Proteolysis and Functional Properties of Mozzarella Cheese as Affected by Refrigerated Storage

Chaves, W.H. Viotto, and C.R.F. Grosso

ABSTRACT

Each of three cheese batches was randomly divided into two parts, one was subjected to slow freezing for 15 days and the other maintained under refrigeration. Thereafter the cheeses were tempered under refrigeration for 1 mo and evaluated weekly. In general, cheeses presented low melting capacity as a result of low levels of proteolysis. This low intensity of proteolysis was probably a function of the destruction of the lactic starter and partial inactivation of the coagulant during stretching of the cheese. Free oil content increased during storage for both treatments, but more in the tempered samples.

Key Words: Mozzarella, proteolysis, refrigerated storage, melting capacity

INTRODUCTION

THE QUALITY OF MOZZARELLA CHEESE DEPENDS ON PHYSICAL properties, especially melting capacity, elasticity, color and production of free oil. Mozzarella can be produced using mesophilic or thermophilic starter culture bacteria. Variations in manufacturing conditions, temperature and storage time can influence the functional properties of mozzarella. For use in pizza, the cheese should be produced using a thermophilic starter, capable of resisting heat treatment (stretching). Together with the residual coagulant, this confers desirable changes in the texture and functional properties. Much mozzarella cheese is produced using mesophilic starters, to make it more acceptable for direct consumption. In general, it is sold as slices and should not therefore show great adhesivity, avoiding the sticking together of slices. Mozzarella cheese produced with mesophilic starters has been used mainly for cheese to direct consumption (sliced) and for use as a culinary ingredient (melted).

The growth in consumption of mozzarella cheese and the increase in production have been rapid. The instability of some physical properties (such as melting capacity and stretchability) and the seasonality of milk production have led large-scale consumers and producers to freeze the cheese for storage (Alvarez, 1986; Pilcher and Kindstedt, 1990). The low temperatures suspend or reduce biochemical modifications which would occur during refrigerated storage.

Few studies have been carried out to investigate the effects of freezing and conditions of thawing on mozzarella cheese. In studies conducted with mozzarella, immediately after thawing, the cheese showed high fat leakage, low cohesiveness, free-surface moisture, bleached discoloration and poor melting (Dahlstrom, 1978). All these alterations were reverted after 3 wk refrigerated storage (4.4°C). Tunick et al. (1991) froze mozzarella cheese at −20°C for 8 wk. After thawing, they tempered the cheeses at 4°C for 3 wk and observed that they showed a greater melting capacity than the nonfrozen control. Bertola et al. (1996) reported that frozen mozzarella produced with a thermophilic starter, and subsequently tempered for 14 to 21 days, showed the same quality as refrigerated cheese.

Evaluation of the extent of physical changes caused by freezing of mozzarella is needed. A study of the extent and depth of proteolysis of mozzarella produced with mesophilic cultures could contribute to a better understanding of changes occurring during refrigerated and frozen storage.

Our objective was to study the effects of slow freezing and the time of tempering (time under refrigeration after thawing) on characteristics of mozzarella produced with a mesophilic starter. We evaluated proteolysis during storage, the physico-chemical characteristics of the raw cheese and functional properties of the melted cheese.

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MATERIALS & METHODS

Milk and mozzarella manufacturing procedure

Mozzarella cheese was produced by a local dairy plant. Milk was pasteurized at 72°C for 15s and cooled to 32°C. Direct-vat freeze-dried culture was added as the starter at a concentration of 10^6 CFU/mL of milk. Mesophilic starter used in cheese making was furnished by Christian Hansen® (Roskilde, Danmark) consisting of *Lactococcus lactis* ssp. *lactis and Lactococcus lactis* ssp. *cremoris* (R704). Solutions of $CaCl_2$ (50%, 25 mL/50 kg of milk) and liquid bovine rennet (mixture of 20% of rennet and 80% of pepsin) from Christian Hansen® (Valinhos, Brazil), enough to coagulate the milk in 30 min, were added. Following a 30 min set at 32°C, the milk coagulum was cut with a wire. A part of the whey was removed (~30%) and hot water was added. The curd was then stirred gently at 38°C for 10 min and heated at 42°C, 15 min with continuous agitation. Then whey was drained (draw pH = 6.35), and the curd was pressed (2× curd weight) and piled in plastic shelves at room temperature (~23°C) for 18–24h to allow fermentation. When the curd reached pH 5.10–5.15 the cheese curd was milled. A twin-screw Mozzarella mixer was used to stretch the curd, the stretching water temperature was 75°C and the curd was ~58°C at the exit of the mixer. Stretched cheese was extruded into plastic boxes and set in a cold water bath at 3–5°C for 1h. Blocks of cheese were placed in a saturated NaCl brine (23%) for 24h at 12°C. Each cheese (3.5 kg, 25×12.5×11 cm) was individually vacuum-packed and stored at 5°C. From each batch produced under factory conditions, 10 pieces were randomly chosen to examine. These samples were placed in an isothermic container and transported to the Milk & Dairy Products Laboratory, UNICAMP, a journey of about 6h.

