

Effect of Trisodium Citrate Concentration and Cooking Time on the Physicochemical Properties of Pasteurized Process Cheese

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ABSTRACT

The effects of the concentration of trisodium citrate (TSC) emulsifying salt (0.25 to 2.75%) and holding time (0 to 20 min) on the textural, rheological, and microstructural properties of pasteurized process Cheddar cheese were studied using a central composite rotatable design. The loss tangent parameter (from small amplitude oscillatory rheology), extent of flow (derived from the University of Wisconsin Meltprofiler), and melt area (from the Schreiber test) all indicated that the meltability of process cheese decreased with increased concentration of TSC and that holding time led to a slight reduction in meltability. Hardness increased as the concentration of TSC increased. Fluorescence micrographs indicated that the size of fat droplets decreased with an increase in the concentration of TSC and with longer holding times. Acid-base titration curves indicated that the buffering peak at pH 4.8, which is due to residual colloidal calcium phosphate, decreased as the concentration of TSC increased. The soluble phosphate content increased as concentration of TSC increased. However, the insoluble Ca decreased with increasing concentration of TSC. The results of this study suggest that TSC chelated Ca from colloidal calcium phosphate and dispersed casein; the citrate-Ca complex remained trapped within the process cheese matrix. Increasing the concentration of TSC helped to improve fat emulsification and casein dispersion during cooking, both of which probably helped to reinforce the structure of process cheese.

Key words: pasteurized process Cheddar cheese, emulsifying salt, rheology

INTRODUCTION

Pasteurized process cheese is an important cheese type in the United States, and its production totaled

~1 million tons in 2003 (IDFA, 2004). According to Food and Drug Administration regulations, process cheese types are categorized into pasteurized process cheese, pasteurized process cheese food, and pasteurized process cheese spread based on composition and permitted ingredients such as emulsifying salts (**ES**), dairy powders, and stabilizers (Code of Federal Regulations, 2004). The cheese industry has devoted considerable efforts to controlling the texture and meltability of process cheese products and to developing new products.

Pasteurized process cheese is manufactured with natural cheese, water, and ES. Commonly used ES include several types of sodium phosphates and trisodium citrate (**TSC**). In process cheese manufacturing, grated cheese is mixed with ES and water, and the mixture is heated at a high temperature with mechanical shear. Emulsifying salts are a very important ingredient in process cheese-making because heat treatment of natural cheese causes “oiling off,” which is separation of fat from cheese mass. Emulsifying salts are often considered to act as Ca chelating agents and CN dispersing material (Caric et al., 1985; Shimp, 1985). During cooking, ES help to change some of the CN from an insoluble form to a soluble form, where they can emulsify the fat. It is well known that soluble CN, such as sodium caseinate, are excellent emulsifiers because of their separate hydrophobic and hydrophilic regions on the CN molecules.

It is well recognized that the textural properties of process cheese are influenced by the conditions used for converting natural cheese into process cheese (Thomas, 1973; Shimp, 1985; Berger et al., 1998; Zehren and Nusbaum, 2000; Guinee et al., 2004). It is also well known that the state of Ca in cheese is important for cheese functionality (Lucey and Fox, 1993). Lucey and Fox (1993) suggested that the insoluble Ca associated with CN plays a key role in modulating cheese texture. Hassan et al. (2004) reported that the ratio of soluble Ca to total Ca in Cheddar cheese increased with age. However, the state of Ca in process cheese is unknown. Several studies on milk solutions and process or analog cheeses reported that various types of ES have different

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Ca chelating ability (Nakajima et al., 1975; Guinee et al., 2004). Guinee et al. (2004) summarized the likely chelating ability of ES in pasteurized process cheese as increasing in the following order: polyphosphate > pyrophosphate > orthophosphate > citrate. The various types of ES influence the final characteristics of process cheese (Gupta et al., 1984). Gupta et al. (1984) reported that the use of different types and ES concentrations (ESC) resulted in process cheese that had a wide range of different pH values and degrees of hardness and meltability. However, other studies (Thomas et al., 1980; Cavalier-Salou and Cheftel, 1991) have not reported similar trends for functional properties of individual ES compared with those reported by Gupta et al. (1984), probably because of differences in the experimental cheese-making conditions.

