Effect of Salt on Structure-Function Relationships of Cheese¹

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ABSTRACT

Our objective was to determine the effect of salt on structural and functional properties of cheese. Unsalted Muenster cheese was obtained on 1 d, vacuum packaged, and stored for 10 d at 4°C. The cheese was then cut into blocks that were vacuum packaged. After 4 d of storage at 4°C, cheese blocks were high-pressure injected one, three, or five times, with a 20% (wt/wt) sodium chloride solution. Successive injections were performed 24 h apart. After 40 d of storage at 4°C, cheese blocks were analyzed for chemical, structural, and functional attributes. Injecting sodium chloride increased the salt content of cheese, from 0.1% in the control, uninjected cheese to 2.7% after five injections. At the highest levels, salt injection promoted syneresis, and, after five injections, the moisture content of cheese decreased from 41 to 38%. However, the increased salt content caused a net weight gain. Cheese pH, soluble nitrogen, and total and soluble calcium content were unaffected. Cheese injected five times had a 4% increased area of cheese occupied by protein matrix compared with uninjected cheese. Hardness, adhesiveness, and initial rate of cheese flow increased, and cohesiveness decreased upon salt injection. However, the final extent of cheese flow, or melting was unaffected. We concluded that adding salt to cheese alters protein interactions, such that the protein matrix becomes more hydrated and expands. However, increasing the salt content of cheese did not cause an exchange of calcium with sodium. Therefore, calcium-mediated protein interactions remain a major factor controlling cheese functionality.

(Key words: calcium, high-pressure injection, ionic strength, syneresis)

INTRODUCTION

When considering the available data regarding the effect of salt content on the chemical composition and

functional properties of cheese, it is observed that there are no data reporting on some possible effects of salt (e.g., soluble calcium content), that some results seem to contradict each other (e.g., cheese melting), and that there is not enough information available to determine structure-function relationships of cheese. Following, some of these issues—for which there is not enough information available or that seem to be contradictory—are introduced, and the objectives of our study presented.

Adding salt to milk or casein systems promotes dissociation of calcium and phosphate from within casein micelles and into solution (Casiraghi and Lucisano, 1991; Gatti and Pires, 1995; Gaucheron et al., 2000). It has been suggested that adding salt promotes calcium solubilization from paracasein in casein pellets and cheese (Creamer, 1985; Kindstedt et al., 1992), thus displacing calcium from the protein matrix and into the serum. Paulson et al. (1998) and Schroeder et al. (1988) observed that when salt was added to cheese, calcium content remained the same. However, soluble calcium was not determined. Therefore, whether adding salt to cheese would cause mobilization of calcium from caseins and into solution remains uncertain.

Displacement of calcium from casein micelles by adding salt to milk may cause increased hydration or solvation of caseins (Creamer, 1985). However, in cheese, salt addition normally promotes syneresis and decreases the moisture content of cheese (Kindstedt et al., 1992; Guinee and Fox, 1993; Mistry and Kasperson, 1998; Schroeder et al., 1998). Thus, in contrast to results in milk systems, adding salt to cheese seems to cause decreased hydration of caseins. However, increased salt content has also resulted in no decrease in the moisture content of cheese (Cervantes et al., 1983; Paulson et al., 1998). Therefore, increasing the salt content of cheese may not cause moisture losses of cheese, and whether caseins become less hydrated upon salting of cheese is not clear.

Adding salt to cheese also affects cheese composition by influencing microbial activity (Thomas and Pearce, 1981; Schroeder et al., 1988; Guinee and Fox, 1993). Even though adding low concentrations of salt to milk may promote starter activity, higher concentrations

Received May 3, 2002.

Accepted July 8, 2002.

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¹Contribution number 7450 of the Utah Agricultural Experiment Station. Approved by the director.

have the opposite effect (Irvine and Price, 1961). In addition, salt content may also affect cheese proteolysis by affecting microbial and enzyme activity, with high salt levels decreasing the rate and/or extent of proteolysis (Fox and Walley, 1971; Schroeder et al., 1988; Mistry and Kasperson, 1998). Thus, salt content may affect both cheese pH and proteolysis, which could in turn affect cheese functionality.

