dried at the carbon dioxide critical point. The dried blocks were mounted on aluminum stubs, coated with a thin layer of gold, and examined by secondary electron imaging in a JEOL 840A scanning electron microscope.

Micrographs of samples taken after stretching revealed irregular smooth-surfaced cavities separated by thick and thin fractured faces of casein matrix. Numerous small vesicles, ranging from about 5 μm to less than 50 nm in diameter, were also present. The vesicles and cavities had been occupied by fat globules, which were removed as a result of

the sample preparation technique. The strands visible in some of the cavities are believed to be remnants of fat globule membrane. The cavities also contained many chains of *S. thermophilus* and comparatively few rods of *L. bulgaricus*. Bacteria in cheese tend to congregate at the surface of the fat droplets. The low-fat cheese (Figure 7, upper photo) was prepared at the low temperature, which is more conducive to bacterial survival, and contained about 50% more colonies than the full-fat cheese prepared at the higher temperature (Figure 8, upper photo).

At 6 wk, the cavities typically found in the younger cheese samples were absent from the low-fat cheese. Instead, as can be seen in Figure 7 (lower photo), there were larger, somewhat spherical spaces, often containing chains of *S. thermophilus* attached to the sides. These cavities were apparently due to proteolytic breakdown of the casein network and resulting coalescence of fat globules. The full-fat cheeses displayed similar cavities, visible in Figure 8 (lower photo). At 6 wk, the low-fat cheese contained 25% fewer bacterial colonies than at 0 wk, whereas the full-fat samples showed a 47% decrease. The release of proteolytic enzymes from autolysed bacteria evidently contributed to casein degradation.

All of the results from the electrophoretic, rheological, and microscopic studies indicated that a low-fat Mozzarella cheese with acceptable textural and melting properties can be made if the cooking temperature is kept low and the product is stored long enough to allow proteolysis to partially break down the casein matrix. We then investigated the effects of homogenization of cheese milk on Mozzarella to determine if possible structural changes in the cheese could improve its texture.

EFFECTS OF HOMOGENIZATION

Proteolysis

Homogenization of low-fat cheese milk at 10.3 MPa did not affect breakdown of α_{s1} -casein in the cheeses prepared at 32.4°C (Table 2). However, homogenization at 17.2 MPa retarded proteolysis, possibly as a result of physical changes to the casein at this higher pressure. Cooking at 45.9°C decreased the MNFS and thus the extent of proteolysis, an effect previously observed with the nonhomogenized Mozzarella.

Homogenization of skim milk at 10.3 MPa did not affect proteolysis, as would be expected from the lack of effect on whole cheese milk homogenized at 10.3 MPa.

Hardness and Springiness

Hardness increased with homogenization pressure, and, as indicated earlier, at the higher cooking temperature. Hardness decreased after storage in nonhomogenized cheese (Figure 2), but the decrease was smaller in cheese made from milk homogenized at 10.3 MPa (Figure 9). No decrease in hardness was evident for homogenization at 17.2 MPa (Figure 10). When milk is homogenized, the fat globule membrane is disrupted and replaced, at least in part, by a combination of casein submicelles and fat globule membrane fragments. Interaction of this complex with the protein matrix would make the cheese harder.

As before, springiness increased with decreasing fat content since the elasticity of the casein matrix is lowered by the presence of fat globules. This effect diminished with increasing pressure due to the higher number of fat globules created.

The hardness and springiness of Mozzarellas prepared from skim milk homogenized at 10.3 MPa were no different from those of cheeses made from whole milk homogenized at that pressure. These results indicate that hardness and springiness are influenced by fat content and not fat globule size when moderate homogenization pressures are used.

Storage and Loss Moduli

G' and G'' increased with homogenization pressure, again because of interactions of adsorbed casein with the protein matrix. As before, values decreased with storage time. Homogenization of skim milk had no effect.

