Mozzarella Cheese: Impact of Coagulant Type on Functional Properties[1]

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ABSTRACT

The objective of this study was to determine the impact of coagulant type and refrigerated storage on functional properties of unmelted and melted Mozzarella cheese. A "no-brine" Mozzarella cheese-making method was used to produce cheese with homogeneous chemical composition. Cultured Mozzarella cheeses were made with three different coagulants (*Endothia parasitica* protease, chymosin derived by fermentation, and *Mucor miehei* protease) in 1 d, and cheese making was replicated on 3 different d. During 50 d of storage at 4°C, texture profile analysis parameters (hardness, cohesiveness, and springiness) of unmelted cheese decreased, meltability increased, apparent viscosity of melted cheese decreased, and free oil formation from melted cheese increased. Overall, the Mozzarella cheese made using *E. parasitica* protease was softer in unmelted cheese texture, was more meltable, and had lower apparent viscosity and more free oil release on melting than other cheese. In general, cheeses made with chymosin and *Mucor miehei* protease were similar in functional characteristics.

(**Key words**: Mozzarella cheese, coagulant type, functional properties, apparent viscosity)

Abbreviation key: **AV** = apparent viscosity, **CDF** = chymosin derived by fermentation, **TPA** = texture profile analysis.

INTRODUCTION

A variety of low cost milk coagulants from microbial sources have been used for cheese making (4). The use of microbial coagulants, such as proteases from *Endothia parasitica* and *Mucor miehei*, can affect cheese texture and flavor (2), functional properties (18), and yield (1, 2). Cheeses made with chymosin derived by fermentation (**CDF**) were not distinguishable from those made with calf rennet in quality (20), yield (1), or proteolytic pattern (25, 26).

Evidence of proteolytic activity from residual coagulant during cheese aging was shown in both cultured and direct acid Mozzarella (6, 8, 18, 25, 26). The *E. parasitica* protease is considered to be the most heat sensitive of commercial milk coagulants (9, 21). However, if *E. parasitica* protease is not inactivated during cheese making, this enzyme can be highly proteolytic in Mozzarella (26, 27), Cheddar (7), and Gouda cheese (23).

Proteolytic activity by coagulant in cheese (19, 24, 25, 26) depends on the amount of coagulant retained in the cheese (17) and the amount of inactivation by temperature and pH that occurred during cheese making (9). Creamer (5) reported that rennet (presumably chymosin) remained active and influenced proteolysis in Mozzarella cheese during refrigerated storage when curd temperatures remained <65°C during stretching. Generally, the stretching temperature used in the US is <65°C. Thus, proteolysis in Mozzarella during refrigerated storage can be significant.

In an earlier report, Yun et al. (26) described proteolysis in Mozzarella cheeses (made with *M. miehei* protease, chymosin, and *E. parasitica* protease) that had been cooked to 41°C and

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stretched at 57°C. The *E. parasitica* protease was more proteolytic than *M. miehei* protease or chymosin, but the casein substrate specificity differed with coagulant type. Proteolysis of both α_s-caseins and of β-casein occurred in the Mozzarella cheese made with *E. parasitica* protease during storage at 4°C, but α_s-caseins were hydrolyzed preferentially in cheeses made with chymosin and *M. miehei* protease.

Proteolysis appears to be the primary cause of changes in functional properties of Mozzarella during storage (27, 28). Kiely et al. (11) reported that imitation Mozzarella (made without coagulant) showed little proteolysis of caseins and almost no change in melting properties during 1 mo of refrigerated storage. Because the integrity of α_s-caseins and β-casein is the primary determinant of Mozzarella structure, differences in the rate and extent of their hydrolysis would be expected to yield cheeses with different melting and textural properties.

Therefore, the type of coagulant used for cheese making would be expected to affect functional properties of Mozzarella cheese during refrigerated storage. However, the extent to which different types of milk coagulants contribute to changes in functional properties of Mozzarella cheese is not clear. Thus, the objective of the present study was to determine the impact of three coagulant types (i.e., CDF, *E. parasitica* protease, and *M. miehei* protease) on changes in functional properties of Mozzarella cheese during 50 d of storage at 4°C.

