DAIRY FOODS

Effect of Sodium Chloride on the Serum Phase of Mozzarella Cheese

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ABSTRACT

The objective was to compare changes in the serum phase of brine-salted and unsalted Mozzarella cheeses during aging to elucidate the impact of NaCl on the serum phase and physico-chemical changes. Brine-salted and unsalted blocks of low moisture, part-skim Mozzarella cheese that had been commercially produced were analyzed for expressible serum after 2, 4, 6, 8, and 10 d of storage at 4°C. The amount of expressible serum decreased during aging of both brine-salted and unsalted cheeses; however, amounts were higher for unsalted cheese and decreased more slowly than for brine-salted cheese. The amounts of protein that were insoluble at pH 4.6 in the expressible serum increased dramatically during aging of brine-salted cheeses, but increased only slightly during aging of unsalted cheeses. Urea-PAGE and SDS-PAGE confirmed that intact α_{s1}-casein (CN), α_{s2}-CN, β-CN, and para-κ-CN were present at higher concentrations and increased to a greater extent in the expressible serum of brine-salted than in unsalted Mozzarella cheeses. The data are consistent with the hypothesis that NaCl in the serum phase of Mozzarella cheese promotes microstructural swelling, a concomitant increase in water-holding capacity, and the solubilization of intact caseins from the para-casein matrix.

(**Key words**: Mozzarella cheese, expressible serum)

Abbreviation key: **ES** = expressible serum, **SP** = protein that was soluble at pH 4.6.

INTRODUCTION

It is well established that Mozzarella cheese made by conventional means must undergo a brief period of aging, usually 1 to 3 wk at around 4°C, to attain optimum functionality for use as a pizza ingredient (8, 11). The hydrolysis of intact caseins (i.e., primary proteolysis) by the coagulant is one of the driving forces behind the characteristic changes in functional characteristics during aging (13, 17, 18). The starter culture may also make a significant, although comparatively small, contribution to primary proteolysis and consequent changes in functional characteristics (2). However, the principal contribution of the starter culture to proteolysis occurs in the form of secondary proteolysis, which is important to browning properties (1, 2, 14).

In addition to proteolysis, other factors may also play a role in functional development. For example, the water-holding capacity of Mozzarella cheese increases substantially during aging, which may be symptomatic of physico-chemical and structural changes that may affect functional behavior (9). Guo and Kindstedt (5) reported that the amount of expressible serum (**ES**) that was obtained by centrifuging Mozzarella cheese at 12,500 × *g* for 75 min at 25°C provided a useful parameter of water-holding capacity. Under these conditions, about 30% of the total moisture content of Mozzarella cheese samples was expressed during the first few days after manufacture, but ES decreased to 0 within ca. 2 wk at 4°C because of increased water-holding capacity (5). The ES contained a variety of soluble constituents, including intact (i.e., unhydrolyzed) α_s- and β-CN, both of which increased substantially in concentration as the cheese aged. Guo and Kindstedt (5) concluded that a dynamic relationship exists between the para-casein matrix and the serum phase of Mozzarella cheese whereby intact caseins migrate from the para-casein matrix into the serum phase as soluble species, presumably until equilibrium is established.

Guo and Kindstedt (5) proposed a model to explain the observed physico-chemical changes; the model is based on the peculiar microstructure of Mozzarella cheese (12) and the phenomenon of curd swelling

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[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, University of Vermont, or the Northeast Dairy Foods Research Center.

and peptizing action of NaCl that has been reported by Guerts et al. (3). According to the model, the low concentration of NaCl within the serum phase plays a critical role by promoting the solubilization of intact caseins and the swelling of para-casein fibers within the cheese microstructure. As microstructural swelling proceeds, the large open columns of loosely held serum that are unique to the microstructure of stretched (pasta-filata type) cheese are progressively transformed to a continuous hydrated protein gel (5). The model is consistent with the observed physico-chemical changes, but has yet to be corroborated.

