Use of Fat Replacers in Low Fat Mozzarella Cheese[1]

D. J. McMAHON,* M. C. ALLEYNE,* R. L. FIFE,* and C. J. OBERG†
*Western Center for Dairy Protein Research and Technology,
Department of Nutrition and Food Sciences,
Utah State University, Logan 84322-8700
†Department of Microbiology, Weber State University, Ogden, UT 84404-2506

ABSTRACT

Low fat Mozzarella cheeses with <6% fat were made using fat replacers to increase the moisture content and to improve the functional properties of the cheese. We used two protein-based fat replacers (Simplesse® D100 and Dairy-Lo®) and two carbohydrate-based fat replacers (Stellar™ 100X and Novagel™ RCN-15). Moisture contents of the cheeses were 53.0, 54.3, 55.2, 55.3, and 57.3% for the control, Stellar™, Dairy-Lo®, Simplesse®, and Novagel™ cheeses, respectively. Apparent viscosity of the cheese at 80°C was not significantly affected by addition of fat replacers, but there was a significant effect on meltability. Cheeses made with Stellar™ and Simplesse® had greater overall meltability than cheese made with Dairy-Lo® or the control. The cheese made with Novagel™ contained the most moisture but, from d 1 to d 14, melted to the least extent. By d 21, all cheeses melted to the same extent.

The location of the fat replacers in the cheese structure was examined using scanning electron microscopy. Distribution of the fat replacers within the cheese was influenced by the extent of microparticulation of the fat replacer, size of the fat replacer particles, and processing steps that caused an interaction between the fat replacer and the caseins in milk. Novagel™ was present as the largest particles (up to 80 μm), and their incorporation into the cheese curd resulted in increased openness in the cheese, and large serum channels (up to 300 μm) were formed. None of the other fat replacers increased the openness of the cheese structure. Cheese sample preparation for the electron microscopy caused the Novagel™ particles to shrink and to appear artifactually as solid particles rather than as fibrous particles as shown by freeze-drying. Stellar™ was observed as spheroid particles (0.5 to 1 μm in diameter) embedded within the protein matrix of the cheese as well as being present in the serum channels. No discrete Dairy-Lo® particles were observed in the protein matrix, indicating a low level of microparticulation (particles < 0.2 μm) of the proteins in Dairy-Lo®.

(**Key words**: microstructure, low fat, Mozzarella, fat replacers)

Abbreviation key: $\mathbf{AV_{80}}$ = apparent viscosity at 80°C, **SEM** = scanning electron microscopy.

INTRODUCTION

In recent years, consumer demand for low fat foods has encouraged research on low fat cheese (3, 27), including research on reducing the fat content of Mozzarella cheese (16, 17, 21, 32, 33). Problems of inferior organoleptic and physical properties in these products suggested the use of fat replacers to provide the desirable qualities of traditional cheese (5, 14). When cheese with a fat content ≤10% is manufactured without modification of the regular procedure, that cheese is usually hard, rubbery, and translucent and has poor flavor development. For low fat Mozzarella cheese, a further problem is the poor meltability and performance when cooked on a pizza (17). These properties can be ameliorated if the water content of the cheese is increased by modifying the cheese manufacturing procedure (16, 33), incorporating whey proteins into the cheese curd (16), or by adding ingredients that bind moisture or impart fatlike characteristics when incorporated into the cheese matrix (9, 12, 18, 28). Fat replacers have been used successfully in foods such as salad dressings, bakery products, and processed cheeses (7); however, there are few reports of their use in natural cheese.

Many commercial fat replacers are now available and are promoted for their potential to make superior low fat products (4, 9, 10, 18, 30). Fat replacers that are suggested for use in cheese can be grouped into two categories, microparticulated materials based on protein or microparticulated materials based on carbohydrate. Whey proteins that have been micropartic-

Received July 17, 1995.
Accepted July 9, 1996.
[1]Contribution Number 4648 of the Utah Agricultural Experiment Station, Utah State University, Logan 84322-4810. Approved by the director. Mention of companies or products does not constitute endorsement by Utah State University, Utah Agricultural Experiment Station, or Weber State University over similar products not mentioned.

ulated by heat and mechanical action are the only protein-based fat replacers that have been proposed for use in cheese. In comparison, a variety of carbohydrates have been used to make fat replacers, including starch, pectin, and cellulose, often in combination with plant or bacterial gums such as guar gum and xanthan gum.

Increasing the ratio of water to protein of cheese makes the cheese softer. Water is thought to act as a lubricant or plasticizer between the protein molecules, making the cheese more pliable (7, 33). Fat replacers, because of their particulate nature, can also act as light-scattering centers and increase the opaqueness of low fat cheese. It has been shown that the fat content of cheeses influences cheese microstructure (1, 17), and we have observed that a higher moisture content also helps improve cheese meltability. In this study, we made low fat (≤6% fat) Mozzarella cheese with four different fat replacers and evaluated the location of the fat replacers in the cheese in relation to moisture content and melting properties of cheese.

MATERIALS AND METHODS

Cultures, Rennet, and Milk

Skim milk and 2% milk from the Gary H. Richardson Dairy Products Laboratory at Utah State University were pasteurized at 80°C for 29 s, cooled to 4°C, and mixed to give a 4.2 ratio of casein to fat. Lyophilized direct-set cultures of *Lactobacillus helveticus* LH 100 and *Streptococcus thermophilus* TA 061 and single-strength calf rennet extract were obtained (Rhône-Poulenc Marshall Products, Madison, WI).

Fat Replacers

The fat replacers used were Simplesse® D100 (NutraSweet Co., Deerfield, IL), which is based on whey protein; Dairy-Lo® (Pfizer Inc., New York, NY), a whey protein concentrate containing 35% protein; Stellar™ 100X (A. E. Staley Mfg. Co., Decatur, IL), a fat replacer of modified corn starch plus xanthan gum; and Novagel™ RCN-15 (FCM Corp., Philadelphia, PA), a blend of microcrystalline cellulose and guar gum. The quantity of fat replacer used in making cheese was based on usage levels recommended by the manufacturer: 0.6 g of Simplesse® or Stellar™ kg of milk and 2.5 g of Novagel™ or Dairy-Lo®/kg of milk. Dispersion of fat replacers in milk was also according to manufacturer recommendations. Simplesse® or Stellar™ was added to milk at 4°C and dispersed for 2 min using a high speed mixer (Omni 5000; Omni International, Gainsville, VA); the milk was then warmed to 30°C for cheese making. Novagel™ was similarly dispersed in milk, and then the milk was heated to 63°C and immediately cooled to 30°C. Dairy-Lo® was dispersed in a portion (approximately 30%) of the milk at 4°C, heated to 80°C for 10 min, and then cooled to 30°C; the remainder of the milk was then added.