MATERIALS AND METHODS

Cheese Making

Three vats of cheese were made in 1 d each with a different coagulant using the "no-brine" cheese-making method as previously reported (25, 26). Cheese making was replicated on 3 different d. The type and amount of coagulants used were CDF (Chymax®, double strength), .097 ml/kg of milk; *M. miehei* protease (Morcurd Plus®, double strength), .101 ml/kg of milk; and *E. parasitica* protease (Surecurd®, triple strength), .053 ml/kg of milk. All coagulants were supplied by Pfizer Inc. (Milwaukee, WI), and the amount used in cheese making was calculated based on the relative milk-clotting activity determined by the supplier. All coagulants were selected from lots in commercial use at the time of the study. The cheese in this study had been made for a previous study reporting on the effect of the coagulant type on composition and proteolysis of Mozzarella cheese (26).

Sample Allocation

Six 1.2-kg cylinders (7.5 cm in diameter × 30 cm long) of Mozzarella cheese were produced from each vat, and each cylinder of cheese was assigned a number corresponding to the order in which it was extruded from the mixer. Cheese cylinders 3 and 4 were used for texture profile analysis (**TPA**) and melting test. Each cylinder was sectioned, perpendicular to the longitudinal axis, into three equal segments weighing about 400 g. All six segments were vacuum packaged separately and stored at 4°C until the day of testing.

Cylinders 2 and 5 were used for tests of apparent viscosity (**AV**) and free oil. One day after manufacture, cylinders 2 and 5 from each vat were shipped in insulated containers containing ice packs by overnight delivery to the University of Vermont. Upon receipt, each cylinder was sectioned into six equal segments weighing about 200 g. All segments were vacuum packaged individually and stored at 4°C. One segment from each cylinder from each vat was chosen randomly for helical viscometry (12) and free oil formation test (14).

Functional Property Tests

The TPA (3, 27) of Mozzarella cheese was performed in quadruplicate for each treatment at each refrigerated storage time using the Instron Universal Testing Machine (model TM; Instron Corp., Canton, MA). A modified Schreiber test (15, 27) was used to measure cheese meltability in quadruplicate. The AV of melted Mozzarella cheese was measured in duplicate by helical viscometry (12). Free oil formation by Mozzarella cheese was measured in duplicate using the centrifugation method (14). All functionality tests, except for the AV test, were conducted at 3, 8, 15, 21, 29, and 50 d of storage; the AV test was conducted at 8, 15, 21, 29, and 50 d of storage at 4°C.

Experimental Design and Statistical Analysis

On each day of cheese making, the order of cheese making for the three coagulant types was selected so that the effects of day and

TABLE 1. Statistical model used for data analyses.

Factors	df	Analyzed as
Whole-plot factor		
Coagulant type	2	Classification
Day of cheese making	2	Block
Order of cheese making	2	Block
Error	2	
Subplot factor		
Age	1	Quantitative
Age × age	1	Quantitative
Interaction of coagulant type × age	2	Classification × quantitative
Interaction of coagulant type × (age × age)	2	Classification × quantitative
Error	39[1]	

[1]The degrees of freedom of the error term for the subplot factor error for apparent viscosity results were 30 instead of 39 because only five times of aging (d 8, 15, 21, 29, and 50) were used instead of six.

order of cheese making were blocks in a 3 × 3 Latin square design. To monitor the changes in functional properties of Mozzarella during refrigerated storage, a split-plot design was used in which the whole-plot factor (coagulant type) was replicated in a 3 × 3 Latin Square design. The factors, degree of freedom, and analysis method are shown in Table 1. The PROC GLM of SAS (SAS Institute, Cary, NC) was used for data analysis. The level of significance was $P < .05$ throughout the paper.

RESULTS

Unmelted Cheese Functionality

Coagulant type did not significantly affect TPA hardness and TPA springiness (Table 2). However, the effect on TPA cohesiveness marginally exceeded the .05 significance ($P = .07$) (Table 2). For all cheeses, TPA hardness and TPA springiness decreased with age (Figure 1, A and C, respectively; Table 2). No effect on TPA cohesiveness by the linear term of age was significant, but the quadratic term of age was significant (Figure 1B; Table 2).

Melted Cheese Functionality

Meltability increased with age for all coagulant types (Figure 2A). A significant effect of the interaction of coagulant type × age occurred for meltability (Table 3). The AV decreased with age for all coagulants (Figure 2B). The rate of decrease in AV was faster during the first 3 wk. The effect of coagulant type on AV was not significant ($P > .05$). The

TABLE 2. Mean squares and probabilities of texture profile analysis (TPA) parameters for unmelted Mozzarella cheese stored at 4°C for 50 d.

