Effect of selected Hofmeister salts on textural and rheological properties of nonfat cheese

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ABSTRACT

Three Hofmeister salts (HS; sodium sulfate, sodium thiocyanate, and sodium chloride) were evaluated for their effect on the textural and rheological properties of nonfat cheese. Nonfat cheese, made by direct acidification, were sliced into discs (diameter = 50 mm, thickness = 2 mm) and incubated with agitation (6 h at 22°C) in 50 mL of a synthetic Cheddar cheese aqueous phase buffer (pH 5.4). The 3 HS were added at 5 concentrations (0.1, 0.25, 0.5, 0.75, and 1.0 M) to the buffer. Post-incubation, cheese slices were air dried and equilibrated in air-tight bags for 18 h at 5°C before analysis. Small amplitude oscillatory rheology properties, including the dynamic moduli and loss tangent, were measured during heating from 5 to 85°C. Hardness was determined by texture profile analysis. Acid-base buffering was performed to observe changes in the indigenous insoluble (colloidal) calcium phosphate (CCP). Moisture content decreased with increasing HS concentration. Cheeses incubated in high concentrations of SCN^- softened earlier (i.e., loss tangent = 1) compared with other HS treatments. Higher melting temperature values were observed for cheeses incubated in high concentrations of SO_4^{2-} . Hardness decreased in cheeses incubated in buffers with high concentrations of SCN^- . The indigenous CCP profile of nonfat cheese was not greatly affected by incubation in Cl^- or SCN^- , whereas buffers with high concentrations of SO_4^{2-} reduced the acid-base buffering contributed by CCP. The use of high concentrations (1.0 M) of SCN^- for incubation of cheeses resulted in a softer protein matrix at high temperatures due to the chaotropic effect of SCN^- , which weakened hydrophobic interactions between CN. Cheese samples incubated in 1.0 M SO_4^{2-} buffers exhibited a stiffer protein matrix at high temperatures due to the kosmotropic effect of SO_4^{2-} , which helped to strengthen hydrophobic interactions in the proteins during the heating step. This study showed

that HS influenced the texture and rheology of nonfat cheese probably by altering the strength of hydrophobic interactions between CN.

Key words: Hofmeister series, nonfat cheese, rheology, texture

INTRODUCTION

The per capita natural cheese consumption in the United States exceeded 14.5 kg in 2008 (ERS and USDA, 2010). Cheeses, like Cheddar, though marketed as a good source of dietary calcium, are high in fat (e.g., 32%). The production of reduced- and low-fat varieties has appealed to some health-conscious individuals, yet nonfat (NF) varieties are not very appealing to most consumers. Complete removal of milkfat creates a cheese with numerous defects including pale or translucent color, rubbery texture, dryness, lack of desired flavor, or the presence of off-flavors (Banks, 2004). Fat globules prevent CN proteins from forming a tightly packed network. Removing fat disrupts the fat-protein-moisture ratio within the network, resulting in an increase in both moisture and protein contents. Many methods have been developed to try to produce reduced- and low-fat cheeses but it remains difficult to produce acceptable NF cheese. This research seeks to better understand the protein interactions within the cheese network and how they can be manipulated to alter the textural properties of cheese.

Caseins form the network in cheese, and various types of interactions hold CN micelles together, including hydrophobic interactions, +/– salt bridges, and colloidal calcium phosphate (CCP) crosslinks (Lucey and Fox, 1993; Horne, 1998). These CN interactions can be manipulated by changes in pH, temperature (e.g., heating post-manufacture), and mineral content. Hydrophobic interactions exist as regions of non-polar groups within the CN proteins, shielding themselves from water; moreover water molecules can form hydrogen bonds with the polar regions of CN. Hydrophobic interactions are the main driving force responsible for protein folding. The hydrophobicity of proteins is greatly affected by temperature (e.g., increasing cheese temperature increases hydrophobic interactions), which results in

Received August 8, 2010.

Accepted May 16, 2011.

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the contraction of CN molecules and a decrease in the overall strength of CN gel networks (Lucey et al., 2003). The major minerals found within CN micelles are CCP. These mineral nanoclusters form crosslinks that play a major role in the structure and function of the CN micelles; they also contribute to the structural integrity of the CN network in cheese. Solubilization of CCP affects the texture of cheese (Lucey and Fox, 1993). Lowering pH results in increased solubilization of CCP crosslinks, decreasing the firmness and increasing melt. At very high temperatures, when attractive CN interactions are sufficiently weakened, cheese melts as long as the electrostatic attractions are not too strong (e.g., such as in low-pH cheese; i.e., <5.0) (Lucey et al., 2003). The CN interactions in full-fat cheese networks have been extensively studied (Lucey et al., 2003).

The serum (aqueous) phase may be expressed from cheeses under hydraulic pressure (Morris et al., 1988). The serum phase, although variable among different types of cheeses, manufacture techniques, and with aging, is composed mainly of salts (Ca, Na, P, among others) as well as soluble proteins or peptides. The serum phase of cheese can be altered by immersion of cheese in brine or salt buffer solutions that vary in the amount of NaCl or calcium. O'Mahony et al. (2006) used the buffer immersion approach to alter the mineral content of Cheddar cheese post-manufacture. Increasing the calcium content of cheese post-manufacture decreased melt and increased hardness (O'Mahony et al., 2006). Guinee and Fox (1986) used brine with varying amounts of NaCl to analyze the movement of salt and water in cheese during the brining process. Increasing the salt content of the brine increased the NaCl concentration of the cheese, which caused a decrease in the moisture content (Guinee and Fox, 1986).

The Hofmeister salts (**HS**) are a series of salts classified by their effects on the hydrophobic tendencies of proteins. The Czech scientist, Franz Hofmeister, first studied them in 1888 and is credited for organizing many salts by their effect on egg white gelation (Kunz et al., 2004). These HS influence hydrophobic interactions primarily at high salt concentrations (>0.2 M). At low concentrations of HS, an electrostatic screening effect is observed that is similar for all types of HS. Additions of low concentrations (≤ 0.2 M) of HS (regardless of salt type) screen the charges on protein, which reduces electrostatic interactions between proteins. At high concentrations of HS, charges are already screened and the HS specificity becomes apparent (Zhang and Cremer, 2010). Hofmeister anions have a greater effect on water than cations due to their similar size and charge density as water (Kunz, 2010). Research with HS has primarily focused on protein solubility and the hydrophobic interactions during the gelation of various

globular protein systems (Damodaran and Kinsella, 1982; Babajimopoulos et al., 1983; Arntfield et al., 1990; Mulvihill et al., 1990; Bowland and Foegeding, 1995; Lawal, 2006).