Salt content affects cheese functionality either directly or indirectly by mediating other changes in cheese composition. Increased salt content causes increased hardness and decreased cohesiveness of cheese (Cervantes et al., 1983; Schroeder et al., 1988). However, results that seem to be contradictory have been reported regarding the effect of salt content on cheese melting. Thus, increased salt content decreased the melting of Mozzarella cheese (Olson, 1982), but increased the melting of nonfat Mozzarella cheese (Paulson et al., 1998). Additional work may prove helpful to better understand how salt content affects cheese melting.

The salt content of cheese may also affect cheese structure. Increased salt content of cheese would promote solubilization of caseins (Guo and Kindstedt, 1995; Guo et al., 1997), causing the protein matrix to become more hydrated and to swell (Guo and Kindstedt, 1995; Guo et al., 1997; Paulson et al., 1998). However, very limited data (Paulson et al., 1998) is available for determining the relationships between cheese structure and functionality as affected by salt content of cheese. Determining structure-function relationships of cheese would help better understand the effect of salt on cheese functionality.

The objectives of the present research were to determine the effect of salt on cheese structure and to relate changes in structure to changes in functional properties of the cheese.

MATERIALS AND METHODS

Cheese

Three 3-kg loaves of unsalted Muenster cheese were obtained from a cheese production facility on the day of manufacture, vacuum packaged, and stored for 10 d at 4°C. The loaves were then cut into 0.5- to 0.6-kg blocks that were vacuum-packaged and stored for an additional 4 d at 4°C before injection.

Cheese Injection

A two-stage homogenizer (Crepaco, model 3DDL-3535, Chicago, IL) served as the pump for injection. It had an outlet line that went through a solenoid-operated valve to an injection head, which had 13 nozzles

aligned 1 cm apart from one another. Adjusting the homogenizer valves allowed for changing the pressure of injection, which was set at 17 MPa. The burst duration was controlled via the solenoid-operated valve and set to 1 s. Injected solution flowed through sapphire nozzles (0.02-cm i.d.) and into the cheese. Cheese blocks were accurately weighed and then high-pressure injected one, three, or five times, with a 20% (wt/wt) sodium chloride solution (3.8 *M*). Successive injections were performed 24 h apart and according to an injection pattern of 1 × 1 cm applied to two opposite sides of the cheese block. During injection, only a portion of dispensed solution was effectively retained in the cheese block (visual observation). Therefore, after injection, cheese blocks were blotted with paper towels to remove extraneous fluid, and cheese weight was recorded. The cheese was then vacuum packaged and stored for an additional 40 d at 4°C before analysis.

Chemical Composition

Fat content was determined by using a modified Babcock method (Richardson, 1985), moisture content by using the vacuum oven AOAC method 926.08 (1990), and sodium chloride according to AOAC method 971.19 (model 926 salt analyzer; Corning, Medfield, MA) (1990). Total and soluble calcium was determined by inductively coupled plasma-atomic emission spectroscopy (US Environmental Protection Agency, 1992). To determine soluble calcium, cheese samples (5 g) were blended with 50 g of water using a hand-held, high-speed homogenizer, and transferred to a beaker. The blending container was then rinsed with water (150 g), and the water was transferred to the beaker. After standing for 20 min, the solution was filtered through Whatman #42 filter paper. The filtrate was then analyzed for calcium content. A pH meter (model IQ240, IQ Scientific Instruments, Inc. San Diego, CA), with a stainless steel probe (model PH06-SS, IQ Scientific Instruments, Inc.), was used to determine cheese pH, which was measured by taking a sample from the cheese block and inserting the pH probe into it. Proteolysis was determined by measuring nonprotein nitrogen. Cheese samples (1.5 g) were blended with 30 ml of trichloroacetic acid solution (12%, wt/wt) using a hand-held, high-speed homogenizer, and transferred to a beaker. The blending container was then rinsed with 20 ml of trichloroacetic acid solution, and the solution transferred to the beaker. After standing for 20 min, the solution was filtered through Whatman #42 filter paper, and nitrogen content in the filtrate measured by Kjeldahl method.

Table 1. Cheese Composition.