Cheese Making

A control batch of low fat Mozzarella cheese without fat replacers and four batches containing fat replacers were made using 7.3 kg of milk per batch according to the methods of Merrill et al. (16). Apart from addition of fat replacers, all vats were treated identically. Milk pH was adjusted to 6.0 using lactic acid (EM Industries Inc., Cherry Hill, NJ), then warmed to 34°C, and inoculated with 0.75 g each of *S. thermophilus* and *L. helveticus*. After 45 min of ripening, 3 ml of rennet (diluted to 30 ml with distilled water) were added to each vat of milk, and a firm set was produced in 10 min. Curd was cut with 1.9-cm knives and then allowed to heal for 15 min. The temperature was raised to 38°C over 10 min; then the whey was drained, and curds were cheddared (turning every 20 min) at 38°C until the curd pH reached 5.2. The cheddared curd was cut, hand-stretched in hot water at 83°C until elastic and smooth, placed into stainless steel molds (9 × 9 × 9 cm), and immersed in ice water for 30 min to cool. Cheese loaves were removed from the molds, placed into individual brines (saturated NaCl, pH 5.0, 4°C) for 4 h, then vacuum-packaged, and stored at 4°C.

Chemical Analysis

Cheese pH, moisture, and fat were measured after 1 d. Fat was determined using a modified Babcock method (24). Cheese moisture was determined by microwave oven (model AVC 80; CEM Corp., Matthews, NC) by drying samples to a constant weight (i.e., <0.1 mg change over 10 s) at 50% power. Each sample was measured in triplicate.

Physical Analysis

Meltability and apparent viscosity of melted cheese were measured at 1, 7, 14, and 28 d. Melting was determined using the test tube method of Olson and Price (23) at an oven temperature of 150°C. The apparent viscosity of melted cheese at 80°C ($\mathbf{AV_{80}}$) was measured by the method of Kindstedt and Kiely (11) as modified by Fife et al. (6) and reported as the mean of readings from 0.5 to 1.5 min.

Electron Microscopy

Samples of stretched cheese (3 × 3 × 10 mm) were fixed in 2% glutaraldehyde (Electron Microscopy Sciences, Fort Washington, PA) for 18 h. Fixed samples were then fractured in liquid nitrogen and prepared for examination of microstructure using the high resolution scanning electron microscopy (**SEM**) technique of McManus et al. (15). The samples were dehydrated into 100% ethanol, rehydrated back into distilled water, and then placed in 0.1*M* sodium cacodylate buffer, pH 7.0, for 10 min prior to metal impregnation using 1% (wt/vol) OsO_4 and 1.5% (wt/vol) $K_4[Fe_9(CN)_6]\cdot 3H_2O$ in the same buffer, followed by 2% (wt/vol) tannic acid in buffer, then again in the osmium and ferrocyanide mixture, followed by 1% (wt/vol) hydroquinone in distilled water for 18 h. The samples were then dehydrated back to ethanol, dried to critical point using CO_2 in a 1200 Critical Point Dryer (Polaron, Waterford, England), and mounted on aluminum SEM stubs with fingernail polish.

To gain information on particulation of the fat replacers, 10% (wt/wt) dispersions of each fat replacer in distilled water were prepared and allowed to hydrate for 1 h at 22°C. An air-dried sample was obtained by placing a drop of the dispersion on an SEM stub, spreading the drop into a thin layer, and then allowing it to air-dry at 22°C. A freeze-dried sample was prepared by placing a drop of dispersion on an SEM stub that had been coated with fingernail polish and then freeze-drying it in a liquid nitrogen-cooled Freon 22 slush, transferring to liquid nitrogen, and removing water using a turbo-molecular pump (IBS/TM200S, VCR Group, South San Francisco, CA) at 2×10^{-6} torr for 18 h.

All SEM stubs with affixed samples were coated with 3 ± 1 nm of iridium by ion beam sputtering (VCR Group). Samples were then examined in a S-4000T field emission, SEM (Hitachi Scientific Instruments, Mountain View, CA) at an accelerating voltage of 5 kV. Images were recorded on T-Max 100 film (Kodak, Rochester, NY) at an exposure time of 80 s.

Statistical Analysis

The cheese making was conducted in triplicate. Analysis of variance was performed using SAS® (26), and chemical composition was analyzed as a randomized block design. Physical properties of melt and AV_{80} were analyzed as a split-plot design with storage time as the split plot. Means were compared by the least significant differences method. Significance was at $P \leq 0.05$ unless stated otherwise.

TABLE 1. Least squares means of moisture contents of low fat Mozzarella cheeses made from milk containing a fat replacer.[1]

Cheese	Moisture
	(%)
Control (no fat replacer)	53.0[c]
Stellar™ 100X	54.3[b]
Dairy-Lo®	55.2[b]
Simplesse® D100	55.3[b]
Novagel™ RCN-15	57.3[a]

[a,b,c]Means (n = 3) without a common superscript letter differ ($LSD_{0.05}$ = 1.05).

[1]Stellar™ 100X (A. E. Staley Manuf. Co., Decatur, IL), Dairy-Lo® (Pfizer Inc., New York, NY), Simplesse® D100 (NutraSweet Co., Deerfield, IL), and Novagel™ RCN-15 (FMC Corp., Philadelphia, PA).

RESULTS

Composition

The fat contents of the low fat cheeses were all between 4 and 5% fat, which met the US food labeling requirement of ≤3 g fat/50 g of the reference amount for low fat foods. There were no differences ($P > 0.05$) in fat content among the cheeses.

There was a difference ($P < 0.01$) in the moisture content of the cheeses as a result of adding fat replacers (Table 1). Low fat cheeses made with the whey-based replacers, Dairy-Lo® and Simplesse®, had 2.2 and 2.3% higher moisture contents, respectively, than did the control cheese, which agrees with the observations of Lucey and Gorry (12). The low fat cheeses containing the carbohydrate-based fat replacers Stellar™ and Novagel™ contained 1.3 and 4.3% more moisture, respectively, than the control cheese. Some of these differences in moisture content may reflect the quantity of fat replacer used in cheese manufacture.

Functional Properties

Addition of fat replacers had a significant effect on how the cheeses functioned when they were heated (Table 2). Using the distance that cheese flows when heated as an index of meltability, addition of Stellar™ or Simplesse® increased melt, but addition of Novagel™ or Dairy-Lo® decreased melt (Figure 1). Although no precise definition for "melt" exists, melt has commonly been used (11) to describe flow properties associated with heated Mozzarella cheese in the absence of an applied force and can be considered an indication of how the cheese will perform when baked on a pizza.

