Composition, Microstructure, and Surface Barrier Layer Development During Brine Salting*

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ABSTRACT

The goal of this study was to characterize the changes in chemical composition, porosity, and structure that occur at the surface of a block of brine-salted cheese and their relationship to the rate at which salt is taken up from the brine. To create a difference in composition, salt uptake, and barrier layer properties, identical blocks of Ragusano cheese were placed in saturated and 18% salt brine at 18°C for 12 d. The overall moisture content and porosity decreased, whereas salt and salt in moisture content increased near the surface of blocks of brine-salted Ragusano cheese for all treatments. The general appearance of the microstructure of the surface of the blocks of brine-salted cheese was much more compact than the microstructure 1 mm inside the block at both brine concentrations. Large differences in porosity of the barrier layer were produced by brine-salting cheese in 18% vs. saturated brine, with cheese in saturated brine having much lower porosity at the surface and taking up much less salt during brining. The macro network of water channels within the microstructure of the cheese was less open near the surface of the block for cheese in both saturated and 18% brine after 4 d. However, no large differences in the size of the macro channels in the cheese structure due to the difference in brine concentration were observed by scanning electron microscopy. It is possible that the shrinkage of the much smaller pore structure within the casein matrix of the cheese is more important and will become more limiting to the rate of salt diffusion. Further microstructure work at higher resolution is needed to answer this question. The calculated decrease in porosity at the exterior

1-mm portion of the block was 50.8 and 29.2% for cheeses that had been in saturated vs. 18% brine for 12 d, respectively. The difference in brine concentration had a very large impact on the salt in moisture content of the cheese. The exterior of the cheese in 18% brine reached a salt in moisture content almost identical to that of the brine very quickly (17.3% at 4 d), whereas the salt in moisture content at the surface of the cheese block in saturated brine was only 11.9% at 4 d. There appears to be some critical concentration of salt in brine above which there is a large negative impact on salt uptake due to the creation of a barrier layer at the surface of the block of cheese.

(Key words: brine, barrier layer, porosity, cheese microstructure)

Abbreviation key: NP18%B = no presalting and 18% brine for 8 d followed by 16 d in saturated brine, NPSB = no presalting and saturated brine, P18%B = presalting and 18% brine for 8 d followed by 16 d in saturated brine, PSB = presalting and saturated brine, SEM = scanning electron microscopy.

INTRODUCTION

Many varieties of cheese are salted by immersion in brine instead of by direct salting of the curd during cheese making. Brine salting causes large gradients of salt and moisture to develop within the structure of blocks of cheese (Geurts et al., 1974b; Resmini et al., 1974; Guinee and Fox, 1983, 1986; Zorrilla and Rubiolo, 1991; Turhan and Gunasekaran, 1999). In general, moisture will be low and salt content will be high at the surface of a brine-salted cheese. When salt penetration into a block of Ragusano cheese is too slow and the salt concentration is too low, the frequency of early gas defects increases (Melilli et al., 2004). Factors within a block of cheese that influence the rate at which salt moves from the exterior to the center of the block include porosity of the cheese, tortuosity of the channels of aqueous phase within the structure of the cheese, proportion of water that is bound in cheese, viscosity

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of the aqueous phase of the cheese, and interaction of sodium with the protein matrix. Resmini et al. (1974) reported that lower brine concentration promoted faster salt uptake in Parmigiano-Reggiano cheese. Similar salt uptake behavior at different brine concentrations has been observed in Ragusano cheese (Melilli et al., 2003a). It is thought that a stronger barrier layer caused by decreased moisture and decreased porosity at the surface of blocks of brine-salted cheeses develops very quickly when blocks of cheese are exposed to saturated salt brines vs. brines that are not fully saturated (Melilli et al., 2003a). Brine temperature also influences the rate of uptake of salt from brine, with salt penetration decreasing with decreasing brine temperature. Brine temperature also influences the rate of penetration of salt into cheese, with lower brine temperature increasing the viscosity of the liquid phase within the cheese and reducing the rate of diffusion of salt into a block of cheese (Melilli et al., 2003b). In a study of the combined impact of brine concentration, brine temperature, and presalting, it was found that presalting the curd before stretching delivered 60% of the normal level of salt to the center of the block immediately (Melilli et al., 2003a). Presalting in combination with the temperature and pH of the curd during stretching reduced gas formation by 75% (Melilli et al., 2004) but did not reduce the rate of salt uptake during brining (Melilli et al., 2003a). Reducing the brine temperature from 18 to 12°C had the second largest impact on reducing early gas production but also reduced the rate of salt penetration into the cheese (Melilli et al., 2004). The objective of this research was to determine how brine concentration at constant temperature influences the composition and microstructure of the surface barrier layer that develops in Ragusano cheese during brine salting.