The HS appear to interact with water molecules at the surface of proteins (Zhang and Cremer, 2010). The HS anion series can be divided into 2 categories, which historically have been called kosmotropes (order-maker) that are small, multiply charged ions with a high charge density (e.g., CO_3^{2-} , SO_4^{2-} , H_2PO_4^- , and F^-) and chaotropes (disorder-maker) that are large, singly-charged ions with a low charge density (e.g., Br^- , NO_3^- , I^- , ClO_4^- , and SCN^-). The historical terms, kosmotrope and chaotrope, are usually not considered very accurate, as recent research indicates that these ions do not have any influence beyond the first hydration shell of proteins (Kunz, 2010).

Chaotropes prefer to interact more strongly with the protein than with the water surrounding the proteins, whereas the kosmotropes interact more strongly with the water surrounding the protein than with the protein itself. Hence, HS disrupt the state (folded or unfolded) of proteins by altering the amount of water on the protein surface, due to the tendency of the salts to interact more with the protein (chaotrope or destabilizing; e.g., SCN^-) or with the surface water (kosmotrope or stabilizing, e.g.; SO_4^{2-}). Lyotropic effects of Cl^- have less effect on protein systems, so are often used as a neutral HS when compared with the anions on either end of the HS series (Resch et al., 2005).

It is known that hydrophobic interactions play a key role in the structure of CN micelles as well as the formation of the CN network in cheese (Horne, 1998; Lucey et al., 2003). We wanted to use salts from the Hofmeister series to probe the importance of hydrophobic interactions in the cheese network. We are not aware of any previous published work in relation to the effect of HS on cheese texture. The objective of this study was to investigate the effects of HS on the textural and rheological properties of NF cheese using a simple model buffer system. Several of the HS are not food grade so a buffer system was considered more appropriate.

MATERIALS AND METHODS

Synthetic Cheddar Cheese Aqueous Phase Solutions

Synthetic Cheddar cheese aqueous phase (**SCCAP**) solutions were prepared according to the method developed by Broome and Limsowtin (2002) and as adapted by O'Mahony et al. (2006). Further modifications were made to reflect the composition of NF cheese base used in this study. The original formulation was based on the serum phase of 1-mo-old Cheddar cheese extracted

using the hydraulic press method reported by Morris et al. (1988).

The SCCAP solutions were made using the following procedure: disodium hydrogen phosphate (6.412 g) was dissolved in 700 mL of deionized water (Milli-Q Reagent Water System; Millipore Corp., Bedford, MA). Citric acid-monohydrate (2.300 g), sodium chloride (75.98 g), magnesium sulfate heptahydrate (0.564 g), magnesium chloride hexahydrate (3.296 g), potassium chloride (3.624 g), and sodium acetate trihydrate (0.600 g) were added in that order. Once the salts were dissolved completely (~15 min), sodium lactate (60.056 g of a 60% wt/wt syrup) was added. With continuous stirring, 10 mL of a solution of manganese chloride tetrahydrate (0.025 g/100 mL) was added. Ca chloride dihydrate was added to bring the Ca concentration of the buffer solution to 2.78 g/L because preliminary research on the base cheese demonstrated that at this Ca concentration in the buffer did not alter the insoluble calcium phosphate content in cheese; similar results were reported by O'Mahony et al. (2006). This was followed by 10 mL of a solution of zinc chloride (0.625 g/100 mL). The pH was adjusted to pH 5.4 with hydrochloric acid (0.5 N) and the solution was made up to volume (1 L) with deionized water. The buffer contained 10% NaCl. Separate solutions were prepared fresh daily, differing only in the concentration of HS. Three salts from the HS series were used: sodium thiocyanate (SCN^-), sodium chloride (Cl^-), and sodium sulfate (SO_4^{2-}), and these HS were added at 5 different concentrations (0.1, 0.25, 0.5, 0.75, and 1.0 M) to the SCCAP solution before the final pH adjustment. A total of 16 buffers were prepared, differing in their concentrations of HS. All reagents used were of analytical grade (Fisher Scientific, Fair Lawn, NJ).

Nonfat Cheese Base

Two 9-kg blocks of directly acidified NF cheese were produced by the method described by Brickley et al. (2008) in University of Wisconsin–Madison Babcock Hall Dairy Plant. Citric acid was used to acidify the milk to pH 5.6. The cheese base had a composition of 57% moisture, 37% protein, 4% ash, and 2% fat. The blocks were vacuum-packed and were held at approximately 4°C for 10 d.

Incubation of Cheese in SCCAP Solutions

Cheese samples (discs: 50 × 2 mm) were made using a Hobart deli-slicer and were packaged (stacks of 6 separated by parchment paper) and stored frozen (−20°C) until analysis. Frozen slices were thawed for 2 d at 4°C before use. Samples were wrapped in 1

layer of cheesecloth (to prevent adhesion to the Petri dish surface) and placed in the bottom of a glass Petri dish (100 × 20 mm); 50 mL of SCCAP solution was added. The Petri dishes were covered with the glass lids to prevent evaporation. Fifteen samples of cheese were incubated at room temperature (22°C) for 6 h in the SCCAP solutions for each concentration of salt. Cheeses were also incubated in the control SCCAP solution buffer (i.e., without the extra HS added). The cheese and buffer mixture were continuously agitated on a platform shaker (Lab Line Instruments, Melrose Park, IL) at approximately 15 rpm. Following incubation, the samples were removed from the buffer and patted dry to remove excess moisture with a Kimwipe and air dried for 30 min (inverted after 15 min). The samples were stored in plastic bags (Whirl-Pak; Nasco International Inc., Fort Atkinson, WI) and allowed to equilibrate overnight (18 h) at 4°C. The following day, samples were analyzed for their compositional, textural, and rheological properties. Post-incubation, the SCCAP solutions were pooled from the Petri dishes and analyzed for their mineral composition.