Meltability

Meltability tended to decrease as homogenization pressure increased, apparently because the membrane complex (casein submicelles plus fat globule membrane fragments) prevented the melted fat from spreading out. The effect was most pronounced for the full-fat cheeses, especially at 6 wk (Table 3). However, the low-fat samples prepared at the low temperature from milk homogenized at 10.3 MPa melted as well as their full-fat counterparts, especially at 6 wk. These low-fat samples exhibited about as much formation

of α_{1} -I-casein as the corresponding full-fat samples.

The meltability of the full-fat cheeses homogenized at 10.3 and 17.2 MPa was much lower than the meltability of the full-fat nonhomogenized and homogenized skim milk cheeses. The full-fat homogenized milk Mozzarellas were also the only samples to puff upward to double or triple their original height upon heating. These effects probably resulted from the lower degree of proteolysis and from the presence of casein submicelles in the new fat globule membrane and from possible interactions with the casein matrix.

Microstructure

Samples for transmission electron microscopy were cut, diced, and fixed in the same manner as the scanning electron microscopy samples. The specimens were post-fixed with 2% osmium tetroxide to retain lipids, dehydrated in a graded series of ethanol solutions, and embedded in an epoxy resin mixture. Thin sections were cut and stained with solutions of uranyl acetate and lead citrate, and then examined in a Philips CM-12 electron microscope. Micrographs were taken using a magnification of 167,000 \times .

Micrographs of Mozzarella at 0 wk revealed particles measuring 10-11 nm in diameter. Some spaces 10-20 nm in diameter were present but not evenly distributed (Figure 11, upper photo). After 6 wk storage, there were slightly larger particles clumping in the samples (Figure 11, lower photo). These particles were presumed to be casein micelles or submicelles. The fine structure at 0 wk and the coarse structure at 6 wk were observed in both low-fat and full-fat samples, and are apparently related to proteolytic breakdown. This

rearrangement of micelles may be responsible for observed changes in rheological properties during refrigerated storage.

The nonhomogenized samples had the same appearance as the homogenized skim samples, with some large fat droplets. Full-fat samples homogenized at 10.3 MPa contained numerous fat droplets measuring 60-400 nm in diameter at 0 and 6 wk (Figure 12). At 17.2 MPa, the droplets were even more numerous and were usually 60-280 nm in diameter, both at 0 and 6 wk (Figure 13). The size and distribution of lipid were thus related to homogenization pressure. The surface of the droplets appeared to be covered with submicelles in the homogenized samples, giving evidence to the theory that fat globule membrane is replaced in part by casein submicelles after homogenization.

Free Oil

As part of the homogenization study, free oil was measured on some full-fat cheeses. Free oil was measured by the procedure developed by Kindstedt and Rippe, in which a ground sample is placed in a Paley-Babcock bottle and is subjected to heat, hot water, and centrifugation. Free oil on a fat basis was calculated as percentage of free oil divided by percentage of total fat.

Low-fat Mozzarella exhibited little if any free oil since the FDM was low enough for the fat to be completely emulsified. Cheeses made from milk homogenized at 10.3 MPa also had low levels of free oil because the smaller fat droplets were well emulsified. The levels of free oil on a fat basis in the nonhomogenized cheeses were much higher, and were not

significantly different from the levels in the homogenized skim cheeses (Table 4). The findings indicate that a reduction in the size of the fat droplets was a key to reduction of free oil, but homogenization of protein was not.

A Perkin-Elmer DSC-7 differential scanning calorimeter was used to obtain melting profiles of free oil and of the fat in the original cheese sample (Figure 14). Casein does not exhibit a melting transition by DSC, but the melting of water produces an interference with milk fat melting on a DSC curve. Therefore, cheese samples were first dried in a 130°C oven. All samples were tempered in the instrument by holding at 60°C for 5 min, cooling to -50° at 5°/min, and holding at -50° for 5 min. A DSC curve was then obtained by heating the sample to 50° at 5°/min. The melting point was defined as the temperature at which the melting of fat was complete. The melting point of the free oil from the homogenized cheeses averaged 34.8°C, which was significantly lower than the average from the other cheeses. The smaller droplets in the homogenized fat apparently complete their melting faster than the larger droplets in nonhomogenized milk. The melting point of the cheese fat was significantly higher than the melting point of the corresponding free oil. Enclosure of fat globules by membrane containing casein submicelles was evidently responsible for this late melting. The free oil and the cheese fat exhibited no differences in heat of fusion or in their fatty acid profiles.

