Effect of the Modification of Fat Particle Size by Homogenization on Composition, Proteolysis, Functionality, and Appearance of Reduced Fat Mozzarella Cheese¹

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ABSTRACT

The effect of the homogenization of milk (0.8% fat) and cream (20% fat) on cheese-making performance, composition, proteolysis, functionality, and appearance of reduced fat Mozzarella cheese was determined. Three vats (230 kg of milk per vat) of cheese were made in 1 d using unhomogenized milk (control), homogenized milk, and milk standardized with homogenized cream. Cheese manufacture was repeated on 3 different d using a randomized complete block design. The homogenized milk treatment had a large amount of curd shattering during cheese manufacture, but the control and the homogenized cream treatments did not. The chemical composition of all cheeses was similar, although the moisture contents of the cheese made from the homogenized products tended to be higher. Homogenization of the milk or cream did not have a large effect on unmelted textural properties of the cheese at 10°C. All cheeses had limited free oil release and melt and excessive browning during pizza baking. Homogenization of the milk increased the nitrogen that was soluble at pH 4.6 in the cheese but did not make this cheese more meltable. The most significant difference between treatments was the appearance of the unmelted cheese. Hunter L values and visual appearance indicated that the cheeses made from homogenized milk or cream were significantly whiter and more opaque than the control. Homogenization significantly improved the appearance of unmelted reduced fat Mozzarella cheese; however, because of improved cheese-

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making performance, homogenization is recommended of only the cream portion.

(**Key words**: low fat, Mozzarella cheese, functional properties, homogenization)

Abbreviation key: **AV** = apparent viscosity, **FO** = free oil, **HC** = homogenized cream treatment, **HM** = homogenized milk treatment, **TPA** = texture profile analysis, **VMD** = volume mean diameter.

INTRODUCTION

Effect of Homogenization on Cheese Functionality

Homogenization of milk or cream significantly decreases the fat particle size and changes the milk fat globule membrane (6). As a consequence, cheese made from homogenized milk or skim milk that was standardized with homogenized cream has properties that are different from those of cheese made from unhomogenized milk (10, 20). Homogenization of milk has been used in the cheese industry to increase yield (via lower fat losses in the whey), reduce fat leakage during melting, increase the rate of lipid hydrolysis for certain types of cheese (e.g., Blue cheese), and permit the use of recombined milk for cheese making (22). However, in commercial practice, the widespread use of homogenization in high fat cheese has not occurred. In addition to the extra time and energy required to homogenize milk, homogenization has other drawbacks. Homogenization can adversely affect the structure of the rennet gel, which results in poor functional properties and lower cheese yields because of curd shattering (8). Homogenization of only the cream portion significantly reduces the amount of protein that is subjected to homogenization and may limit some of the adverse effects (20).

Recently, there has been renewed interest in utilizing homogenization as a processing tool to enhance the properties of lower fat cheese. However, Jana and Upadhyay (11) found that homogenization of

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milk (one stage, total of 2.5 or 4.9 MPa) resulted in a Mozzarella cheese (26% fat) that exhibited less meltability than did cheese from unhomogenized milk. Furthermore, Tunick et al. (32) found that homogenization of milk (two-stage, total of 10.4 or 17.3 MPa) produced reduced fat Mozzarella cheese (9% fat) that was harder at 25°C and less meltable than cheese from unhomogenized milk measured after 1 or 6 wk of refrigerated storage.

The filled gel composite model (33) predicts two different types of effects of homogenization, particle size and the interaction of fat with the protein matrix, on the hardness of the composite. First, homogenization greatly decreases the fat particle size and greatly reduces the number of large fat particles. If the fat in the composite gel is completely solid, there should be no difference in the impact of large and small particles on hardness. For partially melted (i.e., 4°C or 25°C) or completely melted (above 38°C) milk fat, the deformability of the fat droplets increases as the proportion of liquid fat and radius increases (33); thus, at a given temperature above 4°C, the large droplet (unhomogenized) should produce a cheese with softer texture. Second, as a result of homogenization, the fat droplets become coated with casein (6). These new interfacial proteins may interact more strongly with the casein matrix of the cheese than with the membrane of the natural milk fat globule. Given an interaction of the fat filler with the casein produced by homogenization, the hardness of the composite may be changed (33). Thus, compared with the hardness of cheese made from unhomogenized milk, cheese hardness will be increased by the interaction of fat with the matrix when the fat is solid and will be decreased when the fat is liquid.

