Mozzarella Cheese: Impact of Nonfat Dry Milk Fortification on Composition, Proteolysis, and Functional Properties[1]

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ABSTRACT

The effects of fortifying milk with low heat NDM on the composition, proteolysis, and functional properties of Mozzarella cheese during 50 d of storage at 4°C were determined. Three vats (180 kg of milk per vat) of cheese fortified at 0, 1.5, and 3% of NDM were made using a stirred-curd, no-brine method. The ratio of casein to fat was held constant by adjusting the fat content of the milk. The cheese making was replicated on 3 different d using a 3 × 3 Latin square design. Total N content in whey and stretching water increased as NDM fortification increased. The calcium content of the cheese increased as NDM fortification increased, and cheese moisture tended to decrease as fortification increased. Nitrogen that was soluble in 12% TCA and acetate buffer at pH 4.6 increased during storage for all cheeses. Fortification with 3% NDM decreased the rate of increase in both types of soluble N during refrigerated storage. Cheese made with 3% NDM fortification had a tendency toward firmer texture of unmelted cheese and higher apparent viscosity of melted cheese. Meltability and free oil formation were not affected by the fortification. As NDM fortification increased, cheese tended to show a slight increase in browning.
(**Key words**: Mozzarella cheese, nonfat dry milk fortification, composition, functional properties)

Abbreviation key: **AV** = apparent viscosity, **TA** = titratable acidity, **TN** = total N, **TPA** = texture profile analysis.

INTRODUCTION

Seasonality in the availability and pricing of fresh milk for cheese making makes the fortification of milk with NDM an attractive option for Mozzarella cheese manufacture (1, 4, 5, 12). When NDM is added to milk, less fresh cream needs to be removed by separation prior to the manufacture of Mozzarella cheese. When the value of milk fat in cream is low relative to the value of milk fat in cheese, cheese factories have an economic incentive to sell more milk fat as cheese than as fresh cream. The addition of NDM to milk for cheese making helps manufacturers accomplish this goal.

Traditionally, NDM has generally been produced in order to store surplus milk solids for later use. With the implementation of Class III-A pricing in US federal milk marketing orders in 1993, economic incentives for the use of NDM to adjust the ratio of casein to fat in milk for the manufacturing of Italian cheese have made NDM use common practice (15). Because Mozzarella cheese production presently constitutes about 80% of total Italian cheese production (9), the use of NDM in Mozzarella cheese making is currently a common practice.

However, the level of fortification and the protocols of incorporating NDM into milk or water may affect the final quality of Mozzarella cheese. The available literature (4, 5, 6, 12, 16) indicates that Mozzarella cheese or Mozzarella-like products with acceptable quality can be made from milk that has been fortified with NDM. However, the systematic information on the age-related changes in functional properties of Mozzarella cheese produced using milk fortified at various percentages of NDM is not available. Therefore, the objective of this research was to determine the composition, proteolysis, and functional properties of Mozzarella cheese fortified at 0, 1.5, and 3% (wt/wt) NDM.

MATERIALS AND METHODS

Cheese Making

A stirred curd, no-brine method for cheese making (2) was used to produce low moisture, part-skim

Received June 10, 1996.
Accepted July 9, 1997.
[1]Use of trade names, names of ingredients, and identification of specific models of equipment is for scientific clarity and does not constitute any endorsement of product by authors, Cornell University, University of Vermont, or the Northeast Dairy Foods Research Center.
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TABLE 1. Statistical model used for data analyses.

Factors	df	Analyzed as
Whole plot		
NDM Fortification (F)	2	Classification
Day of cheese making	2	Block
Order of cheese making	2	Block
Error	2	
Subplot		
Age (A)	1	Quantitative
A × A	1	Quantitative
F × A	2	Classification × quantitative
F × (A × A)	2	Classification × quantitative
Error	39[1]	

[1]For electrophoresis results, 21 dfare instead of 39 df because four instead of six aging times were used.

Mozzarella cheese. Raw skim milk and raw cream were obtained from the Cornell University dairy plant. The NDM (low heat, Grade A) was obtained from Farmers Cooperative Creamery (McMinnville, OR). Protein and casein contents of the batch of NDM were determined prior to use in fortification. A NDM slurry (mixture of 6% NDM powder and 94% skim milk, wt/wt) was prepared and kept overnight at 4°C for hydration. Unfortified skim milk was adjusted to the desired fortification of 0, 1.5, or 3.0% NDM (wt/wt) by adding the appropriate amount of the NDM slurry. The casein to fat ratio was held constant for all percentages of NDM fortification by adding an appropriate amount of cream after the NDM fortification to achieve a casein to fat ratio of about 1.15 in the standardized milk. The standardized milk was pasteurized (model Universal Pilot Plant; PMS Processing Machinery and Supply Co., Philadelphia, PA) at 72°C for 16 s, cooled to 4°C, and stored overnight at 4°C. The next day, the standardized milk (about 180 kg per vat) was poured into a cheese vat (model 4MX; Kusel Equipment Co., Watertown, WI) and heated to 38°C.