Factors	TPA Hardness		TPA Cohesiveness		TPA Springiness	
Whole-plot factor	MS	P	MS	P	MS	P
Coagulant type	321	.19	.003	.07	.093	.23
Day of cheese making (blocked)	5590*	.01	.001	.14	.734*	.04
Order of cheese making (blocked)	133	.35	.005*	.04	.024	.53
Error	73		.0002		.027	
Subplot factor						
Age	3540*	<.01	<.001	.70	2.994*	<.01
Age × age	231*	.02	.009*	.03	.353*	<.01
Interaction of coagulant type × age	43	.35	.002	.35	.041	.24
Interaction of coagulant type × (age × age)	13	.72	<.001	.98	.001	.97
Error	39		.002		.028	
R^2	.918		.443		.832	

*Statistically significant ($P < .05$).

amount of free oil (total weight basis and total fat basis) increased significantly with age (Figure 2, C and D, respectively; Table 3). Initially, the rate of increase was faster. After 3 wk, little change occurred in free oil release from cheeses made using CDF and *M. miehei* protease, but free oil released from cheeses made using *E. parasitica* protease increased continuously. The effect of coagulant type on free oil formation marginally exceeded the .05 significance (P = .07) (Table 3).

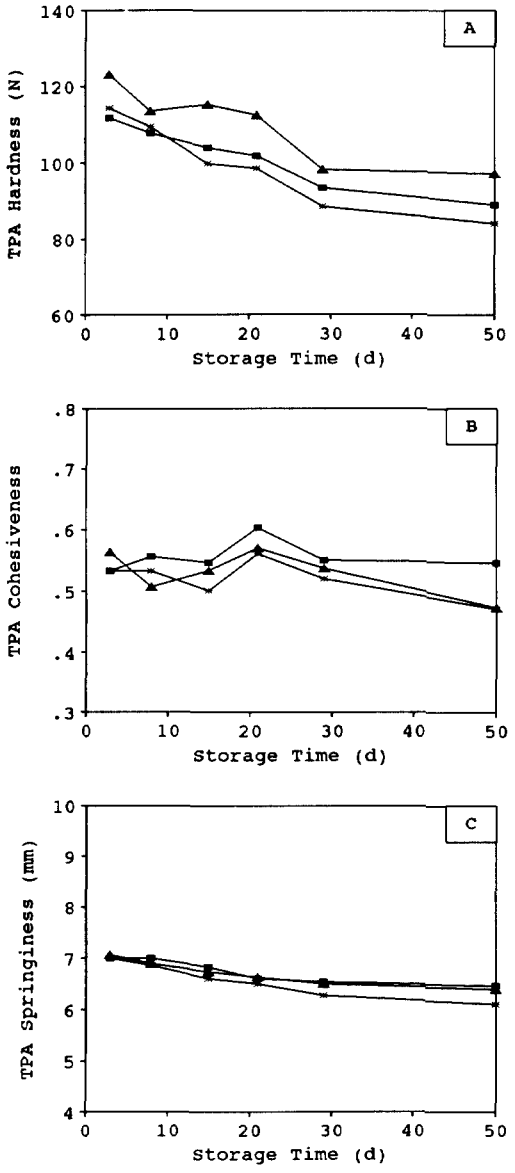

Figure 1. Impact of coagulant type on texture profile analysis (TPA) parameters of Mozzarella cheese during 50 d of storage at 4°C for *Endothia parasitica* protease (*), chymosin derived by fermentation (■), and *Mucor miehei* protease (▲); TPA hardness 1 (A; SEM = 3.6 N), TPA cohesiveness (B; SEM = .07), and TPA springiness (C; SEM = .10 mm).

DISCUSSION

Unmelted Cheese Functionality

Although coagulant types did not significantly affect TPA parameters (P > .05), the cheeses made using *E. parasitica* protease generally tended to be lower in TPA hardness and TPA springiness throughout storage (Figure 1, A and C; Table 2), possibly because of the higher rate of proteolysis in the cheese made using *E. parasitica* protease (26). As in other studies (27, 28), TPA hardness and TPA springiness decreased with storage time as the proteolysis in these cheeses increased during the same period.

Melted Cheese Functionality

The increase in meltability with storage time was generally linear (Table 3; Figure 2). The meltability of the cheese made using *E. parasitica* protease was initially similar to that of cheeses made with the other coagulant types but then increased at a faster rate during storage.