Effect of Homogenization on Cheese Appearance

The appearance of a food is influenced by how it reflects, absorbs, or transmits light, which in turn is related to the physical structure and chemical nature of the food. Appearance is one of the most important attributes of a food and is directly related to consumer acceptance and product quality (23, 24). The lack of whiteness may be a significant defect for unmelted Mozzarella cheese. As a result, the standard of identity for Mozzarella cheese (5) allows the addition of titanium dioxide to increase the whiteness of the cheese. For unmelted reduced fat Mozzarella (ca. 9% fat), Tunick et al. (32) found that cheeses made from homogenized milk were whiter than cheeses made from unhomogenized milk, which were light yellow as determined by visual observation. However, no quantitative data were reported in that study. Furthermore, no information is available in the literature regarding the changes in appearance of reduced fat Mozzarella cheese during refrigerated storage.

The literature (11, 32) proposes that the homogenization of milk at low or high pressure causes Mozzarella cheese hardness to increase and meltability to decrease, which would be undesirable for reduced fat Mozzarella cheese. Homogenization of the cream separately, instead of homogenization of the milk, may minimize this increase in hardness (20) and decrease in meltability. In general, homogenization should increase cheese whiteness, but no quantitative data are available. The objective of this study was to determine the effect of homogenization of milk, of separate homogenization of cream, and of no homogenization of milk on the chemical composition, proteolysis, functional properties, and appearance of reduced fat Mozzarella cheese.

MATERIALS AND METHODS

Homogenization and Cheese Making

Raw skim milk and raw unhomogenized cream (40% fat) were obtained from the Cornell University dairy plant (Ithaca, NY). Fat contents of the raw skim milk [(19); method number 15.8.B.] and cream were determined [(1); method number 33.3.18, 995.18]. The control milk was standardized by combining raw, unhomogenized, 40% fat cream with skim milk, followed by HTST pasteurization (Model Universal Pilot Plant; PMS Processing Machinery and Supply Co., Philadelphia, PA) at 72°C for 16 s. The milk for the homogenized milk treatment (HM) was standardized by combining raw, unhomogenized, 40% fat cream with skim milk, followed by HTST pasteurization and homogenization (model 75E; Manton Gaulin Manufacturing Co., Everett, MA) at about 70°C using first- and second-stage pressures of 13.8 MPa (2000 psi) and 3.45 MPa (500 psi), respectively. Milk for the homogenized cream treatment (HC) was standardized with homogenized 20% fat cream and skim milk followed by HTST pasteurization. The homogenized 20% fat cream was prepared by combining the original 40% fat cream and raw skim milk, batch pasteurizing (65.5°C for 30 min) the mixture, and then homogenizing the mixture at 63°C using first- and second-stage pressures of 13.8 MPa (2000 psi) and 3.45 MPa (500 psi), respectively. A 20% fat cream was used for homogenization because preliminary trials indicated that heavy cream (ca. 40% fat), once homogenized, was too viscous to disperse properly into skim milk. The 20% fat cream was pasteurized prior to homogenization to prevent lipolysis. All standardized milks were cooled to 4°C and were stored overnight at 4°C until used for cheese making the next day. To produce a homogeneous chemical composition, cheese was made using the no-brine, stirred-curd method as described previously (2). For each treatment, about 230 kg of cold, pasteurized, and standardized milk was weighed and poured into a cheese vat (model 4MX; Kusel Equipment Co., Watertown, WI).

Chemical Analyses

Milk, whey, and cheese composition. The titratable acidity of milk and whey and the pH of milk, whey, and cheese were measured as previously described (34). Fat content of the milk and whey was determined by ether extraction [(1); method number 33.2.26, 989.05], and fat content of the cheese was determined by the Babcock test [(18); method number 15.8.2.D.]. All samples were tested for total nitrogen by Kjeldahl [(1); method number 33.2.11, 991.20]. Total N for milk was determined in triplicate, cheese moisture and fat were determined in quadruplicate, and all other analyses were conducted in duplicate.

The preparation of samples for analysis of cheese moisture was performed as previously described (34). The salt concentration was determined by the Volhard test [(19); method number 15.5.B.]. The calcium concentration in the cheese was determined by complexometric titration (13).

Soluble protein. As a measure of proteolysis in the cheese, the N that was soluble in 12% TCA and in pH 4.6 acetate buffer were determined at 1, 16, 30, and 44 d of refrigerated storage as described by Bynum and Barbano (4). The percentages of N were converted to protein equivalents using the 6.38 factor. The soluble N values were expressed as a percentage of the total protein content of the cheese.