Variable	Mean	CV
Fat (%)	29.0	1.0
Moisture (%)	40.8	1.6
pH	5.45	0.2
Calcium (%)	0.7	7.8
Salt (%)	0.08	25

Scanning Electron Microscopy

Cheese samples (approximately $1 \times 1 \times 10$ mm) were taken and fixed in fresh 2% glutaraldehyde solution and stored at 4°C. After refrigerated storage, the samples were processed according to McManus et al. (1993). Samples were frozen in liquefied Freon 22 (-159°C) (Mallinckrodt Inc., Paris, KY), transferred to liquid nitrogen, cryofractured perpendicular to their long axis, and thawed in 2% glutaraldehyde. They were then dehydrated in a graded ethanol series followed by fat extraction with Freon 113 (Mallinckrodt Inc., Paris, KY). After overnight storage in Freon 113 at 4°C, the samples were rehydrated, by reversing the graded ethanol series, and washed with a 0.1 M sodium cacodylate buffer (Electron Microscopy Sciences, Fort Washington, PA), pH 7.2. The samples were then post-fixed for 2 h with a solution containing 1% OsO₄ (Electron Microscopy Sciences) and 1.5% K₄Fe(CN)₆·3H₂O (Fisher Scientific Co., Fair Lawn, NJ). This solution was replaced by a 2% tannic acid (Mallinckrodt Inc.) solution in cacodylate buffer, and the samples were left for 3 h at 20°C. The tannic acid solution was then replaced with the solution of osmium tetroxide and potassium ferrocyanate, and samples were left for 4 h. This solution was later replaced with an aqueous solution of 1% hydroquinone (Mallinckrodt Inc., Paris, KY), and samples were left overnight. After postfixing, the samples were washed with distilled water, dehydrated in a graded ethanol series, and air dried. Samples were then coated with a gold-iridium mix using a sputter coater (model

108, Kurt J. Lesker, PA). After coating, samples were viewed in a field emission scanning electron microscope operated at 3 kV. Images from each sample, at 1500× magnification, from three fields were recorded on Kodak TMX 120 film, and digitally using Spectrum 2.0 software (The Dindima Group Pty. Ltd., Ringwood, Victoria, Australia). Fields were randomly selected from areas of the sample that exhibited good quality planes of fracture.

Image Analysis

Digital images, with pixels in the gray scale 0 to 255 (from black to white) were uploaded into Adobe Photoshop 4.0. The images were then converted from their gray-scale values to binary images in which gray pixels were converted to either white or black pixels by applying the threshold function. In the original digital images, dark pixels corresponded to areas of the micrograph occupied by pockets that originally contained fat and/or serum, while light pixels corresponded to areas occupied by protein matrix. Then, when thresholding, pixels having a gray value lower than the threshold level were converted to black pixels, while those having a gray value higher than the threshold level were converted to white pixels. A threshold level of 95 allowed a precise differentiation between dark and light areas as determined by visually matching the original and binary images. The proportions of black and white pixels, and the areas occupied by them were then determined by applying the histogram function. Thus, the area of cheese matrix occupied by fat/serum pockets (dark areas) and protein matrix (light areas) was determined.

Cheese Functionality

After 40 d of storage at 4°C, cheese was removed from its packaging, dried with paper towels, and reweighed. Melting was performed using the UW Meltmeter (University of Wisconsin-Madison, WI). Duplicate cheese samples, 3 cm in diameter and 0.7 cm in height, were tested at 60°C, with the height of cheese recorded every 0.2 s for 40 s. Initial rate of cheese flow was defined as the rate (mm/s) at which cheese height decreased during the first 2 s of the test. Also, the final extent of cheese flow (decrease in height) at 40 s was determined. Texture profile analysis was performed using a two-bite compression test run on a texture profile analyzer (Model Instron 5542, Canton, MA). The compression factor was 75%, and the crosshead speed was set at 20 mm/min. Samples, 20 mm long × 16 mm in diameter, were taken from the cheese immediately after removal from the refrigerator, and tested at approximately 5°C.