Direct-to-vat frozen starter cultures, Thermococcus C120® (*Streptococcus thermophilus*) and Thermorod R160® (*Lactobacillus delbrueckii* ssp. *bulgaricus*) from Rhône-Poulenc (Madison, WI) were added (0.50 ml of each culture/kg of milk), and the milk was ripened for 60 min at 38°C. At the end of ripening, chymosin derived by fermentation (Chymax®; double strength; Pfizer Inc., Milwaukee, WI) was added (0.10 ml/kg of milk). Cheese-making procedures were similar to those described previously (2): pH of 6.20 at whey drainage, salting pH of 5.65, salting rate of 2.2% (wt/wt), and pH of 5.30 at stretching of curd. A twin-screw, pilot-scale Mozzarella mixer (model 640; Stainless Steel Fabricating, Columbus, WI) was used to stretch the curd. Salted curd was fed into the pilot-scale mixer (about 2 kg/min) and was stretched in hot (57°C) circulating water containing 6% salt (wt/wt). The screw speed of the mixer was 12 rpm.

TABLE 2. Mean (n = 3) composition of milk, whey, and stretching water for three percentages of NDM fortification.

	Fortification				
Component	0% NDM	1.5% NDM	3.0% NDM	SEM	LSD[1]
Milk					
Fat, %	2.09[c]	2.39[b]	2.70[a]	0.003	0.02
TN,[2] %	3.10[c]	3.55[b]	4.03[a]	0.007	0.04
NCN,[3] %	0.73[c]	0.82[b]	0.92[a]	0.005	0.03
CN,[4] %	2.37[c]	2.73[b]	3.10[a]	0.002	0.01
CN/TN, %	76.53[b]	76.85[ab]	77.03[a]	0.074	0.45
CN/Fat	1.14	1.15	1.15	0.004	0.02
Whey					
Fat, %	0.22	0.23	0.26	0.007	0.04
TN, %	0.91[c]	1.02[b]	1.16[a]	0.009	0.05
Stretching water					
Fat, %	0.50[b]	0.66[ab]	0.89[a]	0.055	0.33
TN, %	0.08[b]	0.11[ab]	0.14[a]	0.006	0.04

[a,b,c]Means within the same row without a common superscript differ ($P < 0.05$).

[1]$P = 0.05$.

[2]Total N.

[3]Noncasein N.

[4]Casein N.

TABLE 3. Mean (n = 3) initial composition of Mozzarella cheeses made with three percentages of NDM fortification.

	Fortification				
Component	0% NDM	1.5% NDM	3.0% NDM	SEM	LSD[1]
Moisture, %	48.40	47.65	46.72	0.364	2.22
Fat, %	19.71	20.13	19.88	0.127	0.77
FDB,[2] %	38.21	38.44	37.30	0.352	2.14
Protein, %	26.20	26.37	26.97	0.330	2.01
Salt, %	1.76	1.65	1.75	0.075	0.46
S in M,[3] %	3.65	3.47	3.74	0.182	1.11
Calcium, %	0.69[b]	0.76[a]	0.78[a]	0.009	0.06
Calcium, % of P[4]	2.64	2.89	2.92	0.054	0.33

[a,b]Means within the same row without a common superscript differ ($P < 0.05$).

[1]$P = 0.05$.

[2]Fat content on a dry weight basis.

[3]Salt concentration in the water phase of the cheese.

[4]Calcium as a percentage of protein content of the cheese.

Stretched cheese was extruded into stainless steel, cylindrical tubes (7.5 cm diameter × 30 cm long). The first 1 kg of extruded cheese from each vat was removed and discarded. The tubes, filled with 1.4 kg of cheese, were placed in ice water. After 60 min of cooling, the internal temperature of the cheese reached 20°C. The cheese was removed from the tube and vacuum-packaged (Multi Vac model 160; Koch, Kansas City, MO) in a barrier bag (model B150; Cryovac, Duncan, SC) and stored at 4°C. Eight cylinders of cheese were made per vat. The third and fourth cylinders (sequence of extrusion) were used for chemical analyses; the second and fifth cylinders were used for functionality tests.