Analysis of Cheese Composition

Cheese slices were analyzed before and after incubation for moisture (IDF, 1982), fat (Marshall, 1992), and total protein using the Kjeldahl method (IDF, 1986). Both cheese slices and buffers were analyzed for mineral composition (Ca, P, Na, and S) using inductively coupled plasma optical emission spectrometry (Park, 2000). The compositional results of the buffers after incubation were compared with the original buffer formulation. The pH values were determined by making a slurry (4 g of cheese and 8 g of deionized water using a mortar and pestle for 2 min) and measuring with a pH probe (pH meter 420A; Orion Research Inc., Beverly, MA). The cheese base was analyzed after 10 d of storage. The incubated slices were analyzed 1 d after storage. The acid-base titration method described by Hassan et al. (2004) was used to determine the buffering properties. The buffering due to residual CCP was estimated as the area between the acid and base titrations over the pH limits 5.2 to 4.1 (Hassan et al., 2004; O'Mahony et al., 2006). Values were normalized using protein content (i.e., buffering area under curve/100 g of protein) to account for differences in the protein content between cheeses (O'Mahony et al., 2006). All analyses were performed in triplicate.

Determination of Rheological Properties

Small Deformation Rheological Properties. Small amplitude oscillatory shear (SAOS) properties were measured on a controlled-stress rheometer (Paar

Physica UDS 200; Physica Messtechnik, Stuttgart, Germany) using a 50-mm serrated parallel plate. Samples (diameter: 50 mm, height: 2 mm) were mounted on the peltier (heating) plate, which had been cooled to 5°C. The samples were loaded based on normal force readings to approximately 1.7 N and were allowed to relax until a relatively constant (~1.0 N) force was attained. This method ensured that the plate had proper, uniform contact with the sample during the entirety of the SAOS test. A thin layer of vegetable oil was dispensed around the exposed area of the sample to prevent it from drying out. The sample was heated from 5 to 85°C at a constant rate of 1°C/min. A frequency of 0.1 Hz and a strain of 0.5% (which was within the linear viscoelastic region for our treatments) were used for SAOS tests. The rheological parameters, storage modulus (G'), loss modulus (G''), and loss tangent (LT) were measured at 1-min intervals and reported as a function of temperature for each sample. All analyses were performed in triplicate.

Large Deformation Rheological Properties.

Texture profile analysis was performed using a TA.XT2 Texture Analyzer (Texture Technologies Corp., Scarsdale, NY) using a 35-kg load cell. To prepare samples, 6 incubated cheese slices (diameter: 50 mm, height: 2 mm) were stacked on top of each other and stored in a Whirl-Pak bag overnight in the refrigerator (4°C for 18 h) to allow the stack to fuse together (O'Mahony et al., 2006). Cylindrical samples were obtained using a stainless steel cork borer (i.d.: 13 mm) and plugs were extruded using a plunger. Six samples (diameter: 13 mm, height: 12 mm) were obtained from each stack and allowed to equilibrate in a sealed plastic bag for 30 min (at 4°C) until tests were performed. The samples were compressed twice to 38% of their original heights at a rate of 1 mm/s. Hardness was defined as the maximum height (force) of the first peak (Bourne, 1978). Analyses were performed in triplicate.

Statistical Data Analysis

Statistical analyses were performed using JMP software (Version 8.0.2; SAS Institute Inc., Cary, NC). Results were analyzed using one-way ANOVA. The level of significant difference was determined at $P < 0.05$. When the effects of treatments were statistically significant, the differences between means were analyzed using the Tukey least significant difference test for multiple comparisons of means.

RESULTS

Composition

The composition of the control cheese (i.e., cheeses incubated in 10% NaCl SCCAP solution without ad-

ditional HS) and the cheeses incubated in SCCAP solutions containing 10% NaCl and various concentrations of NaSCN, NaCl, or Na₂SO₄ are shown in Figure 1. Another series of SCCAP solutions was made with 3% NaCl for various concentrations of Na₂SO₄. Cheeses incubated with high concentrations of SO₄²⁻ had significantly lower moisture contents (e.g., 20% lower than the control) compared with other treatments, therefore making textural comparisons between HS treatments impossible. The use of 3% NaCl in the SCCAP solutions resulted in cheese samples incubated with the 1.0 M Na₂SO₄ having comparable moisture contents to cheeses incubated with 1.0 M Cl⁻, SCN⁻, and control (Figure 1a). Therefore, the results from the Na₂SO₄ treatment in the 3% NaCl SCCAP solutions were compared and discussed with the other HS. For comparison purposes, the SCCAP buffer with 10% NaCl concentration for Na₂SO₄ is also presented in Figures 1 to 5 and in Table 1.

The moisture contents of all of the cheeses incubated in different types of HS decreased with an increasing concentration of salt (Figure 1a). The cheeses incubated in 0.1 M Cl⁻ or SCN⁻ had similar moisture contents to the control. As the concentration of salt increased (from 0.25 to 1.0 M), cheeses incubated in Cl⁻ or SCN⁻ had significantly lower moisture contents compared with the control, but were not significantly different from each other. At low HS concentrations (0.1 to 0.5 M), cheeses incubated in SO₄²⁻ (for the 3% NaCl buffer) had significantly higher moisture contents than the control or other experimental cheeses. At high HS concentrations (0.75 to 1.0 M), the cheeses incubated in SO₄²⁻ (3% NaCl buffer) had comparable moisture contents to the cheeses incubated in similar concentrations of Cl⁻ and SCN⁻ salts (Figure 1a). The trends for protein content of cheeses (Figure 1c) varied inversely to those observed for the moisture content (i.e., protein content increased with a decrease in moisture content, as expected).

The pH values for the cheeses varied depending on the type of HS used for incubation (Figure 1b). Cheeses incubated at different concentrations of Cl⁻ or SCN⁻ did not significantly differ in pH from the control. The pH values of cheeses incubated in all concentrations of SO₄²⁻ (3% NaCl solutions) were significantly higher than the control.