We have concluded that homogenization of cheese milk for low-fat Mozzarella manufacture should be limited to no more pressure than is required to recombine ingredients. Since proteolysis is decreased at high pressure, homogenization can be used to extend shelf life.

IMPACT OF THE RESEARCH

The results of the studies on Mozzarella have provided new insights on proteolysis, texture development, and changes in microstructure. Breakdown of α_{s1} -casein in low-fat Mozzarella during refrigerated storage results in a weakening of the cheese structure, and the peptides produced act as filler, which compensates for the lack of fat. Therefore, a low-fat Mozzarella with desirable textural properties can be manufactured if steps are taken to insure some survival of starter microorganisms and retention of rennet activity.

With these facts in mind, Mozzarella cheeses containing 7-11.5% fat have been commercially manufactured, processed into pizzas, and served in the National School Lunch Program. Tests were conducted at several schools in the Philadelphia area as well as eight schools across the country. The high level of acceptance by the students has prompted the USDA's Food and Consumer Service to order the purchase of low-fat Mozzarella for school lunch pizzas. Low-fat pizza is expected to become a permanent part of school lunches in the near future.

ACKNOWLEDGEMENTS

The authors thank Dr. John Phillips for statistical design, Dr. James Shieh for dynamic rheological analyses, Brien Sullivan for SDS-PAGE, and Joseph Uknalis for transmission electron microscopy.

TABLE 1. Moisture, moisture in nonfat substance (MNFS), and fat in dry matter (FDM) in Mozzarella cheeses.

| Fat Level | Cook Temp. (°C) | Moisture (%) | MNFS (%) | FDM (%) |
|------------------|-----------------|--------------|----------|---------|
| 0 MPa | | | | |
| Low | 45.9 | 52.5 | 59.0 | 23.3 |
| Low | 32.4 | 54.1 | 60.4 | 22.6 |
| High | 45.9 | 46.3 | 62.4 | 48.2 |
| High | 32.4 | 49.5 | 65.2 | 47.2 |
| 10.3 MPa | | | | |
| Low | 45.9 | 55.1 | 60.8 | 20.9 |
| Low | 32.4 | 56.6 | 62.4 | 21.3 |
| High | 45.9 | 47.9 | 65.5 | 51.7 |
| High | 32.4 | 48.2 | 65.5 | 51.0 |
| 10.3 MPa (Skim*) | | | | |
| Low | 32.4 | 53.6 | 60.1 | 23.4 |
| High | 32.4 | 45.7 | 62.9 | 50.4 |
| 17.2 MPa | | | | |
| Low | 45.9 | 53.9 | 60.5 | 23.5 |
| Low | 32.4 | 56.8 | 63.7 | 24.7 |
| High | 45.9 | 46.8 | 64.8 | 52.2 |
| High | 32.4 | 47.0 | 64.6 | 51.3 |

*Skim milk homogenized at 10.3 MPa before cream added back.