The objective of the present study was to compare changes in the serum phase of brine-salted and unsalted Mozzarella cheeses during aging in order to elucidate further the impact of NaCl on the serum phase and physico-chemical changes.

MATERIALS AND METHODS

Cheese Samples

On three separate occasions, two unsalted blocks (2.3 kg) of low moisture, part-skim Mozzarella cheese were obtained simultaneously from the processing line of a commercial cheese plant. The cheeses were manufactured using a thermophilic starter culture (*Streptococcus thermophilus* and *Lactobacillus helveticus*; Chr. Hansen's Laboratories, Inc., Milwaukee, WI) and coagulant derived from *Mucor miehei* (double-strength; Rhône-Poulenc, Marschall Products, Madison, WI). Milk for cheese making was standardized by removal of cream. A milled-curd procedure was used for cheese manufacturing; stretching pH was 5.15, and curd temperature was ca. 55°C at the exit of the stretcher. The blocks were obtained in sequence after molding but before brining. Blocks produced from curd at the extreme beginning or extreme end of the vat were avoided during sampling. The blocks were immediately placed in barrier bags and transported on ice to the university (ca. 30 min). Upon arrival, one block was immersed in salt brine containing 18% (wt/wt) NaCl and 0.06% Ca at 4°C for 14 h; the second block was vacuum-packaged at ca. 900 mbar and immersed in water at 4°C for 14 h. Then, brine-salted and unsalted blocks were each sectioned entirely into cubes (1 × 1 × 1 cm), mixed thoroughly to provide a representative sample, and divided into five equal subsamples. Each subsample was vacuum-packaged at ca. 900 mbar, to facilitate fusion and equilibration of salt among the cubes, and stored at 4°C until analysis at 2, 4, 6, 8, and 10 d after manufacture. On each analysis day, randomly chosen subsamples of brine-salted and unsalted cheeses were grated entirely and mixed thoroughly; a representative portion was centrifuged (12,500 × *g* for 75 min at 25°C) to obtain ES as previously described (5). The volume and weight of ES were measured, and the ES was stored at –40°C until chemical analysis.

Chemical Analysis

All chemical analyses were conducted in duplicate. Total moisture in cheese was determined by drying in a forced-draft oven at 100°C for 24 h. Fat content of cheeses was determined by the Babcock method (15), and CP content was determined by the Kjeldahl method, using a semi-micro block digestion method (7). The NaCl content of cheeses was determined by a selective ion electrode method (10), and pH was determined using a Beckman Φ™ 12 pH/ISE Meter (Beckman Instruments, Inc., Fullerton, CA), by direct immersion of a Xerolyt electrode (model HA405; Ingold, Wilmington, MA) into a ground cheese sample at ambient temperature.

The CP content in the ES was determined by Kjeldahl, using a semi-micro block digestion method (7). The protein that was soluble at pH 4.6 of the ES (**SP**) was determined in filtrates that had been prepared according to the methods of the International Dairy Federation (6). Urea-PAGE was used to evaluate the proteins and peptides in the ES using a discontinuous system that has been described previously (5). Proteins and peptides in the ES were also evaluated by SDS-PAGE by a method described elsewhere (4).

Contents of Ca, P, Mg, K, Na, and Zn in cheese and ES were measured using an inductively coupled plasma atomic emission spectrometer (Plasma Spec 2.5; Leeman Labs, Lowell, MA) after the samples were digested in a mixture of HNO_3 and $HClO_4$ (5:1, vol/vol) on a hot plate until the digests were clear (4).

Statistical Analysis

Effects of treatments (brine-salted vs. unsalted cheese), storage time (2, 4, 6, 8, 10 d), and the

TABLE 1. Initial chemical composition (n = 3) of brine-salted and unsalted Mozzarella cheeses.

Component	Brine-salted	Unsalted
Moisture, %	49.38	50.87
Fat, %	20.0	20.0
FDB,[1] %	39.51	40.71
Protein, %	25.24	25.33
Salt, %	1.36	0.13

[1]Fat content on a dry weight basis.