The total calcium content of the cheese samples incubated in HS is shown in Figure 1d. These data were expressed per 100 g of protein to normalize the differences in protein content between treatments. The calcium content of cheeses incubated in Cl⁻ buffers decreased at high concentrations (>0.8 M) of HS in the SCCAP solution; a concomitant increase occurred in the calcium concentration of these SCCAP buffers. The total calcium content of samples incubated with

Figure 1. The moisture content (a), pH (b), protein (c), calcium (d), sodium (e), and sulfur (f) content of nonfat cheese slices after incubation for 6 h at 22°C in 10% NaCl synthetic Cheddar cheese aqueous phase (SCCAP) solutions: control cheese (●), or cheese incubated in different concentrations of Cl^- (▽), SCN^- (◆), SO_4^{2-} (■), or an SCCAP solution containing 3% NaCl and varying concentrations of SO_4^{2-} (□). Error bars indicate ± 1 standard deviation ($n = 3$).

various concentrations of SCN^- or SO_4^{2-} (3 or 10% NaCl buffers) were not significantly different from the control cheese.

The total sodium content of cheese samples (expressed per 100 g of protein) incubated with HS is shown in Figure 1e. The sodium content of cheese increased with increasing concentration of Cl^- or SO_4^{2-} (3% NaCl) in the SCCAP solution; a concomitant decrease occurred in the sodium concentration of the SCCAP buffer (results not shown). This was expected as Na^+ concentration increased with increasing HS concentration (Na^+ is the counter ion used for the HS series). In buffers with 1.0 M Cl^- , the sodium concentration of cheese was twice (7.92 g/100 g of protein) that of the control (3.91 g/100

g of protein). The sodium content of cheeses incubated in varying concentrations of SCN^- was not significantly different from the control at any HS concentration.

The total sulfur content of the cheese samples incubated with HS is shown in Figure 1f. An increase in sulfur concentration occurred with increasing concentrations of SCN^- and SO_4^{2-} (3% NaCl) in the SCCAP solution; a concomitant decrease occurred in the sulfur concentration of the SCCAP buffer (results not shown). This trend was expected, as sulfur is present in both SCN^- and SO_4^{2-} HS. Cheese samples incubated in SCCAP solutions containing 1.0 M SCN^- or SO_4^{2-} (3% NaCl) had total sulfur concentrations of 1.45 and 3.56 g/100 g of protein, respectively. No significant change

Table 1. Small deformation rheological properties of nonfat cheese slices after incubation in synthetic Cheddar cheese aqueous phase (SCCAP) solutions containing 1.0 *M* concentrations of several Hofmeister salts for 6 h at 22°C¹

Treatment	Storage modulus		Loss modulus		Loss tangent (LT)		LT at 85°C (LT ₈₅)	Temperature where LT = 1 (Melt _T ; °C)
	10°C (kPa)	70°C (Pa)	10°C (kPa)	70°C (Pa)	10°C	70°C		
Control	10.2 ± 2.9 ^a	485 ± 1.44 ^a	10.2 ± 2.3 ^a	156 ± 67 ^a	0.25 ± 0.01 ^a	3.28 ± 0.52 ^{ac}	3.90 ± 0.51 ^a	56.6 ± 1.5 ^a
Cl ⁻	13.4 ± 3.6 ^a	696 ± 240 ^a	13.4 ± 3.6 ^{ab}	428 ± 118 ^b	0.26 ± 0.02 ^a	1.61 ± 0.11 ^b	2.28 ± 0.18 ^b	67.0 ± 0.8 ^b
SCN ⁻	11.5 ± 4.5 ^a	228 ± 42 ^b	11.5 ± 4.5 ^a	59 ± 16 ^c	0.37 ± 0.07 ^b	3.97 ± 0.45 ^c	3.18 ± 0.24 ^c	44.1 ± 0.7 ^c
SO ₄ ²⁻ (3% NaCl)	57.7 ± 38.3 ^b	863 ± 327 ^a	133 ± 69.6 ^{bc}	236 ± 47 ^a	0.39 ± 0.07 ^b	3.00 ± 0.15 ^a	3.63 ± 0.33 ^{ac}	56.8 ± 0.8 ^a
SO ₄ ²⁻ (10% NaCl)	107 ± 64.8 ^b	704 ± 106 ^a	219 ± 122.8 ^c	878 ± 333 ^b	0.44 ± 0.12 ^b	0.98 ± 0.03 ^d	1.31 ± 0.09 ^d	75.4 ± 1.4 ^d

^{a-d}Means within a column with different superscripts differ [Tukey Honestly Significant Difference (HSD); *P* < 0.05].

¹Values are means ± standard deviation from 3 replicates.

occurred in total sulfur content in the cheeses incubated in buffers with various concentrations of Cl⁻ compared with control cheeses. Cheeses incubated in 1.0 *M* SO₄²⁻ (10% NaCl) had lower sulfur content, although this may have been due to variable composition in this sample due to the greatly decreased moisture content.

Acid-Base Buffering Curves

The acid-base buffering curves of the cheese samples incubated in SCCAP solutions for the control cheese and cheeses incubated with 1.0 *M* of the HS are shown in Figure 2. Cheeses incubated in 1.0 *M* SO₄²⁻ (both 3 and 10% NaCl solutions; Figure 2d,e) produced very different shaped curves when compared with the other treatments. Control, 1.0 *M* Cl⁻, and 1.0 *M* SCN⁻ cheese samples had buffering peaks at a pH of approximately 4.8 on acidification from the initial pH to pH 3.0 (Figure 2a,b,c). The buffering areas due to CCP for cheeses incubated in 1.0 *M* Cl⁻ and 1.0 *M* SCN⁻ (Figure 2b,c) were visually larger than the control (Figure 2a). The normalized CCP buffering area of the control cheese (i.e., cheeses incubated in SCCAP solution containing no additional HS) and the cheeses incubated in SCCAP solutions containing 0.1, 0.25, 0.5, 0.75, or 1.0 *M* HS are shown in Figure 3. When normalized by protein content, the buffering area due to CCP solubilization for cheeses incubated in 1.0 *M* Cl⁻ and SCN⁻ solutions were not significantly different from the control (Figure 3). The buffering areas for cheeses incubated in all concentrations of SO₄²⁻ (3% and 10% NaCl solution) were significantly smaller than the control (Figure 3). The normalized CCP buffering areas of cheeses incubated in Cl⁻ and SCN⁻ buffers decreased with increasing HS concentration up to 0.75 *M*, but were still larger than the control (Figure 3).

Rheological Properties

Small Deformation Rheological Properties.