MATERIALS AND METHODS

Experimental Procedures

Part 1 - Impact of presalting and brine concentration. The goal of part 1 was to determine the impact of brine concentration (18% vs. saturated) and presalting (2% dry salt added directly to the curd before stretching) on the microstructure of Ragusano cheese. Four treatments were included: 1) the traditional method using no presalting and saturated brine (**NPSB**), 2) presalting and saturated brine (**PSB**), 3) no presalting and 18% brine (**NP18%B**) for 8 d followed by 16 d in saturated brine, and 4) presalting and 18% brine (**P18%B**) for 8 d followed by 16 d in saturated brine. The temperature for both brines was 18°C, which is a typical brine temperature for Ragusano cheese (Melilli et al., 2003b). Cheese manufacture was replicated 3 times. The 4 treatments were made from the

same milk on each day of cheese manufacture. Blocks of cheese were kept fully submerged in brine, the blocks were turned several times daily, the brine was stirred, and the brine concentration was adjusted once per day as described in Melilli et al. (2003a). Cheese blocks were sampled immediately before brine salting and after 1, 4, 8, 16, and 24 d of brine salting for each of the 4 treatments. A more complete description of the experiment and the impact of brine concentration and presalting on cheese composition and salt uptake were reported previously (Melilli et al., 2003a). Data for cheese microstructure will be presented in the current paper.

Part 2 - Impact of brine concentration on barrier layer. The goal of part 2 was to measure the differences in composition and microstructure in more detail near the surface of blocks of cheese brined in 18% and saturated brine. Ten experimental 3.8-kg blocks of Ragusano cheese were manufactured using the procedures described by Melilli et al. (2003a) without presalting. After forming, 2 of the 10 blocks were analyzed before brining. The remaining 8 blocks were divided in 2 groups. One group was placed into saturated salt brine and the other into nonsaturated (18% wt/vol NaCl) brine. The temperature for both brines was kept at 18°C. The cheeses were left submerged in brine; 2 blocks were removed from each brine at 4 d, and 2 removed from each brine at 12 d. Two blocks of cheese at each sampling time were used for microstructure and composition analysis near the surface (i.e., the outer 1.6 cm divided into sixteen 1-mm thick slices) of the block and the other 2 blocks were used for chemical composition of the P1, P2, P3, and P4 portions of the total block (portions described below). Moisture and salt data were analyzed using the GLM procedure of SAS (version 8, 1999, SAS Institute, Cary, NC). The ANOVA model used for analysis is provided in Table 1. Slice (i.e., distance from the surface of the block) was treated as a continuous variable in the split-plot ANOVA models; therefore, the linear and quadratic terms of slice would be correlated. Distortion of the ANOVA by multicollinearity of these terms in the model was minimized by centering the slice variable using a mathematical transformation (Glantz and Slinker, 2001). Slice data was transformed as follows: slice = slice number - [(highest slice number - lowest slice number)/2]. This transformation made the data set orthogonal with respect to slice number. This transformation directs the ANOVA model to consider the effect of brine type and time of brining in the whole plot at the midpoint of slice number (i.e., the midpoint of the distance in this zone from slice 1 to 16). The impact of brine concentration (18% vs. saturated) and brining time (4 vs. 12 d) on mean moisture, salt, and salt in moisture in the cheese were calcu-

Table 1. Sums of the squares (Type III) and probability values (in parentheses) for the ANOVA of the impact of brine concentration (saturated vs. 18%), time of brining (4 and 12 d), and slice (1 to 16) on moisture, salt, and salt in moisture content of cheese.

Factors ¹	df	Moisture	Salt	Salt in moisture	Analyzed as	Error term
Whole plot						
B	1	10.57* (0.01)	15.85* (<0.01)	129.99* (<0.01)	Category	Model error
t	1	129.11* (<0.01)	24.80* (<0.01)	363.59* (<0.01)	Category	Model error
B × t	1	30.83* (0.01)	0.15 (0.06)	0.21 (0.71)	Interaction	Model error
Subplot						
S	1	1444.21* (<0.01)	74.51* (<0.01)	1110.75* (<0.01)	Continuous	Model error
S × B	1	38.81* (<0.01)	1.95* (<0.01)	NS	Interaction	Model error
S × t	1	8.23* (0.03)	1.22* (<0.01)	7.43* (0.03)	Interaction	Model error
S × S	1	241.93* (<0.01)	1.30* (<0.01)	104.67* (<0.01)	Interaction	Model error
S × S × B	1	28.62* (0.01)	0.19* (0.03)	15.94* (0.01)	Interaction	Model error
S × S × t	1	NS	1.46* (<0.01)	NS	Interaction	Model error
Error	55	91.26	...	...		
	54	...	2.20	...		
	56	...	...	86.67		
R ²		0.95	0.99	0.95		

¹B = Brine concentration; t = time of brining; S = slice of cheese.

*Statistically significant ($P < 0.05$).

lated for these category variables in the ANOVA and the means were compared with a *t*-test.