TABLE 2. Percentages of α_{s1} -caseins in Mozzarella cheeses during refrigerated storage. Differences due to cooking temperature and storage time were significant ($P < .001$).

| Fat Level | Cook Temp. (°C) | α_{s1} -CN 1 wk (%) | α_{s1} -CN 6 wk (%) | α_{s1} -I-CN 6 wk (%) |
|------------------|-----------------|----------------------------------|----------------------------------|------------------------------------|
| 0 MPa | | | | |
| Low | 45.9 | 46.8 | 46.6 | 6.0 |
| Low | 32.4 | 45.2 | 24.0 | 22.4 |
| High | 45.9 | 39.8 | 33.1 | 13.8 |
| High | 32.4 | 40.4 | 18.9 | 23.6 |
| 10.3 MPa | | | | |
| Low | 45.9 | 47.0 | 39.1 | 9.9 |
| Low | 32.4 | 45.0 | 22.5 | 27.0 |
| High | 45.9 | 44.2 | 39.7 | 7.8 |
| High | 32.4 | 43.0 | 18.4 | 29.0 |
| 10.3 MPa (Skim*) | | | | |
| Low | 32.4 | 42.4 | 33.7 | 16.2 |
| High | 32.4 | 44.4 | 34.6 | 17.3 |
| 17.2 MPa | | | | |
| Low | 45.9 | 43.8 | 42.3 | 10.0 |
| Low | 32.4 | 57.3 | 43.7 | 4.2 |
| High | 45.9 | 45.8 | 39.7 | 12.5 |
| High | 32.4 | 47.3 | 31.6 | 16.3 |

*Skim milk homogenized at 10.3 MPa before cream added back.

TABLE 3. Meltability of Mozzarella cheeses. Differences due to homogenization pressure, fat in dry matter, cooking temperature, and storage time were significant ($P < .001$).

| Fat Level | Cook Temp. (°C) | Meltability 1 wk | Meltability 6 wk |
|------------------|-----------------|------------------|------------------|
| 0 MPa | | | |
| Low | 45.9 | 0.9 | 1.6 |
| Low | 32.4 | 1.3 | 1.5 |
| High | 45.9 | 2.4 | 3.0 |
| High | 32.4 | 2.7 | 3.2 |
| 10.3 MPa | | | |
| Low | 45.9 | 1.2 | 1.4 |
| Low | 32.4 | 1.2 | 1.7 |
| High | 45.9 | 1.0 | 0.9 |
| High | 32.4 | 1.1 | 1.2 |
| 10.3 MPa (Skim*) | | | |
| Low | 32.4 | 1.3 | 1.6 |
| High | 32.4 | 2.8 | 3.0 |
| 17.2 MPa | | | |
| Low | 45.9 | 0.9 | 1.4 |
| Low | 32.4 | 1.1 | 1.3 |
| High | 45.9 | 1.1 | 0.9 |
| High | 32.4 | 1.0 | 0.9 |

*Skim milk homogenized at 10.3 MPa before cream added back.

TABLE 4. Free oil on a fat basis (FOFB) and melting point (MP) of Mozzarella cheeses. Differences in FOFB due to milk treatment and storage time were significant ($P < .05$). Average MP of cheese homogenized at 10.3 MPa was significantly different ($P < .05$) from other two averages.

| Week | Non-Homogenized | Homogenized Skim* | Homogenized at 10.3 MPa |
|-----------------------|-----------------|-------------------|-------------------------|
| FOFB (%) | | | |
| 1 | 34.4 | 43.3 | 0.6 |
| 6 | 50.8 | 59.3 | 3.1 |
| MP of FO (°C) | | | |
| 1 | 36.9 | 36.2 | 34.9 |
| 6 | 37.1 | 36.8 | 34.6 |
| Avg. | 37.0 | 36.5 | 34.8 |
| MP of Cheese Fat (°C) | | | |
| 1 | 38.5 | 39.0 | 40.1 |
| 6 | 38.9 | 38.5 | 40.6 |

*Skim milk homogenized at 10.3 MPa before cream added back.

LEGENDS FOR FIGURES

Figure 1. SDS-PAGE of Mozzarella, illustrating the caseins present and the proteolysis of α_{s1} - to α_{s1} -I-casein during refrigerated storage. Lane 1: molecular weight standard (Phos b = phosphorlyase B, BSA = bovine serum albumin, OvA = ovalbumin, CAse = carbonic anhydrase, STI = soybean trypsin inhibitor, α -La = α -lactalbumin); lanes 2-4: low-fat Mozzarella after 1, 3, and 6 wk storage; lanes 5-7: full-fat Mozzarella after 1, 3, and 6 wk storage.