The changes in rheological properties of cheeses during

heating from 5 to 85°C are shown in Figure 4. Rheological properties are summarized in Table 1. At low temperatures (e.g., 5°C), cheeses incubated in SO₄²⁻ (both 3 and 10% NaCl) solutions had much higher *G'* values than did the other treatments. At 60°C, cheeses incubated in the Cl⁻ solution had similar *G'* values compared with the cheeses incubated in SO₄²⁻ (3% NaCl) solutions. At temperatures ≥60°C, the Cl⁻ and SO₄²⁻ (10% NaCl buffer) cheeses had higher *G'* values compared with the other treatments. At 5°C, the control, SCN⁻, and Cl⁻ cheeses had similar *G'* values. At >10°C, cheeses incubated in SCN⁻ solutions had significantly lower *G'* values than did other treatments over the rest of the heating range (Figure 4a).

It should be noted that the moisture content of the cheeses incubated in 1.0 *M* SO₄²⁻ (3% NaCl solutions), Cl⁻, or SCN⁻ were similar; however, the cheeses incubated in SCN⁻ solutions had significantly lower *G'* values at all temperatures compared with the other cheeses.

At temperatures <50°C, cheeses incubated in SO₄²⁻ (i.e., both 3 and 10% NaCl) solutions had much higher *G''* values than did the other treatments (Figure 4b). Cheeses incubated in SCN⁻ and Cl⁻ had higher *G''* values than did the control at low temperatures (≤20°C). At high temperatures (i.e., >65°C), control cheeses or cheeses incubated in SCN⁻ had lower *G''* values than did cheeses incubated in Cl⁻ or SO₄²⁻ solutions.

At low temperatures (i.e., 5–40°C), the LT values for the different treatments were similar (Figure 4c). The LT values for cheeses incubated in SCN⁻ solutions started to increase at lower temperatures (~35°C) compared with other treatments. The LT values for the control and SO₄²⁻ (3% NaCl solutions) cheeses only began to increase at >50°C. Cheeses incubated in Cl⁻ and SO₄²⁻ (10% NaCl solutions) buffers did not demonstrate an increase in LT until >60°C (Figure 4c).

Cheeses incubated in 1.0 *M* SCN⁻ exhibit an LT_{max} (Figure 4c); at high temperatures (>70°C) the *G'* curve began to flatten, whereas *G''* values continued to decrease. The other treatments did not demonstrate

Figure 2. Acid-base buffering curves of nonfat cheese slices after incubation for 6 h at 22°C in a 10% NaCl synthetic Cheddar cheese aqueous phase (SCCAP) solution, control (a), or containing 1.0 M Cl^- (b), SCN^- (c), SO_4^{2-} (d); or in a 3% NaCl SCCAP solution containing 1.0 M SO_4^{2-} (e). Cheese samples were titrated from their initial pH to pH 3.0 with 0.5 N HCl and then back-titrated to pH 7.0 with 0.5 N NaOH. Representative curves for each treatment are shown.

an LT_{max} (at least not at the measured frequency of 0.1 Hz). At 85°C, the LT values (LT_{85}) for the control and other 1.0 M HS treatments (excluding SCN^-) were still increasing (Table 1; Figure 4c). Large LT_{85} values indicate a high degree of fluidity and rapid relaxation of protein-protein bonds, which facilitates rearrangement within the CN matrix (O'Mahony et al., 2006). The LT_{85} values for cheeses were in the following order: control > SO_4^{2-} (3% NaCl solution) > SCN^- > Cl^- > SO_4^{2-} (10% NaCl solution).

An LT value = 1 (i.e., when the ratio of $G''/G' = 1$) is sometimes (Sutheerawattananonda and Bastian, 1998) considered the melting temperature (Melt_T) of cheese (Table 1). It should be noted that for cheese the temperature where $G''/G' = 1$ depends on the applied frequency (Udayarajan et al., 2005). Cheeses incubated in 1.0 M SCN^- solutions had lower Melt_T (~44°C) than the other treatments and higher LT values at 70°C than the cheeses incubated in SO_4^{2-} or Cl^- solutions. The Melt_T for cheeses were in the following order: SO_4^{2-} (10% NaCl solution) > Cl^- > SO_4^{2-} (3% NaCl) = control > SCN^- .

Large Deformation Rheological Properties. The large deformation rheological properties of the cheese samples incubated in SCCAP solutions with various types of HS are shown in Figure 5. Hardness increased with an increase in the HS concentration for Cl^- and SO_4^{2-} . This trend was possibly due to a decrease in moisture content with an increase in HS concentration (Figure 1a). Cheese samples incubated in 1.0 M Cl^- or SO_4^{2-} solutions were significantly harder than the control. Cheeses incubated in high concentrations of both SO_4^{2-} treatments were significantly harder than cheeses incubated in Cl^- , although under these conditions (i.e., > 0.8 M), the SO_4^{2-} (3% NaCl solution) cheese had a similar moisture content to the Cl^- cheese (Figure 1a). Cheese samples incubated in solutions with high concentrations of SCN^- (>0.5 M) were significantly softer than the control, although they had lower moisture contents than the control (Figure 1a). Cheeses incubated in low (≤ 0.5 M) SO_4^{2-} (3% NaCl) concentrations were softer than the control cheese probably due to the samples having high moisture contents (Figure 1a).

DISCUSSION

In this study, the 3 HS demonstrate a significant effect on the compositional, textural, and rheological properties of NF cheese. It is likely that these effects are due to the kosmotropic and chaotropic nature of these HS, especially at high concentrations (e.g., 1.0 M). The dehydration effect of sodium on the cheese matrix (Monib, 1962) also contributes to the observed physical and compositional changes. At low HS concentrations

Figure 3. Normalized buffering capacity from acid-base buffering titrations of nonfat cheese slices after incubation for 6 h at 22°C in 10% NaCl synthetic Cheddar cheese aqueous phase (SCCAP) solutions: control cheese (●), or cheese incubated in different concentrations of Cl^- (▽), SCN^- (◆), SO_4^{2-} (■), or an SCCAP solution containing 3% NaCl and varying concentrations of SO_4^{2-} (□). Error bars indicate ± 1 standard deviation ($n = 3$). Buffer capacity was estimated as the area between the acid and base titrations in the pH range 4.1 to 5.2. CCP = colloidal calcium phosphate.