Functional Properties of Unmelted and Melted Cheese

Texture profile analysis (**TPA**), as described by Bourne (3), was performed on the unmelted cheese using an Instron Universal Testing Machine (model TM; Instron Corp., Canton, MA) after 30 d of storage at 4°C (35). A modified Schreiber test (15) was used to quantify cheese meltability and was determined after 2, 8, 16, 30, and 44 d of storage at 4°C. Apparent viscosity (**AV**) was determined on the melted cheese using helical viscometry as described by Kindstedt and Kiely (12). Free oil (**FO**) was determined on the melted cheese using the centrifugation method described by Kindstedt and Rippe (14). Both AV and FO were determined after 3, 16, 30, 44, and 56 d of storage at 4°C. The pizza bake test (25) was used to evaluate the functionality (shred melting and browning) of the cheese as a pizza topping after 30 d of storage at 4°C. All tests were performed using the procedures previously described (35).

Fat Particle Size Analysis

Fat particle size in the milk. The size of the milk fat globule was determined using a particle size analyzer (Mastersizer E, model E; Malvern Instruments, Worchestershire, United Kingdom). The system was configured to evaluate the size of milk fat globules using a 45-mm focus, 2.4-mm beam length, polydisperse model, and volume result. Distilled water at 40°C was the dispersive medium. Prior to analysis, the milk samples were heated to 40°C. The calibration was determined as previously described (29) to evaluate the size distribution of the milk fat globules. The measurements were performed in duplicate at 20% obscuration. From the distribution, we calculated the volume mean diameter (VMD), the volume to surface average diameter (Sauter mean), and the mean diameter below which 90% of all fat volume is contained.

Fat particle size in the cheese. The distribution and size of the fat particle in the cheese were qualitatively determined using scanning electron microscopy. The procedure used has been described by Oberg et al. (21).

Appearance Analysis

The appearance of the milk and cheese was quantitatively determined using a Macbeth Color-Eye Spectrophotometer (model 2020; Kollmorgen Instruments Corp., Newburgh, NY). The L, a, and b values correspond to whiteness, greeness or redness, and vellowness or blueness, respectively. These values were calculated using illuminant A (incandescent lamp). The color measurements were performed on fresh milk samples, tempered to 25°C, and done in triplicate. The cheese appearance was determined by tempering to 4°C a cheese cylinder (3.6 cm diameter \times 5 cm long) that was cut from the middle of the original cheese cylinder. Immediately prior to color measurement, the cylinder was cut into 5-mm high discs using a wire. A freshly cut cheese disc at 4°C was placed in the spectrophotometer viewing port for color determination. The color measurements of the cheese were done in quadruplicate after 2, 8, 16, 30, and 44 d of storage at 4°C.

Experimental Design and Statistical Analysis

A 3 \times 3 randomized complete block design was used. On each of the 3 d of cheese manufacture, the control, HM, and HC cheeses were made. Changes in proteolysis, functional properties (melt, AV, and FO), and appearance during refrigerated storage were monitored using a split-plot design in which the whole-plot factor (fat level) was replicated in a 3 \times 3 randomized complete block design. For the whole-plot factor, treatment was analyzed as a class variable, and the day of cheese manufacture was blocked. For the subplot factor, age and age \times age were analyzed as quantitative variables. The degrees of freedom in the statistical model were the same for the functional properties and for appearance. The PROC GLM of SAS[®] (28) was used for all data analyses.

RESULTS

Composition of Milk, Whey, and Cheese

The composition, particle size, and color data for milk and the composition data for whey are shown in Table 1. As expected, the fat and protein contents of

the standardized milks were not significantly different. Why the pH of the HM was higher than the control and HC milks is not clear; however, the difference was small and did not affect the manufacture of cheese. As expected, homogenization significantly reduced the size of the fat globules. The VMD of the HM sample decreased over threefold compared with the VMD of the control. The values for the VMD, Sauter mean, and the mean diameter below which 90% of all of the fat volume for the HC is contained all were significantly smaller than the values for the control, although not as small as the HM (Table 1). The difference between the HM and HC was likely from the fat particle clustering that occurred in the HC (30). As expected, HM or HC significantly increased the milk whiteness (Table 1). The a and b values for the HM and HC did not differ, but the control milk was greener (greater negative a value) and less yellow (smaller positive b value) than was the milk for HM and HC.

The protein contents of the whey were the same for all treatments, but the fat contents were different (Table 1). Fat losses to the whey were greater with HM than with the control or HC. Fat losses to the whey were lowest for the HC.

Chemical composition of cheese is shown in Table 2. No significant differences were detected in the

TABLE 1. Mean (n = 3) composition, particle size, and color of the unhomogenized milk (control), homogenized milk treatment (HM), and milk standardized with homogenized cream treatment (HC) and composition of the whey.