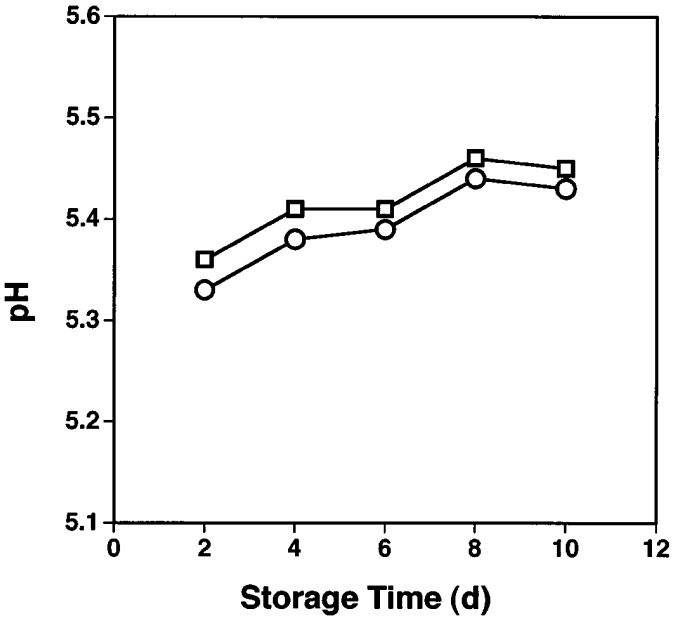

Figure 1. Changes in the pH of brine-salted (□) and unsalted (o) Mozzarella cheeses (n = 3) during storage at 4°C.

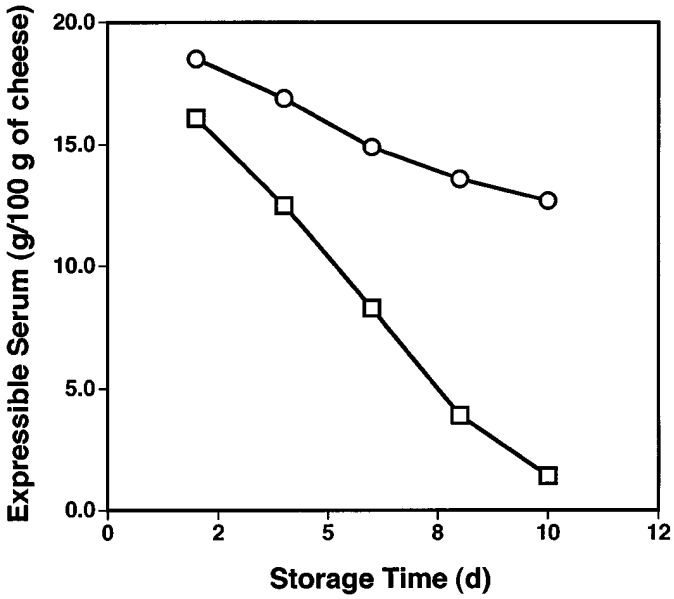

Figure 2. Changes in the quantity of expressible serum obtained from brine-salted (□) and unsalted (o) Mozzarella cheeses (n = 3) during storage at 4°C.

interaction of treatment and storage time on pH of cheese, amount of ES, and contents of CP, SP, and minerals in the ES were evaluated. A split-plot design was employed, and treatment was assigned in a randomized block as the main plot factor; storage time and the interaction of storage time and treatment were subplot factors. The data were analyzed by ANOVA using PROC GLM of SAS (16). The level of significance was $P \leq 0.05$.

RESULTS AND DISCUSSION

The chemical composition of brine-salted and unsalted cheeses is compared in Table 1. Fat and moisture contents were within the standards of identity for low moisture, part-skim Mozzarella cheese. Brine-salted cheeses had lower moisture contents than did unsalted cheeses because of the moisture loss from the block during brining. The pH of cheeses (Figure 1) increased significantly during the 10-d storage period, but brine-salted and unsalted cheeses did not differ significantly (Table 2).