(<0.2 M), an electrostatic screening effect should be observed (von Hippel and Wong, 1964; Damodaran and Kinsella, 1982). At high HS concentrations (>0.2 M), a concomitant increase occurred in the kosmo- or chaotropic effects of the HS on the cheese protein network. Salt-specific trends were observed for the compositional (Figure 1), rheological (Figure 4), and textural (Figure 5) properties of cheese at low or high concentration of HS.

Thiocyanate (SCN^-)

Chaotropic salts decrease the hydrophobic driving force for protein folding and decrease surface tension (by exposing hydrophobic residues) due to the increase in the number of salt molecules (decrease in water molecules) at the surface of the protein (Zhang and Cremer, 2010). A common effect of SCN^- , a chaotropic HS, is increased protein solubility or swelling due to decreased hydrophobic interactions at the protein surface (Arntfield et al., 1990). Protein swelling can also be caused by the movement of Na^+ and Cl^- ions from the cheese matrix into the brine, if the brine has a low Ca^{2+} concentration (Geurts et al., 1972). Lowering the concentration of Na^+ or Cl^- salts in a brine solution also leads to the depletion of the serum-phase Na^+ and Cl^- in the cheese, resulting in swelling of the cheese matrix (Geurts et al., 1972; Guinee, 2004). Calcium and sodium contents did not change, whereas sulfur content increased in cheeses incubated in buffers with

Figure 4. Storage modulus (a), loss modulus (b), and loss tangent (c) as a function of temperature of nonfat cheese slices after incubation for 6 h at 22°C in 10% NaCl synthetic Cheddar cheese aqueous phase (SCCAP) solutions: control cheese (●), or cheese incubated in different concentrations of Cl^- (▽), SCN^- (◆), SO_4^{2-} (■), or an SCCAP solution containing 3% NaCl and varying concentrations of SO_4^{2-} (□). Cheeses were heated from 5 to 85°C at a rate of 1°C/min and a frequency of 0.1 Hz. Error bars indicate ± 1 standard deviation ($n = 3$).

Figure 5. Hardness from texture profile analysis of nonfat cheese slices after incubation for 6 h at 22°C in 10% NaCl synthetic Cheddar cheese aqueous phase (SCCAP) solutions: control cheese (●), or cheese incubated in different concentrations of Cl[−] (▽), SCN[−] (◆), SO₄^{2−} (■), or an SCCAP solution containing 3% NaCl and varying concentrations of SO₄^{2−} (□). Error bars indicate ± 1 standard deviation (n = 3).

high concentrations of SCN[−] (Figure 1d–f). A corresponding decrease occurred in sulfur in the SCCAP buffers (results not shown). During the incubation of slices in SCCAP solutions containing SCN[−], the ion diffused into the moisture phase of the NF cheese as the sulfur content increased (Figure 1f).

Arntfield et al. (1990) reported that the association of SCN[−] to protein molecules results in a net-negative charge on the protein, which destabilizes (weakens) the network. On a molecular level, chaotropic salts allow the buried hydrophobic residues to be exposed, resulting in the loss or disruption of the native (folded) structure of protein (Lawal, 2006). For globular proteins, chaotropic salts induce denaturation (exposure of buried hydrophobic groups) but because CN micelles do not have highly ordered secondary structures, the term denaturation may not be very appropriate.

Thermal denaturation temperature (T_d) of protein is related to the thermal energy required to exceed the hydrophobic (entropic) driving force for folding. Various studies have reported that SCN[−] decreases the T_d of globular proteins (von Hippel and Wong, 1964; Babajimopoulos et al., 1983; Arntfield et al., 1990; Wang and Damodaran, 1991). von Hippel and Wong (1964) reported that SCN[−] decreased the melting temperature for the helix to coil transition of ribonuclease. The Melt_T of cheese is related to the amount of thermal energy needed to overcome the attractive interactions responsible for the elasticity of the matrix. We believe that SCN[−] could cause a decrease in T_d and lower Melt_T for protein gels, as both properties are related to a weakening of hydrophobic interactions that contribute to the

structure/integrity of the protein structure. A weakening of the CN-CN interactions in cheeses incubated in high concentrations of SCN[−] was demonstrated by the decreased hardness (Figure 5), as well as high LT values at 70°C (Table 1). Arntfield et al. (1990) also found that SCN[−] resulted in heat-induced gels with high LT values. Cheeses incubated in 1.0 M SCN[−] SCCAP solutions had significantly lower Melt_T than the control, 1.0 M Cl[−], or SO₄^{2−} (3 and 10% NaCl solutions).

Chaotropes (i.e., SCN[−]) decrease hydrophobic interactions within the protein network by perturbing the first layer of water surrounding the proteins (Lawal, 2006; Zhang and Cremer, 2006), thus weakening hydrophobic interactions between CN. Rheological profiles (Figure 4) support this suggestion because cheeses incubated in 1.0 M SCN[−] had lower G' values than the control, 1.0 M Cl[−], or SO₄^{2−} (3 and 10% NaCl)-incubated cheeses over the temperature range (10–85°C) despite having significantly lower moisture content than the control (Figure 1a). Sanchez and Burgos (1997) reported that heat-induced gels made from hydrolyzed sunflower protein isolates had a decrease in G' values with the addition of high concentrations of SCN[−].

The 1.0 M SCN[−] was the only treatment that exhibited an LT_{max} (at ~70°C) over the temperature range 5 to 85°C (Figure 4c). Hydrophobic interactions increase up to a maximum at about 60 to 70°C, at which point their strength begins to diminish (Lucey et al., 2003). The presence of an LT_{max} could indicate a weakening in the strength of the hydrophobic interactions between CN particles (Figure 4c), which could have also contributed to the low G' values observed at >60°C (Figure 4a).

The increase in LT values at temperatures >40°C occurred sooner (i.e., at a lower temperature) in cheese incubated in 1.0 M SCN[−] than in other treatments (Figure 4c). Arntfield et al. (1990) reported that the addition of 0.5 M NaSCN to ovalbumin and vicilin greatly decreased the T_d compared with the addition of 0.5 M Na₂SO₄.

The microstructure of protein gels is also altered by the use of high concentrations of SCN[−] (Arntfield et al., 1990; Bowland and Foegeding, 1995). The addition of high levels of SCN[−] modified our cheese properties as it was visually obvious that the cheese became very pliable. The physical structure of the cheeses incubated in 1.0 M SCN[−] SCCAP solutions demonstrated decreased hardness at low (4°C) temperatures (Figure 5) and at high temperatures (10 to 85°C) low G' values (Figure 4a) were observed indicative of a decrease in hydrophobic interactions between CN (Lucey et al., 2003).