It is known that pH has an important influence on physicochemical properties of process cheese (Marcheseau et al., 1997). Cavalier-Salou and Cheftel (1991) reported that the influence of ES (at 3% wt/wt usage level) on pH of analog cheese increased in the following order: tetrasodium pyrophosphate > TSC > disodium phosphate > sodium phosphate. Thomas (1973) reported that the pH of pasteurized process cheese should be ≥ 5.6 to have suitable textural attributes. However, there has been little published research on the effect of ESC on process cheese properties when cheese pH was kept constant; this information is essential for a better understanding of the effects of ES on the functionality of process cheese.

Cooking time also affects the textural properties and size of fat droplets of process cheese (Rayan et al., 1980). Process cheese manufacturers have noted that the prolongation of cooking time and the use of particular types of ES may cause a change in the viscosity of the melted cheese, which is the so-called "creaming" or creaming reaction (Zehren and Nusbaum, 2000). It is known that the creaming reaction affects the characteristics of the final product (Berger et al., 1998). Excessively long cooking time can result in the formation of a pudding-like gel of melted cheese mass in the cooker (i.e., over-creaming) or a crumbly, dry texture in the final product; both are considered defects. Berger et al. (1998) suggested that the creaming reaction only occurs in the presence of fat in cheese. Conversely, in a recent study, Lee et al. (2003) showed that the creaming reaction could be observed in a fat-free process cheese analog. However, the mechanism responsible for the creaming reaction is not yet very clear.

Trisodium citrate is commonly used as an ES in process cheese; it is often used in slice applications. There have been several studies on the effects of various concentrations of TSC on textural and rheological properties of process or analog cheese (Templeton and Som-

mer, 1932; Thomas, 1973; Gupta et al., 1984; Cavalier-Salou and Cheftel, 1991; Gupta and Reuter, 1993; Suthierawattananonda et al., 1997). There were conflicting results from these studies, presumably because of differences in the experimental conditions, such as raw ingredients (e.g., cheese), pH, composition, and cooking method. There does not appear to be any published study of the impact of TSC concentration on the properties of process cheese where pH was kept constant, and there are no reports on the state of Ca phosphate in these cheeses.

The objectives of this study were to investigate the effect of different concentrations of TSC and cooking times on the textural and rheological properties of pasteurized process Cheddar cheese that was made with a constant pH (~5.6) and to investigate how TSC influenced the state of Ca and phosphate in process cheese. The rheological properties of hot process cheese were also studied to provide information on possible structural changes during the cooking process.

MATERIALS AND METHODS

Materials

The natural cheese used as raw material was 4-month-old Cheddar cheese that was always obtained from the same cheese factory (Alto Dairy Cooperative, Waupun, WI). This cheese was selected because it had a consistent composition (moisture, 37%; fat, 34%; protein, 26%; 669 mg of Ca/100 g of cheese; mean of $n = 3$). The ES used was TSC dihydrate (Fisher Scientific, Fair Lawn, NJ). pH modifiers used were 50% sodium hydroxide (Fisher Scientific) or 88% lactic acid (Brenntag Great Lakes, LLC, Wauwatosa, WI).

Process Cheese Manufacture

Process cheeses were prepared with a Blentech twin-screw cooker (Blentech Corp., Rohnert Park, CA) equipped with direct and indirect steam injection. Cheddar cheese was tempered overnight at room temperature (-24°C) and grated using a meat grinder (Biro Manufacturing, Marblehead, OH). The process cheese cooker was preheated to 80°C for 1 min; then, 8.0 kg of grated cheese, distilled water, and TSC (0.25 to 2.75% on an anhydrous basis) were added to the cooker and mixed at 50 rpm for 40 s. During mixing, 50% sodium hydroxide or 88% lactic acid was added to adjust the pH of the final product to approximately 5.6. Preliminary experiments were conducted to determine the amount of alkali or acid needed to keep the process cheese pH at ~5.6. After mixing, the cheese was stirred at 100 rpm and heated by direct steam injection (87 kPa) for 100 s; after that, indirect steam was used to continue heat-

ing to 80°C. Holding times at 80°C ranged from 0 to 20 min depending on the experimental design. Vacuum pressure (1.3 kPa) was applied during the last 15 s to help remove air bubbles. Cheese was poured into 0.9-kg pouches and stored at 5°C overnight. Process cheese blocks were then repacked, vacuum-sealed, and stored again at 5°C. The textural, rheological, and compositional analyses were determined 7 d after manufacture.