Chemical Analyses and Functional Tests

Titratable acidity (**TA**) and pH of milk, whey, and curd were monitored during cheese making (17). Milk, whey, stretching water, and cheese samples were analyzed, and proteolytic changes of the cheese (i.e., intact α_{s1}-CN plus α_{s2}-CN and β-CN as well as N soluble in pH 4.6 acetate buffer and in 12% TCA)

TABLE 4. Mean squares and probabilities (in parentheses) for pH, titratable acidity (TA), and indices of proteolysis of Mozzarella cheese during 50 d of storage at 4°C.

Factors	pH	TA	pH 4.6-Soluble N	12% TCA-Soluble N	α_{s1}-CN and α_{s2}-CN	β-CN
Whole plot						
Fortification (F)	0.0006	0.0030	6.16	1.27	3.9	0.4
	(0.54)	(0.18)	(0.22)	(0.12)	(0.92)	(0.78)
Day of cheese making (block)	0.0228*	0.0579*	2.49	0.18	8.1	8.8*
	(0.03)	(0.01)	(0.41)	(0.49)	(0.84)	(0.10)
Order of cheese making (block)	0.0052	0.0061	0.80	0.08	5.3	4.2
	(0.12)	(0.10)	(0.68)	(0.69)	(0.89)	(0.23)
Error	0.0007	0.0007	1.71	0.17	43.3	1.7
Subplot						
Age (A)	0.0198*	0.0786*	327.63*	81.35*	2520.4*	37.9*
	(<0.01)	(<0.01)	(<0.01)	(<0.01)	(<0.01)	(<0.01)
A × A	0.00018	0.0103*	17.92*	3.44*	144.7*	7.8
	(0.30)	(0.03)	(<0.01)	(<0.01	(<0.01)	(0.10)
F × A	0.0001	0.0030	1.72*	0.34*	2.0	0.1
	(0.87)	(0.24)	(<0.01)	(<0.01)	(0.70)	(0.95)
F × (A × A)	0.0002	0.0006	0.30	0.09*	4.5	1.4
	(0.72)	(0.74)	(0.12)	(0.05)	(0.45)	(0.59)
Error	0.0007	0.0020	0.14	0.03	5.4	2.6
R^2	0.800	0.757	0.988	0.990	0.967	0.623

*$P \leq 0.05$.

were monitored up to 50 d of storage at 4°C (17). Functional properties of the cheese were determined by texture profile analysis (**TPA**), a modified Schreiber test for meltability, helical viscometry for apparent viscosity (**AV**), and a centrifugation method for free oil, as previously described (18). To determine the browning characteristics of cheese upon baking, three color indices (2), L value (light to dark), a value (red to green), and b value (yellow to blue) were obtained for each sample in duplicate. All functionality tests were conducted at 3, 8, 15, 21, 29, and 50 d of storage at 4°C. The SDS-PAGE for proteolysis and the baking test for color measurements of the cheese were made after 3, 15, 29, and 50 d of cheese storage at 4°C.

Experimental Design and Statistical Analysis

Three vats of cheese, each using three different percentages of NDM fortification, were made on 1 d from one batch of milk. The cheese making was replicated on 3 different d. On each day, the order of cheese making for the three NDM fortification percentages was changed so that the effects of day and order of cheese making would be blocked in a 3×3 Latin square design. Changes in proteolysis and functional properties during refrigerated storage were assessed using a split-plot design with NDM fortifications as a whole-plot factor. The factors, degrees of freedom, and the statistical model are shown in Table