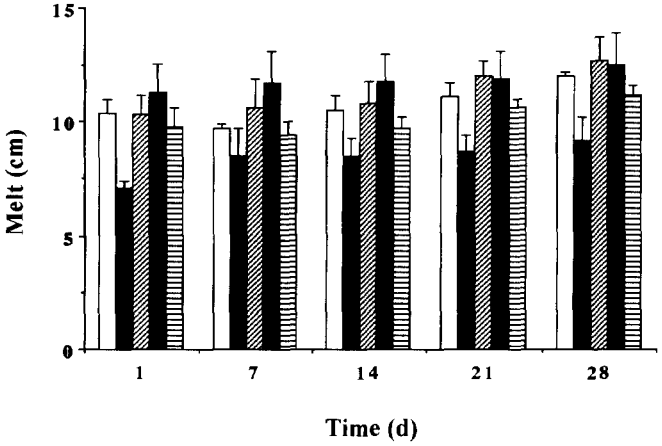


Figure 1. Mean (±SEM) measurements of melt of low fat Mozzarella cheese made with milk containing no fat replacer (open bar), Novagel™ (FMC Corp., Philadelphia, PA) (solid bar), Simplesse® (NutraSweet Co., Deerfield, IL) (diagonal striped bar), Stellar™ (A. E. Staley Manuf. Co., Decatur, IL) (gray bar), and Dairy-Lo® (Pfizer Inc., New York, NY) (horizontally striped bar).

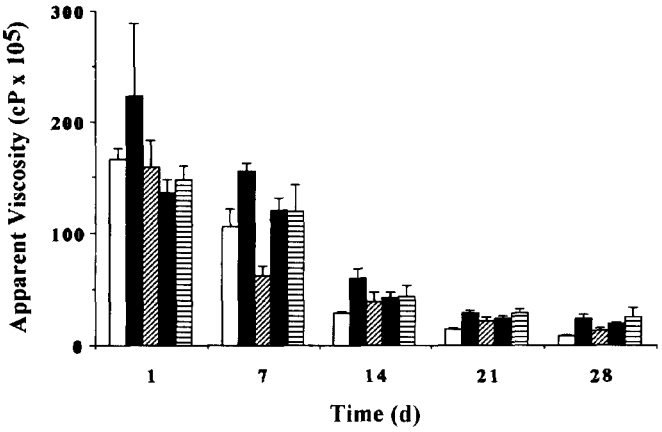


Figure 2. Mean (± SEM) measurements of apparent viscosity at 80°C of low fat Mozzarella cheese made with milk containing no fat replacer (open bar), Novagel™ (FMC Corp., Philadelphia, PA) (solid bar), Simplesse® (NutraSweet Co., Deerfield, IL) (diagonal striped bar), Stellar™ (A. E. Staley Manuf. Co., Decatur, IL) (gray bar), and Dairy-Lo® (Pfizer Inc., New York, NY) (horizontally striped bar).

The typical pattern of increased melt during storage of part-skim Mozzarella cheese (11, 20, 21, 22) and reduced fat (10% fat) Mozzarella cheese (16) was observed for the low fat cheeses; this effect of storage time on melt was very highly significant ($P \leq 0.001$). There were also differences between individual treatments ($P \leq 0.05$) at specific storage times (Figure 1). At d 1, 7, and 14, the cheese containing Novagel™ melted less ($P < 0.05$) than the control cheese or other cheeses containing fat replacers. On d 21 and 28, there were no significant differences between cheeses.

Addition of a fat replacer did not have a significant effect on AV_{80} (Table 2). During storage, all of the cheeses followed the same pattern of decreasing AV_{80} as has been previously shown (20, 21, 22). There were large variations in AV_{80} measurements, which may have precluded determination of significance between the various treatments (Figure 2).

Microstructure

Control Cheese. Low fat Mozzarella cheese, made without any fat replacer, had the typical microstructure of Mozzarella cheese after hot water stretching: a continuous protein matrix interspersed with serum channels (Figure 3), although, compared with mechanically stretched cheese (19), the protein

TABLE 2. The ANOVA for melt and apparent viscosity at 80°C (AV_{80}) for low fat Mozzarella cheeses containing fat replacers and for a control cheese without fat replacer, as a function of fat replacer and storage time.

		Mean squares	
Source of variation	df	Melt	AV_{80}
Replicate	2	29.91	4.20×10^{13}
Fat replacer (FR)	4	26.25*	2.74×10^{13} NS[1]
Error A	8	4.54	2.42×10^{13}
Storage time (T)	4	7.56***	57.29×10^{13}***
FR × T	16	0.47NS	2.00×10^{13} NS
Error B	40	0.65	1.25×10^{13}
Corrected total	74		

[1]$P > 0.05$.

*$P < 0.05$.

***$P < 0.001$.

strands in the hand-stretched cheeses were less unidirectionally oriented. During SEM preparation, the cheese samples were fractured perpendicularly to the protein fibers so that the serum channels were mostly seen in cross-section and were about 5 to 15 μm in width. Compared with low moisture, part-skim Mozzarella cheese (which contains 15 to 22% fat) (13), serum channels between the protein strands were fewer in the experimental cheeses, which was expected, because those cheeses contained <6% fat.

In Mozzarella cheese, the serum channels are the location of the fat globules and most of the bacteria (13, 19), which are lost from the serum channels after the sample is fractured during SEM preparation. Fat is also extracted during dehydration with ethanol, and, although some bacteria were observed in the serum channel, most of them would have been washed from the fracture zone. Occasionally, bacteria and other debris were observed on the fracture surface of the samples, probably as a result of electrostatic attraction after being displaced from the serum channels during sample preparation. Collapsed fat globule membranes or their fragments were also observed occasionally in the serum channels.

Stellar™. Addition of Stellar™ did not increase the openness of the cheese structure (Figure 4) compared with that of the control cheese. Small spheroid particles (0.2 to 0.5 μm diameter) were embedded in the protein matrix and also were lying on the fracture surface. When the cheese samples were fractured during SEM preparation, the contents of any exposed serum channels would have been released, and some of these materials adhered to the newly formed fracture surface. Thus, any materials seen on the fracture surface would most likely have originated from the serum channels. Stellar™ consists of microparticu-