Table 2. Statistical results for the effect of adding sodium chloride on chemical and functional properties of unsalted Muenster cheese after 40 d of storage at 4°C

Source	Model ¹
Variable	<i>P</i>
Salt	0.0001
Moisture	0.0454
Weight	0.0002
Hardness	0.0008
Adhesiveness	0.0364
Cohesiveness	0.0003
Flow rate	0.0001

¹ $Y_j = \mu + T_j + e_j$, where *Y* is the variable of interest, μ is the overall mean, *T* is the treatment effect, and *e* is the error term.

Figure 1. Salt content of unsalted Muenster cheese injected with a sodium chloride solution (20% wt/wt) and stored for 40 d at 4°C. Successive injections performed 24 h apart. Error bar = LSD/2.

Hardness, cohesiveness, and adhesiveness were determined by analyzing the data according to Bourne (1978).

Experimental Design and Statistical Analysis

The experiment was conducted in triplicate as a completely randomized design. Three treatments, corresponding to number of injections one, three, or five, along with a control, uninjected cheese, were considered in the experiment. Two cheese samples were analyzed for each variable except weight, soluble and total calcium, and soluble nitrogen, and their mean considered for analysis of variance. For scanning electron microscopy, three cheese samples from one replication were analyzed. Thus, each sample was considered as a replicate for analysis. Statistical analysis (GLM and LSD) was performed using SAS (1999).

RESULTS

Cheese Composition

The moisture content of cheese was in compliance with the standard of identity for Muenster cheese, lower than 46%; however, the fat content as a percent of solids was lower than required, 49% compared with 50% in dry basis (FDA, 1991) (Table 1). Calcium and sodium chloride content were as expected.

In accordance with previous results (Pastorino et al., 2001), injecting a concentrated solution of sodium chloride significantly increased the salt content of cheese (Table 2). Each injection increased the salt content of cheese by 0.5% on average, and after five injections,

Figure 2. Moisture content of unsalted Muenster cheese injected with a sodium chloride solution (20% wt/wt) and stored for 40 d at 4°C. Successive injections performed 24 h apart. Error bar = LSD/2.

the salt content increased from 0.1% in the uninjected cheese to 2.7% (Figure 1).

Salt injection promoted syneresis, and after refrigerated storage, drops of serum were observed inside the package of cheese blocks injected three and five times. After each injection, the cheese had been blotted, so the serum in the package was serum expelled from the cheese rather than residual injectant not incorporated into the cheese block. Thus, the moisture content of cheese significantly decreased after five injections (Table 2), from 41% in the control cheese to 38% (Figure 2). Accordingly, adding salt normally promotes syneresis and decreases the moisture content of cheese (Schroeder et al., 1988; Kindstedt et al., 1992; Guinee and Fox, 1993; Mistry and Kasperson, 1998). However, even though injecting salt at the highest levels caused moisture losses, the increased salt content resulted in a significant increase in cheese weight (Table 2), with a net weight gain of 1.9% after five injections (Figure

Figure 3. Weight change of unsalted Muenster cheese injected with a sodium chloride solution (20% wt/wt) and stored for 40 d at 4°C. Successive injections performed 24 h apart. Error bar = LSD/2.

3). In contrast, in brine-salted cheese the weight of cheese blocks normally decreases upon salting (Geurts et al., 1972; Guinee and Fox, 1993).

In agreement with previous studies (Cervantes et al., 1983; Kindstedt et al., 1992; Guo et al., 1997), and in contrast to the results of Thomas and Pearce (1981), increased salt content of cheese did not affect cheese pH, and the pH of cheese remained at 5.5. Also, the increased salt content had no effect on the content of soluble nitrogen, an indicator of extent of proteolysis. Similarly, Kindstedt et al. (1992), observed no differences in proteolysis between sections of low-moisture and low-moisture part-skim Mozzarella cheese with high- and low-salt content (2 to 3% compared with 0.4%). As previously reported (Schroeder et al., 1988; Paulson et al., 1998), salt content had no effect on total calcium content of cheese. In addition, soluble calcium was unaffected by adding salt to cheese and remained at approximately 50% of total calcium.