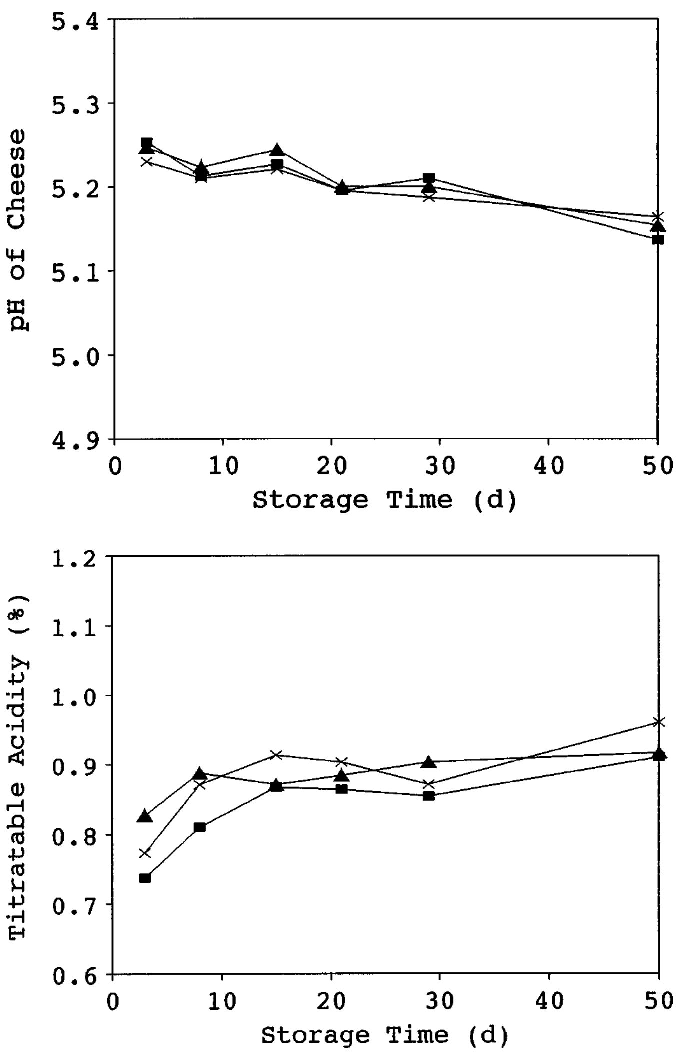

Figure 1. Cheese pH (A; SEM = 0.02) and titratable acidity (B; SEM = 0.03%) during storage at 4°C of Mozzarella cheese made from milk fortified with 0% NDM (■), 1.5% NDM (x), and 3.0% NDM (▲).

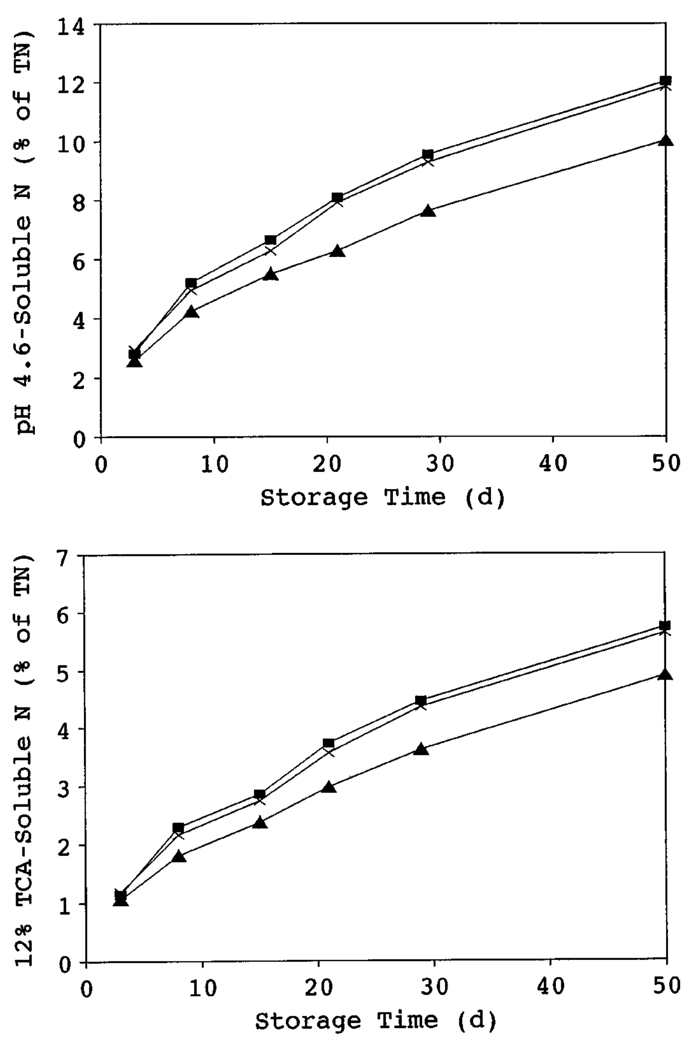

Figure 2. Nitrogen soluble in acetate buffer at pH 4.6 (A; SEM = 0.21%) and in 12% TCA (B; SEM = 0.03%) during storage at 4°C of Mozzarella cheese made from milk fortified with 0% NDM (■), 1.5% NDM (x), and 3.0% NDM (▲).

