

## Effects of Skim Milk Homogenization on Proteolysis and Rheology of Mozzarella Cheese

### ABSTRACT

*The influence of milk homogenization on proteolysis and functional properties of Mozzarella cheese was examined in an effort to develop low-fat Mozzarella with desirable textural and melting properties. Two-stage homogenization of skim milk at 6870/3430 kPa prior to the addition of nonhomogenized cream did not inhibit the meltability of full-fat or low-fat cheeses, when compared with cheeses made from nonhomogenized milk, but the meltability of full-fat cheese made from homogenized milk was greatly decreased. Homogenization of whole or skim milk had no significant effect on other rheological parameters or on proteolysis of  $\alpha_s$ -casein in full-fat and low-fat cheeses. Refrigerated storage at 4°C caused meltability and proteolysis to increase and the other parameters to decrease. Differences in fat content caused significant changes in hardness, springiness and meltability. Fat globule size and homogenization of protein affected proteolysis and rheology much less than fat content and storage time.*

### INTRODUCTION

The expanding popularity of pizza has caused Mozzarella cheese consumption to increase dramatically in the US over the past 20 years. The demand for Mozzarella would be even larger if not for the apprehension by many consumers about dietary fat. These concerns have led to the development of Mozzarella containing 9–11% fat (Tunick *et al.*, 1991, 1993a, b), which is less than half the fat of normal full-fat Mozzarella (USDA, 1976). This low-fat (LF) Mozzarella could be labeled in the US as 'lite' according to new regulations (Mermelstein, 1993).

Homogenization of milk intended for Mozzarella has been used to improve the yield of the cheese and impart a white color to the product (Breene *et al.*, 1964; Quarne *et al.*, 1968), eliminating the need to add titanium dioxide as a whitening agent. Mistry and Anderson (1993) have suggested that homogenization could improve the texture of a reduced-fat cheese since the surface area of the fat within the protein matrix would be increased. Lelievre *et al.* (1990) found that meltability and stretchability of full-fat Mozzarella decreased with homogenization; they theorized that this was a result of interactions of casein that had been adsorbed onto the fat droplets. A previous study in the authors' laboratory compared the rheology and proteolysis of LF and high-fat (HF) Mozzarella made from homogenized and nonhomogenized milk (Tunick *et al.*, 1993b). Meltability and proteolysis of  $\alpha_{s1}$ -casein ( $\alpha_{s1}$ -CN) decreased and hardness, elastic modulus and viscous modulus increased with homogenization pressure in both LF and HF Mozzarella. However, reduction of the curd cooking temperature from 45.9 to 32.4 °C increased proteolytic breakdown of  $\alpha_{s1}$ -CN to  $\alpha_{s1}$ -I-casein ( $\alpha_{s1}$ -I-CN) resulting in reduced hardness and improved meltability.

Meltability is an important property of Mozzarella cheese that is intended for pizza; over 75% of the Mozzarella made in the US is used for this purpose (Kindstedt, 1993). Homogenization may inhibit melting, but homogenization of skim milk followed by the addition of nonhomogenized cream might not. Such treatment of milk would provide an independent measure of the effect of homogenization on protein components of milk. In this paper, we compare HF and LF Mozzarella cheeses prepared from homogenized skim milk to those prepared from homogenized whole milk and nonhomogenized milk.

## MATERIALS AND METHODS

### Milk preparation

LF and HF 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 HF cheese milk was standardized with cream (35% fat) or skim milk (0.14% fat) to 3.5% milk fat and the LF 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 in a Manton-Gaulin (Everett, MA, USA) homogenizer at 63 °C at 6870 and 3430 kPa. The fat from the milk intended for homogenized-skim milk cheese was separated before the cream and skim milk were pasteurized at 63 °C for 30 min. The skim was homogenized as above and the cream was then added back to the milk to achieve the desired fat content.

### Cheese preparation

Mozzarella cheese was prepared according to the procedures previously described (Tunick *et al.*, 1993a, b). Cheese milk was held at 32.4 °C and inoculated by direct

addition of 125 mL of CR7 starter culture<sup>†</sup> (Marschall-Rhône Poulenc, Madison, WI, USA), described by the manufacturer as 50% *Streptococcus thermophilus* and 50% *Lactobacillus bulgaricus*. After the pH decreased 0.1 unit, 4.4 g of #01034 single strength calf rennet (Chr. Hansen's Laboratory, Milwaukee, WI, USA) were added and the milk was held for 35 min. The curd was cut, held for 15 min, stirred at 32.4 °C for 10 min, and held at that temperature for 90 min. The whey (pH 6.3–6.4) was then drained and the curd rinsed and cut into slabs. When the pH decreased to 5.2–5.3, the slabs were covered in cheesecloth and placed in ice overnight.