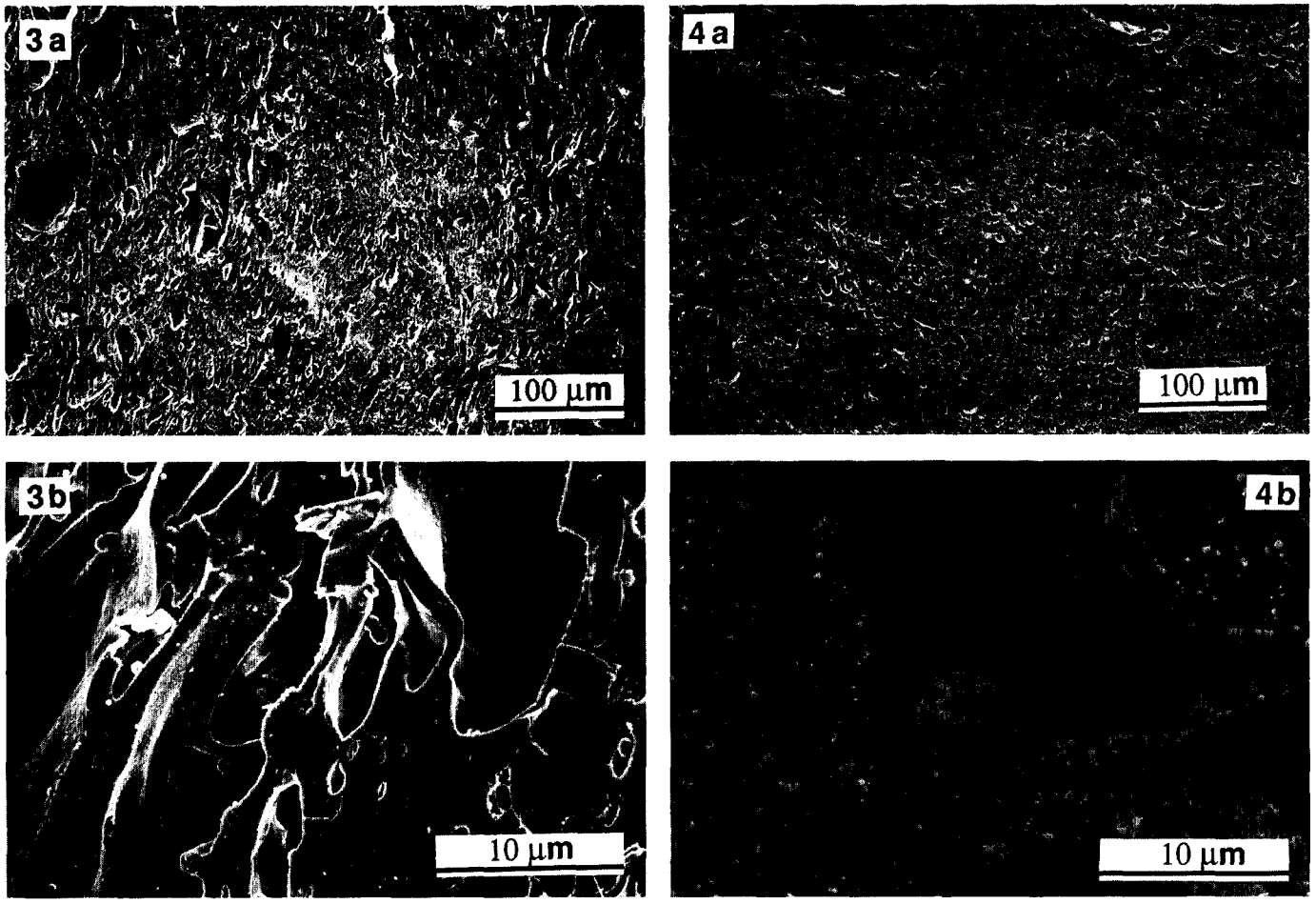


Figure 3. Scanning electron micrographs of control low fat Mozzarella cheese at low (a) and high (b) magnifications.

Figure 4. Scanning electron micrographs of low fat Mozzarella cheese containing Stellar™ (A. E. Staley Manuf. Co., Decatur, IL) at low (a) and high (b) magnifications.

lated material and nonparticulated material (Figure 5), but any nonparticulated material intermixed with the proteins would be indistinguishable, given the resolution level of SEM and the metal coating applied to the sample as part of SEM preparation.

Novagel™. Addition of Novagel™ to the low fat cheese increased the openness of the cheese structure (Figure 6). These cheeses contained large amorphous fat replacer particles that were much greater than the serum channels observed in the control cheese. The size of the cavities introduced by the Novagel™ particles ranged from 30 to 300 μm. Many of these serum channels still contained Novagel™ particles and large numbers of bacteria. Bacilli and cocci (presumably from the *L. helveticus* and *S. thermophilus* starter cultures), as well as nonstarter bacteria (possibly enterococci and streptococci, based upon their morphology), were often observed in the same serum channels as the Novagel™ particles.

The Novagel™ particles (Figure 7) were much larger than the Stellar™ particles (Figure 5) and ranged in size from 10 to 100 μm. When Novagel™ microparticles were air-dried from aqueous dispersion (Figure 7) or were dehydrated by transfer into ethanol (Figure 6), they had the appearance of being solid particles. This appearance was similar to the partially collapsed spheres of spray-dried gum arabic microcapsules that were observed by Rosenberg et al. (25), and so it was assumed that the Novagel™ particles had undergone a similar collapse. Their solid-like appearance was thus assumed to be an artifact of SEM preparation. When the aqueous Novagel™ dispersion was freeze-dried, a completely different structure was apparent, and the Novagel™ particles were observed as a loose network of fibrous material (Figure 8). Such a fibrous nature would be expected of a particle consisting of microcrystalline cellulose combined with guar gum. Upon further examination of cheese samples containing Novagel™, some Novagel™ particles that had been fractured were observed (Figure 9), which confirmed their fibrous nature. It was also apparent in Figure 9 that the Novagel™ particles had occupied the entire serum cavity within the cheese but that they collapsed during the dehydration process of SEM preparation.

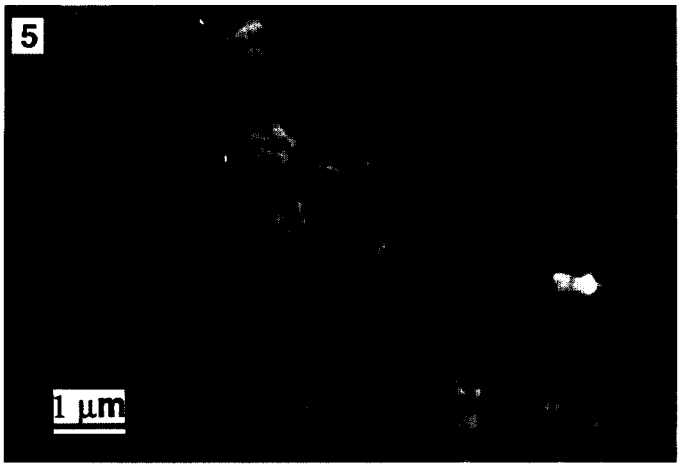


Figure 5. Scanning electron micrograph of air-dried Stellar™ (A. E. Staley Manuf. Co., Decatur, IL) microparticles.