The results of the ANOVA for the various parameters of the ES are summarized in Table 2. The quantities of ES that were obtained from brined-salted and unsalted cheeses during the first 10 d of storage are compared in Figure 2. The mean level of ES from brine-salted cheeses decreased from around 16 g/100 g of cheese at 2 d after manufacture to <1 g/100 g of cheese by d 10, which was consistent with earlier observations (5). In contrast, concentrations of ES from unsalted Mozzarella decreased only slightly during the same period. The ANOVA con-

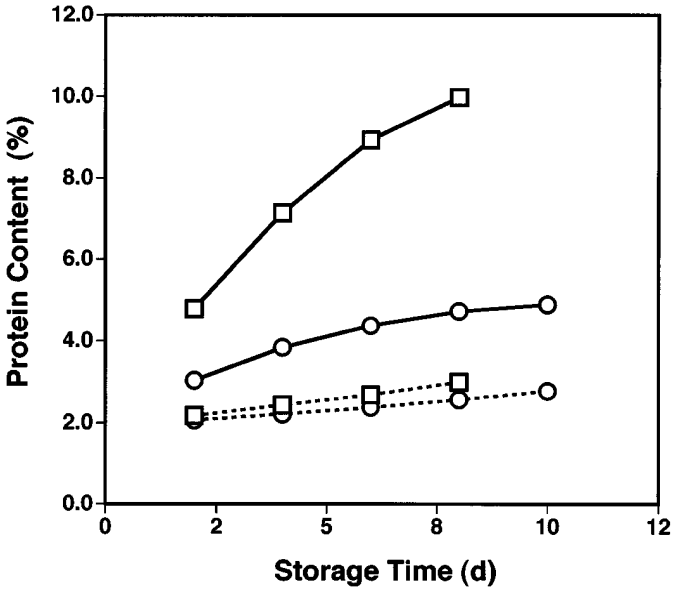

Figure 3. Changes in the contents of CP (solid lines) and protein that was soluble at pH 4.6 (broken lines) of the expressible serum obtained from brine-salted (□) and unsalted (o) Mozzarella cheeses (n = 3) during storage at 4°C.

TABLE 2. The ANOVA for pH of cheese, quantity of expressible serum (ES), and contents of CP, protein that is soluble at pH 4.6 (SP), and Ca, P, K, Mg, Na, and Zn in the ES as a function of brining treatment and storage time at 4°C.

		MS									
Source of variation	df	pH of Cheese	Quantity of ES	CP	SP	Ca	P	K	Mg	Na	Zn
Whole plot											
Treatment (T)	1	0.0043	291*	83*	0.383	6923	3042	321,335*	2426	36,831*	5.22
Error	4	0.0013	5	7.2	0.399	7578	617	23,876	928	502	2.38
Subplot											
Storage time (ST)	1	0.0416*	529*	39.7*	1.317*	1752*	833*	602	4961*	2	11.21*
ST × T	1	0.0001	40*	10.4*	0.109*	10	261	11,102	161	1	2.61*
Error	22[1]	0.0013	3	0.2	0.010	203	107	6663	237	67	0.19

[1]For the subplot factor for CP and SP, degrees of freedom of the error term was 16 instead of 22, because only four aging times were used instead of five. For the subplot factor for Ca, P, K, Mg, Na, and Zn, degrees of freedom of the error term was 10 instead of 22, because only three aging times were used instead of five.

*$P \leq 0.05$.