The next day, the curd was divided into eight parts and stretched and kneaded multidirectionally by hand for 7 min in 70–80 °C water. 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 six weeks. Two cheese types were prepared each week over a period of several months according to a 3 × 2 factorial arrangement in a completely random design. The types consisted of LF and HF from each of the three milk treatments. There were three replicates of each type. Each sample was analysed after one and six weeks of storage.

### Compositional analyses

After one week, moisture was determined by the forced-draft oven method (AOAC, 1990) and fat by the modified Babcock test (Kosikowski, 1982). Percentage of NaCl was determined after at least 3 weeks for selected samples by chloride ion electrode (Orion Research, Inc., Boston, MA, USA). Three replicates of each sample were analysed.

### Electrophoresis

Cheese samples were grated for protein analysis, and 2 g were added to 5 mL buffer (0.166 mM Tris, 1 mM EDTA, pH 8.0), in a 30-mL cup of a Model 23 Virtis homogenizer (Virtis Co., Gardiner, NY, USA). The sample was mixed at 70% of full speed for 15 min, 5 mL of 7% sodium dodecyl sulfate in buffer was added, the sample was mixed at 50% of full speed for 5 min, 2 mL of 10 mM dithiothreitol in buffer was added, and the sample was mixed well and held in an ice bath 20–30 min. Samples were then centrifuged for 1 h at 4–5 °C at 18 000 rpm in an SS34 rotor of a Sorvall RC5C centrifuge (DuPont Co., Newtown, CT, USA). The supernatant was filtered through laboratory wipe tissues and lyophilized. The resulting extracts were stored at –20 °C prior to polyacrylamide gel electrophoresis with SDS (SDS-PAGE), which was performed with the PhastSystem (Pharmacia LKB Biotechnology, Piscataway, NJ, USA) using 20% gels (Tunick *et al.*, 1993a, b). Gels were stained with Coomassie blue R250, destained and dried. A Bio-Rad Model 620 Video Densitometer (Bio-Rad Laboratories, Richmond, CA, USA) interfaced with a computer and 1D Analyst II (Version 3.10) software (Bio-Rad) was used to scan the gels and integrate peak areas. Percentages of individual caseins were expressed as per cent of total  $\alpha$ - and  $\beta$ -casein.

<sup>†</sup>Use of brand or firm name does not constitute endorsement by the USDA over others of a similar nature not mentioned.

## Rheological analyses

Texture profile analysis was performed at one and six weeks as previously described (Tunick *et al.*, 1993a, b), with hardness and springiness being determined at 25 °C using an Instron Universal Testing Machine Model 4201 (Instron, Inc., Canton, MA, USA). The elastic modulus ( $G'$ ) and viscous modulus ( $G''$ ) were determined at one and six weeks with a Rheometrics Dynamic Analyzer RDA-700 (Rheometrics, Inc., Piscataway, NJ, USA) as previously described (Tunick *et al.*, 1993a, b). Meltability, a unitless parameter, was determined as before at one and six weeks by the Schreiber test, in which the expansion of a disk of cheese (18 mm diameter, 5 mm thick) is measured on a target graph of numbered concentric circles after 5 min in a 232 °C oven (Kosikowski, 1982; Tunick *et al.*, 1993a, b).

## Statistical analyses

The data were analysed by the Statistical Analysis System—General Linear Models procedure (SAS, 1987). The compositional, rheological and electrophoretic data were analyzed by factorial analysis of variance to examine the main effects and interactions of fat, milk treatment, and time factors. The main factors and their interactions are described as significant only when  $P < 0.05$ .

# RESULTS AND DISCUSSION

## Composition

Table 1 shows the percentages of moisture, fat in dry matter (FDM), and NaCl in the cheeses. Moisture in nonfat substance (MNFS), which is equal to % moisture/(100 – % fat), is also calculated. The reduced fat content of LF cheeses leads to a higher moisture content than HF cheeses. No significant effects attributable to MNFS were observed in the statistical analyses. The cheeses prepared from homogenized milk exhibited curd shattering, but loss of solids to whey was minor.