On reception, each batch was randomly separeted into 2 groups, each group (the entire block of mozzarella cheese) being subjected to one of the following treatments: (1) maintained under refrigeration (T=6–8°C) as a control; (2) slow freezing in a cold chamber (T= −20°C, thus simulating the way in which the product is commercially frozen). After 15 days frozen storage, the frozen pieces of cheese were transferred to a refrigerated chamber and maintained there for 29 days at 6–8°C. This period of refrigerated storage after thawing was termed the "tempering time". This experiment was carried out in triplicate, using 3 batches manufactured on 3 separate days.

Chemical analysis

Each block of mozzarella cheese was sectioned, perpendicular to the longitudinal axis, into 3 equal segments weighting about 1200 g. All segments were vacuum packed and stored at 6–8°C until the day of testing. The central segment was used for chemical analysis, titratable acidity and proteolysis. The left side was used to determine expressible serum, water activity and free oil, and the right side for melting capacity and color. For all analyses, except melting capacity, the cheese samples were ground in a blender to provide a particle size of about 2–3 mm. Ground samples were packed in a 50 mL plastic snap-lid vial, without headspace, to minimize moisture loss during storage at 6–8°C. Salt content was determined using the Volhard method (Richardson, 1985). Fat content was determined by the Gerber method (Instituto Adolfo Lutz, 1985), total nitrogen by the macro-Kjeldahl method (AOAC, 1995) and titratable acidity according to Richardson (1985). All analyses were done in triplicate, except moisture and ash contents which were determined in quadruplicate according to AOAC (1995). Titratable acidity was determined after 1, 8, 15, 22, and 29 days of storage at 6–8°C.

Proteolysis

Amounts of nitrogen soluble in pH 4.6 acetate buffer and in 12% TCA were determined after 1, 8, 15, 22, and 29 days storage, to evaluate the extent and depth of proteolysis, using the methodology of Bynum and Barbano (1985). Results were expressed as % total nitrogen content (TN) of the cheese.

Urea gel electrophoresis was carried out on the control and tempered samples after 1, 8, 15, 22, and 29 days refrigerated storage, according to the methodology of Farkey et al. (1991), using a concentration of 12% acrylamide. The gels were stained with G250 Coomassie Brilliant Blue according to Blakesley and Boezi (1977), and the samples were prepared according to Shalabi and Fox (1987).

Functional properties

All tests on melted and unmelted cheeses were conducted after 1, 8, 15, 22, and 29 days storage at 6–8°C. Expressible serum was determined in triplicate (Guo and Kindstedt, 1995) to evaluate changes due to freezing and during refrigerated storage. Water activity was determined in duplicate using the Aqualab CX-2 Decagon devices.

The color of the cheese, both raw and after melting, was determined using a portable Minolta colorimeter connected to a DP-301 data processor, determining values for L, a, and b (Hunter Lab color space). The color of the cheese after melting was determined in triplicate according to the methodology of Barbano et al. (1993).

The melting capacity of the mozzarella was determined in quadruplicate by Schreiber's method as modified by Kosikowski (1982). Free oil formation in the mozzarella cheese was determined in quadruplicate by the Gerber method as modified by Kindstedt and Fox (1991).

Experimental design and statistical analysis

A complete factorial experimental design was used to study the following factors: (1) storage conditions (treatment of sample–freezing/tempering and refrigerated control); (2) storage time and (3) block (each block representing a day of manufacture). The experiment was randomized in blocks. The statistical analysis was carried out using version 6.11 of the SAS® System statistical package (SAS Institute, Inc., Cary, NC). The results were analyzed using the General Linear Models Procedure PROC GLM to check the isolated effect of the factors studied (block, time and storage conditions) as well as the interaction between factors. Significance of differences was defined at $p \leq 0.05$.