Component	Control	HM	HC	SEM	LSD ¹
Milk					
pH	6.60 ^b	6.64 ^a	6.59 ^b	0.00	0.01
Fat, %	0.83	0.83	0.83	0.01	0.03
Protein, %	3.14	3.16	3.17	0.02	0.06
d43, ² μm	2.73 ^a	0.84 ^c	1.23 ^b	0.01	0.04
$d32^{3}_{,3}$ µm	0.79 ^a	0.51 ^c	0.58 ^b	0.00	0.02
d0.9, ⁴ µm	5.54 ^a	1.55 ^c	2.10 ^b	0.03	0.14
Hunter value ⁵					
L	76.92 ^c	78.84 ^a	78.57 ^b	0.04	0.16
а	-5.52 ^b	-4.85 ^a	-4.92 ^a	0.02	0.09
b	1.86 ^b	2.25 ^a	2.21 ^a	0.01	0.04
Whey					
Fat. %	0.13 ^b	0.19 ^a	0.11 ^c	0.00	0.01
Protein, %	0.90	0.89	0.90	0.02	0.06

^{a,b,c}Means within the same row without a common superscript differ (P < 0.05). Lack of superscripts in a row indicates no significant differences among means.

 $^{1}P = 0.05.$

²Volume mean diameter of fat particles.

³Sauter mean diameter of fat particles.

⁴Fat particle diameter below which 90% of fat volume is contained.

 $^5\mathrm{The}$ L, a, and b values correspond to whiteness, greeness or redness, and yellowness or blueness, respectively.

Component	Control	HM	HC	SEM	LSD ¹
pH	5.23 ^b	5.21 ^b	5.27 ^a	0.01	0.02
Moisture, %	53.56	54.13	54.31	0.60	2.37
Fat, %	8.97 ^{ab}	8.14 ^b	9.14 ^a	0.23	0.88
FDB, ² %	19.34 ^{ab}	17.76 ^b	19.99 ^a	0.48	1.89
Protein, %	31.51	31.35	30.21	0.46	1.81
M:P ³	1.70	1.73	1.80	0.05	0.18
MNFS,4 %	58.84	58.93	59.78	0.63	2.48
Salt, %	1.44	1.69	1.59	0.07	0.27
S in M, ⁵ %	2.68	3.13	2.94	0.15	0.58
Ca, %	0.94	0.92	0.92	0.02	0.06
Ca, % of Protein	2.99	2.95	3.03	0.03	0.13

TABLE 2. Mean (n = 3) initial composition of reduced fat Mozzarella cheese made from unhomogenized milk (control), homogenized milk treatment (HM), and milk standardized with homogenized cream treatment (HC).

^{a,b}Means within the same row without a common superscript differ (P < 0.05). Lack of superscripts in a row indicates no significant differences among means.

 ${}^{1}P = 0.05.$

²Fat content on a dry weight basis.

³Ratio of moisture to protein.

⁴Moisture in the nonfat substance of the cheese.

⁵Salt in the moisture phase of the cheese.

levels of moisture, protein, moisture in the nonfat substance, or moisture to protein ratio. Although the moisture content, moisture in the nonfat substance, and moisture to protein ratio of the HC tended to be higher, the differences were not significant (P > 0.05). The salt content, ratio of salt to moisture, calcium content, and calcium content as a percentage of protein content of the cheese did not differ. The final pH of the cheeses and fat contents were different among treatments. The control and HC cheeses contained more fat than the HM cheese (Table 2), which was consistent with the lower concentration of fat in the whey for the control and HC (Table 1).

Proteolysis

The treatment had a significant effect on the content of pH 4.6-soluble N of the cheese (Table 3); however, only the soluble N of the HM was significantly greater than that of the control (Figure 1). No differences between treatments were detected for the 12% TCA- soluble N (Table 3). Age had a large impact on soluble N (Table 3). During storage at 4°C, both the pH 4.6-soluble N and the 12% TCA-soluble N significantly increased (Figures 1 and 2). Furthermore, the interaction of treatment and age was significant (Table 3), which was caused by the greater increase in pH 4.6-soluble N in HM cheese during aging (Figure 1).

Functional Properties

Unmelted cheese. After 30 d of storage at 4°C, no significant differences in TPA hardness and springiness (measured at 10°C) from treatment were detected. The TPA cohesiveness tended to be higher for the HM and HC than for the control; however, only

TABLE 3. Mean squares, probabilities (in parentheses), and degrees of freedom for indices of proteolytic changes (as a percentage of total N) of reduced fat Mozzarella cheese during 44 d of storage at 4° C.

Factor	df	pH 4.6- Soluble N	12% TCA- Soluble N
Whole plot			
Treatment (T)	2	6.08*	0.278
		(0.04)	(0.14)
Day of cheese	2	4.26	0.695*
manufacture (blo	cked)	(0.06)	(0.03)
Error	4	0.703	0.078
Subplot			
Age (A)	1	336.89*	77.92*
0		(<0.01)	(<0.01)
$A \times A$	1	9.40*	0.665*
		(<0.01)	(0.02)
$T \times A$	2	1.47*	0.165
		(0.02)	(0.24)
$T \times (A \times A)$	2	0.679	0.016
		(0.11)	(0.86)
Error	21	0.286	0.109
R ²		0.985	0.974

*P < 0.05.