Sampling

Part 1. The sampling of cheese for chemical analysis has been presented previously (Melilli et al., 2003a). Cheeses were sampled at time 0 (before brining), 1, 4, 8, 16, and 24 d. Each experimental block ($15 \times 15 \times 15$ cm) of Ragusano cheese, at the sampling day, was weighed and divided in 4 portions of approximately equal weight: P1, P2, P3, and P4. The exterior portion (P1) represented all 6 faces of the block (approximately 0.6 cm thick). The P2 portion was removed (approximately 1 cm thick) after removal of the P1 portion, from all 6 faces of the block. The P3 portion (approximately 1 cm thick) was removed next, and the cube remaining (about $10 \times 10 \times 10$ cm) was the central portion (P4). A visual representation of this procedure was provided previously (Melilli et al., 2003a). A sample of cheese was selected from each portion (P1, P2, P3, and P4) for scanning electron microscopy (SEM) analysis.

Part 2. Two blocks of cheese were sampled at time 0 (before brining), 2 after 4 d, and 2 after 12 d of brining in saturated brine at 18°C. Each experimental block of cheese was accurately weighed on the sampling day. One of the 2 blocks was divided into 4 portions (P1, P2,

P3, and P4) as described by Melilli et al. (2003a); these samples were used for chemical analysis to confirm that the blocks of cheese in part 2 had similar differences in salt uptake and moisture loss as those produced in part 1. For the second block (at 0, 4, and 12 d), 2 slabs of cheese, 3 cm thick, were cut from 2 opposite faces of the block of cheese. From this $15 \times 15 \times 3$ cm slab, the center region ($7 \times 7 \times 3$ cm) was removed. One cylinder (3 cm thick $\times$ 1 cm diameter) was removed from the center of each $7 \times 7 \times 3$ cm thick slab with a cork borer. Each 1-cm diameter cylinder was placed on a cutting device and sixteen 1-mm slices were removed from the cylinder of cheese starting at the original outside surface of the block to a distance of 1.6 cm into the block. The distance of 1.6 cm (in 1-mm increments) corresponds exactly to the sum of thickness of the P1 and P2 portions used in the other method for sampling the full block. Each 1-mm slice of the cheese core represented locations from 0 to 16 mm, starting from the surface of the slice nearest the exterior of the block (i.e., slice 1). Two cores were removed from each block and sliced. The total number of slices (1 mm thick) obtained for SEM was 32 from each block, (i.e., 16 slices per core).

After removal of the 3×1 cm core, the remaining two $7 \times 7 \times 3$ cm pieces of cheese were used for further sampling for chemical analysis. Each $7 \times 7 \times 3$ cm piece of cheese was sliced into 1-mm thick zones starting

from the original external surface of the block. This was done progressively to a depth of 16 mm into the block to provide samples for a more detailed chemical analysis of the composition within the P1 and P2 zones and corresponded to the slices taken for SEM analysis.

Chemical Analysis

The cheese from the P1, P2, P3, and P4 portions of the first block and the cheese from the 1-mm slices removed from the second block of cheese were ground and analyzed separately. Moisture content was determined by drying all the samples in a forced air oven at 100°C for 24 h (AOAC, 2000; method number 33.2.44; 990.20), and the salt content was determined by the Volhard method (AOAC, 2000; method number 33.7.1; 935.43).

SEM

Two approaches were used for sampling and sample preparation to examine the microstructure of cheese slices. In part 1 of the experiment, the exterior surface of P1, P2, P3, and P4 portions was removed from the portion and prepared for SEM as described below for surface structure of slices. In part 2, samples of each of the first 3 slices (0 to 1, 1 to 2, and 2 to 3 mm) starting at the brine-cheese interface were prepared using a freeze-fracture method to allow examination of the internal structure of the cheese near the surface of blocks held in 18% and saturated brines for 4 d.

Surface structure of slices. Each sample representing the P1, P2, P3, and P4 portions removed from each block of cheese in part 1 of the experiment was prepared for SEM by modified version of the procedure of Rousseau (1988). The sample was fixed in 2.5% (wt/vol) glutaraldehyde in 0.1 M sodium cacodylate buffer (Sigma-Aldrich, Steinheim, Germany), pH 7.2 for 24 h at 4°C. Fixed samples were rinsed with the cacodylate buffer for 10 min at 20°C. This step was repeated 3 times. Dehydration was carried out using a graded ethanol (FLUKA, Sigma-Aldrich) series (10, 25, 50, 75, 85, and 95% vol/vol) for 10 min in each bath at 20°C and then absolute ethanol for 1 h at 4°C. After these steps, absolute ethanol was removed and new absolute ethanol was added for maintenance, at 20°C. Samples were dried by the critical-point method in CO₂, using a Polaron CPD 7501 (Polaron, Watford, UK). Dried samples were mounted on SEM aluminum stubs, using a carbon adhesive (SPI Supplies Structure Probe, West Chester, PA). Mounted samples were coated (approximately 18 nm thick) with gold-palladium in a Polaron SC7620 mini sputter coater in argon medium. The specimens were examined in an JEOL JSM-5900-LV scanning

electron microscope (JEOL, Tokyo, Japan) operated at 15 or 20 kV and a working distance of 20 to 22 mm. Images were recorded at 400× magnification.