Cheeses incubated in SCCAP solutions with high (>0.8 M) concentrations of SCN[−] demonstrate similar effects (weakening of the protein matrix, swelling, loss

of structure, and low temperature softening) to those previously been reported in various globular protein gel systems. We propose that during the incubation of cheese in high concentrations of SCN^- , the SCN^- diffuses into the serum phase of the NF cheese. At such high HS concentrations, the SCN^- ions interact with each other (rather than with water molecules) and remove the monolayer water near the surface of CN that are in the vicinity of the serum. The removal of surface water allows a decrease in hydrophobic interactions between CN, which disrupts the hydrophobic interactions that are one of the key crosslinking agents for CN molecules (Horne, 1998). The decreased hydrophobic interactions between CN are responsible for the softer, more easily meltable network observed for cheeses treated with SCN^- .

Calcium, because it is involved in CCP crosslinks, plays a key role in the CN network of cheese (Lucey and Fox, 1993; Lucey et al., 2003). Solubilization of Ca from the cheese network results in the swelling of the para-casein matrix (Geurts et al., 1972). O'Mahony et al. (2006) found an inverse relationship between CCP concentration and moisture content (i.e., a decrease in moisture content in cheeses with high Ca buffers that increased the CCP concentration of the incubated cheese). Incubated cheese slices decreased in moisture content with increasing HS in the buffers (Figure 1a), yet total calcium content did not vary with increasing HS concentration (Figure 1d). The total sulfur content of the cheeses increased with increasing concentration of SCN^- (Figure 1f), indicating that the HS had diffused into the moisture phase of cheese slices. The shape of the buffering curve of the 1.0 M SCN^- cheeses (Figure 3c) was similar to that of the control (Figure 3a); likewise, the normalized buffering area was not significantly different from that of the control (Figure 2). The buffering results suggest that SCN^- had little effect on the CCP content in our cheese.

Sulfate (SO_4^{2-})

High concentrations of SO_4^{2-} in the SCCAP buffer with 10% NaCl resulted in cheeses with very low moisture content ("salting out"; Figure 1a) and these cheeses had greatly increased hardness (Figure 5). Sulfate salts, such as $(\text{NH}_4)_2\text{SO}_4$, are often used to salt out proteins and sulfate has historically been used to precipitate CN from solutions (Zittle, 1966). Composition and textural analysis for this cheese were difficult due to its variable composition (Figure 1d-f; Figure 5). Arntfield et al. (1990) reported that the addition of 0.5 M SO_4^{2-} to ovalbumin resulted in protein aggregation, possibly due to enhanced hydrophobic interactions. Decreasing the NaCl concentration from 10 to 3% in the SO_4^{2-} SC-

CAP buffer increased the moisture content for the 1.0 M SO_4^{2-} cheeses and allowed for comparison with other HS treatments with similar moisture contents (Figure 1a). Geurts et al. (1974) reported that the use of lower NaCl content brines resulted in cheeses with increased moisture contents.

On formulating the 1.0 M SO_4^{2-} (10% NaCl) SCCAP solutions, it was noted that it was not possible to exceed a Na_2SO_4 concentration of 1.0 M, as precipitation of the buffer rapidly occurred. After incubation (6 h) of cheeses in the 1.0 M SO_4^{2-} (10% NaCl) solutions, the buffer was cloudy and a white precipitate settled out at the bottom of the beaker after collecting the pooled buffer.

At low SO_4^{2-} concentration (<0.6 M), samples incubated in 3% NaCl SCCAP solutions had very high moisture contents (Figure 1a). Visually, the cheeses lost much of their structure. Guinee and Fox (1986) reported a similar trend for Romano cheeses that were incubated in low NaCl brines [6.5%; i.e., moisture content increased above the control ("salting-in") due to increased protein solubility, resulting in swelling of the CN matrix]. Sodium content and swelling greatly increased when cheeses were incubated in the low (3%) NaCl buffer for SO_4^{2-} (Figure 1e). This was due to the removal of NaCl from the buffer and transfer to the cheese (Geurts et al., 1972; Geurts et al., 1974; Guinee and Fox, 1986). Geurts et al. (1972) and Monib (1962) reported swelling in cheeses and para-casein systems in the presence of low NaCl buffer/brine solutions. At high concentrations of SO_4^{2-} (>0.6 M) no swelling occurred as the moisture content decreased (Figure 1a) and hardness increased (Figure 5).

The pH increase in SO_4^{2-} cheeses incubated in 3% NaCl SCCAP buffers was probably due to the replacement of Na^+ for H^+ in the cheese. Additional textural tests were performed on cheeses incubated in 1.0 M SO_4^{2-} SCCAP solutions where the pH was adjusted (i.e., to become similar to the other treatments). The texture tests were of cheeses with the modified pH was similar to those with the unmodified pH (results not shown). Changes in the acid-base buffering curves for cheeses incubated in 1.0 M SO_4^{2-} (3 and 10% NaCl solutions; Figure 2d,e) may be related to Na^+ and H^+ ion exchange in the native CCP.

At low temperatures, (5°C) the G'' and G' values for cheeses incubated in 1.0 M SO_4^{2-} (3% NaCl) were significantly higher than other treatments (Table 1; Figure 4a,b). Some studies on the formation of heat-induced gels from globular proteins have reported that high SO_4^{2-} concentrations produce weak gels (Babajimopoulos et al., 1983; Arntfield et al., 1990; Sanchez and Burgos, 1997). The addition of SO_4^{2-} increases the T_d of globular proteins due to the enhancement

of hydrophobic interactions; thus, higher temperatures are required to overcome the hydrophobic interactions that stabilize the protein structure (von Hippel and Wong, 1964; Babajimopoulos et al., 1983; Arntfield et al., 1990). The formation of heat-induced gels from globular protein requires that the protein is first denatured and then the unfolded proteins can aggregate by various interactions, including hydrophobic interactions. Thus, the addition of SO_4^{2-} increases the T_d , which could inhibit the initial denaturation step but enhances hydrophobic interactions that could increase the strength of the heat-induced gel. Several studies have indicated that the addition of SO_4^{2-} enhanced the strength (O'Riordan et al., 1989) or elasticity (Mulvihill et al., 1990) of heat-induced gels. Very high levels of SO_4^{2-} result in salting out of proteins and this prevents heat-induced gelation.