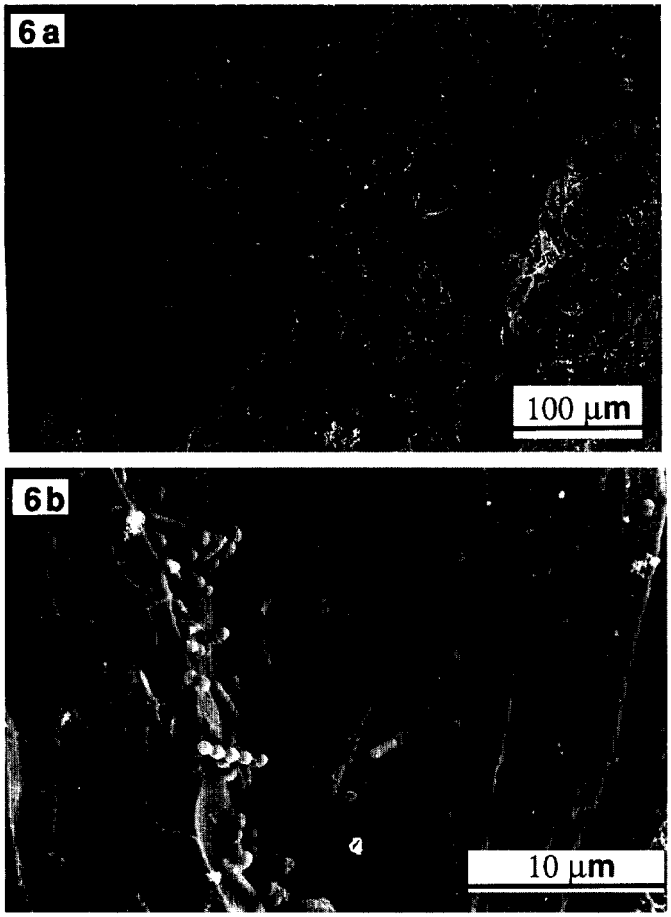


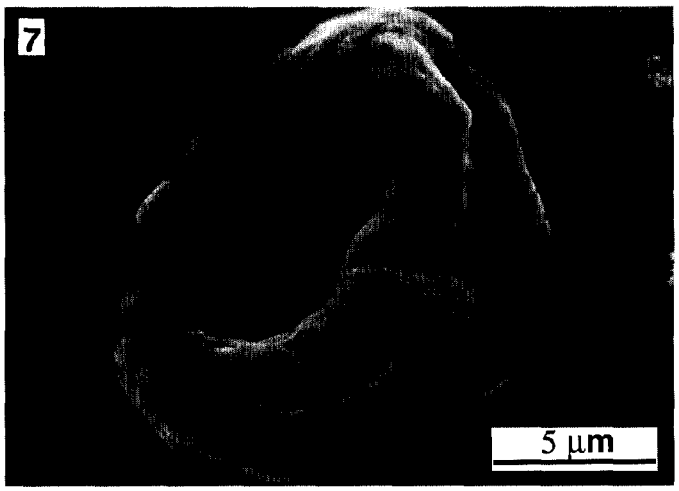
Figure 6. Scanning electron micrographs of low fat Mozzarella cheese containing Novagel™ (FMC Corp., Philadelphia, PA) at low (a) and high (b) magnifications.

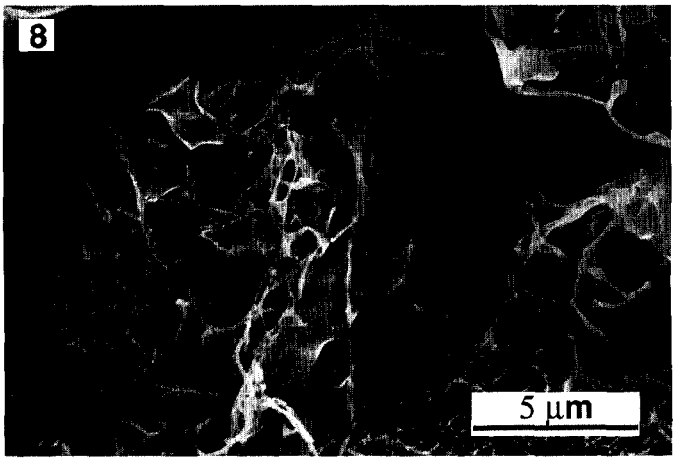
Simplesse®. Addition of Simplesse® did not increase the openness of low fat Mozzarella cheese because no differences were observed between the number or size of the serum channels (Figure 10a) compared with those of the control cheese (Figure 3). At higher magnification, spherical particles approximately 0.5 to 1.0 μm diameter were observed embedded within the cheese protein matrix and also lying

on the fracture surface. The size of these particles observed in cheese (Figure 10b) and from aqueous dispersion (Figure 11) corresponded to the structure of Simplesse® microparticles shown using atomic force microscopy (8) and were within the expected size range of 0.1 to 2.0 μm (28, 29). Thus, the Simplesse® particles were approximately twice the size of the Stellar™ particles.

The Simplesse® particles observed on the fracture surface would originally have been contained in the serum channels, indicating that Simplesse® was distributed between the protein matrix and the serum. However, unlike Novagel™, the Simplesse® particles were too small to influence the size of the serum channels. The morphology of the Simplesse® particles are shown in Figure 12, and, of the four fat replacers tested in this experiment, the Simplesse® particles were the most spherical and were present as particles of a relatively narrow size distribution. This figure also shows the location of Simplesse® within the protein matrix and how any debris in the fluids used during SEM preparation can become attached to the fracture surface. It was also interesting to note the size of Simplesse® particles relative to bacteria (presumably *L. helveticus*). The presence of what appeared to be a partially degraded Simplesse® particle attached to a bacterial cell (Figure 12b) implied that the denatured whey proteins of Simplesse® were susceptible to bacterial enzymes and could act as an additional nitrogen source for the bacteria.

Dairy-Lo®. Addition of Dairy-Lo® did not increase openness of the cheese (Figure 13a), and identification of this fat replacer in the cheese was problematic. Even at high magnification, there was little difference between the Dairy-Lo® cheese and the control cheese (Figure 13b). There were some clumps of material (about 5 to 10 μm size) within the serum channels and on the fracture surface. Smaller particles (<0.5 μm) were also observed within the protein surface of the serum channels, although they were not apparent within the fracture surface. The serum channels in the control cheese typically had smooth surfaces, and so these small particles were assumed to be Dairy-Lo® microparticles. The Dairy-Lo® fat replacer had a much lower microparticulation size (Figure 14) than Simplesse® (Figure 11), and, at the resolution available using SEM, nonparticulated material was difficult to differentiate from other proteins contained in the cheese matrix. The high proportion of nonparticulated material in Dairy-Lo® was confirmed when a freeze-dried sample of the Dairy-Lo® dispersion was examined (data not shown).





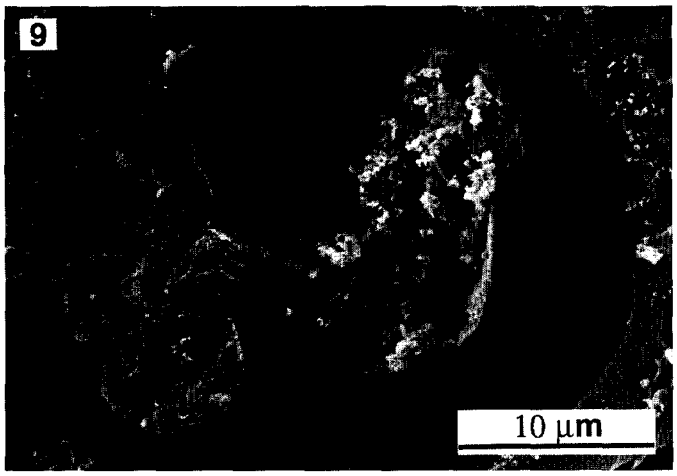


Figure 7. Scanning electron micrograph of air-dried Novagel™ (FMC Corp., Philadelphia, PA) microparticle.