Figure 1. Effect of treatment on pH 4.6-soluble N, as a percentage of the total N (TN), in the reduced fat Mozzarella cheese made from unhomogenized milk (control; \blacksquare), by homogenized milk treatment (HM; +), or by homogenized cream treatment (HC; *) during storage at 4°C.

the HC had TPA cohesiveness that was significantly greater than that of the control (Table 4).

Melted cheese. Treatment had no effect on meltability or AV, but age had a large impact on the meltability and AV of the cheese (Table 5). During storage at 4° C from 2 to 44 d, the mean values for meltability of the cheeses increased from 40 to 47

TABLE 4. Parameters of reduced fat Mozzarella cheese made from unhomogenized milk (control), homogenized milk treatment (HM), and milk standardized with homogenized cream treatment (HC) using texture profile analysis (TPA) measured at 10° C and 50% compression after 30 d storage at 4° C.

TPA Parameter	Control	HM	НС	SEM	LSD ¹
Hardness, N	73.98	68.16	68.93	6.44	20.59
Cohesiveness	0.65 ^b	0.77 ^{ab}	0.78 ^a	0.04	0.13
Springiness, mm	5.26	5.56	5.54	0.37	1.18

^{a,b}Means within the same row without a common superscript differ (P < 0.05). Lack of superscripts in a row indicates no significant differences among means.

 $^{1}P = 0.05.$

mm; the AV decreased from about 7000 to 1000 Pa \times s during storage at 4°C from 3 to 56 d, which is consistent with previous results (26) for reduced fat (ca. 9% fat) Mozzarella cheese. The treatment had a significant effect on the FO released by the cheese. Although the control cheese released significantly more fat as a percentage in the cheese (Figure 3), the amount of fat released was low compared with that of higher fat cheese. For example, as the fat content of the cheese in previous studies (27), increased from 15 to 25%, the FO release (expressed as a percentage of fat in the cheese) increased from about 10 to 45% compared with 3.5% for the control cheese of this study (Figure 3). There was also a significant inter-



TABLE 5. Mean squares, probabilities (in parentheses), and degrees of freedom of the meltability, apparent viscosity, and free oil of reduced fat Mozzarella cheese during 44 d of storage at 4°C.

Factors	df	Melt	Apparent viscosity	Free oil
			(×10 ⁶)	(% fat)
Whole plot				
Treatment (T)	2	2.20	0.131	13.11*
		(0.60)	(0.87)	(<0.01)
Day of cheese	2	52.59*	0.466	2.99
manufacture (blocked)		(0.02)	(0.64)	(0.09)
Error	4	3.76	0.940	0.648
Subplot				
Age (A)	1	306.71*	192.03*	3.92*
0 ()		(<0.01)	(<0.01)	(<0.01)
$\mathbf{A} \times \mathbf{A}$	1	0.211	`59.09 [*]	3.18 [*]
		(0.76)	(<0.01)	(<0.01)
$T \times A$	2	5.63	0.802	4.27*
		(0.10)	(0.33)	(<0.01)
$T \times (A \times A)$	2	0.169	0.292	0.780
		(0.93)	(0.67)	(0.15)
Error	30	2.23	0.706	0.391
R ²		0.871	0.928	0.858

Figure 2. Effect of treatment on 12% TCA-soluble N, as a percentage of the total N (TN), in the reduced fat Mozzarella cheese made from unhomogenized milk (control; \blacksquare) by homogenized milk treatment (HM; +), or by milk standardized with homogenized cream treatment (HC; *) during storage at 4°C.

 $^{\ast}P<0.05.$

Journal of Dairy Science Vol. 81, No. 8, 1998



Figure 3. Effect of treatment on free oil release, as a percentage of cheese fat, in the reduced fat Mozzarella cheese made from unhomogenized milk (control; \blacksquare), by homogenized milk treatment (HM; +), or by homogenized cream treatment (HC; *) during storage at 4°C.

action of treatment and age (Table 5), indicating that the difference in FO caused by differences in the extent of increase of FO among treatments increased during aging (Figure 3).

Treatment had an effect on the melting and browning of the cheeses during pizza baking (Figure 4). The HM and HC cheeses had almost no melt or fusion of shreds and had severe browning and scorching of many intact shreds. The shreds of the control cheese exhibited more melt and fusion and less scorching than did the HM and HC; however, the shred melt, fusion, and browning of the control cheese was unacceptable compared with that of higher fat cheese (25). When the control cheese cooled to room temperature, it became translucent, and the redness of the tomato sauce could be seen through the cheese.