Our study involved the addition of HS to an existing gel network (cheese), which is a very different situation to that in previous studies on the solubility of protein solutions or heat-induced protein gelation. Due to numerous interactions that contribute to the structure of the cheese matrix, the addition of SO_4^{2-} to the buffer did not precipitate CN, rather the SO_4^{2-} ions may have increased hydrophobic interactions between CN, resulting in the loss of water and a localized rearrangement of the CN in the matrix (as we observed visible changes in the appearance of the cheese). Previous studies have indicated that the addition of high concentrations of SO_4^{2-} during the formation of heat-induced protein gels altered the microstructure (Arntfield et al., 1990; Bowland and Foegeding, 1995).

Texture analysis results (Figure 5) agreed with these rheological trends (Figure 4); at low temperatures (5°C), samples incubated in 1.0 M SO_4^{2-} (3% NaCl solutions) had significantly higher hardness values than other treatments (Figure 5). Hydrophobic interactions in cheese are weak at low temperatures (Lucey et al., 2003). The moisture content for SO_4^{2-} (3% NaCl solution) was significantly lower than the control (Figure 1a), but did not differ significantly from the SCN^- or Cl^- treatment, indicating that this type of HS still affects the cheese network even when hydrophobic interactions were weak. At low temperatures, other forces (i.e., electrostatic interactions or CCP crosslinks) are still present in the CN network (Horne, 1998; Lucey et al., 2003) and appear to be modified by the presence of HS, as hardness followed the trend: $\text{SO}_4^{2-} > \text{Cl}^- > \text{SCN}^-$ (Figure 5). Because SO_4^{2-} was added in the form Na_2SO_4 , compared with the other HS, which were monovalent HS, the ionic strength was higher for SO_4^{2-} buffers.

The Melt_T for the SO_4^{2-} (3% NaCl solution) did not differ significantly from the control, yet the LT_{85} value

was lower than that of the control, indicating that at higher temperatures this cheese did not soften as much as the control (Table 1; Figure 4c). During heating, the increased strength in hydrophobic interactions typically reduces the contact between CN molecules, which reduces overall gel strength (Lucey et al., 2003). It is possible that SO_4^{2-} enhanced the hydrophobic interactions within the CN network and helped maintain gel stiffness during the heating step.

We propose that the use of high concentrations of SO_4^{2-} results in the sulfate ions diffusing into the moisture phase of NF cheese. Once incorporated into the matrix, the SO_4^{2-} ions prefer to interact with other SO_4^{2-} ions, which forces water molecules to the CN surface. This causes an enhancement of hydrophobic interactions between CN that is responsible for the increased hardness (Figure 5) and stiffness during the heating process (Figure 4). The high Melt_T for cheese with SO_4^{2-} (Table 1) is similar to the increased T_d , reported for proteins (von Hippel and Wong, 1964). Very high SO_4^{2-} levels resulted in considerable moisture loss from cheese, which is a form of salting out of the CN system.

Chloride (Cl^-)

Within the HS series, Cl^- is classified as a weak kosmotrope (Kunz, 2010). This description appeared to be accurate for the NF cheese system. The relationship between NaCl and cheese has been thoroughly investigated (see the review by Guinee and Fox, 2004). Geurts et al. (1974) modeled the transport of NaCl into cheese during the brining of Gouda cheese; they calculated the rate of diffusion of NaCl was approximately $2 \text{ mm}^2/\text{d}$. After the 6-h incubation period, cheeses were stored for another 18 h, which should have allowed the ions sufficient time to reach equilibrium, as the thickness of the cheese slices was 2 mm.

Increasing the concentration of Cl^- in the SCCAP solution resulted in cheeses with a decrease in moisture content and increased protein content (Figure 1a,c). These results are in agreement with those of Geurts et al. (1980) who reported that NaCl uptake in cheese increased with an increase in the concentration of NaCl in the brine. The water lost (lower final moisture content) is greater than the NaCl gained due to the difference in the molecular size of H_2O compared with Na^+ and Cl^- ions (Guinee and Fox, 1986).

The hardness of globular protein gels increases with an increase in the concentrations of Cl^- (Arntfield et al., 1990; Mulvihill et al., 1990; Wang and Damodaran, 1991), which is in agreement with the increased hardness of cheeses with increasing Cl^- concentration (Figure 5). The G' and G'' values over the temperature

range from 5 to 85°C for cheeses incubated in 1.0 M Cl⁻ (Figure 4a,b) decreased less than in other treatments, which indicates that Cl⁻ cheeses do not soften as much with increasing temperature. The Melt_T for 1.0 M Cl⁻ cheeses was much higher than that in the control and 1.0 M SCN⁻ treatments. At high NaCl levels, greatly increased electrostatic screening of charges occurred between CN, which should enhance hydrophobic interactions, according to the dual-bonding CN approach of Horne (Horne, 1998).

CONCLUSIONS

Hofmeister salts are known to influence hydrophobic interactions in globular proteins. Although CN are not globular proteins, hydrophobic interactions do play a major role in the formation of CN micelles and greatly influence the properties of CN-based networks, such as cheese. Salts from the HS influence the hydrophobic interactions within protein networks: increasing (kosmotropes; i.e., SO₄²⁻) or decreasing (chaotropes; i.e., SCN⁻) hydrophobic interactions. The HS effect was concentration dependent. At low HS concentrations (e.g., 0.1 M), electrostatic interactions were weakened but the cheese matrix remained intact due to the presence of other (non-electrostatic) types of interactions (e.g., CCP crosslinks, hydrophobic interactions). The HS influence on hydrophobic interactions became species dependent as the concentration approached 1.0 M. The strength of the NF cheeses matrix incubated in 1.0 M of different types of HS increased in the order SO₄²⁻ > Cl⁻ > SCN⁻, which was similar to the effect of these salts on decreasing hydrophobic interactions. The results of this study are consistent with the view that the main CN interactions in cheese are hydrophobic and electrostatic interactions, as well as CCP crosslinks. These results provide a better understanding of the role of hydrophobic interactions in CN networks, which might assist in improving NF cheese texture.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the financial support of this research by the Dairy Research Institute (Rosemont, IL).

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