The decrease in AV with increasing storage time was consistent with the increase in meltability (Figure 2B). Initially, the AV decreased rapidly and then more slowly after wk 3 of storage. On average, AV of the cheeses made using *E. parasitica* protease were lower than the AV of other cheeses throughout the refrigerated storage.

The amount of free oil released from the cheeses made using *E. parasitica* protease increased continuously, but free oil of the cheeses made with other coagulants became constant after 3 wk of refrigerated storage. The changes in free oil based on total fat tended to be similar to the changes of the free oil based on total weight. Functional properties of Mozzarella cheeses made in this study were similar to those of cheeses from previous studies (11, 14, 22, 27, 28).

Proteolysis and Functionality

Cheese compositions were virtually identical for all three coagulant types (26). Therefore, the differences among cheeses were due to the coagulant activities. Proteolysis in all cheeses during storage, as measured by soluble nitrogen (in pH 4.6 acetate buffer or in 12% TCA) and SDS-PAGE, was significant (26). Soluble nitrogen increased, and residual α_s-caseins decreased, in all cheeses during 50 d of storage at 4°C. A significant effect caused by coagulant type existed on the extent and pattern of proteolysis. Proteolysis of cheese made using *E. parasitica* protease was initially similar to that of other cheeses but then increased at a faster rate. Proteolysis of α_s-caseins by *E. parasitica* protease was not faster than that of CDF (26). However, the SDS-PAGE results showed that proteolysis of β-casein occurred only in the cheese made using *E. parasitica* protease, not in the cheeses made using CDF or *M. miehei* protease during 50 d of storage at 4°C (26).

Functional properties of all Mozzarella cheeses changed significantly during storage and were influenced by coagulant type. During refrigerated storage, the extent of proteolysis by *E. parasitica* protease was higher than that of CDF or *M. miehei* protease (26). These differences in the extent of proteolysis were reflected in the AV characteristics of the cheese (Figure 2B). Initially, the functional characteristics of all cheeses were similar. However, the rate of change in functionality was faster in the cheese made using *E. parasitica* protease than in other cheeses. As a result, the meltability and free oil became higher, and AV became lower, during storage in the cheese