Figure 2. Linear regressions of hardness vs. moisture in nonfat substance (MNFS) of low-fat and full-fat Mozzarellas after 1 wk storage ($R^2 = 0.716$) and 6 wk storage ($R^2 = 0.730$). Hardness decreased with fat content, storage time, and MNFS.

Figure 3. Linear regressions of springiness vs. MNFS of low-fat and full-fat Mozzarellas after 1 wk storage ($R^2 = 0.704$) and 6 wk storage ($R^2 = 0.782$). Springiness increased with fat content and decreased with storage time and MNFS.

Figure 4. Linear regressions of elastic modulus G' ($R^2 = 0.698$) and viscous modulus G'' ($R^2 = 0.748$) vs. MNFS of low-fat and full-fat Mozzarellas after 1 wk storage. G' and G'' decreased with fat content and MNFS.

Figure 5. Schreiber test of Mozzarella cheese, showing unmelted and melted samples,

and the target graph used to determine results.

Figure 6. Linear regressions of meltability vs. MNFS of low-fat and full-fat Mozzarellas after 1 wk storage ($R^2 = 0.761$) and 6 wk storage ($R^2 = 0.632$). Meltability increased with fat content, storage time, and MNFS.

Figure 7. Scanning electron micrographs of low-fat Mozzarella after 0 wk storage (upper photo) and 6 wk storage (lower photo). Arrows point to colonies of *S. thermophilus*. Fat droplets formerly occupied the cavities (c) and vesicles (v) dispersed throughout the casein matrix (m). Bar at lower right indicates 10 μm .

Figure 8. Scanning electron micrographs of full-fat Mozzarella after 0 wk storage (upper photo) and 6 wk storage (lower photo). Arrows, letters, and bar as in Figure 7.

Figure 9. Linear regressions of hardness vs. MNFS of low-fat and full-fat 10.3-MPa Mozzarellas after 1 wk storage ($R^2 = 0.656$) and 6 wk storage ($R^2 = 0.619$). Hardness decreased with fat content and MNFS, and decreased somewhat with storage time.

Figure 10. Linear regressions of hardness vs. MNFS of low-fat and full-fat 17.2-MPa Mozzarellas after 1 wk storage ($R^2 = 0.548$) and 6 wk storage ($R^2 = 0.759$). Hardness decreased with fat content and MNFS, but not with storage time.

Figure 11. Transmission electron micrographs of full-fat nonhomogenized Mozzarella after 0 wk storage (upper photo) and 6 wk storage (lower photo), showing the change from fine to coarse structure. Micelles/submicelles appear as small black dots. Bar at lower right indicates 100 nm.

Figure 12. Transmission electron micrographs of low-fat 10.3-MPa Mozzarella after 0 wk storage (upper photo) and 6 wk storage (lower photo), showing the change from fine to coarse structure and the appearance of new membranes around the fat globules. Same scale as Figure 11.

Figure 13. Transmission electron micrographs of full-fat 17.2-MPa Mozzarella after 0 wk storage (upper photo) and 6 wk storage (lower photo), showing the change from fine to coarse structure and the appearance of new membranes around the fat globules. Same scale as Figure 11.