RESULTS & DISCUSSION

Chemical composition of the cheese

The average results for the chemical composition of the three batches of mozzarella cheese were 44.3% moisture, 29.0% fat, 22.9% total nitrogen, 1.45% salt, 3.28% salt in moisture, 3.0% ash, and 52.1% FDM (Fat in Dry Matter). The pH was 5.19.

Proteolysis

Proteolysis was measured as a function of the chemical levels of soluble nitrogen and the electrophoretic profile. We made a statistical analysis of the levels of proteolysis (Table 1). There was no difference between the two treatments with respect to extent of proteolysis, indicating therefore, no difference in primary proteolysis due to coagulant.

The nitrogen solubles in 12% TCA quantify the depth of proteolysis, measuring the activity of the lactic starter enzymes. A significant difference occurred between the two treatments (Table 1), the refrigerated samples showing great intensity. This was probably due to the growth of nonstarter lactic acid bacteria (NSLAB) which had survived the pasteurization, since the values for titratable acidity remained practically constant during storage (Fig. 1), showing that the lactic starter was inactivated during stretching. Progression of the levels of nitrogen solubles at pH 4.6 and in 12% TCA was followed (Fig. 2) throughout the storage time. The values for soluble nitrogen were extremely low, and similar to those reported by Barbano et al. (1993) for mozzarella produced without lactic starter during the same storage time.

Electrophoresis demonstrated the hydrolysis of α_{s1}-casein to α_{s1-1}-casein, showing the activity of the coagulant during refrigerated storage. Although demonstrating hydrolysis, it was not possible to discern differences from the electrophoretic profiles with respect to storage time or sample treatment. In our experiment, the water temperature (75°C) and pH (5.15) of stretching could have partly inactivated the coagulant and destroyed the mesophilic starter. The appearance of γ_1, γ_2, and γ_3 casein bands, indicated the possible activity of plasmin in the degradation of the β-casein (Fig. 3,4).

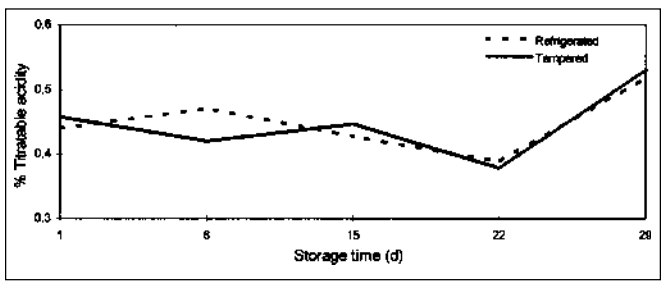

Fig. 1—Effect of tempering and refrigeration on titratable acidity during 29 days storge at 6-8°C.

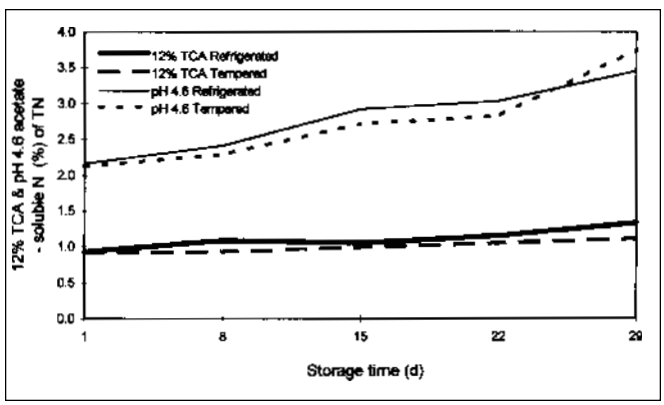

Fig. 2—Effect of tempering and refrigeration on nitrogen solubles at pH 4.6 and in 12% TCA during 29 days storage (6-8°C).

Table 1—Mean square and probabilities for indices of proteolytic changes of mozzarella during 29 days storage at 6- 8 C[a]

Factors		Titratable acidity		pH 4.6		12% TCA	
	DF	P	Ms	P	Ms	P	Ms
Block	2	0.050*	0.045	0.002*	1.565	0.000*	0.298
Treatments	1	0.986	0.000	0.676	0.020	0.010*	0.094
Time	4	0.304	0.015	0.001*	1.908	0.005*	0.074
Interaction (treatment X time)	4	0.974	0.001	0.670	0.065	0.387	0.010
Error	8						
R-Square		0.754		0.933		0.949	

[a]Treatments: Tempered and refrigerated. P = probabilities; Ms = Mean square; DF = Degrees of freedom.
*Statistically significant at P≤0.05.