Cheese Microstructure

The control, uninjected cheese had a structure typical of a stirred/pressed-curd cheese, with protein matrix interspersed with areas that originally contained fat and/or serum (Figure 4A). The structure of salt-injected cheese looked similar to that of the control cheese, with fat/serum pockets ranging in size between 1 and 11 μm in diameter or length observed throughout the cheese matrix (Figure 4B). Applying the threshold function of the software allowed binary images of the original micrographs to be obtained (Figure 5). In these images, fat/serum pockets (black areas) were clearly differentiated from the protein matrix (white areas). For the control cheese, the protein matrix occupied 84% of the cheese matrix, with fat/serum pockets occupying the remaining 16% (Figure 5A). Although only significant at $P < 0.1$, cheese injected five times had a 4% increased area of cheese occupied by protein matrix compared with the uninjected cheese (Figure 5B). Thus, after five injections the protein matrix occupied 88% of the cheese matrix, with fat/serum pockets occupying the remaining 12%. This is in agreement with the results of Paulson et al. (1998), who observed salted nonfat Mozzarella to have a more homogeneous cheese matrix, with increased area occupied by protein matrix compared with unsalted cheese.

Cheese Functionality

Salt injection significantly affected the hardness of cheese (Table 2). In agreement with previous studies (Cervantes et al., 1983; Schroeder et al., 1988; Mistry and Kasperson, 1998), increased salt content caused

Figure 4. Scanning electron micrographs of Muenster cheese after 40 d of storage at 4°C. A: unsalted cheese (uninjected); B: salt-injected cheese (5 injections). Bar = 10 μm .

increased hardness of cheese, but no further increase was observed after three injections (Figure 6). During the analysis, cheese blocks injected five times partially collapsed, losing structural integrity when compression approached 70%. Cheese adhesiveness and cohesiveness were also significantly affected by salt injection (Table 2). Injected cheese had increased adhesiveness, but there was no further difference after one injection (Figure 7). Also, in agreement with Cervantes et al. (1983) and Schroeder et al. (1988), increased salt content of cheese decreased cheese cohesiveness (Figure 8).

Injecting salt significantly affected the initial rate of cheese flow (Table 2). Even though salt injection increased the initial rate of flowing, no further increase was observed after three injections, and cheese injected five times had decreased flow rate compared with cheese injected once (Figure 9). In previous studies, increased salt content decreased the melting of young Mozzarella cheese (Olson, 1982) and increased the melting of nonfat Mozzarella cheese (Paulson et al.,

Figure 5. Binary image of scanning electron micrographs 4A and 4B after thresholding. Bar = 10 μm .

1998). In contrast, in the present experiment, the final extent of cheese flow, or melting, was unaffected by the increased salt content of cheese.

DISCUSSION

Chemical Composition

Moisture retention. In previous work (Pastorino et al., 2003), injection of a calcium solution into cheese induced contraction of the protein matrix with concomitant release of serum and loss of moisture, which resulted in a less hydrated protein matrix. Thus, in the present experiment, the occurrence of syneresis and moisture losses of cheese after three injections was an indicator of a possibly less hydrated protein matrix. But determining whether salting of cheese by injecting a concentrated salt solution caused decreased hydration of the protein matrix requires further considerations.

When salt is added to an aqueous solution, the volume occupied by that solution increases. In cheese, serum represents an aqueous solution, and injection of a con-

Figure 6. Hardness of unsalted Muenster cheese injected with a sodium chloride solution (20% wt/wt) and stored for 40 d at 4°C. Successive injections performed 24 h apart. Error bar = LSD/2.

centrated brine solution increased the salt content of cheese. Considering that salt mostly remains in the water phase of cheese, salt injection resulted in increased salt concentration in the serum. Then, as a result of increased salt concentration, the volume of serum would increase, and the magnitude of this change could be estimated based on the increase of salt-in-water concentration in the cheese. Because the increase in volume is not strictly equivalent to the increase in salt content, in particular, at a salt-in-water concentration of 6.9% (that achieved after five injections), the volume occupied by cheese serum would increase by 2% compared with the 0.2% salt-in-water content of the uninjected cheese. However, the volume of pockets in cheese for allocating serum and the water-holding capacity of the protein matrix is limited. Considering water to be uniformly distributed throughout the cheese matrix, and that the cheese remains with no deformation or increase in its total volume, if the protein matrix expands, serum must be displaced from within pockets.