Rheological Measurements

Rheological properties of pasteurized process cheese were measured by dynamic small amplitude oscillatory rheology. A Paar Physica UDS 200 controlled-stress rheometer (Anton Paar, Ashland, VA) with 50-mm serrated parallel plate geometry was used, as described by Lucey et al. (2005). Cheese disks, which were cut to 50 mm diameter and 3 mm in thickness, were heated from 5 to 85°C at a heating rate of 1°C/min. A frequency of 0.08 Hz and a strain of 0.5% were applied to measure the storage modulus (G') and loss tangent (LT).

Rheological Measurements of Hot Process Cheese

Some (~300 g) of the melted process cheese was poured into an insulated stainless steel container after cooking for 10 min (80°C). Cheeses were quickly transported to the rheometer, where the bottom plate and a 50-mm serrated plate probe were already warmed up to 80°C. Some of the molten cheese was transferred to the rheometer plate using a spoon. The 50-mm serrated probe was lowered until a gap of 1 mm was attained. The excess cheese outside the plate was removed by a spatula. Vegetable oil was applied to the outside edges of the sample to help prevent moisture losses. Rheological measurements on melted cheese samples were started exactly 4 min after the cheese was removed from the cooker. A frequency of 0.2 Hz and a strain of 0.3% were applied for 20 min at a constant temperature (80°C) to measure the changes in G' and LT over time.

Acid-Base Titration

Acid-base titrations were performed as described by Hassan et al. (2004). Process cheese homogenates were prepared for titration by dispersing 8.0 g of grated process cheese in 40 mL of distilled water. This dispersion was titrated from its initial pH to pH 3.0 with 0.5 *N* hydrochloric acid and then from pH 3.0 to 8.0 with 0.5 *N* sodium hydroxide. Buffering indices were calculated for each addition of titrant, and buffering curves were prepared by plotting these indices as a function of pH (Hassan et al., 2004).

Texture Profile Analysis and Uniaxial Compression

Texture profile analysis and uniaxial compression were measured using a TA.XT2 Texture Analyzer (Texture Technologies Corp., Scarsdale, NY) equipped with a 25-kg load cell. Samples with a diameter of 16 mm and a height of 17.5 mm were cut from process cheese blocks using a cork borer. For the texture profile analysis test, cheese was compressed to 20% of the original height by a 50-mm aluminum cylinder test probe with a cross-head speed of 0.8 mm/s. For uniaxial compression, cheese was compressed to 80% of original height using the same test probe and cross-head speed as for the texture profile analysis test. All tests were performed at 5°C, and tests were replicated at least 5 times.

Meltability

Meltability was measured by the modified Schreiber test and by the University of Wisconsin Meltprofiler as described by Muthukumarappan et al. [1999a, b (respectively)]. For the Schreiber test, cheese disks of 5 mm thickness and 40 mm in diameter were put on glass petri dishes and heated in an oven at 232°C for 5 min. The area of spread was measured using a computer imaging system, which was equipped with a CCD camera (model VDC 3874; Sanyo, San Diego, CA). For the University of Wisconsin Meltprofiler test, cheese disks of 7 mm thickness and 30 mm in diameter were used. These tests were replicated 4 times.

Soluble Ca and P Content of Cheese

We attempted to express cheese “juice” from process cheese using the technique described by Morris et al. (1988). A small quantity of juice could be obtained from process cheese with the 0.25% tetrasodium pyrophosphate; no juice was obtained with higher ESC. An alternative procedure was developed to quantify water-soluble Ca in process cheese. Cheese juice, by the method of Morris et al. (1988), was prepared from process cheese made with the lowest ESC, and this juice was used for comparison with the soluble Ca content determined from the new water soluble extraction method. Table 1 shows the soluble Ca content in process cheese as a percentage of total Ca content using the cheese “juice” method and new extraction method with various dilution factors. There was no significant difference between the soluble Ca content of cheese juice and a 1:1 dilution (cheese:water). However, when the 1:1 dilution was used, no water extract was obtained from process cheese made with some ($\geq 1.5\%$) ESC. Therefore, the 2:3 dilution (cheese:water) was selected for this extraction method.

Table 1. Soluble Ca as a percentage of total Ca. Comparison between the “cheese juice” method and new water extraction method with various dilution factors ($n = 2$). Cheese used was process cheese with 0.25% tetrasodium pyrophosphate

Cheese:water	Soluble Ca (% of total Ca)
“Cheese juice”	26.1 ^a
1:1	27.3 ^{ab}
1:1.5	30.1 ^{bc}
1:2	31.6 ^c
1:4	63.7 ^d

^{a-d}Means within the same row not sharing a common superscript are different ($P < 0.05$).