Table 2 —Mean square and probabilities for functional properties changes of mozzarella during 29 days storage at 6- 8 C[a]

Factors		Meltability		Free oil		Expressible serum	
	DF	P	Ms	P	Ms	P	Ms
Block	2	0.281	1.143	0.157	2.081	0.346	0.509
Treatments	1	0.227	1.310	0.057*	4.347	0.473	0.238
Time	4	0.513	0.679	0.085*	2.678	0.000*	18.85
Interaction (treatment X time)	4	0.033*	3.468	0.463	0.880	0.541	0.349
Error	8						
R-Square		0.814		0.812		0.961	

Treatments: Tempered and refrigerated. P = probabilities; Ms = Mean square; DF = Degrees of freedom.
*Statistically significant at P≤0.1.

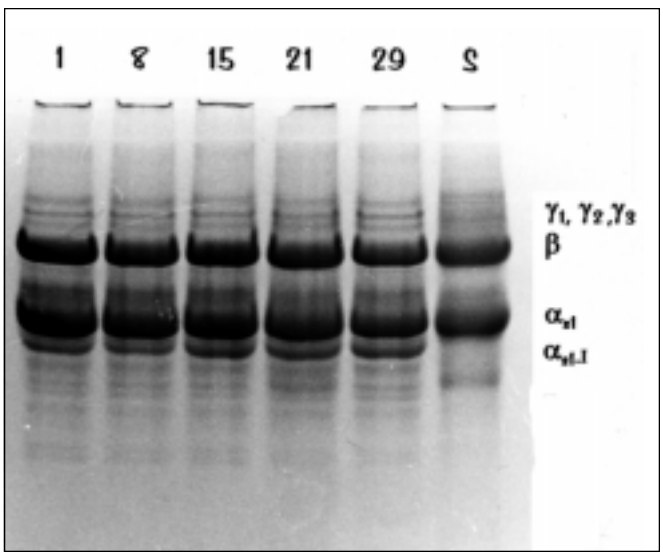

Fig. 3—Electrophoretogram of refrigerated mozzarella: Band 1-28=storage days of mozzarella; Band S=sodium caseinate (standard).

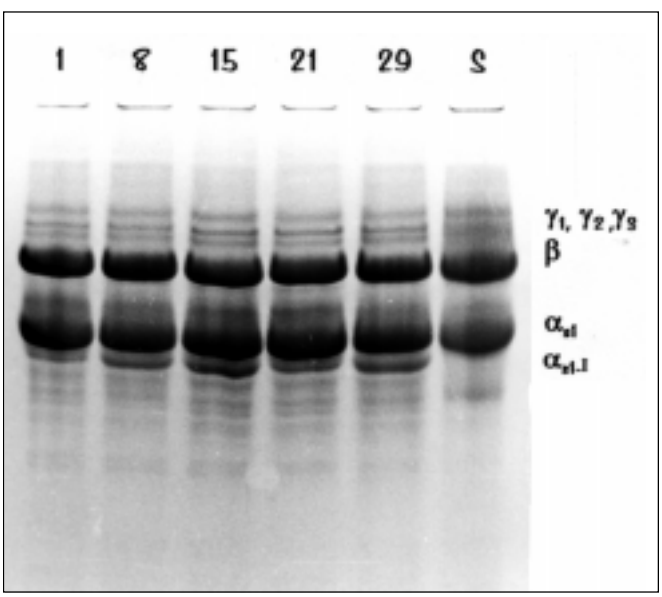

Fig. 4—Electrophoretogram of tempered mozzarella: Band 1-28=storage days of mozzarella; Band S=sodium caseinate (standard).

Functional properties

With respect to water activity and the color of cheese before and after melting, no difference was observed for any factors studied. However, changes occured in the amount of expressible serum in the refrigerated and tempered cheeses during storage (Fig. 5). For all cheeses, expressible serum diminished progressively and intensively during the first days continuing to the tenth day of storage in all cases. Results indicated that, after the tenth day neither the frozen nor refrigerated mozzarella cheeses should present the free moisture problems normally found in fresh mozzarella cheese. There was an increase in water holding capacity as evidenced by the decrease of expresssible serum shown during these first ten days of storage. This would probably facilitate the shredding and slicing of mozzarella cheese.