Figure 8. Scanning electron micrograph of freeze-dried Novagel™ (FMC Corp., Philadelphia, PA).

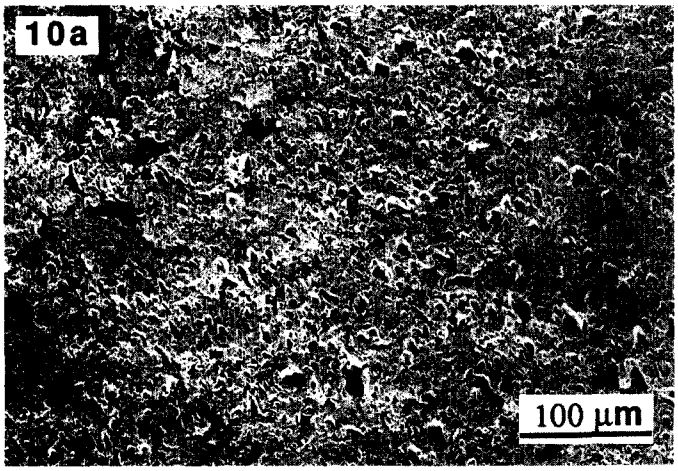
Figure 9. Scanning electron micrographs of Novagel™ (FMC Corp., Philadelphia, PA) microparticles in low fat Mozzarella cheese showing the collapse of particles that occurred during sample preparation.

DISCUSSION

There are a number of considerations when a fat replacer is being selected to use in the manufacture of low fat cheeses. These include level of microparticulation, size of the microparticles, interaction of the fat replacer with caseins, quantity of fat replacer used, distribution of the fat replacer between casein matrix and serum within the cheese curd, and distribution (and, hence, yield) of the fat replacer between curd and whey. For the four fat replacers used in this work (Stellar™, Novagel™, Simplesse®, and Dairy-Lo®), differences were observed in their morphology, their location in the cheese, their impact on cheese microstructure, their effect on cheese moisture content, and their effect on melted cheese functionality.

We did not measure the distribution of fat replacers between curd and whey, but, assuming 70% retention in the curd of carbohydrate material in Novagel™ and Stellar™ and 70% retention of protein material from Simplesse® and Dairy-Lo®, relative quantities of the fat replacers in the cheeses could be approximated. Novagel™ cheese would have contained about 1.7% fat replacer, Dairy-Lo® cheese about 0.6% fat replacer (Dairy-Lo® powder contains only 35% protein), Stellar™ cheese about 0.4% fat replacer, and Simplesse® cheese about 0.2% fat replacer (Simplesse® powder contains 53% protein). Therefore, the Novagel™ cheese would be expected to show the highest increase in moisture content because it was present at the highest level. However, the functionality of the fat replacer may also be important because Simplesse® cheese had 1% more moisture than Stellar™ cheese even though there should have been twice as much Stellar™ in the cheese. Similarly, the two fat replacers that were based on whey protein (Dairy-Lo® and Simplesse®) produced cheeses with the same moisture content (55%), even though more Dairy-Lo® was used.

Simplesse® was observed as microparticles embedded within the casein matrix, which may allow greater moisture retention than does Dairy-Lo®, which was present as smaller particles. Simplesse® is made by a proprietary process involving high shear, high temperature treatment to generate microparticles (28); Dairy-Lo® is produced by ultrafiltering sweet whey followed by heating to denature the whey proteins partially. Loss of the Dairy-Lo® from the curd may have been greater because of its smaller size. Recommended usage of Dairy-Lo® required a heat treatment at 80°C for 10 min (in a portion of the milk) to bind the whey proteins in Dairy-Lo® to the casein micelles in milk through bonding with κ-CN. However, we did not analyze the milk after heat treatment to determine whether sufficient additional whey protein denaturation had occurred to bring about bonding of β-LG and κ-CN. Such bonding of



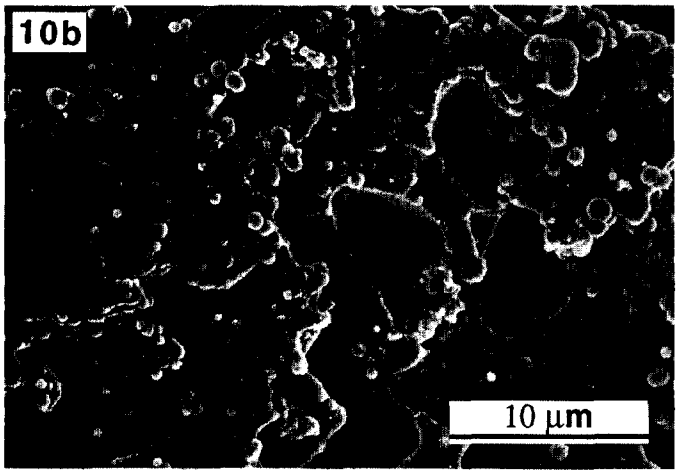


Figure 10. Scanning electron micrographs of low fat Mozzarella cheese containing Simplesse® (NutraSweet Co., Deerfield, IL) at low (a) and (b) high magnification.

Figure 11. Scanning electron micrograph of air-dried Simplesse® (NutraSweet Co., Deerfield, IL) microparticles.

denatured β-LG to casein micelles in milk has been shown to reduce curd syneresis during cheese making, but it was not determined whether this factor contributed to the increased moisture of the Dairy-Lo® or whether it was solely a function of the water-holding ability of the whey proteins incorporated into the cheese curd.

The increased moisture content of low fat cheese made using fat replacers suggested that curd syneresis was retarded during cheese making, which can occur as a result of water being bound directly to the fat replacer, or the fat replacer may interfere with shrinkage of the casein matrix, thus lowering the driving force involved in expelling water from the curd particles. The effectiveness of Novagel™ in increasing moisture content can be attributed to the increased openness of cheeses containing Novagel™. One of the functions of fat in Mozzarella cheese is the formation of serum channels. If all of the fat is removed, no serum channels were observed (D. J. McMahon and C. J. Oberg, 1996, personal observations), and fewer serum channels were observed in low fat Mozzarella cheese than in part-skim Mozzarella cheese (21).