Freeze fracture. In part 2 of the experiment, the exterior three 1-mm thick slices were selected for microstructure determination. This represented a total distance of 3 mm into the block corresponding to the outer 3 mm of the 6-mm thick P1 portion evaluated in part 1. A rectangular strip of cheese sample (approximately 1 × 3 × 8 mm) was cut from the center of each of the 3 circular (1 cm diameter) slices of cheese nearest the surface of the block. The 3 slices represented a distance from the original surface of the block in contact with the brine to a depth of about 3 mm into the block of the cheese. The surface of each rectangular strip closest to the exterior of the block of cheese was marked using a thin marker. One block before brining, 1 block kept in the saturated brine for 4 d, and 1 block kept in 18% brine for 4 d were analyzed. The samples were processed according to McManus et al. (1993), differing in the following steps: after 1 h in 2% aqueous glutaraldehyde (Sigma-Aldrich) solution at 20°C, the glutaraldehyde solution was changed, and the samples were stored in new solution for 3 d at 5°C. To maintain the identity of the surface of the rectangular strip of cheese that was originally oriented toward the brine, the cryo-fractured samples were pierced with a syringe-cleaning wire (0.076 mm o.d.). The wire was placed far from the point at which the fractured face would be created. Samples were dried by the critical-point method in a Polaron CPD 7501 (Polaron) with CO₂. Dried samples were mounted on aluminum stubs, using a carbon adhesive (SPI Supplies Structure probe) and gold-palladium coated for 15 s in a Polaron SC 7620 mini sputter coater in argon medium. Samples were viewed in a JEOL JSM-5900-LV scanning electron microscope (JEOL) operated at 15 kV. A series of sequential images was recorded from the fracture of each rectangular strip, at 400× magnification, starting from the most exterior surface to the most interior of the cheese strip. The total distance into the block that was observed was about 3 mm.

RESULTS AND DISCUSSION

Impact of Presalting and Brine Concentration (Part 1)

The impact of presalting and brine concentration on salt uptake and moisture gradient was presented previously (Melilli et al., 2003a). Presalting delivered 60% of the normal level of salt to the center of the block before brine salting without decreasing the rate of uptake of salt from saturated (PSB) or 18% brine (P18%B). Use of 18% brine for the first 8 d of 24 d of brine salting increased the rate of salt uptake, compared with 24 d

in saturated brine (i.e., NP18%B and P18%B vs. NPSB and PSB). The increased rate of salt uptake with 18% brine compared with saturated brine was related to the impact of salt brine on the moisture content and porosity of the cheese near the surface of the block (portion P1). Brine with higher salt content caused a rapid loss of moisture from cheese near the surface of the block. Moisture loss caused shrinkage of the cheese structure and decreased porosity presumably near the surface, which impeded moisture movement out of and salt movement into the block. The use of 18% salt brine delayed the moisture loss and shrinkage at the exterior of the block and allowed greater salt penetration. Therefore, it was concluded (Melilli et al., 2003a) that the surface porosity of the cheese was higher for the cheeses in 18% brine, whether they were presalted or not presalted.

The process of salt uptake during brine salting has been studied for other cheese varieties. Geurts et al. (1974b, 1980) characterized moisture loss and salt uptake in brine-salted Gouda cheese. Geurts et al. (1974b) found that salt penetrates into cheese, inducing a weight gain, and moisture exits the cheese causing a weight loss during brining for a net loss of weight during brine salting. Similar results were reported for Ragusano cheese (Melilli et al., 2003a). Moisture content is one of the factors that influences porosity of cheese. In 2 cheeses of same type, the cheese with higher moisture content absorbed salt more rapidly (Geurts et al., 1974b), because it had a higher porosity. The use of 18% brine kept the moisture content of cheese higher at the surface layer than did saturated brine (Melilli et al., 2003a), and the uptake of salt was higher and the loss of moisture was lower for cheeses in 18% brine. Cheeses that have a net loss of weight during brine salting will contract (Geurts et al., 1974b; Luna and Chavez, 1992), but the contraction is not uniform throughout the block. The contraction of the cheese matrix (and moisture loss) is the greatest near the surface of the block and this would be expected to produce a compact barrier layer at the surface of the block.