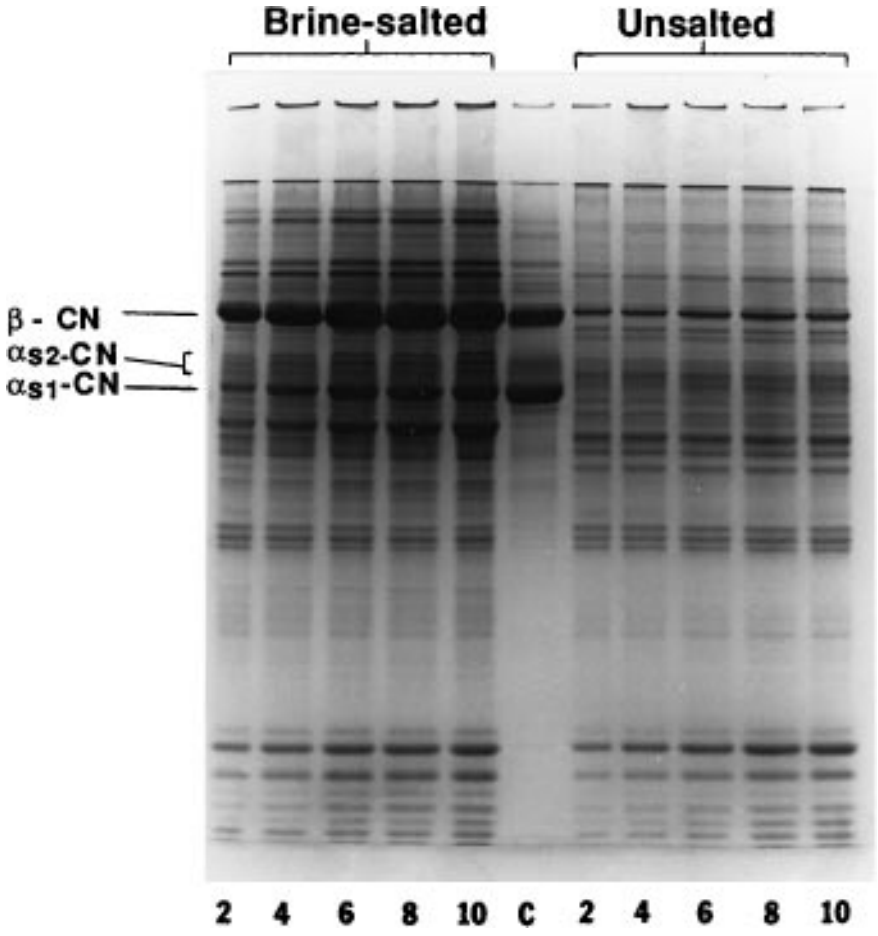

Figure 4. The urea-PAGE gel of the expressible serum that was obtained from brine-salted and unsalted Mozzarella cheese during storage at 4°C. Lanes 1 through 5, expressible serum from brine-salted cheese on d 2, 4, 6, 8, and 10, respectively; lane 6, unsalted cheese (C) on d 2; and lanes 7 through 11, expressible serum from unsalted cheese on d 2, 4, 6, 8, and 10, respectively.

TABLE 3. Concentration of selected minerals in brine-salted and unsalted Mozzarella cheeses (n = 3) and in the expressible serum from the cheeses during storage at 4°C.

Minerals in cheese	Cheese	Storage				
		2 d	4 d	6 d	8 d	10 d
	(mg/kg)					
Brine-salted cheese						
Ca	6490	3150	3,246	3513	. . .	. . .
P	4246	1619	1,587	1543	. . .	. . .
K	715	989	971	942	. . .	. . .
Mg	257	229	256	277	. . .	. . .
Na	5549	9601	11,191	9351	. . .	. . .
Zn	34	2.1	4.2	5.3	. . .	. . .
Unsalted cheese						
Ca	6688	3621	3,633	3739	3899	3709
P	4378	1475	1,278	1218	1183	1097
K	725	1212	1,204	1287	1186	1248
Mg	252	263	274	296	297	287
Na	574	1100	1,114	1136	1137	1134
Zn	33	2.2	2.5	3.0	3.5	4.2