The expressible moisture in Mozzarella cheese is contained in these serum channels (13), and, because Novagel™ particles are large, they are able physically to prevent fusion of the casein fibers during stretching of the cheese, allowing greater retention of serum not only because of the water-holding abilities of individual Novagel™ particles, but also because the creation of new serum channels would allow retention of serum adjacent to the fat replacer particles. When such large serum channels are distributed throughout the casein matrix, the cheese is softer and more plia-

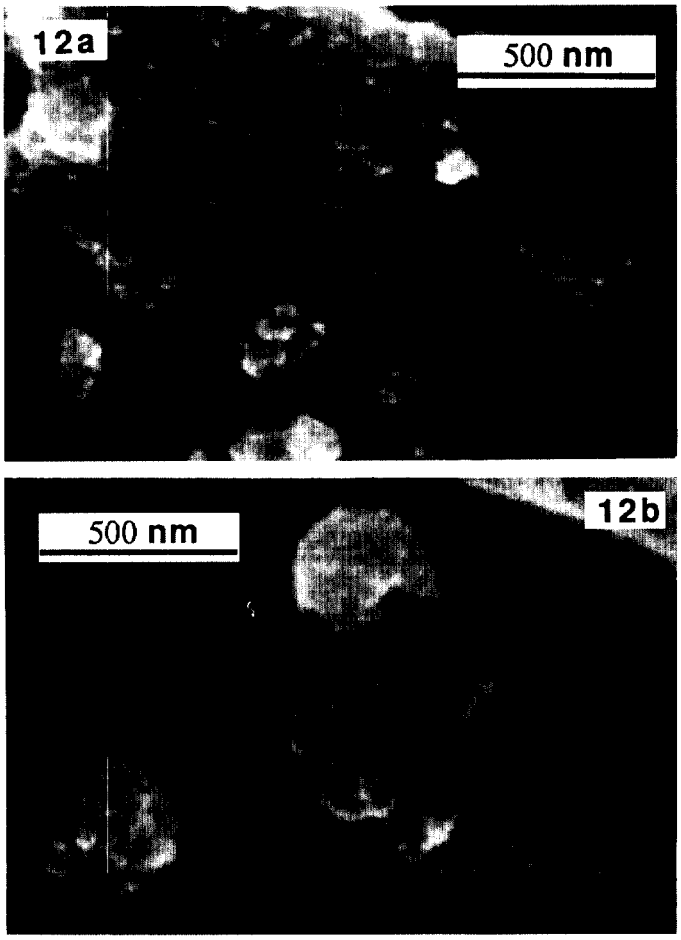


Figure 12. Scanning electron micrographs of Simplesse® (NutraSweet Co., Deerfield, IL) microparticles imbedded in protein matrix (a) and attached to bacterial cell (b) in low fat Mozzarella cheese.

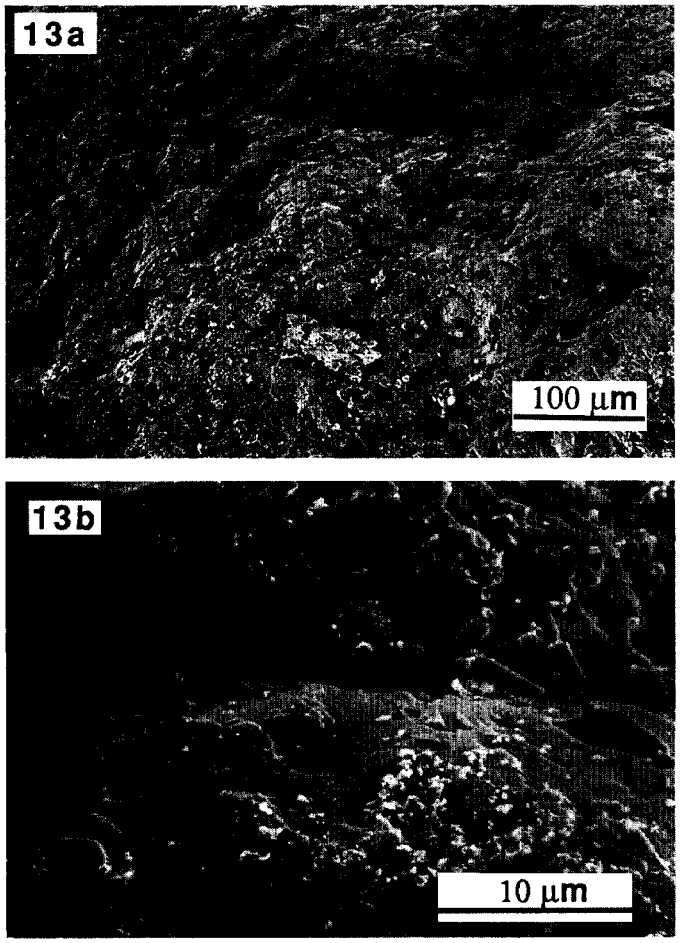


Figure 13. Scanning electron micrographs of low fat Mozzarella cheese containing Dairy-Lo® (Pfizer Inc., New York, NY) at low (a) and high (b) magnifications.

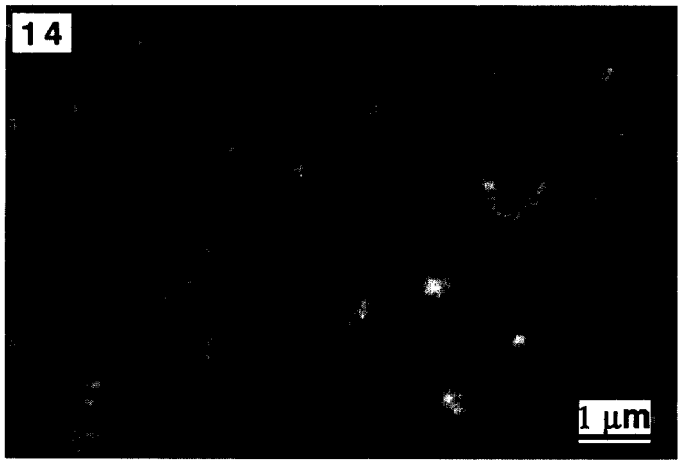


Figure 14. Scanning electron micrograph of air-dried Dairy-Lo® (Pfizer Inc., New York, NY) microparticles.

ble because of the increased moisture and impeded coalescence of the protein strands as shown by Taneya et al. (31). The other fat replacers (Stellar™, Simplesse®, and Dairy-Lo®) did not increase curd openness, so any increase in moisture would be a result of water-holding abilities of the individual fat replacer particles that were retained in the cheese curd.

We have observed a strong correlation between moisture content and meltability of Mozzarella cheese (data not shown), and so it was surprising that in this experiment the cheese with the highest moisture content (Novagel™ cheese) melted the least. A possible explanation is the large size and fibrous nature of the cellulose particles in Novagel™ may have impeded slippage of the proteins, retarding movement of the cheese when it was heated. Cheese containing Stellar™ had the most melt, suggesting that the starch component of Stellar™ provided lubrication, allowing protein strands to flow more easily. Dairy-Lo® cheese also reduced melt; perhaps the bonding of β-LG to caseins also impeded flow of the protein strands.

The observation of Stellar™ particles in the protein matrix as well as on the fracture surface suggested a distribution of Stellar™ between the protein matrix and the serum channels as described for Simplesse®. This result agrees with observations made using confocal scanning laser microscopy; when Stellar™ was used to manufacture low fat cheese, it was distributed evenly between the protein matrix and serum channels (Khalid Shammet, 1996, personal communication).