Selected scanning electron micrographs of the cheeses made in the study by Melilli et al. (2003a) are presented in Figure 1. Overall, for all the presalting and brine treatments, the structure in the P1 portion was more compact (Figure 1a, c, e, and g) than in the P2 portion (Figure 1b, d, f, and h). The porosity of the cheese structure for all the P3 and P4 portion for all treatments appeared similar to the P2 portion by visual examination of the micrographs (data not shown). The differences in cheese porosity seen in Figure 1 are consistent with the moisture data for these cheeses reported earlier (Melilli et al., 2003a). The cheese was more compact near the surface (P1) and more porous

in P2, P3, and P4. The thickness of the P1 portion of the cheese was 0.6 cm. Therefore, if there was a gradient of composition and porosity of microstructure within the P1 or P2 portions, we could not determine these gradients from the data available from part 1. As a result, a study of the composition and microstructure of cheese within the P1 and P2 portions was conducted and reported in part 2.

Impact of Brine Concentration on Barrier Layer (Part 2)

Moisture. Part 2 of this study was designed to provide more detail about the exterior of the cheese, portions P1 (first 0.6 cm from the surface) and P2 (0.6 to 1.6 cm from the surface), where a barrier layer develops impeding the entry of salt into the block. The samples taken at the center (7×7 cm) of 2 faces of a $15 \times 15 \times 15$ -cm block of cheese were sectioned into 16 slices, each 1 mm thick, starting from the surface of the block and ending 1.6 cm into the block. The moisture content of each slice is shown in Figure 2 for cheese at d 0 (before brining) and after 4 and 12 d of brining. Moisture content of the cheese decreased ($P < 0.01$) with increased time of brining (Tables 1 and 2), but on average, in the total 1.6-cm exterior zone of the block of cheese, the moisture content was not different between the cheese in saturated brine vs. 18% brine (Table 2). However, the distribution of moisture within the exterior 1.6-cm zone was very different for the cheese in saturated brine vs. 18% brine. Slice (i.e., distance from the surface) had the biggest impact (i.e., high sum of squares for both the linear and quadratic term of slice, S and $S \times S$ in Table 1) on moisture content (Table 1 and Figure 2). The 2-way interaction of brine concentration with both the linear and quadratic terms for slice ($P \leq 0.01$) indicates (Table 1) that the rate of change of moisture content from a depth of 1.6 cm inside the block to the exterior surface is different for 18% vs. saturated brine (Figure 2). The moisture decreases much more rapidly near the surface of the block for the cheese kept in saturated brine (Figure 2). This is consistent with a less porous structure of the cheese near the surface of the block (Figure 1a, c, e, and g) for cheese in part 1. In addition, a portion of the water within the pores is tightly bound to the protein matrix and this has been estimated to be roughly 10% of the moisture in the cheese (Geurts et al., 1974a,b). The bound water is not available to act as a solvent and transmission medium for salt penetration during brining. The constant amount of bound water becomes an increasing proportion of the total moisture remaining in the cheese structure near the surface of the block during brining. If 10% of the original 42% moisture in slice 1 (i.e., 4.2%)

Figure 1. Scanning electron microscopy images at 4 d: a) portion 1, treatment NPSB (no presalting, saturated brine), b) portion 2, treatment NPSB, c) portion 1, treatment NP18%B (no presalting, 18% brine), d) portion 2, treatment NP18%B, e) portion 1, treatment P18%B (presalting, 18% brine), f) portion 2, treatment P18%B, g) portion 1, treatment PSB (presalting, saturated brine), and h) portion 2, treatment PSB.

Figure 2. Percentage moisture content in cheese before brining (▲), after 4 d brining in saturated brine (■) and 18% brine (●), and after 12 d brining in saturated brine (□), and 18% brine (○).

Figure 3. Percentage salt content in cheese before brining (▲), after 4 d brining in saturated brine (■) and 18% brine (●), and after 12 d brining in saturated brine (□) and 18% brine (○).

at the beginning of brining (Figure 2) is bound to the cheese protein matrix, then at 12 d when the moisture in slice 1 has decreased to 20%, the moisture in slice 1 available for salt transfer is about 15 to 16%, when it was about 37 to 38% at the beginning of brining. This decrease in available water becomes a barrier to movement of salt into the block.

Salt. The salt content in the total 16-mm exterior portion of the block of cheese increased ($P < 0.01$) with time of brining (Tables 1 and 2), and the cheese in 18% brine took up more salt than cheese in saturated brine (Table 2). Salt content of cheese increased ($P < 0.01$) mostly in a linear manner, going from an internal depth of 1.6 cm in the cheese to the surface (Table 1, Figure 3). The salt content at the surface of the block (i.e., slice 1) reached a maximum value for cheese in 18% brine at 4 d, whereas at 12 d, the cheese in saturated brine had still not reached this level of salt at the surface of the block (Figure 3). More salt penetrated into the cheese at all locations from 1 to 16 mm at 12 d for the 18% brine than for the saturated brine (Figure 3). At

12 d of brining, the exterior 1-mm slice of cheese was still lower in salt (Figure 3) content (and lower in moisture content, Figure 2) for cheese held in saturated brine than for the cheese in 18% brine, indicating a more porous structure for the cheese in 18% brine.