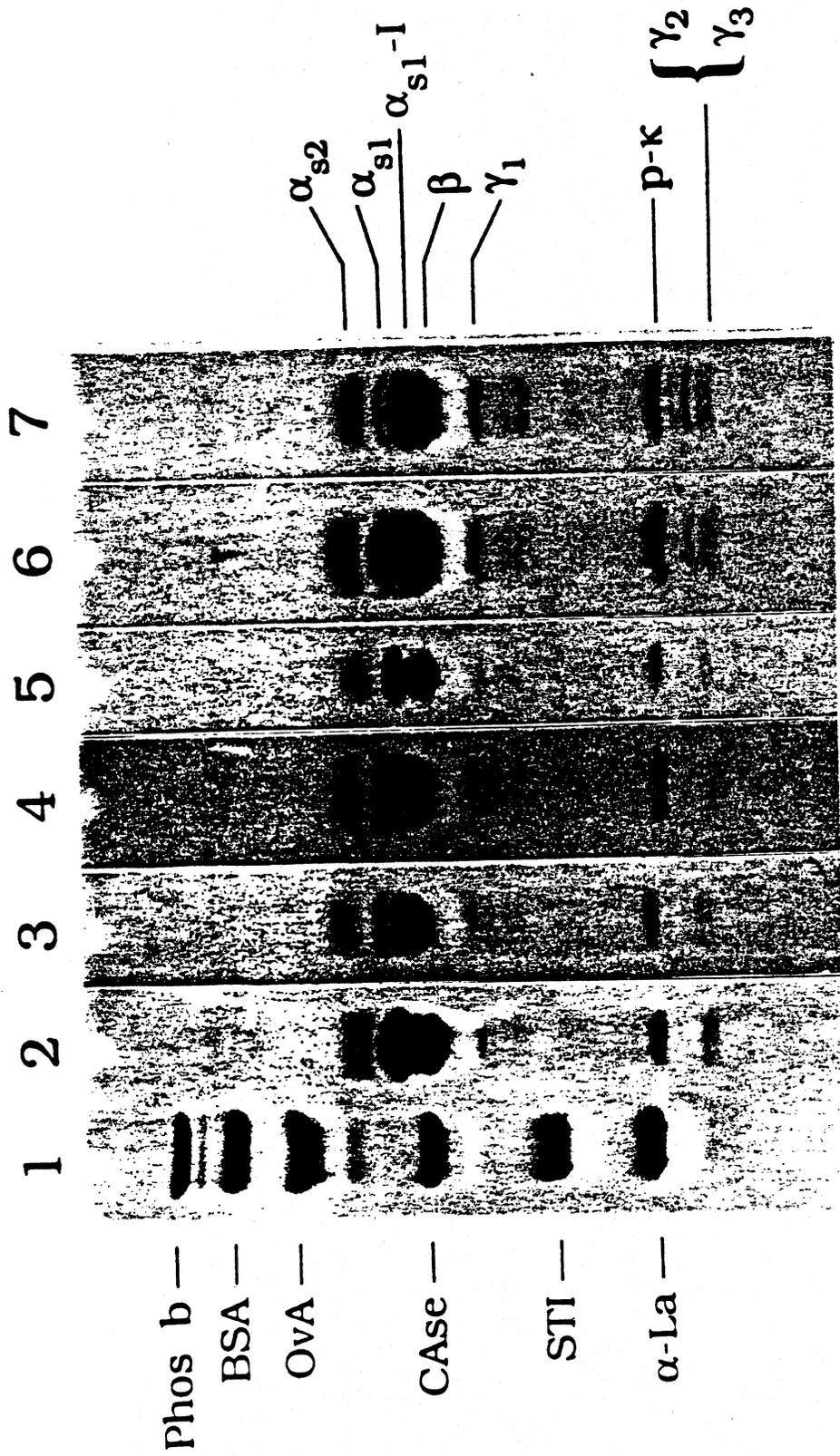
Figure 14. DSC melting profiles of 3.72 mg of free oil (solid line) and 9.32 mg of dried cheese (dashed line) from a full-fat 10.3-MPa Mozzarella after 1 wk storage. The MP of the free oil is 40.1°C and the MP of the fat in the dried cheese is 34.9°.