Twelve grams of cheese and 18 g of deionized water were added to stomacher bags (31 × 18 cm; Seward Ltd., Norfolk, UK) and homogenized by a Stomacher 400 Circulator (Seward Ltd.) at 260 rpm for 5 min at room temperature. Cheese dispersion was then held in a water bath at 40°C for 60 min. The mixture was transferred to a 250-mL centrifuge tube (Nalgene Brand Products, Rochester, NY) and centrifuged at 3,000 × g for 30 min at 4°C. The top layer (fat) was removed from the tube. The supernatant was filtered through Whatman No. 113 filter paper and stored at 5°C overnight. Because some of the Ca and P in the supernatant could be associated with soluble proteins, a centrifugal UF unit was used (Ultrafree-MC NMWL, 5-kDa cut-off membrane, Millipore Corp., Billerica, MA) to retain all protein-bound Ca and P; the permeate was used to determine the water-soluble Ca and P. Two hundred fifty microliters of the supernatant were added to the membrane unit at room temperature (~24°C), and then it was centrifuged at 4,500 × g for 90 min at room temperature. The Ca and P contents of the cheese and UF permeate (diluted with 0.2 N nitric acid) were measured by inductively coupled argon plasma emission spectroscopy (Vista-MPX Simultaneous ICP-OES, Varian, Inc., Palo Alto, CA). Wavelengths of plasma emission used to measure the Ca and P content were 317.9 and 214.9 nm, respectively. The approach used to estimate the percentage of insoluble Ca and P in process cheese as a percentage of total cheese Ca or P was similar to the approach described by Kuchroo and Fox (1982) for determining the pH 4.6 water-soluble N in cheese. The equation used is as follows:

Insoluble Ca or P (%) in cheese as a percentage of total Ca or P =

$$100 - \left(\frac{\text{moisture content of cheese (\%)}}{100} + D \right) \times \frac{M_p}{M_t} \times 100, \quad [1]$$

where M_p is the concentration of Ca or P in the permeate (mg/100 g of solution), M_t is total amount of Ca or P in cheese (mg/100 g of cheese), and D is the dilution factor, which was 1.5. Tests were replicated as least twice.

Composition Analyses

Cheeses were analyzed for moisture (IDF, 1982), fat (Marshall, 1992), protein (IDF, 1986), and pH by insertion of a pH probe (pH meter 420A, Orion Research, Beverly, MA) into cheese.

Fluorescence Microscopy

The procedure described by Sutheerawattananonda et al. (1997) was used for obtaining micrographs of fat droplets in process cheese. Cheese samples were cut from the center of each cheese block. Samples were sectioned using a sharp razor blade. Cheese sections were stained with 0.1% (wt/vol) Nile Blue A (Sigma-Aldrich, St. Louis, MO) for 1 min, rinsed with milliQ water, and mounted in water. The stained sections were held at 4°C and analyzed within 3 h using a fluorescence microscope (Axioskop 2 plus, Carl Zeiss, Eching, Germany) fitted with a 10× objective (Achromplan 10×/0.25, Carl Zeiss).

Experimental Design and Statistical Analyses

The effects of ESC and holding time on the rheological, physical, and chemical characteristics of process Cheddar cheese were investigated using a central composite rotatable design (Mullen and Ennis, 1979) and response surface methodology (Lucey et al., 1998; Montgomery, 2001). A 2-level factorial experimental design was chosen to study the effects of the independent variables (ESC and holding time) with 2 star points ($\alpha = 1.414$) and 4 replicates of the center point (Table 2). The coded variables were related to the real units by Equations 2 and 3:

$$\text{Coded ESC} = \frac{\text{ESC}(\%) - 1.5}{0.884} \quad [2]$$

$$\text{Coded holding time} = \frac{\text{holding time (min)} - 10}{7.071} \quad [3]$$

Results were analyzed by multiple (stepwise) regression and response surface methodology using the Statgraphics program (version 5.1 plus, Manugistics, Rockville, MD). Dependent variables were estimated using second-order (polynomial) models to provide a good description of the geometric slope of response surfaces. Stepwise regression was used to eliminate insignificant

Table 2. Values of independent variables of each experiment in coded and actual values for the central composite experimental design