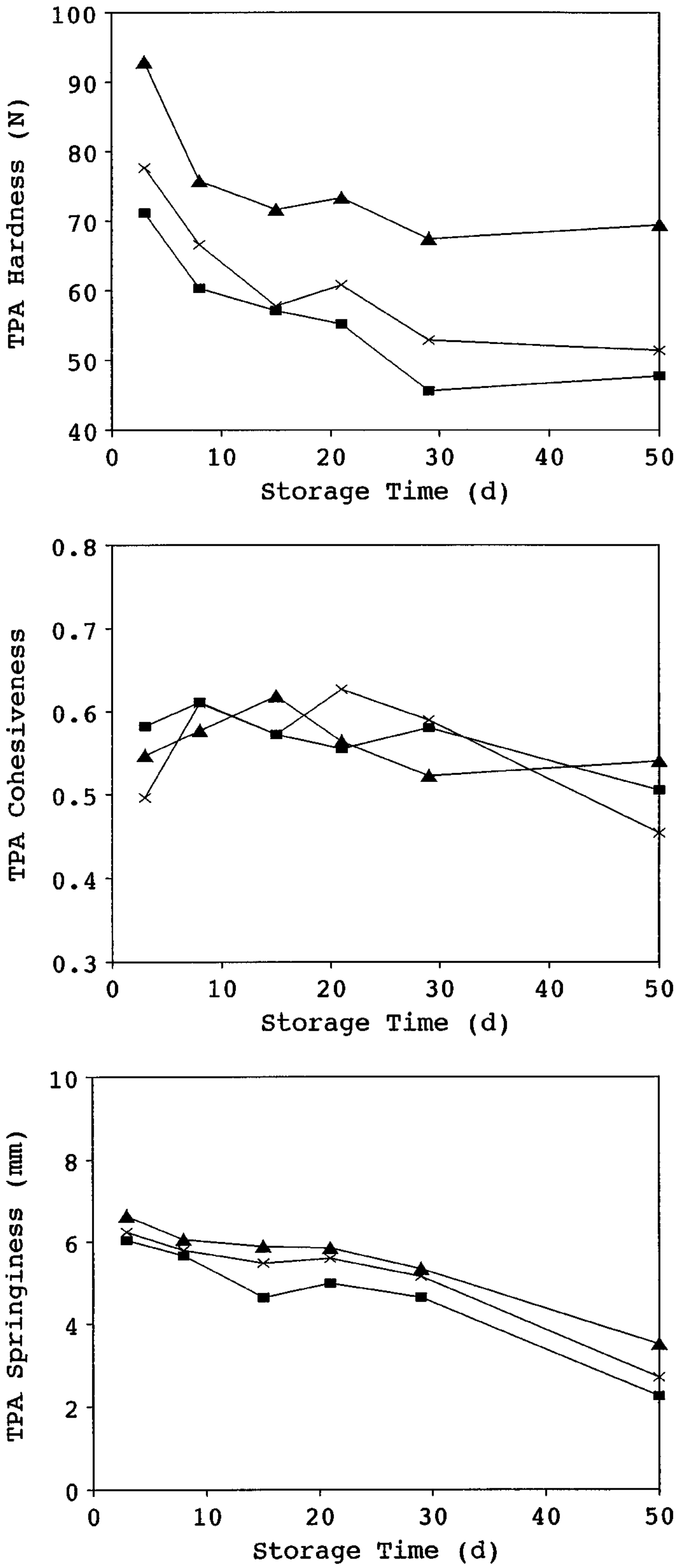

Figure 3. Texture profile analysis (TPA) parameters during storage at 4°C of Mozzarella cheese made from milk fortified with 0% NDM (■), 1.5% NDM (x), and 3.0% NDM (▲): TPA hardness (A; SEM = 3.3N), TPA cohesiveness (B; SEM = 0.03), and TPA springiness (C; SEM = 0.25 mm).

1. The PROC GLM of SAS (11) was used for data analysis. The statistical significance was determined at $P < 0.05$.

RESULTS AND DISCUSSION

Composition of Milk, Whey, Stretching Water, and Cheese

With the addition of NDM to milk, total N (**TN**), noncasein N, and casein contents in milk increased significantly (Table 2). However, the same casein to fat ratio was maintained at about 1.15 by adding the appropriate amount of cream to increase the fat con-