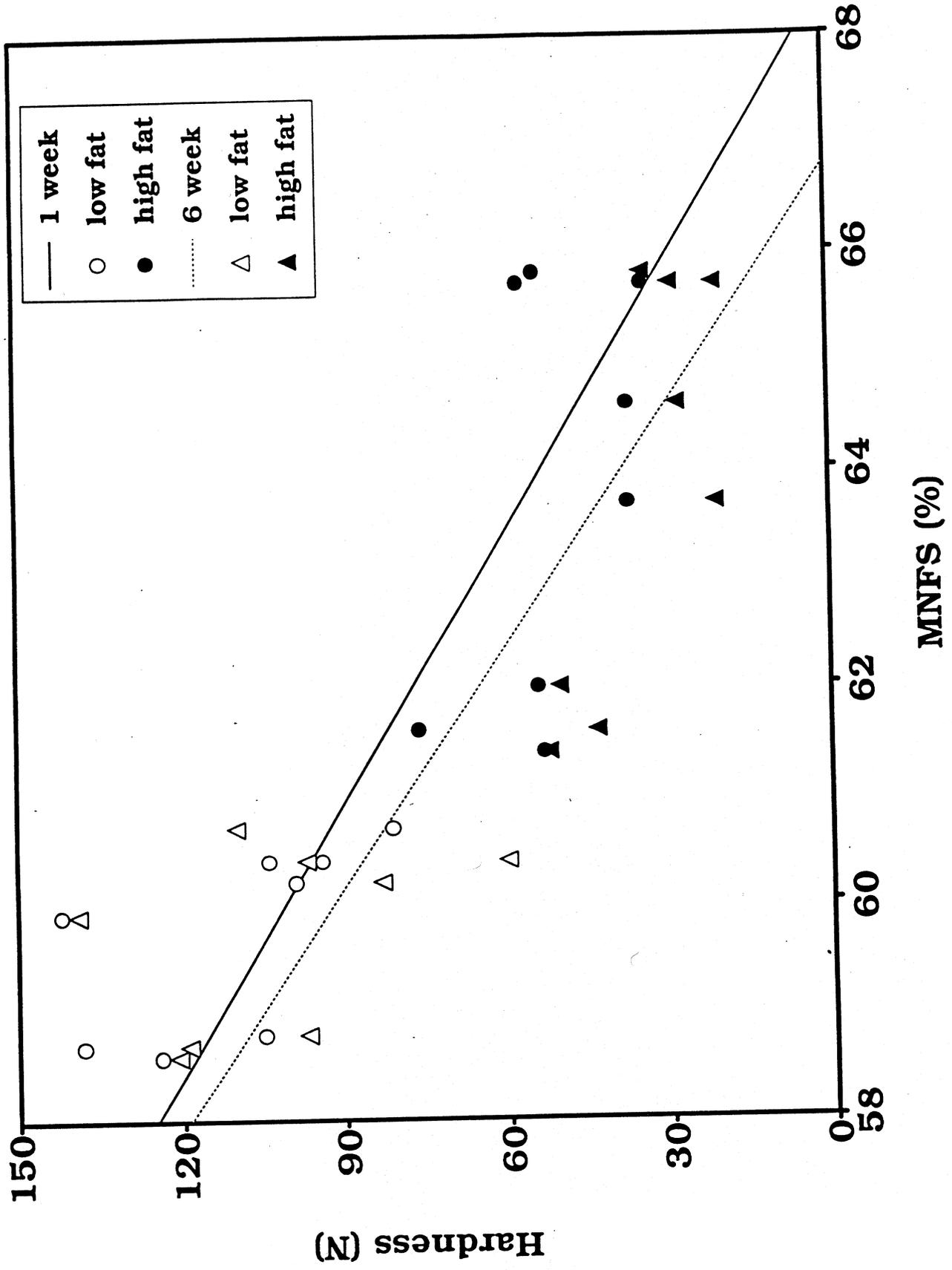
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