Appearance and Microstructure

The treatment had a significant effect on the appearance of the unmelted cheese (Table 6). The HM and HC cheeses had higher Hunter L values and were, therefore, whiter than the control cheese (Figure 5). The whiteness of all of the cheeses significantly decreased over 44 d of refrigerated storage, and the difference between HM and HC compared with the control increased as time of storage increased, as indicated by the significant interaction of treatment and age (Table 6). All of the cheeses seemed to become slightly redder (increasing positive a value) over 44 d of refrigerated storage (Figure 6), but the

magnitude of the change was small (i.e., less than 1 unit). Treatment, but not age, had a significant effect on the Hunter b value (Table 6). The mean Hunter b value over 44 d of refrigerated storage at 4°C was 8.73, 7.57, and 8.16 for the control, HM, and HC cheeses, respectively. Visual observation of the unmelted cheeses confirmed these results as the HM and HC cheeses appeared to be significantly whiter and less yellow than the control (results not shown).

Homogenization had a significant impact on the microstructure of the cheese (Figure 7). The HM or HC significantly reduced the fat particle size in the milk for cheese manufacture (Table 1). Apparently, smaller voids (fat particles) were embedded in the protein matrix (gray areas) for the HM and HC cheeses (Figure 7, B and C, respectively) than for the control cheese (Figure 7A). Therefore, cheese made from milk with smaller fat particles, caused by homogenization (HM or HC) had significantly smaller fat particles dispersed throughout than did cheese made from unhomogenized milk with larger fat particles (control). Also, there was a clear tendency for the fat particles to form aggregates of varying size and number in the control cheese (Figure 7A), which was not observed for the HM and HC cheeses (Figure 7, B and C, respectively).

DISCUSSION

In general, HM or HC cheese had similar characteristics, but were different from the control cheese



Figure 4. Appearance of a pizza topped with reduced fat Mozzarella cheese made from unhomogenized milk (control, upper left), by homogenized milk treatment (HM, bottom center), or by homogenized cream treatment (HC, upper right) after 30 d of storage at 4° C.



Figure 5. Effect of treatment on the Hunter L value (whiteness) of the reduced fat Mozzarella cheese made from unhomogenized milk (control; \blacksquare), by homogenized milk treatment (HM; +), or by homogenized cream treatment (HC; *) during storage at 4°C.

(unhomogenized milk). Therefore, the discussion focuses on characteristics of the unhomogenized control cheese versus those of cheese made from HM or HC. However, there were important differences between the cheeses made from HM and HC.

Homogenization and Chemical Composition

Homogenization did not seem to have a large impact on the chemical composition of the cheese (Table 2). However, HC resulted in better fat retention than did HM, probably because of the adverse effect of homogenization (13.8 MPa) on the rennet gel structure (evident by more curd shattering during cheese manufacture for the HM). Gilles and Lawrence (8) reported that milk that had been homogenized at pressures greater than 5.5 MPa formed weak coagula. Tunick et al. (32) also observed curd shattering during cheese manufacture when using homogenized milk (10.3 MPa and 17.2 MPa) to manufacture reduced fat Mozzarella cheese. Because only about 3% of the total protein in the HC cheese had been subjected to the large shear forces of homogenization (because of the separate homogenization of the 20% fat cream), compared with 100% for the HM cheese, no curd shattering was observed during the manufacture of HC cheese.

The HM cheese (homogenization pressure of 13.8 MPa) produced a small increase in the rate of proteolysis compared with the proteolysis of the control and HC cheeses (Table 3 and Figure 1). Malin and Tunick (18) found that homogenization of milk at 10.3 MPa had little effect, but, at 17.2 MPa, homogenization significantly reduced the amount of proteolysis in reduced fat Mozzarella cheese. More work needs to be done to identify clearly the effect of homogenization on proteolysis in reduced fat Mozzarella cheese.

Homogenization and Functional Properties

Because homogenization did not have a large effect on the chemical composition and proteolysis of the cheeses, homogenization was not expected to have a large effect on the functional properties.

Unmelted cheese. Measured at 10°C and 50% compression, homogenization had no effect on the TPA hardness and springiness; HM and HC tended to increase the TPA cohesiveness of the cheese. Tunick et al. (32) compared the rheology of reduced fat Mozzarella cheeses prepared from homogenized and unhomogenized milks. Those researchers found that homogenization of Mozzarella cheese milk at 10.3 MPa and higher usually resulted in a product that had greater TPA hardness, measured at 25°C and 75% compression, than did cheese made from unhomogenized milk. They (32) hypothesized that this result was due to crosslinking (chemical bonding) of the casein on the newly formed fat globule to the casein in the cheese matrix. This explanation was also reported by Lelievre et al. (16).