Salt in moisture. The salt in moisture content of the cheese was much higher for the cheese in 18% brine than saturated brine, and increased with time of brining (Tables 1 and 2). Salt in moisture content of cheese increased ($P < 0.01$) with a linear (i.e., S in Table 1) and a quadratic component (i.e., $S \times S$ in Table 1) going from an internal depth of 16 mm to the surface (Figure 4). The maximum salt in moisture was in slice 1 (surface of the block) for cheeses in 18% brine (17.3% at 4 d, and 18.7% at 12 d). The behavior of salt in moisture for the cheese in saturated brine was very different from that in cheese in 18% brine. At 4 d, the salt in moisture in slice 1 (i.e., 11.9%) for the saturated brine was much lower than salt in moisture for cheese in the 18% brine (Figure 4). The salt in moisture increased more rapidly near the surface for the cheese in satu-

Table 2. Mean values and least significance difference (LSD) of moisture, salt, and salt in moisture content (%) in cheese, by brine concentration (18% vs. saturated) and by time of brining (4 vs. 12 d).

	Moisture		Salt		Salt in moisture	
	Mean	LSD	Mean	LSD	Mean	LSD
Brine						
Saturated	39.62 ^a	NS ¹	1.82 ^b	0.10	5.38 ^b	0.62
18%	39.61 ^a	NS	3.09 ^a	0.10	8.32 ^a	0.62
Time						
4 d	41.59 ^a	0.65	1.68 ^b	0.10	4.48 ^b	0.62
12 d	37.64 ^b	0.65	3.23 ^a	0.10	9.21 ^a	0.62

^{a,b}Means within a column that do not share a common superscript are different at $P < 0.05$.

¹NS = Not significant.

Figure 4. Percentage salt in moisture content in cheese before brining (▲), after 4 d brining in saturated brine (■) and 18% brine (●), and after 12 d brining in saturated brine (□), and 18% brine (○).

rated brine at both 4 and 12 d and the concentrations of salt in moisture in slices 2 through 16 were always lower for the cheese in saturated brine (Figure 3). The more rapid decrease in salt in moisture content for the cheese in saturated brine going from slice 1 inward and the lower level of salt in moisture in slices 2 through 16 for the cheese in saturated brine is consistent with the idea that a stronger barrier layer is blocking salt penetration in the blocks in saturated brine compared with those in 18% brine. After 12 d of brining, the salt content of the exterior 1 mm slice of cheese in saturated brine (Figure 3) was still lower than for the cheese in 18% brine, even though the salt in moisture content of the cheese in saturated brine was higher (Figure 4). Geurts et al. (1974b, 1980) found that at the very high salt in moisture contents (15% or higher) that occur near the rind, the cheese matrix may undergo a spontaneous shrinking or contraction, and some moisture might be squeezed out of the structure. These conditions exist near the surface of the block in Ragusano cheese. In addition, salt in the aqueous phase of the cheese would promote solubilization of caseins (Guo et al., 1997) causing the protein concentration in the water phase of the cheese to increase. This would increase the viscosity of the aqueous phase of the cheese near the surface of the block and provide additional resistance to diffusion of salt through this zone of the cheese.

Microstructure. Scanning electron microscopy images are shown in Figure 5 for the surface (nearest the exterior of the block) of slices 1, 2, and 3 for the part 2 cheeses at d 0 before brining (Figure 5a, b, and c) and after being held in saturated (Figure 5d, e, and f) and 18% brine (Figure 5g, h, and i). In Figure 5, the areas that have the appearance of holes are areas that contained fat, water, or both in the original cheese. This

is because fat and water in the cheese structure are removed during preparation of the samples for SEM. The matrix of the structure in Figure 5 is composed of protein and minerals. The structure of the cheese at the surface (i.e., the outer 1 mm) of the block before brining (Figure 5a) is porous, and the holes that were occupied by water and fat are round or irregular shaped and uniformly distributed throughout the cheese. These holes seen in the freeze-fracture face (Figure 5a) range (cross-sectional size) from about 2 to 25 μm , and represent columns of fat and water of varying length within the 3-D structure of the cheese. This observation of the microstructure in our study is similar to that for low and high fat Mozzarella shortly after manufacture by Cooke et al. (1995). Cooke et al. (1995) reported a broad range of openings 10 to 20 μm wide or larger, and of smaller, more circular openings.