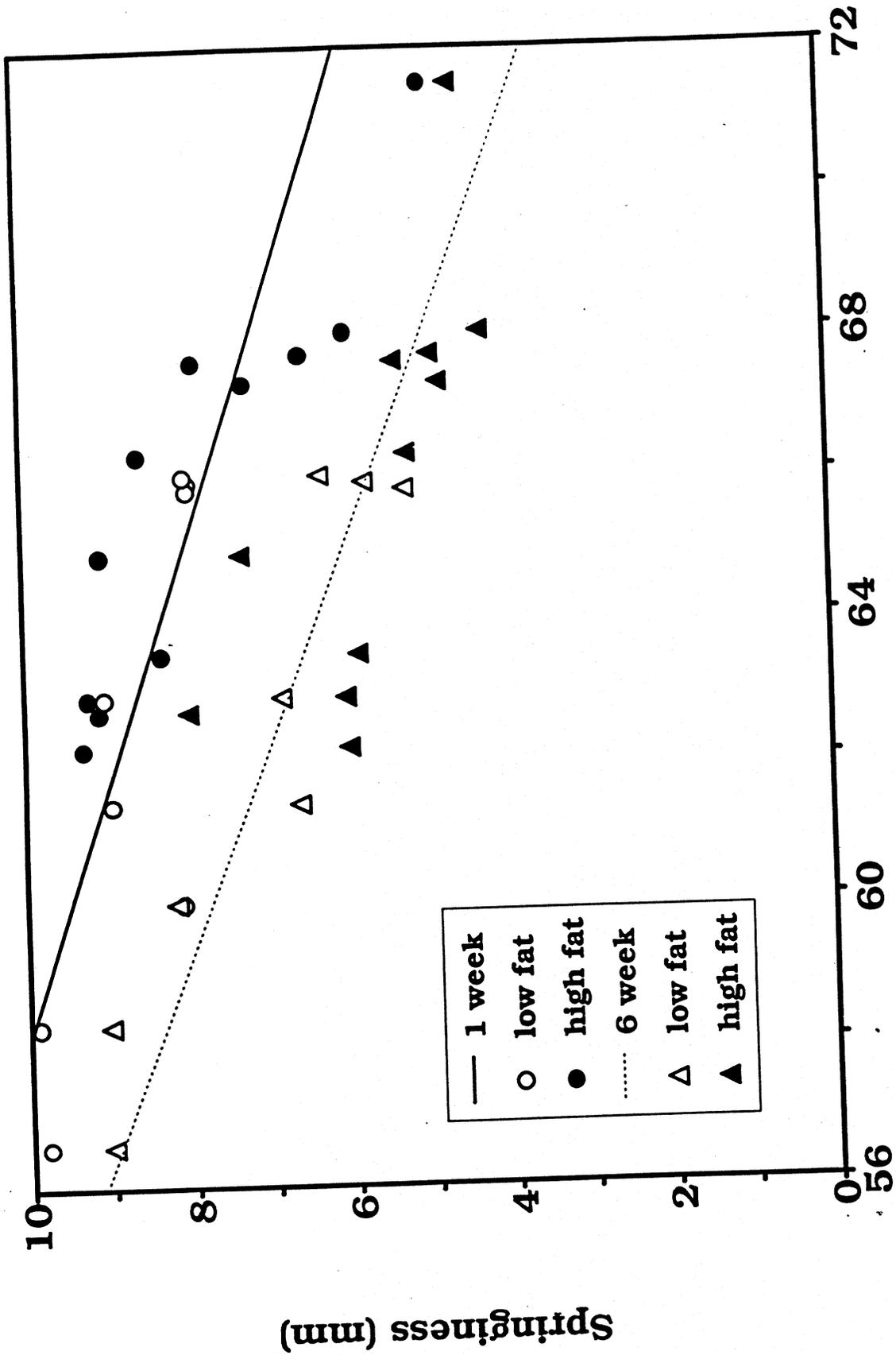
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Springiness (mm)

% MNFS