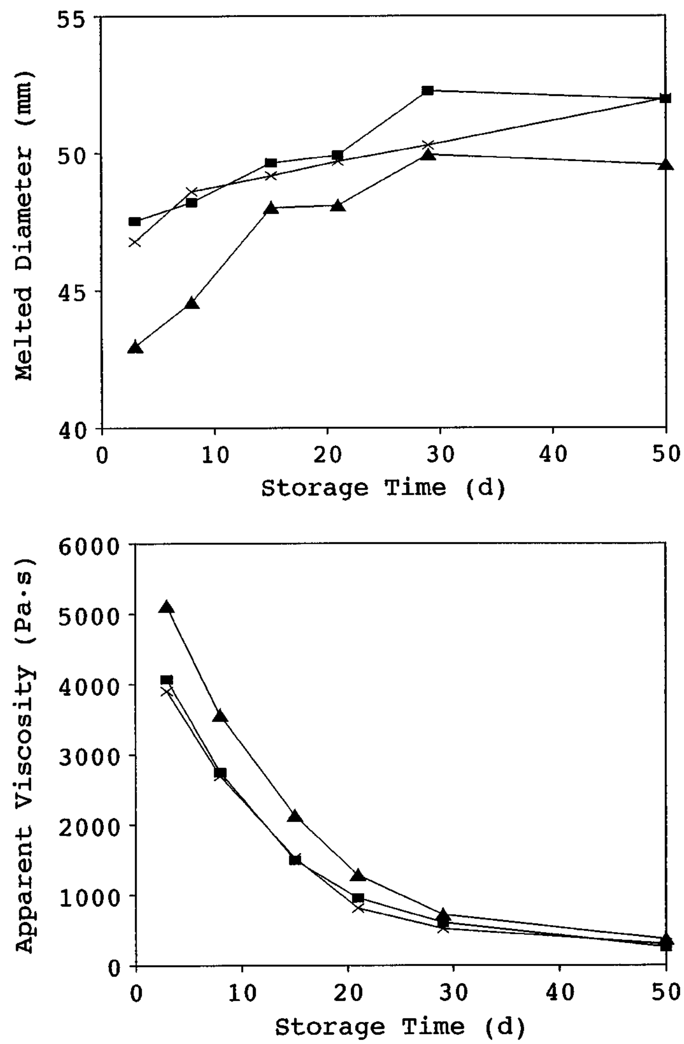

Figure 4. Meltability (A; SEM = 1.2 mm) and apparent viscosity (B; SEM = 225 Pa·s) during storage at 4°C of Mozzarella cheese made from milk fortified with 0% NDM (■), 1.5% NDM (x), and 3.0% NDM (▲).

TABLE 5. Mean squares and probabilities (in parentheses) of texture profile analysis (TPA) parameters, meltability, apparent viscosity, and free oil of Mozzarella cheese during 50 d of storage at 4°C.

	TPA					
Factors	Hardness	Cohesiveness	Springiness	Meltability	Apparent viscosity (10^6)	Free oil
Whole plot						
Fortification (F)	730 (0.38)	0.0052 (0.61)	1.62 (0.51)	8.4 (0.75)	0.41 (0.65)	0.02 (0.58)
Day of cheese making (blocked)	414 (0.52)	0.0010 (0.89)	1.07 (0.61)	19.6 (0.56)	1.63 (0.32)	1.33* (0.02)
Order of cheese making (blocked)	356 (0.56)	0.0009 (0.55)	2.09 (0.45)	26.0 (0.49)	1.36 (0.36)	0.55 (0.06)
Error	447	0.0081	1.70	25.3	0.78	0.03
Subplot						
Age (A)	3389* (<0.01)	0.0014 (0.45)	28.02* (<0.01)	184.7* (<0.01)	106.38* (<0.01)	37.51* (<0.01)
A × A	983* (<0.01)	0.0251* (<0.01)	3.17* (<0.01)	33.4* (0.01)	27.17* (<0.01)	41.52* (<0.01)
F × A	3 (0.92)	0.0043 (0.20)	0.28 (0.24)	6.4 (0.23)	0.78* (0.01)	0.02 (0.91)
F × (A × A)	5 (0.86)	0.0116* (0.02)	0.08 (0.65)	5.4 (0.29)	0.14 (0.41)	<0.01 (0.98)
Error	32	0.0025	0.19	4.2	0.15	0.18
R^2	0.874	0.523	0.910	0.726	0.953	0.881

*$P \le 0.05$.

tent. Casein as a percentage of TN increased as NDM fortification increased because casein as a percentage of TN was higher in the NDM than in the fresh milk. The NDM fortification caused an increase in the TN content of whey and stretching water and fat content in stretching water (Table 2).