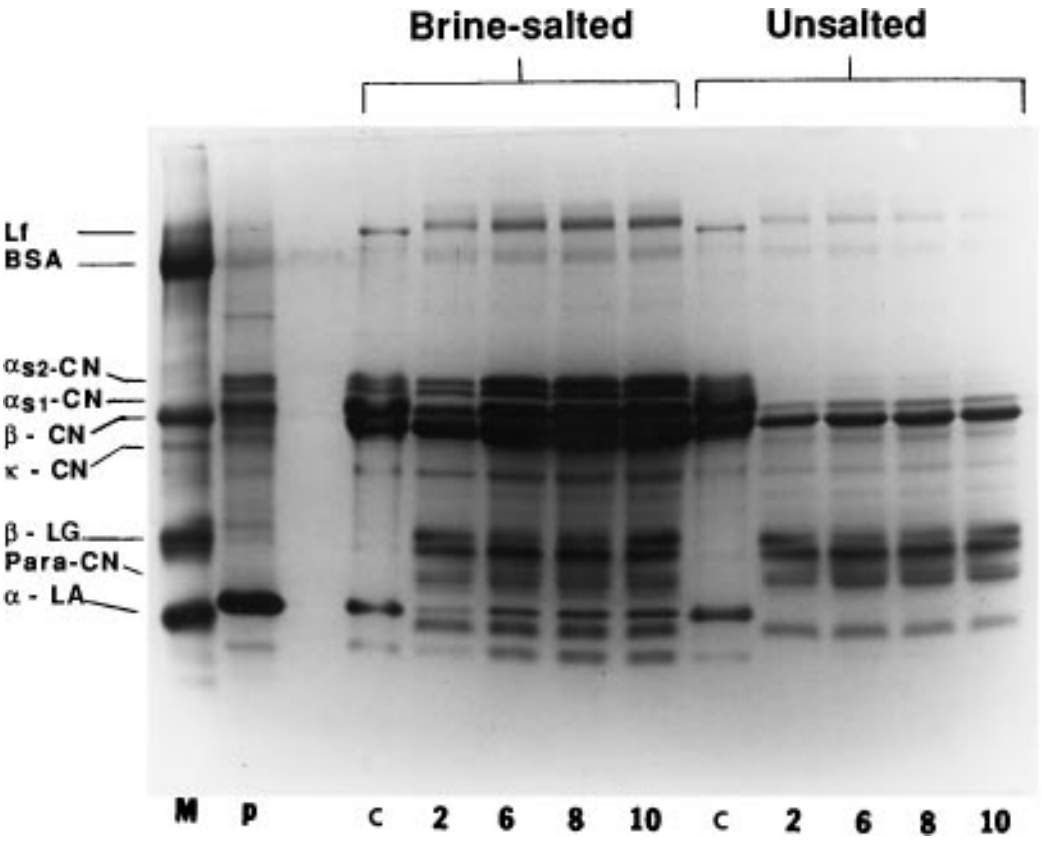

Figure 5. The SDS-PAGE gel of the expressible serum that was obtained from brine-salted and unsalted Mozzarella cheese during storage at 4°C. Lane 1, molecular mass markers (M) for lactoferrin (Lf), BSA, β-CN, κ-CN, β-LG, and α-LA. Lane 2, isolated para-κ-CN (p); lane 3, unused; lane 4, brine-salted cheese (c) on d 2; lanes 5 through 8, expressible serum from brine-salted cheese on d 2, 6, 8, and 10, respectively; lane 9, unsalted cheese (c) on d 2; and lanes 10 through 13, expressible serum from unsalted cheese on d 2, 6, 8, and 10, respectively.

firmed that the effect of treatment and the interaction of treatment and time on the quantity of ES were significant (Table 2). Thus, unsalted Mozzarella showed little increase in water-holding capacity compared with the water-holding capacity of brine-salted Mozzarella during the first 10 d of storage at 4°C.