**TABLE 1**  
Percentages ( $\pm$  Standard Deviations) of Moisture, Fat in Dry Matter (FDM), NaCl and Moisture in Nonfat Substances (MNFS) in Mozzarella Cheeses

Type	Moisture	FDM	NaCl	MNFS
Nonhomogenized LF	54.0 $\pm$ 0.4	21.5 $\pm$ 1.4	2.8 $\pm$ .2	60.0 $\pm$ 0.8
Homogenized LF	55.9 $\pm$ 1.2	21.5 $\pm$ 0.4	3.0 $\pm$ .3	61.8 $\pm$ 1.1
Homogenized skim LF	53.6 $\pm$ 0.4	23.4 $\pm$ 1.8	2.6 $\pm$ .1	60.1 $\pm$ 0.5
Nonhomogenized HF	48.5 $\pm$ 2.0	47.3 $\pm$ 0.6	2.6 $\pm$ .2	64.1 $\pm$ 1.5
Homogenized HF	47.4 $\pm$ 2.2	50.4 $\pm$ 0.9	2.8 $\pm$ .2	64.5 $\pm$ 2.3
Homogenized skim HF	45.7 $\pm$ 1.4	50.4 $\pm$ 0.6	2.4 $\pm$ .3	62.9 $\pm$ 1.5

## Proteolysis

Rennet, which retains some activity during manufacture of Mozzarella (DiMatteo *et al.*, 1982; Tunick *et al.*, 1991), initially cleaves  $\alpha_{s1}$ -CN to form  $\alpha_{s1}$ -I-CN and a smaller peptide (McSweeney *et al.*, 1993a);  $\alpha_{s1}$ -CN is also susceptible to cleavage by plasmin (McSweeney *et al.*, 1993b). Breakdown of  $\alpha_{s1}$ -CN to  $\alpha_{s1}$ -I-CN during storage was observed in all samples (Table 2). No significant correlations with fat content or treatment of milk were found (Table 3), although the homogenized-skim milk cheeses appeared to undergo less proteolysis of  $\alpha_{s1}$ -CN.

Percentages of  $\alpha_{s2}$ -CN in all samples averaged  $13.3 \pm 3.2$  at one week and  $12.7 \pm 4.4$  at six weeks. Average percentages of  $\beta$ -CN in all samples were  $42.5 \pm 2.7$  at one week and  $36.3 \pm 6.0$  at six weeks. Extensive proteolytic breakdown of these caseins did not take place since they are not attacked by rennet (Fox, 1989). Any degradation of  $\alpha_{s2}$ -CN or  $\beta$ -CN that did occur may have been due to the action of plasmin (Farkye *et al.*, 1991; Farkye & Fox, 1992). Another recent study of proteolysis in Mozzarella also showed that  $\alpha_{s1}$ -CN degrades, and  $\alpha_{s2}$ - and  $\beta$ -CN do not, when rennet is used as the coagulant (Yun *et al.*, 1993).

## Hardness and springiness

Reduction of fat in cheese leads to a denser and more elastic protein network. As a result, the hardness and springiness value of the LF cheeses were significantly higher than those of the HF cheeses (Tables 2 and 3). The proteolytic breakdown of the protein matrix during storage led to decreases in hardness and springiness with time. The homogenization treatment used did not significantly affect hardness or springiness, which indicates that these properties are influenced by fat content and not fat globule size.

## Meltability

In most cases, meltability increased with fat content and storage time (Tables 2 and 3). A disk of Mozzarella spreads when it melts because the base of the disk flows under the weight of the upper layer (Lelievre *et al.*, 1990). Meltability is dependent on FDM (Kindstedt, 1991) and on proteolysis, since fat is released when casein is broken down into peptides.

The meltability of the HF homogenized-milk cheeses was much lower than the meltability of the HF nonhomogenized- and homogenized-skim milk cheeses, accounting for the significant fat-milk interaction in Table 3. The HF homogenized-milk cheeses were also the only samples that puffed upward to double or triple their original height upon heating. These effects were probably caused by an increase in fat emulsification within the casein matrix. Homogenization of whole milk ruptures the fat globule membranes; casein micelles or submicelles are then adsorbed onto the lipid droplets as pseudo-fat globule membrane, hindering the spread of the melted fat (Lelievre *et al.*, 1990). Free oil formation in Mozzarella is also decreased by homogenization and resulting emulsification (Tunick, 1994). In addition, the higher NaCl concentration in the HF homogenized-milk cheese probably enhanced emulsification. Kindstedt *et al.* (1992) have proposed that  $\text{Na}^+$  exchanges with casein-bound  $\text{Ca}^{2+}$  in Mozzarella cheese; this enhances the emulsification properties of casein and therefore decreases

**TABLE 2**  
Effect of Milk Treatment on  $\alpha_{s1}$ -Casein,  $\alpha_{s1}$ -I-Casein, Hardness, Springiness, Meltability, Elastic Modulus, and Viscous Modulus of Mozzarella Cheeses after One or Six Weeks. Averages of Three Replicates