Treatment no.	Coded values		Actual values	
	Concentration of ES ¹	Holding time	Concentration of ES (%)	Holding time (min)
1	0	0	1.50	10.0
2	-1	-1	0.62	2.90
3	0	0	1.50	10.0
4	+1	+1	2.38	17.1
5	+1	-1	2.38	2.90
6	0	+ α^2	1.50	20.0
7	0	- α	1.50	0.0
8	- α	0	0.25	10.0
9	0	0	1.50	10.0
10	+ α	0	2.75	10.0
11	-1	+1	0.62	17.1
12	0	0	1.50	10.0

¹ES = Emulsifying salts.² $\alpha = 1.414$.

factors (F -value < 4) using backward selection, which begins with all of the variables in a model and removes them one at a time to simplify the model (Montgomery, 2001).

RESULTS

Composition

The pH values for the various process cheese samples were similar (range = 5.58 to 5.76; mean = 5.67; Table 3). The moisture contents were also similar (range = 38.2 to 40.1; mean = 39.1). Both pH and moisture content influence the texture of process cheese (Templeton and Sommer, 1930, 1932; Lee et al., 1996; Marchesseau et al., 1997); however, the small pH and moisture variation obtained in these experimental cheeses suggested that these 2 factors should not have a major impact on the response variables of this design. Sutherland and Bastian (1998) reported that variations in

the moisture content between 38.6 and 39.8% did not significantly influence the melting transition temperature during the heating of process Cheddar cheese made with TSC or disodium phosphate.

Rheological Properties

The effects of ESC on rheological properties during heating from 5 to 85°C of process cheese made with various concentrations of TSC are shown in Figure 1 (a and b). The rheological properties of the natural Cheddar cheese, used as the raw material for process cheese manufacture, are also shown for comparison purposes. The G' values of all cheeses decreased as cheese temperature increased (Figure 1a). Higher G' values were obtained at all temperatures with higher ESC (e.g., 2.75%). This was especially obvious at higher temperatures (i.e., $\geq 40^\circ\text{C}$). The G' values of natural cheese appeared to exhibit a similar trend to process cheese made with the 1.5% TSC concentration during heating until $\sim 65^\circ\text{C}$. Thereafter, the G' values of natural cheese did not decrease; actually, its value slightly increased at higher temperatures (between 65 and 85°C). A similar profile for Cheddar cheese was recently reported by Udayarajan et al. (2005). Process cheese made with higher ESC had lower LT values between 40 and 55°C (Figure 1b). However, at higher temperatures ($\geq 70^\circ\text{C}$) the LT values were higher for process cheeses made with high ESC. All process cheeses made with TSC had higher maximum LT (**LTmax**) values (from 2.3 to 3.3) compared with the raw material, natural Cheddar cheese (~ 1.5).

The response surface plots for LT values at 50°C and the G' values at 70°C are shown in Figure 2 (a and d, respectively). The corresponding prediction models are

Table 3. Composition of process cheese for each experimental treatment

Treatment no.	pH	Moisture (%)	Fat (%)
1	5.63	40.14	32.52
2	5.66	38.98	32.05
3	5.62	38.72	32.54
4	5.59	38.84	31.62
5	5.58	38.77	31.45
6	5.61	39.60	31.48
7	5.76	38.72	32.20
8	5.74	39.11	32.87
9	5.73	38.80	32.06
10	5.65	38.18	31.75
11	5.75	38.51	32.73
12	5.71	38.54	32.05