Size and extent of fat replacer microparticulation, as well as interaction between the fat replacer and caseins, affected location of the fat replacer in the cheese structure. Fat replacer particles such as Novagel™, which were larger than the individual protein strands in Mozzarella cheese, were too large to be embedded in the protein; instead they created large serum channels in the cheese. Smaller particles (such as Simplesse® and Stellar™) could be distributed between the protein matrix and the serum channels but had little effect on openness of the cheese microstructure. Each of the four fat replacers used in this work contained both particulated material and nonparticulated material, and the nonparticulated material was not observed, given the resolution and imaging constraints of SEM. Sample preparation prior to SEM examination must also be considered when SEM images are being interpreted, because changes in fat replacer morphology (such as shown for Novagel™) can occur.

ACKNOWLEDGMENTS

This research was performed in the Electron Microscopy Laboratory at Utah State University and supported by the National Dairy Protein Promotion and Research Board and the Utah Agricultural Experiment Station. The authors thank William McManus for helpful advice on electron microscopy and Gary Pederson for photographic work.

REFERENCES

1 Anderson, D. L., and V. V. Mistry. 1994. Reduced fat Cheddar cheese from condensed milk. 2. Microstructure. J. Dairy Sci. 77: 7.
2 Barbano, D. M., K. Y. Chu, J. J. Yun, and P. S. Kindstedt. 1993. Contribution of coagulant, starter, and milk enzymes to proteolysis and browning in Mozzarella cheese. Page 65 *in* Proc. 30th Annu. Marschall Italian Cheese Seminar, Madison, WI.
3 Duxbury, D. D. 1989. US domestic cheese trends. Continued market growth encourages cheese technology research. Food Process. 50:33.
4 Duxbury, D. D. 1992. Super stabilizer versatile as fat replacer. Food Process. 53:39.
5 Emmons, D. B., M. Kalab, E. Larmond, and R. J. Lowrie. 1980. Milk gel structure. X. Texture and microstructure in Cheddar cheese made from whole milk and from homogenized lowfat milk. J. Texture Stud. 11:15.
6 Fife, R. L., D. J. McMahon, and C. J. Oberg. 1996. Functionality of low fat Mozzarella cheese. J. Dairy Sci. 79:1903.
7 Glicksman, M. 1991. Hydrocolloids and the search for the "Oily Grail". J. Food Technol. 10:94.
8 Heertje, I. 1993. Structure and function of food products: a review. Food Struct. 12:343.
9 Hewitt, L. 1993. The low fat no fat boom. Food Manuf. 68:23.
10 Hoffmeister, A. 1993. A new milk protein concentrate for lowfat foods. Food Marketing Technol. 7:4.
11 Kindstedt, P. S., and L. J. Kiely. 1990. Physical and chemical methods for controlling the body and texture of Mozzarella cheese. Page 1 *in* Ninth Bienn. Cheese Ind. Conf., Logan, UT.
12 Lucey, J. A., and C. Gorry. 1993. Effect of Simplesse® 100 on the manufacture of low fat Cheddar cheese. Page 439 *in* Cheese

Yield and Factors Affecting Its Control. Int. Dairy Fed. Seminar, Int. Dairy Fed., Brussels, Belgium.
13 McMahon, D. J. 1995. How the microstructure of Mozzarella cheese is related to functional properties. Page 18 *in* Proc. 32nd Annual Marschall Italian and Specialty Cheese Seminar. Marschall Products, Rhône-Poulenc, Madison, WI.
14 McMahon, D. J., C. J. Oberg, and W. McManus. 1993. Functionality of Mozzarella cheese. Aust. J. Dairy Technol. 48:99.
15 McManus, W., D. J. McMahon, and C. J. Oberg. 1993. High resolution scanning electron microscopy of milk products: a new sample preparation procedure. Food Struct. 12:475.
16 Merrill, R. K., C. J. Oberg, and D. J. McMahon. 1994. Physical properties of Mozzarella cheese. A method for manufacturing reduced fat Mozzarella cheese. J. Dairy Sci. 77:1783.
17 Mistry, V. V., and D. L. Anderson. 1993. Composition and microstructure of commercial full-fat and low-fat cheeses. Food Struct. 12:259.
18 Mitchell, H. L. 1993. Novel milk protein concentrate for low-fat foods. Int. Food Ingred. 5:25.
19 Oberg, C. J., W. R. McManus, and D. J. McMahon. 1993. Microstructure of Mozzarella cheese during manufacture. Food Struct. 12:251.
20 Oberg, C. J., R. K. Merrill, R. J. Brown, and G. H. Richardson. 1992. Effect of milk-clotting enzymes on physical properties of Mozzarella cheese. J. Dairy Sci. 75:669.
21 Oberg, C. J., R. K. Merrill, W. R. McManus, M. Kalab, and D. J. McMahon. 1996. Microstructure and physical properties of a reduced fat Mozzarella cheese made using *Lactobacillus casei* adjunct culture. Food Sci. Technol. 29:721.
22 Oberg, C. J., A. Wang, L. V. Moyes, R. J. Brown, and G. H. Richardson. 1991. Effect of thermolactic culture proteolytic activity on physical properties of Mozzarella cheese. J. Dairy Sci. 74:389.
23 Olson, N. F., and W. V. Price. 1958. A melting test for pasteurized process cheese spread. J. Dairy Sci. 41:999.
24 Richardson, G. H. ed., 1985. Page 351 *in* Standard Methods for the Examination of Dairy Products. 15th ed. Am. Publ. Health Assoc., Washington, DC.
25 Rosenberg, M., Y. Talmon, and I. J. Kopelman. 1988. The microstructure of spray-dried microcapsules. Food Struct. 7:15.
26 SAS® User's Guide: Statistics, Version 6.1 Edition. 1991. SAS Inst., Inc., Cary, NC.
27 Simard, R. E. 1991. Evaluation of lowfat cheese problems. Page 37 *in* Proc. Cheese Res. Tech. Conf., Ctr. Dairy Res., Madison, WI.
28 Singer, N. S., and J. M. Dunn. 1990. Protein microparticulation: the principle and the process. J. Am. Coll. Nutr. 9:388.
29 Singer, N. S., S. Yamamoto, and J. Latella, inventors. 1988. Protein product base. US Pat. No. 4,734,287.
30 Staley Manufacturing Co. 1991. New starch-based fat replacer-ITF post-show report. Food Eng. 63:26.
31 Taneya, S., T. Izutsu, T. Kimura, and T. Shioya. 1992. Structure and rheology of string cheese. Food Struct. 11:61.
32 Tunick, M. H., K. L. Mackey, J. J. Shieh, P. W. Smith, P. Cooke, and E. L. Malin. 1993. Rheology and microstructure of low-fat Mozzarella cheese. Int. Dairy J. 3:649.
33 Tunick, M. H., K. L. Mackey, P. W. Smith, and V. H. Holsinger. 1991. Effects of composition and storage on the texture of Mozzarella cheese. Neth. Milk Dairy J. 45:117.