Cheese Type <sup>a</sup>	$\alpha_{s1}$ -CN (%) <sup>b</sup>		$\alpha_{s1}$ -I-CN (%) <sup>b</sup>		Hardness (N)		Springiness (mm)		Meltability		$G'$ (N/cm <sup>2</sup> )		$G''$ (N/cm <sup>2</sup> )	
	1	6	1	6	1	6	1	6	1	6	1	6	1	6
<i>Nonhomogenized</i>														
LF	44.0	28.4	3.2	19.5	105	83	8.59	7.66	1.2	1.4	6.40	6.85	1.98	1.77
HF	41.7	30.8	4.6	14.0	49	37	7.94	5.52	2.6	3.2	5.41	4.49	1.74	1.53
<i>Homogenized</i>														
LF	43.8	27.9	1.2	22.5	95	70	8.85	7.15	1.2	1.7	7.18	4.64	2.27	1.44
HF	44.7	30.9	2.1	18.6	61	54	8.29	6.71	1.0	1.1	7.58	6.64	2.10	1.93
<i>Homogenized skim</i>														
LF	42.4	33.7	0	16.2	109	77	8.55	7.10	1.3	1.6	7.65	6.50	2.39	2.12
HF	44.7	34.6	0	17.3	49	47	7.73	6.49	2.8	3.0	5.74	6.06	1.93	2.05
RMSE <sup>c</sup>	7.36		6.72		18.6		0.540		0.184		1.46		0.362	

<sup>a</sup>LF = Low fat; HF = high fat.

<sup>b</sup>As percent of total  $\alpha$ - and  $\beta$ -casein.

<sup>c</sup>RMSE = Root mean square for error.

**TABLE 3**  
Statistically Significant Main Factors and Interactions in Experimental Mozzarella Cheeses

<i>Source</i> <sup>a</sup>	<i>DF</i> <sup>b</sup>	$\alpha_{s1}$ -CN		$\alpha_{s1}$ -I-CN		<i>G'</i>		<i>G''</i>	
		<i>MS</i> <sup>c</sup>	<i>P</i>	<i>MS</i>	<i>P</i>	<i>MS</i>	<i>P</i>	<i>MS</i>	<i>P</i>
Week	1	1352	***	2275	***	9.495	*	0.6038	*
Residual	23	54.10		45.12		1.588		0.1314	

  

<i>Source</i>	<i>DF</i>	<i>Hardness</i>		<i>Springiness</i>		<i>Meltability</i>	
		<i>MS</i>	<i>P</i>	<i>MS</i>	<i>P</i>	<i>MS</i>	<i>P</i>
Fat	1	13 030	***	6.803	***	7.200	***
Milk	2	NS		NS		3.201	***
Fat-milk	2	NS		NS		3.498	***
Week	1	1 892	*	21.64	***	1.034	***
Residual	24	346.5		0.2920		0.0339	

<sup>a</sup>Effects and interactions of fat level (fat), treatment of milk (milk), and weeks of storage (week) tested against residual error.

<sup>b</sup>DF = Degrees of freedom.

<sup>c</sup>MS = Type I mean squares.

\* $P < 0.05$ ; \*\*\* $P < 0.0001$ ; NS = not significant ( $P \geq 0.05$ ).

meltability (Kindstedt, 1993), but apparently does not affect proteolysis (Farkye *et al.*, 1991). Salt gradients, which are commonly found in brine-salted Mozzarella (Kindstedt, 1993), were not present in any of the samples tested, presumably because the sample blocks were relatively small.

#### Elastic and viscous moduli

Values of  $G'$  and  $G''$  decreased with storage time in most of the samples (Tables 2 and 3) due to degradation of the casein matrix. Fat content and homogenization had no significant effect. As with any viscoelastic solid,  $G'$  of each sample was greater than  $G''$ . The loss tangent, which is  $G''/G'$ , ranged from 0.27 to 0.34 in the one-week samples; the values were 5–10% higher in the six-week samples. This increase with refrigerated storage in the contribution of the viscous modulus, which has been observed by others and attributed to proteolysis (Diefes *et al.*, 1993), is an indication that Mozzarella softens during storage and becomes less elastic.

### CONCLUSIONS

Mozzarella cheeses prepared from homogenized whole milk, homogenized skim milk, and nonhomogenized milk displayed no significant differences in hardness, springiness,  $G'$ ,  $G''$  or degradation of  $\alpha_{s1}$ -CN to  $\alpha_{s1}$ -I-CN. The meltability of HF cheeses made from homogenized whole milk was significantly lower than the meltability of the other HF cheeses. The results appear to indicate that except for meltability, neither fat globule size nor two-stage homogenization of casein at 6870 + 3430 kPa affect rheology or proteolysis of Mozzarella cheese.

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