Figure 6. Effect of treatment on Hunter a value (greeness or redness) of the reduced fat Mozzarella cheese made from unhomogenized milk (control; \blacksquare), by homogenized milk treatment (HM; +), or by homogenized cream treatment (HC; *) during storage at 4°C.

TABLE 6. Mean squares and probabilities (in parentheses) for Hunter indices of color changes of reduced fat Mozzarella cheese during 44 d storage at 4° C.

Factors	L	а	b
Whole plot			
Treatment (T)	78.74*	0.157	1.92*
	(0.01)	(0.27)	(0.01)
Day of cheese	22.02	0.041	0.276
manufacture (blocked)	(0.10)	(0.65)	(0.23)
Error	5.04	0.084	0.125
Subplot			
Age (A)	179.27*	2.23*	0.217
0	(<0.01)	(<0.01)	(0.09)
$\mathbf{A} \times \mathbf{A}$	33.40*	0.522*	0.161
	(<0.01)	(<0.01)	(0.14)
$T \times A$	4.08*	0.071*	0.049
	(0.02)	(0.01)	(0.50)
$T \times (A \times A)$	1.57	0.035	0.055
	(0.21)	(0.10)	(0.46)
Error	0.946	0.014	0.069
R ²	0.957	0.904	0.852

¹The L, a, and b values correspond to whiteness, greeness or redness, and yellowness or blueness, respectively.

*P < 0.05.

In the composite model for the filled gel, HM or HC produces at least two separate changes in the curd structure that may influence the unmelted cheese characteristics. These are a reduction in the particle size of the fat filler and an interaction of the fat filler with the gel (i.e., casein matrix). Each of these changes could have a separate effect on the unmelted cheese characteristics.

The model would predict that, if homogenization produced a strong interaction between the fat (filler) and the casein matrix, then the mechanical properties of the composite would be a reflection of the fat and the matrix (33). Therefore, at low temperatures (e.g., 10°C when milk fat is semi-solid and very hard), the fat bound to the matrix would increase the hardness; at higher temperatures (e.g., 25°C, when the milk fat is mostly liquid and soft), the fat bound to the matrix would reduce the hardness of the composite. The composite model for the filled gel would predict a separate effect of fat filler particle size that is independent of the interaction with the matrix. Small particles versus large particles would increase the hardness of the cheese when the fat is more liquid (e.g., 25°C), but not when the fat is more solid (e.g., 10°C).

Our results showed that there was no change in the TPA hardness (measured at 10° C) that was due to homogenization; therefore, the interaction of the fat and casein matrix caused by homogenization does



Figure 7. Scanning electron micrographs of control reduced fat Mozzarella cheese (A), reduced fat Mozzarella cheese made by homogenized milk treatment (C), or reduced fat Mozzarella cheese made by homogenized cream treatment (C) after 2 d of storage at 4°C. Arrows indicate the fat globule aggregates (A) and the individual fat globules (B and C). Scale bar = 10 μ m.

not have a significant influence on the hardness of reduced fat Mozzarella cheese. Because the measurements of TPA hardness by Tunick et al. (32) were conducted at 25°C, a temperature at which a much higher percentage of the milk fat would be liquid, the cheese structure with the larger fat particles would be expected to have a lower TPA hardness than one with the smaller fat particles because of the increased deformability of the large versus small fat particles. Therefore, the composite model for the filled gel apparently can accurately explain the results in our study and those of Tunick et al. (32). Furthermore, the interaction of the fat and casein matrix appear to be less important for the hardness of unmelted cheese than for the fat particle size when the effect of the temperature on fat hardness is considered.

Melted cheese. Among the measured functional properties of the melted cheese, FO release exhibited one of the largest differences between treatments. The HM and HC cheeses expressed virtually no FO, but the control cheese had about 3% FO (as a percentage of fat in the cheese). These results are consistent with those of Tunick (31) who found that homogenization of the milk used to make Mozzarella cheese (ca. 25% fat) significantly decreased the amount of FO released. Those results (31) indicated that the reduction in fat particle size (not the exposure of casein to homogenization) and the subsequent changes in the cheese structure were responsible for the change in FO release.

Electron micrographs showed that fat particles tended to form aggregates in the control cheese, but not in the HM and HC cheeses. In our experience, these aggregates form because of fat coalescence during stretching. Such aggregates suggest that the total fat in the control cheese has much less surface area available to interact with the protein phase than the total fat in the HM and HC cheeses. This result may have implications for emulsion stability and may lead to the higher FO release observed for the control cheese (Table 5; Figure 3). Furthermore, full fat Mozzarella cheese has greater aggregation of fat particles (our unpublished results) and FO release (27) than does reduced fat cheese, suggesting that the fat particle aggregation and FO release may be related. Although the FO release for the control cheese was higher than that in the HM and HC cheeses, it was still well below the amount of FO released by higher fat Mozzarella cheeses (35).