In contrast to the cheese before brining (Figure 5a), the cheese near the surface (i.e., outer 1 mm) of the block in saturated brine (Figure 5d) and 18% brine (Figure 5g) after 4 d show a more compact structure, with the smaller holes elongated in a direction parallel to the surface of the block of cheese. Once the block is in the brine, water moves out of the block into the brine from this region, and salt moves into the block from the brine (Melilli et al., 2003a). The fat does not exit the block into the brine. This results in a net loss in weight from the block and most of this weight loss happens at the surface (Melilli et al., 2003a). As a result, there is physical shrinkage of the block. The shrinkage puts force on the structure of the block that is perpendicular to the surface and is likely the reason why the holes in the structure of cheese near the surface of the block (Figures 5 d and g) are "flattened" compared with the hole structure before brining. In addition, careful examination of the shape and size of the holes (in Figures 5 d and g) reveals chains of flattened, small, spherical holes (formerly fat globules) linked together in a row parallel to the surface of the block. These structures are areas where water has migrated out of the original cavity in the structure of the cheese into the brine and left behind only the fat droplets that were originally in that area of the structure. This type of structure with chains of fat globules was also observed in 6-wk-old Mozzarella cheese by Cooke et al. (1995). The change in structure as the Mozzarella cheese aged from 0 to 6 wk in the data of Cooke et al. (1995) may represent the movement of protein from the casein matrix into the expressible water (Guo and Kindstedt, 1995; Guo et al., 1997) and movement of water into the matrix. This is consistent with the decrease in expressible moisture that occurs during refrigerated storage of Mozzarella cheeses (Guo and Kindstedt, 1995) and Ragusano cheese (Melilli et al., 2003a). The decrease in express-

Figure 5. Scanning electron microscopy images of cheese from part 2. (a) d 0, slice 1; (b) d 0, slice 2; (c) d 0, slice 3; (d) d 4, saturated brine (SB), slice 1; (e) d 4, SB, slice 2; (f) d 4, SB, slice 3; (g) d 4, 18% brine, slice 1; (h) d 4, 18% brine, slice 2; and (i) d 4, 18% brine, slice 3.

ible serum occurs sooner and to a greater extent in the P1 portion than in the P4 portion of Ragusano cheese during brining (Melilli et al., 2003a). When this occurs, the area in the microstructure that was formerly occupied by water will be occupied by enough protein plus water that the removal of water no longer leaves a cavity in the microstructure and only the original fat droplets are removed during the defatting and dehydration sample preparation steps for freeze-fracture SEM (McManus et al., 1993).

The microstructure of the cheese 2 and 3 mm into the block from the surface gradually changed from a structure that contained elongated openings parallel to the original surface of the block (Figure 5b, e, and h) to a structure with larger, more irregular openings (Figure 5c, f, and i) at a depth of 3 mm from the surface of the block. This corresponds to an increase in moisture

content of the cheese and an increase in porosity as the moisture increases from slice 1 to slice 3.

When comparing the SEM images of the slices from the block held 4 d in saturated brine (Figure 5 d, e, f) with images from cheese held 4 d in 18% brine, it was difficult to detect any large microstructure differences in the thickness of a more compact barrier layer that would explain the higher rate of salt uptake in the blocks in 18% vs. saturated brine. However, the pores visible in these SEM images are not the only pores in the microstructure of the cheese. The areas of the structure that appear to be solid protein matrix contain a network of much smaller pores that cannot be seen by SEM at this resolution. Lee et al. (1980) investigated the diffusion of salt, fatty acids, and esterases in Mozzarella cheese and determined the impact of the orientation of the fibrous structure of the protein matrix of the

cheese created by the pasta-filata process. They found that the orientation of matrix fibers had no impact on diffusion of salt through the cheese structure because the NaCl molecule was so small relative to the size of pores both within and between fibers of the casein matrix. Thus, it needs to be recognized that as well as the macro network of porosity that is visible in Figure 5, there is a network of smaller pores in the matrix that is not visible. The influence of differences in brine concentration on the micro network of pores in the matrix is not known, but the contraction of the protein matrix near the surface of the cheese could have an impact on the macro pore structure of the cheese and the network of pores within the casein matrix itself. It is possible that the high concentration of salt in the saturated brine caused a reduction in diffusion of salt through the casein matrix portion of the cheese structure during brine salting. Further microstructure work would be needed at higher resolution to determine if brine concentration changed the porosity of the pore structure within the hydrated casein matrix.

Porosity Difference and Shrinkage due to Moisture Loss

As cheese loses moisture from its structure, the cheese shrinks and becomes less porous (Geurts et al., 1974b, 1980). The largest decrease in moisture occurs near the surface of the block and therefore it would be expected that the exterior portion of the block would experience the largest shrinkage. Payne and Morison (1999) estimated shrinkage and reduction in porosity of cheese based on the assumption that all shrinkage arose from the change in volume of the solution within the pores. Payne and Morison (1999) estimated, from the data of Geurts et al. (1974b), that the cheese shrank to 86% of its original volume near the surface and that porosity was reduced from about 45 to 36%. Melilli et al. (2003a) estimated shrinkage to about 89.6% of the original size for the cheeses used in part 1 of the present study. Assuming that the volume of the cheese structure contained inside the pores is related directly to moisture content of the cheese, an estimate of the percentage of decreasing volume of pore structure by slice (i.e., from 0 to 16 mm into the cheese) was calculated for our cheeses and is shown in Figure 6. To allow for a comparison of the relative difference in porosity between the cheese in 18% and saturated brine, within d 4 and within d 12, the moisture difference at slice 16 was used to eliminate the influence of block to block variation in moisture content of the cheese that existed before the initiation of brine salting. The cheese in slice 1 for the block in saturated brine lost 50% of its moisture (i.e., a decrease in porosity of 50%) between d 0 and d

Figure 6. Percentage decrease in volume of pores within the exterior 1.6 cm of the cheese blocks taken in 1-mm slices for cheese at 4 d (■) and 12 d (□) of brining in saturated, and at 4 d (●) and 12 d (○) of brining in 18% brine at 18°C.