Assuming the data on micrograph area corresponds with the volume of cheese occupied by protein matrix or serum/fat pockets, the volume of cheese occupied by protein matrix increased by 4%. Thus, upon addition of salt, the protein matrix became more hydrated. However, the protein matrix has limited water-holding capacity, and, while there was some migration of serum into the protein matrix, there was also migration of excess water out of the cheese. This was observed as syneresis and the cheese had a net loss of moisture (1.6 g/100 g of cheese after five injections).

In general, when cheese is brined, there is a decrease in the moisture content of cheese. However, when cheese was brined, so as to produce a final salt content

Figure 7. Adhesiveness of unsalted Muenster cheese injected with a sodium chloride solution (20% wt/wt) and stored for 40 d at 4°C. Successive injections performed 24 h apart. Error bar = LSD/2.

Figure 9. Initial rate of flow of unsalted Muenster cheese injected with a sodium chloride solution (20% wt/wt) and stored for 40 d at 4°C. Successive injections performed 24 h apart. Error bar = LSD/2.

of 1.1 and 1.8%, there was no difference in the moisture content of cheese (Cervantes et al., 1983). In some cases, salting may even result in cheese with higher moisture content than unsalted cheese. Paulson et al. (1998) made nonfat Mozzarella with final salt contents of 0.1 to 2.2%, and observed no decrease in the moisture content of cheese. The only difference in moisture content was between unsalted cheese and cheese that was dry-salted before cooking and stretching, the latter having increased moisture content. However, the cheese was made according to a direct-acid cheese-making procedure that promoted solubilization of calcium from casein and into solution. As a result, the cheese had low calcium content, 0.4%, which would decrease interactions between proteins, enhancing the effect of salt in promoting protein-to-water interactions. Thus, the wa-

ter-holding capacity of cheese curd increased when salt was added, and syneresis was inhibited, resulting in cheese with increased moisture content. In addition, increasing the salt content of cheese above about 0.5%, by using hot brine solution during cooking/stretching of the curd, had no further influence on moisture content. Therefore, whether salting decreases or increases the moisture content of cheese depends on the amount of salt added, method of salting (e.g., dry-salting, brining, or injection of salt solution), and probably on the chemical composition of cheese (e.g., low or high calcium content).

pH and proteolysis. Salting of cheese can influence cheese pH through its effect on microbial activity (Thomas and Pearce, 1981; Guinee and Fox, 1993). Adding low levels of salt to milk (up to 1.5%) may promote starter activity, but higher levels (2.5% and above) have the opposite effect (Irvine and Price, 1961). In cheese, depressed microbial activity at high salt levels (above 6% salt-in-water) leads to increased concentration of residual lactose and higher cheese pH (Thomas and Pearce, 1981). However, the level of salt at which starter activity decreases depends on bacterial specie and strain, and moisture content of cheese (Guinee and Fox, 1993). In the present experiment, salt was not injected into cheese until 14 d after manufacture. Generally, there is no residual lactose in cheese after 2 wk of ripening (McSweeney and Fox, 1993), so that injecting salt would not cause a change in pH by altering microbial usage of sugar and amount of acid produced in cheese during further storage.

The pH of cheese during long-term storage typically increases as a result of proteolysis, by formation of NH_3 (Fox et al., 1993). Salt content may influence cheese proteolysis by affecting microbial and enzyme activity,

Figure 8. Cohesiveness of unsalted Muenster cheese injected with a sodium chloride solution (20% wt/wt) and stored for 40 d at 4°C. Successive injections performed 24 h apart. Error bar = LSD/2.

with high salt levels decreasing the rate and/or extent of proteolysis (Fox and Walley, 1971; Schroeder et al., 1988; Mistry and Kasperson, 1998). However, in the present experiment there were no differences in proteolysis (measured as TCA-soluble nitrogen) based in salt content of cheese. In agreement with our results, Kindstedt et al. (1992) also observed no differences in proteolysis between sections of Mozzarella cheese with high- and low-salt content.