Functionality during pizza baking is the ultimate test for any prototype for lower fat Mozzarella cheese. The results of Fife et al. (7) suggested that, in addition to fat content of the cheese, the ratio of moisture to protein might also be important for desirable functionality during melting. However, in the present study, cheeses with the same fat content and the same ratio of moisture to protein behaved differently during pizza baking. Therefore, more work is needed for better understanding of the factors contributing to the proper melting and browning of Mozzarella cheese during pizza baking. Finally, no differences were detected in the meltability (as determined by Schreiber melt test) and AV among the cheeses that were due to homogenization, but meltability was different during pizza baking. The usefulness of these tests for lower fat Mozzarella cheeses may be limited because they do not appear to correlate directly with the meltability of cheese on pizza.

Appearance

The largest effect of homogenization was on the characteristics of the physical distribution of the fat in the milk and cheese. The HM or HC significantly reduced the fat globule size (Table 1), which resulted in a change in the microstructure of the unmelted cheese (Figure 7). This change in structure manifested itself in a change in cheese appearance (Table 6). A reduction in the fat particle size made the unmelted cheese whiter (initially and during storage) and less yellow. Lemay et al. (17) demonstrated that unmelted full fat Cheddar cheeses made from microfluidized milk or microfluidized cream were whiter than the control cheese (no microfluidization). They (17) found that the Hunter L value significantly increased from 82.8 to 85.4 to 86.5, and hypothesized that the decreased milk fat globule size and the increased number of globules increased the light scattering of the cheese, leading to an increase in whiteness. Our results were consistent with those.

However, the whiteness of all the unmelted cheeses decreased significantly during storage at 4°C (Figure 5). What change caused the decrease in whiteness? To help answer this question, a fat-free Mozzarella cheese was made from skim milk using the same procedures as described in Materials and Methods. The L value of the fat-free Mozzarella significantly decreased from 64.8, 59.5, 56.4, and 53.2 to 51.7 after 2, 8, 16, 30, and 44 d of storage at 4°C, respectively. The magnitude of the decrease in the L value over the time of refrigerated storage for the fat-free Mozzarella cheese was larger than that for the reduced fat Mozzarella cheese. Therefore, changes in the skim phase of the cheese (mainly, protein and moisture) that increase the absorption of light, and not changes in the fat phase, appear to be responsible for the decrease in whiteness over time. The rate of decrease in the L value over time was not as large for cheese made from HM or HC because of the larger number of small fat globules near the surface of the cheese. These small fat globules decrease the depth of penetration of incident light compared with that of cheese with the same fat content but without homogenization.

Proteolytic and physicochemical changes in the skim phase during aging may have contributed to the decrease in whiteness that was observed in this study. For example, significant proteolysis occurred in all cheeses (Figures 1 and 2), resulting in the accumulation of soluble peptides in the serum phase, which may have changed its light absorption characteristics. Furthermore, intact caseins may also have become soluble and accumulated in the serum phase during aging. Evidence of the solubilization of intact caseins in full fat Mozzarella was reported in a previous study that examined the changes in the expressible serum obtained from cheese by high speed centrifugation (8). In the present study, expressible serum was obtained from cheeses during the first 10 d of aging (data not shown). Changes in the protein and mineral contents of the expressible serum will be the subject of a separate report.

Because the browning was so severe for the homogenized treatments in the pizza bake test (Figure 4), it is not clear whether the increased whiteness of the unmelted cheese will also increase the whiteness of the melted cheese. Ultimately increasing the whiteness and opacity of both unmelted and melted reduced fat Mozzarella cheeses would be desirable.

CONCLUSIONS

Homogenization of the cream, instead of the milk, improved the cheese-making performance by reducing the amount of curd shattering and fines, and by reducing the amount of fat lost during the cheesemaking process. The HM or HC did not have a large effect on the unmelted textural properties of reduced fat Mozzarella cheese at 10°C. However, the physical changes in the cheese structure because of a reduction in fat particle size significantly increased the whiteness of the unmelted cheese, which might be expected to improve consumer acceptance of a reduced fat Mozzarella cheese. This change could be a significant step toward developing a reduced fat Mozzarella cheese of high quality. However, the reduced fat (ca. 9% fat) Mozzarella cheese produced in this study had limited melt and excessive browning during pizza baking for the control, HM, and HC. The production of pH 4.6-soluble N was higher in the HM, but this production did not make the HM cheese more meltable than the HC or control cheese. Further work is needed to understand how to increase the meltability and decrease the browning of reduced fat Mozzarella cheese during pizza baking.

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