Percentages of CP and SP in the ES are shown in Figure 3. Analyses of CP and SP from brine-salted cheese were not performed on d 10 because the amount of ES obtained was too small. The effects of storage time and the interaction of treatment and storage time on SP were significant (Table 2). Increased SP in the ES suggested that the soluble peptides resulting from proteolysis accumulated in the serum phase of the cheeses during aging. The significant interaction of treatment and storage time (Table 2) suggested that brine-salted cheese underwent greater proteolysis than did unsalted cheese, although differences between the treatments were small (Figure 3).

Percentages of CP in the ES differed significantly for brine-salted and unsalted cheeses, and the effects of storage time and the interaction of treatment and storage time were also significant (Table 2). However, in contrast to SP, percentages of CP were much higher and increased much faster in brine-salted cheese than in unsalted Mozzarella (Figure 3). The difference between percentages of CP and SP represents protein in the ES that is insoluble at pH 4.6, which consist largely of intact (i.e., unhydrolyzed) caseins (5). Thus, the ES from brine-salted cheeses showed a rapid increase in the levels of intact caseins, but the ES from unsalted cheeses showed correspondingly little increase (Figure 3).

Differences in levels of intact caseins in the ES of brine-salted and unsalted Mozzarella were confirmed using urea-PAGE (Figure 4). Consistent with earlier observations (5), brine-salted cheeses had comparatively high concentrations of α_{s1}-CN, α_{s2}-CN, and β-CN in the ES, which appeared to increase greatly during storage. In contrast, unsalted cheese had much lower concentrations of intact caseins, which appeared to increased only slightly during storage. Similar results were obtained by SDS-PAGE (Figure 5). With SDS-PAGE, it was also possible to identify para-κ-CN, which was present and increased over time in the ES of brine-salted Mozzarella, but could not be detected in the ES of unsalted Mozzarella (Figure 5). Finally, the whey proteins α-LA and β-LG were readily identified by SDS-PAGE in the ES of both brine-salted and unsalted cheeses (Figure 5). Levels of these proteins in the ES of brine-salted cheese appeared to increase only slightly or not at all during aging, in contrast to the caseins. In summary, α_{s1}-CN, α_{s2}-CN, β-CN, and para-κ-CN were present at higher concentrations and increased more in the ES of brine-salted than unsalted Mozzarella cheese.

The data in Figures 2 through 5 are consistent with the hypothesis proposed earlier (5) that NaCl in the serum phase of Mozzarella cheese promotes the microstructural swelling with a concomitant increase in water-holding capacity and the solubilization of intact caseins from the para-casein matrix.

Concentrations of selected minerals in the cheeses and in the ES during storage are summarized in Table 3. Analyses of ES from brine-salted cheeses were not performed on d 8 and d 10 because the amount of ES collected was too small. Salt treatment significantly affected the concentrations in the ES of Na and K, but did not affect the concentrations of the other minerals (Table 2). Concentrations of Ca, P, Mg, and Zn were significantly affected by storage time (Table 2); Ca, Mg, and Zn increased, but P decreased (Table 3).

A previous study (5) found that concentrations of Zn increased in the ES of brine-salted Mozzarella during storage. In the present study, Zn concentrations increased in the ES of both brine-salted and unsalted cheeses, but at a faster rate in the former (Tables 2 and 3). Zinc was presumed to be largely associated with the para-casein matrix; therefore, the presence of NaCl may have enhanced the migration of Zn from the para-casein matrix to the serum phase. Calcium and Mg also increased in the ES over time, suggesting a movement of these minerals from the colloidal to serum phases; presumably as dissociated cations or in association with the soluble caseins.

CONCLUSIONS

Results of the present study suggest that NaCl is critical to the development of water-holding properties and the solubilization of intact caseins during the early stages of Mozzarella cheese aging. These data are consistent with the model proposed earlier that NaCl promotes swelling at the microstructural level and casein solubilization through its peptizing action. Increased water-holding capacity, microstructural swelling, and casein solubilization may be important factors in the development of desirable functional properties. The water-holding capacity of the cheese may influence dehydration and blistering and burning properties during baking, and the microstructural swelling and casein solubilization may be important to the attainment of desirable melted consistency.

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