12 (Figure 2). The porosity of cheese in saturated brine decreased by 36.5 and 50.8% at 4 and 12 d of brining (compared with the porosity in slice 16 at d 4), respectively, in the outermost 1 mm, compared with a decrease in porosity of 22.9 and 29.2% at 4 and 12 d of brining, respectively, in the outermost 1 mm of the cheese in 18% brine (Figure 6). During the first 4 d of brining, the largest loss in porosity occurred in the outer 1 mm (i.e., slice 1) in the cheese in saturated brine (Figure 6) and this is consistent with the appearance of the cheese in this zone, Figure 5d. From d 4 to 12, there was a further decrease in porosity in slices 1 to 5 for the cheese in 18% brine, but the continued decrease in porosity for these same positions for the cheese in saturated brine was very large and the difference in porosity between the cheese in 18% brine and saturated brine became larger (Figure 6). The impact of the less porous structure for the cheese in saturated brine on movement of moisture out of the block and salt into the block is seen very clearly in Figures 7 and 8, respectively. The moisture content of the P1 portion for the cheese in saturated brine was lower at 4 and 12 d brining than the moisture content of the P1 portion for cheese in 18% brine (Figure 7). The less porous barrier layer (P1) for the cheese in saturated brine blocked movement of water from the interior of the block outward and movement of salt from the exterior of the block inward. This effect on moisture movement out of the block can be seen by comparing the moisture content of the P2, P3, and P4 portions for the cheese in saturated brine with the moisture content of the P2, P3, and P4 portions for the cheese in 18% brine (Figure 7). Although the exterior of the block in saturated brine was dry and less porous, the interior portion of the block

Figure 7. Moisture content of cheese in portions P1, P2, P3, and P4 within a 3.8-kg block of Ragusano cheese after 0, 4, and 12 d in saturated brine (SB) and 18% brine (18%B) at 18°C.

had higher moisture content in the same positions than the cheese in the 18% brine (Figure 7). The opposite behavior can be seen for salt migration into the blocks from brine with less salt penetrating into the P2, P3, and P4 portions of the cheese in saturated brine than for cheese in 18% brine (Figure 8). This clearly indicates that there was a much stronger barrier layer at the surface of the blocks of cheese kept in saturated brine vs. those kept in 18% brine.

Further work on microstructure is needed using other approaches to understand differences in the structure of the barrier layer for brine-salted cheeses and the status of water in the region near the surface of the block of cheese. A better understanding of the dynamics of salt uptake during brine salting of cheese will allow development of strategies to achieve more rapid pene-

Figure 8. Salt content of cheese in portion P1, P2, P3, and P4 within a 3.8-kg block of Ragusano cheese after 0, 4, and 12 d in saturated brine and in 18% brine at 18°C.

tration of salt into the central portion of cheese blocks with the goal of reducing the incidence of early gas production in brine-salted cheeses.

CONCLUSIONS

Moisture content and porosity decreased, whereas salt and salt in moisture content increased near the surface of blocks of brine-salted Ragusano cheese. The general appearance of the microstructure of the surface of the blocks of brine-salted cheese was much more compact than the microstructure 1 to 2 mm inside the block for both brine concentrations. Large differences in porosity and strength of barrier layer were produced by brine-salting cheese in 18% vs. saturated brine, with cheese in saturated brine having much lower porosity at the surface and taking up much less salt during brining. The macro network of water channels within the microstructure of the cheese was less open near the surface of the block for cheese in both saturated and 18% brine after 4 d. However, no large differences in the size of the macro channels in the cheese structure due to the difference in brine concentration were observed by SEM. It is possible that the shrinkage of the much smaller pore structure within the casein matrix of the cheese is more important and will become more limiting to the rate of salt diffusion. Further microstructure work at higher resolution is needed to answer this question. The calculated decrease in porosity at the exterior 1-mm portion of the block was 50.8 and 29.2% for cheeses in saturated vs. 18% brine at 12 d, respectively. The difference in brine concentration had a very large impact on the salt in moisture content of the cheese at the exterior surface, with the cheese in 18% brine reaching a salt in moisture content almost identical to that of the brine very quickly (17.3% at 4 d), whereas the salt in moisture content at the surface of the cheese block in saturated brine was only 11.9% at 4 d. It appears that there may be some critical concentration of salt in brine above which there is a large negative impact on salt uptake due to the creation of a barrier layer at the surface of the block of cheese.

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