Soluble calcium. It has been proposed that adding salt promotes calcium solubilization from the paracasein matrix of rennet-treated casein pellets and cheese (Creamer, 1985; Kindstedt et al., 1992). Calcium can be lost from cheese during brining if the brine solution contains low calcium content (e.g., 0.1%) or no calcium at all. However, this is not related to increasing the salt content of cheese because when enough calcium is added to the brine (0.6% calcium content) there is no loss of calcium from the cheese (Geurts et al., 1972). In the present experiment, the content of total and soluble calcium was unaffected by the increased salt content of cheese. Thus, solubilization of calcium in cheese is independent of salt content.

Cheese Microstructure

It has been proposed that sodium chloride in the serum phase of Mozzarella cheese would promote solubilization of caseins and increased protein-to-water interactions. Thus, the protein matrix becomes more hydrated and swells, occupying an increased area of cheese matrix, becoming more continuous and homogeneous in appearance (Guo and Kindstedt, 1995; Guo et al., 1997; Paulson et al., 1998). Our results support this role of salt increasing the hydration of proteins by altering protein interactions. This occurs by increased salt content impairing interactions between proteins and promoting protein-to-water interactions. Thus, a partial relaxation of the protein matrix would occur that would allow water from within pockets to migrate into the protein matrix. As a result, the protein matrix became more hydrated and swelled, occupying increased area of cheese matrix.

Cheese Functionality

Hardness. Increasing the salt content of cheese increases cheese hardness, but the cheese becomes more brittle (Olson, 1982; Cervantes et al., 1983; Mistry and Kasperson, 1998). According to Cervantes et al. (1983), salt affects cheese hardness by promoting interactions between proteins. In the present experiment, cheese hardness increased after one injection of salt, but after further injections (i.e., with salt content greater than

0.5%), the effect of salt on cheese hardness was confounded with decreased moisture content of cheese. However, it is unclear why interactions between proteins that affect hardness could increase when our analysis of microstructural data suggests impaired protein-to-protein interactions and increased protein-to-water interactions when the salt content of cheese is increased.

Cheese can be considered as a gel that results from the effective interaction of proteins to form aggregates leading to the initial formation of protein strands, and then of a matrix that entraps serum, fat, and bacteria. However, proteins in cheese not only interact among themselves, but also with water, fat, and salts, the nature and extent of these interactions depending on the ionic environment of cheese and processing conditions. Adding sodium chloride to protein suspensions or cheese increases the ionic strength of the system. In general, increasing the ionic strength of water causes "salting-in" or increased solubility of proteins. It seems that a similar phenomenon happens in cheese because the protein matrix swelled, suggesting increased protein-to-water interactions in salted cheese. This was also observed by Paulson et al. (1998). Such an increased hydration would increase the thickness of strands that make up the internal structure of the protein matrix of cheese. Swelling of the strands in the protein matrix may then compensate for decreased interactions between proteins, and the protein matrix would have increased capacity to withstand deformation during compression, so that cheese hardness increases.

At high ionic strength, the solubility of proteins frequently decreases, and proteins come out of solution. In this experiment, after five injections the protein matrix appeared more hydrated compared with unsalted cheese, indicating that "salting-out" of proteins in cheese requires a salt-in-water content greater than 7%.

Cohesiveness and adhesiveness. According to Cervantes et al. (1983), salt content affects cheese cohesiveness independently of other variables by modifying interactions with other cheese constituents. In our experiment, the effect of salt decreasing cheese cohesiveness was confounded with decreased moisture content of cheese, which may also affect cheese cohesiveness (Tunick et al., 1991). Increasing ionic strength by injecting salt can affect protein interactions at more than one level. For example, more extensive short-range interactions, such as those involving increased hydration and thickness of strands in the protein matrix, could contribute to increased hardness of cheese. In contrast, weaker and/or decreased long-range interactions, and water loss from pockets throughout the cheese may lead

to a less elastic cheese matrix, resulting in decreased cheese cohesiveness. In addition to changes in cohesiveness, anything that changes the ability of the proteins to interact with water or other proteins can also influence cheese adhesiveness. Injecting salt increased cheese adhesiveness, which may be a result of increased ability of proteins to interact with water and other nonprotein elements. However, increasing the salt content of cheese from 0.5 to 2.7% did not cause any further increase in adhesiveness.