No significant differences were detected in the composition of the cheese because of the NDM fortification except calcium content. However, moisture content tended to be lower ($P < 0.10$) as fortification increased (Table 3). Calcium content in cheese was higher as fortification level increased (Table 3) because of the higher concentration of calcium in the whey at draining, which increased the calcium content of the serum phase. Although no significant difference in the ratio of calcium to protein was detected, the values for calcium to protein ratio tended to increase as the percentage of NDM fortification increased (Table 3).

The mean moisture, fat, protein, and salt contents of the cheeses made using different NDM fortification were within the specifications for low moisture, part-skim Mozzarella (3, 14). The TA increased, and pH decreased slightly, during 50 d of refrigerated storage, but the pH and TA of the cheeses at all three percentages of NDM fortification were similar (Figure 1 and Table 4) There was no indication by TA or pH of a larger amount of acid production in cheeses produced from milks fortified with NDM.

Proteolysis

The N that was soluble in acetate buffer at pH 4.6 and in 12% TCA increased significantly during storage (Table 4 and Figure 2), and there was a significant interaction of NDM fortification with age.

TABLE 6. Mean squares and probabilities (in parentheses) for L, a, and b values[1] of baked Mozzarella cheese stored at 4°C up to 50 d.

	L	a	b
Whole plot			
Fortification (F)	38.64 (0.06)	18.20 (0.12)	0.40 (0.33)
Day of cheese making (blocked)	39.93 (0.06)	7.90 (0.24)	0.15 (0.58)
Order of cheese making (blocked)	0.63 (0.80)	3.46 (0.42)	0.49 (0.29)
Error	2.47	2.47	0.20
Subplot			
Age (A)	0.89 (0.75)	228.01* (<0.01)	40.73* (<0.01)
A × A	59.81 (0.01)	173.53* (<0.01)	25.30* (<0.01)
F × A	1.79 (0.81)	2.09 0.45)	1.10 (0.48)
F × (A × A)	0.30 (0.96)	0.28 (0.90)	0.17 (0.89)
Error	8.35	2.55	1.46
R^2	0.544	0.779	0.457

[1]Color indices were L value (light to dark), a value (green to red), and b value (blue to yellow).

The impact of 3% NDM fortification reduced (Table 4) the depth (i.e., 12% TCA-soluble N) and the extent of proteolysis (i.e., pH 4.6 acetate-soluble N). The lower soluble N formation in the 3% NDM cheese may have been caused by its slightly lower moisture content.

For all treatments, the amount of intact residual α_s-CN decreased (from about 39% of total protein to about 15% of total protein for all treatments) as storage time at 4°C increased, but no significant influence of NDM fortification was detected (Table 4). Electrophoresis and the amount of N that was soluble in acetate buffer at pH 4.6 do not measure the same thing and therefore would not be expected to give identical results. Electrophoresis has indicated that the same amount of intact α_s-CN remained in the cheese regardless of NDM fortification level as α_s-CN decreased with storage time; therefore, the initial proteolytic cleavage of intact casein by residual chymosin was probably not influenced by fortification. However, the release of N soluble in acetate buffer at pH 4.6 and in 12% TCA was influenced by the fortification level, which indicated that an increased level of fortification had a negative influence on the subsequent proteolysis of the fragments of α_s-CN. The amount of intact residual β-CN decreased slightly (from 35 to 32% of total protein) for all cheeses during 50 d of storage (Table 4).