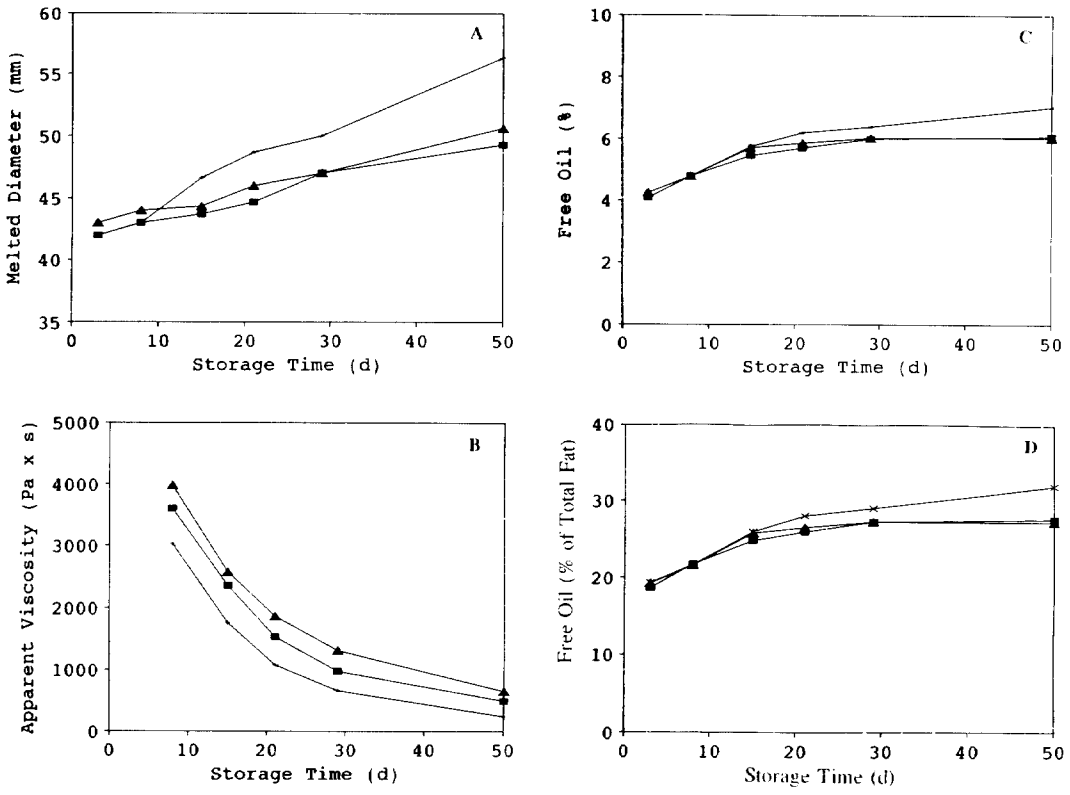

Figure 2. Impact of coagulant type on meltability (A; SEM = .87 mm), apparent viscosity (B; SEM = 158 Pa × s), free oil release (C; SEM = .22%), and free oil release as a percentage of total fat (D; SEM = 1.0%) of Mozzarella cheese during 50 d of storage at 4°C for *Endothia parasitica* protease (*), chymosin derived by fermentation (■), and *Mucor miehei* protease (▲).

TABLE 3. Mean squares and probabilities of meltability, apparent viscosity, free oil, and free oil as a percentage of total fat for Mozzarella cheese stored at 4°C for 50 d.

Factors	Meltability		Apparent viscosity		Free oil		Free oil, fat basis	
	MS	P	MS	P	MS	P	MS	P
Whole-plot factor								
Coagulant type	22.4	.16	876,266	.25	.29	.07	5.0	.24
Day of cheese making (blocked)	57.8	.07	3,966,932	.07	4.61*	<.01	40.8*	.04
Order of cheese making (blocked)	1.7	.71	140,372	.68	.03	.42	.7	.70
Error	4.1		293,555		.02		1.6	
Subplot factor								
Age	370.4*	<.01	46,647,417*	<.01	31.16*	<.01	644.5*	<.01
Age × age	.4	.55	9,156,103*	<.01	7.31*	<.01	150.1*	<.01
Interaction of coagulant type × age	23.2*	<.01	54,196	.49	.29	.15	5.9	.16
Interaction of coagulant type × (age × age)	.9	.66	13,618	.83	.02	.90	.4	.89
Error	2.3		75,010		.15		3.1	
R^2	.905		.965		.884		.871	

*Statistically significant ($P < .05$).

made using *E. parasitica* protease. These results indicate that the extent of proteolysis and, especially, β-casein breakdown significantly influenced functional properties such as meltability, AV, or free oil release of Mozzarella cheese.

Practical Implications

Coagulant retained in the cheese influences proteolysis and functional properties of Mozzarella cheese during refrigerated storage. Consequently, cheese-making parameters that affect the amount or activity of coagulant retained in the cheese may also influence functionality through their influence on the coagulant. Factors such as pH at drawing whey (10, 17), cooking temperature (28), salt in the aqueous phase of the cheese (13), mixing temperature (5), and heat-induced interaction of whey proteins (16) may influence the extent or pattern of proteolysis and the resulting changes in functional properties. Interactions of all of these factors may cause confusion for cheese makers when several factors vary simultaneously. For example, a cheese maker can achieve either the highest or lowest extent of proteolysis using *E. parasitica* protease as a coagulant. If use of *E. parasitica* protease is combined with a high drawing pH and high cooking temperature, proteolysis caused by the coagulant will likely be minimized. However, the same coagulant combined with a low drawing pH and low cooking temperature would allow maximal proteolysis and the corresponding changes in functionality. Control of functional properties of Mozzarella cheese requires an understanding not only on the effect of many individual parameters, but also on their interactions in the cheese-making process.

CONCLUSIONS

In all cheeses, TPA hardness of unmelted cheese decreased, meltability increased, AV decreased, and free oil formation increased during 50 d of storage at 4°C. During this time, coagulant type affected texture of unmelted Mozzarella cheese and functional characteristics of melted cheese. Compared with the cheeses made with *M. miehei* protease or CDF under the cheese-making conditions used in this study, the Mozzarella cheese made with *E. parasitica* protease had softer unmelted cheese

texture, was more meltable, and released more free oil upon melting. The differences in the rate of changes in functional properties of Mozzarella cheese during storage appear to be related to the differences in the proteolysis caused by the coagulant type.

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