Melting. According to Olson (1982), higher salt content (2% compared with 1%) decreases the melting of young Mozzarella cheese. In contrast, Paulson et al. (1998) reported increased melting when the salt content of nonfat Mozzarella cheese increased from 0.14 to 0.4%, with further increases in salt content, up to 2.2%, having no effect on cheese melting. Also, salting increased the melting of cheese on d 1, but had no effect on the melting of cheese on d 24. In the present experiment, salt content had no effect on the final extent of cheese flow, or melting. The different results between these studies can be better understood when differences in the chemical composition and age of the cheeses are considered.

Olson (1982) studied cheese with higher fat and calcium content than did Paulson et al. (1998), but both reported on young cheese. At the level of salt reported by Olson (1982) to decrease cheese melting, Paulson et al. (1998) observed no effect on cheese melting. Adding salt to cheese increases the ionic strength and, in cheese with relatively high calcium content (0.7% for Mozzarella made by standard procedures), such as in the study of Olson (1982), this may lead to increased interactions between proteins that decrease cheese melting. However, in the study of Paulson et al. (1998), similar increase in salt content was at lower ionic strength because the cheese had low calcium content (0.4%). As a result, interactions between proteins did not increase significantly, and cheese melting was unaffected. In addition, at lower salt levels than those reported by Olson (1982), when the salt content of cheese increased from 0.14 to 0.4%, Paulson et al. (1998) observed increased cheese melting. Thus, a relatively small increase in the salt content of unsalted cheese with low calcium content seems to promote protein-to-water interactions. As a result, interactions between proteins are impaired and protein hydration increases, which results in increased melting of young cheese.

In the present experiment, the cheese also had higher fat and calcium content compared with the cheese used by Paulson et al. (1998). However, the results agree for cheeses more than 24 d old, and over a wide range of salt content (from 0.1 to 2.2%), with increased salt content having no effect on cheese melting. When these

observations are compared with those reported by Olson (1982) and Paulson et al. (1998) in young cheese, it seems that changes associated with the aging of cheese inhibited the effect of salt content on cheese melting regardless of the difference in fat and calcium content of cheese.

Even though salt injection had no effect on the final extent of cheese flow, it increased the initial rate of cheese flow. After one injection of salt, there was an initial increased flow rate, but no increase was observed after further injections. This suggests that increased hydration of the protein matrix favored protein-to-water interactions. Therefore, by impairing interactions between proteins and promoting protein-to-water interactions, salt injection caused partial relaxation of the protein matrix that initially favored the flowing of cheese, but that had no significant effect on the final extent of flow of a full-fat cheese whose calcium content of 0.7% remained the same.

In summary, the effect of salt on cheese melting varies according to cheese age (e.g., few days compared with several weeks old), the content of salt (e.g., 0.5% compared to 2.0%), and probably with cheese composition (e.g., calcium content: 0.4% compared to 0.7%). From our observations in this experiment and previous studies (Paulson et al., 1998; Pastorino et al., 2003), salt content affected cheese functionality to a lower extent than did calcium content, and we agree with Lawrence et al. (1983) that calcium is a primary determinant of cheese functionality. In addition, calcium content appears to limit the extent to which changes in salt content can affect cheese structure, and may determine whether adding salt increases or decreases the melting of young cheese. Discussing the effect of chemical composition on cheese functionality by considering first the effect of a changing ionic environment on protein interactions, protein aggregates, and cheese structure helps to better understand and integrate observations.

CONCLUSIONS

Adding salt increases the ionic strength in cheese, which promotes increased solvation of proteins, thus altering protein interactions. Such increased protein-to-water interactions cause partial relaxation of the protein matrix, which becomes more hydrated and swells. The influence of salt on cheese functionality is most prevalent in the range of 0 to 0.5%, in which case adding salt increases cheese hardness, adhesiveness, and the initial rate of cheese flow. At salt contents above 0.5%, salt appears to further increase hardness and decrease cohesiveness of cheese. Increasing the salt content of cheese did not, however, affect cheese melting, although this may not be the case for young cheese.

In addition, increased salt content did not cause an exchange of calcium with sodium, and soluble calcium remained constant. Therefore, calcium-mediated protein interactions remain a major factor controlling the functionality of cheese.

ACKNOWLEDGMENTS

This research was funded by Dairy Management Incorporated and the Utah Agricultural Experiment Station.

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