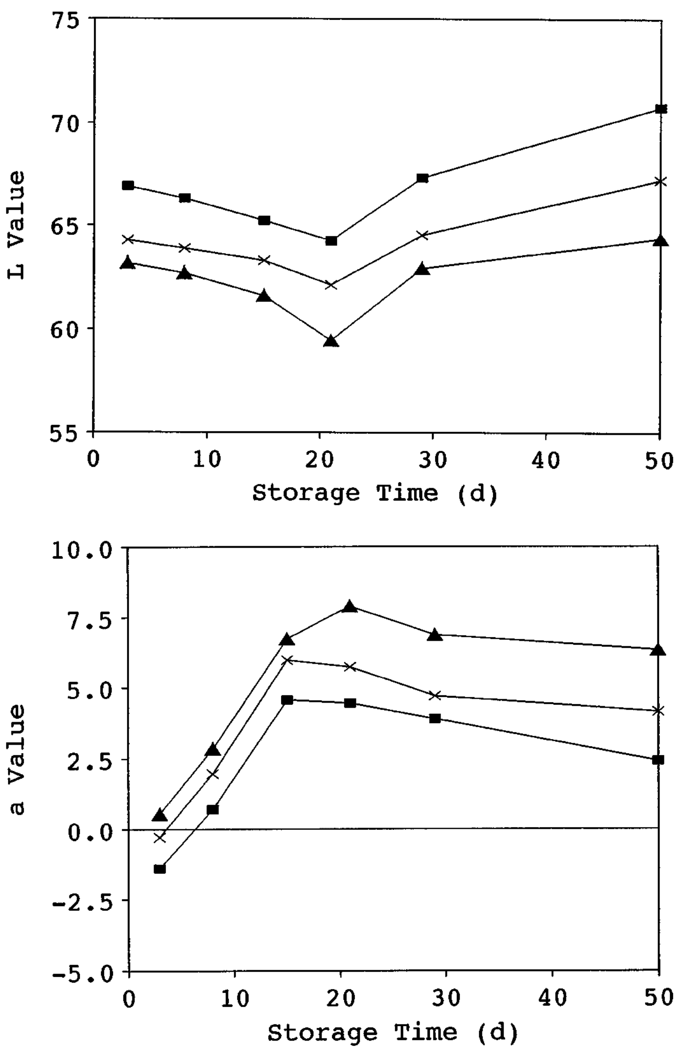

Figure 5. The L values (A; SEM = 1.67) and a values (B; SEM = 0.98) of Mozzarella cheese made from milk fortified with 0% NDM (■), 1.5% NDM (x), and 3.0% NDM (▲) and baked after 15 d of storage at 4°C. The L value is a measure from light to dark, and the a value is a measure from red to green.

Texture and Functionality

For all cheeses, all TPA parameters changed because of age during refrigerated storage (Figure 3 and Table 5). The TPA hardness of the cheese made from milk fortified with 3% NDM appeared to be slightly higher throughout the storage period (Figure 3A), but differences were not significant (Table 5). The slightly firmer texture might be the combined result of slightly lower moisture, higher calcium, and slower proteolysis in the cheese made from milk fortified with 3% NDM. Variations in mineral content of Mozzarella cheese can affect the cheese texture significantly (7).

Meltability of all cheeses increased significantly during refrigerated storage (Tables 5 and Figure 4A). Although addition of 3% NDM seemed to reduce meltability, no statistically significant differences were detected in meltability caused by NDM fortification. The AV decreased significantly during refrigerated storage for all cheeses. The 3% NDM fortification resulted in a higher AV(i.e., significant interaction of NDM and age), especially during the early stage of storage (Figure 4B). No significant differences were detected in free oil formation because of NDM fortification (Table 5). Free oil release was about 3% on d 3 of storage and increased to about 5% fat from d 15 to 50.

The addition of 3% NDM caused slightly more browning (Figure 5), as was indicated by a lower (P = 0.06) L-value (darker) and a numerically higher a value (more red), which might have been caused by the increase in lactose content in the milk and higher lactose or galactose in the cheese. Excessive browning could be a major concern for cheese made from NDM fortified milk because of the potential for increased residual lactose. However, when the cheeses were shredded and baked on a pizza, there was only a slight difference in browning among the three treatments, which was consistent with the trend for the L and a values. No statistically significant differences in the color values were related to fortification, but a and b values changed with age ($P < 0.01$) (Table 6).

Cheeses in this study showed typical patterns of change in functional properties during refrigerated storage (8, 10, 13, 18). The NDM fortification had only minor impact on unmelted cheese texture and melting characteristics during storage, especially fortification with 1.5% NDM.

CONCLUSIONS

Although TN content in whey and stretching water increased as fortification increase, the composition of the fresh cheese was not affected by fortification, except that calcium concentration was slightly higher. At 3% NDM fortification, moisture content tended to be lower, but not statistically significant, in this experiment. Calcium content of cheese was higher in cheese made from milk fortified with 3% NDM. Nitrogen that was soluble in 12% TCA and in acetate buffer at pH 4.6 increased during storage for all cheeses. However, 3% NDM fortification reduced the rate of increase in both measurements of soluble N. Cheese made from milk fortified with 3% NDM also tended to have slightly firmer texture as unmelted cheese and to have higher apparent viscosity when melted. Meltability and free oil formation were not affected by the fortification. The fortification by 3% NDM only slightly increased browning in the baked cheese. Overall, the changes in functional properties of the cheese that were caused by increasing fortification with NDM appeared to be relatively small.

ACKNOWLEDGMENTS

The authors thank Robert Rasmussen, Wen-I Tsai, Maureen Chapman, Patricia Nelson, Joanna Lynch, Shirley Koslowski, George Houghton, and Scott Hawks for their technical assistance. Financial support was provided by Northeast Dairy Foods Research Center, Dairy Management Inc., and Hatch Project 143-444.

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