Freezing did not affect the amount of expressible serum of the mozzarella (Table 2) and no difference was observed between the treatments. Moisture loss by evaporation and the level of NNP in the cheese may influence the decrease in a_w during storage of mozzarella cheese (Marcos, 1993). The moisture content of our cheeses remained practically constant during storage, due to the use of plastic packaging, which avoided the loss of water by evaporation. The NNP level was extremely low and practically constant during storage, independent of treatment (Fig. 2). Variations in measurement of the expressible serum did not appear to influence the a_w values. Changes in expressible serum during early stages of cheese ripening are due to a redistribution of moisture within the cheese (Rankumar et al. 1997). These are related to the basic properties of the protein matrix and the transient effects of curd salting, rather than a direct consequence of glycolytic and proteolytic changes.

In general, the melting capacity of all the cheeses (Fig. 6) was relatively low due to low levels of proteolysis. The recently thawed samples showed greater melting capacity, probably due to some alteration in protein structure during freezing which resulted in a reduction of cohesiveness. This observation was in confirmation of the results

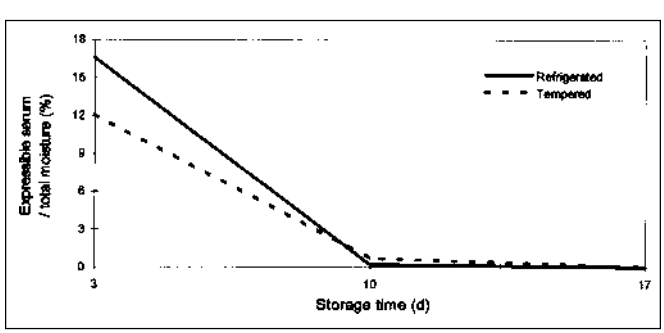

Fig. 5—Effect of tempering and refrigeration on expressible serum during storage (6-9°C).

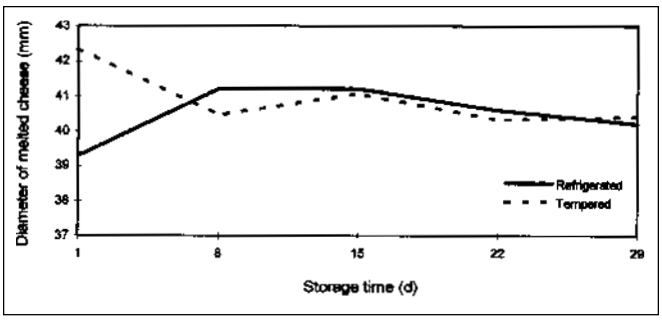

Fig. 6—Effect of tempering and refrigeration on melting capacity of mozzarella cheese during 29 days storage at 6°C.

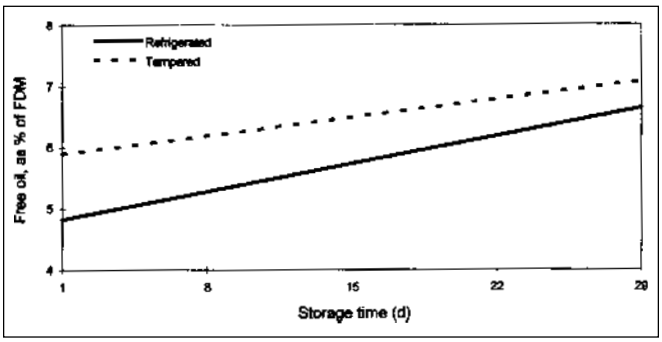

Fig. 7—Effect of tempering and refrigeration on separation of free oil during 20 days storage at 6-8°C.

of Tunick et al. (1991). After one week tempering, no difference was observed between treatments with respect to melting capacity.

All cheeses showed the tendency of increased oil separation with storage time (Fig. 7). The formation of free oil was greater in tempered samples. Freezing affected the separation of free oil, probably due to some protein denaturation or the growth of ice crystals. Also the rupture of the lipoprotein membrane of the fat globules, which could favor the coalescence of fat and the formation of larger agglomerates, could lead to a greater separation of free oil.Thus, the cheeses submitted to freezing followed by tempering showed a greater formation of free oil during storage. Samples freshly thawed showed a greater melting capacity, but after one week there was no difference between refrigerated samples and those tempered after thawing.

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