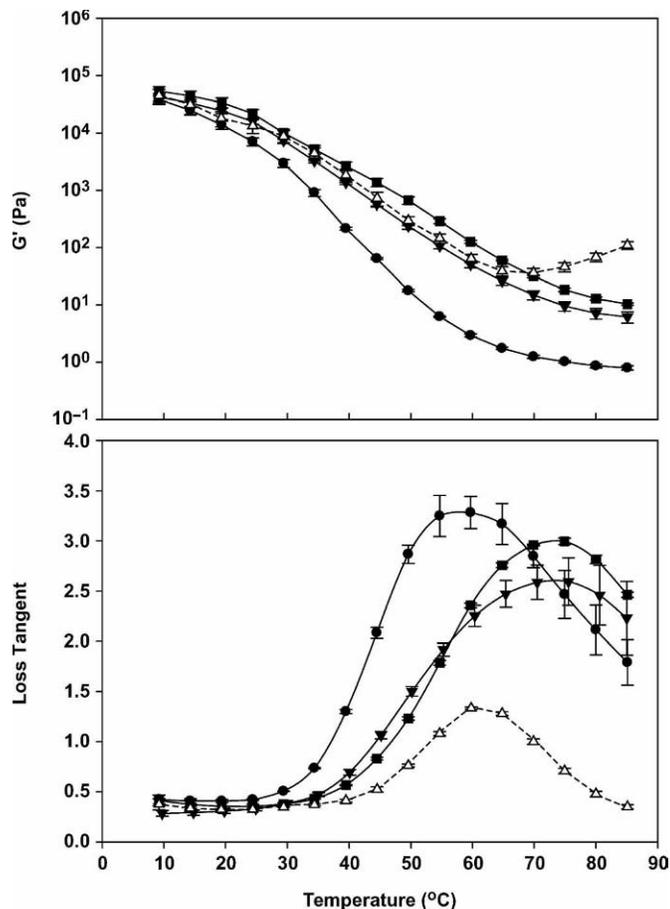


Figure 1. Storage modulus, G' (a), and loss tangent (b), as a function of temperature for process cheeses made with various concentrations of trisodium citrate (TSC) and the natural Cheddar cheese used in process cheese-making (Δ). TSC concentrations: 0.25% (\bullet), 1.50% (\blacktriangledown), and 2.75% (\blacksquare). Holding time was 10 min for all process cheese samples. Data represent means ($n = 3$); error bars represent standard deviations.

shown in Table 4 with R^2 values of 0.98 and 0.80 for the LT value at 50°C and G' value at 70°C, respectively. The prediction model for LT value at 50°C was negatively affected by ESC, and the holding time and the model also included the quadratic term for ESC. The prediction model for the G' value at 70°C had positive ESC and holding time terms. The LT values at other temperatures (e.g., 60°C) were tested, but the LT temperature that gave the prediction model with the highest R^2 was 50°C. The LT values at 50°C increased as ESC decreased or holding times decreased (Figure 2a). The G' values at 70°C increased with increasing ESC and longer holding times (Figure 2d).

Textural Properties

Hardness values derived from compression of cheese to 20% of original height increased with increasing ESC

and longer holding times (Figure 2c). A highly significant prediction model was obtained for hardness and ESC and holding time, the interaction between ESC and holding time and the quadratic term for ESC were all significant terms, and all positively affected the G' value (Table 4). The hardness value of the natural Cheddar cheese sample ($3,539 \pm 235$ g) was higher than any process cheese sample. Chewiness of process cheese showed a similar trend as hardness (Table 4). Uniaxial compression profiles indicated that process cheese made with TSC did not exhibit a distinct fracture (Figure 3). However, as the ESC increased, there was a slight inflection point, and the value for the force at this inflection point increased with increasing ESC, which suggested that some fracture might have occurred during compression.

Meltability

In Figure 4, the changes in cheese height profile during melting, obtained by the University of Wisconsin Meltprofler, are shown for process cheeses made with various concentrations of TSC. The profile for the natural cheese used for process cheese-making is also shown for comparison purposes. Cheese with the lowest ESC (0.25%) initiated flow at a low temperature. Process cheese with the highest ESC (2.75%) exhibited the lowest meltability. In the natural cheese, the time where maximum flow occurred, which is the inflexion point in the curve, was longer than all of the process cheeses made with TSC, even though the final degree of flow value of natural cheese was close to that of process cheese made with 1.5% ES. In Figure 2b, the response surface plot for degree of flow is shown. Meltability (degree of flow) decreased as the ESC increased and holding times increased (Figure 2b). A highly significant ($R^2 = 0.94$) prediction model was obtained for degree of flow; ESC, holding time, and a quadratic term for ESC were the significant terms (Table 4). Schreiber melt area showed a similar trend as the degree of flow, but the prediction model had a lower R^2 value (0.66) (Table 4). In the prediction models for degree of flow and Schreiber melt, the ESC and holding time were negative terms, indicating that meltability decreased with increasing ESC and longer holding times.

Insoluble Ca and P

The response surface plots for insoluble Ca as a function of total Ca in cheese is shown in Figure 2f. A highly significant ($R^2 = 0.86$) prediction model was obtained for insoluble Ca; ESC, holding time, and the quadratic term for holding time were all significant terms (Table 4). As ESC increased, the percentage of insoluble Ca

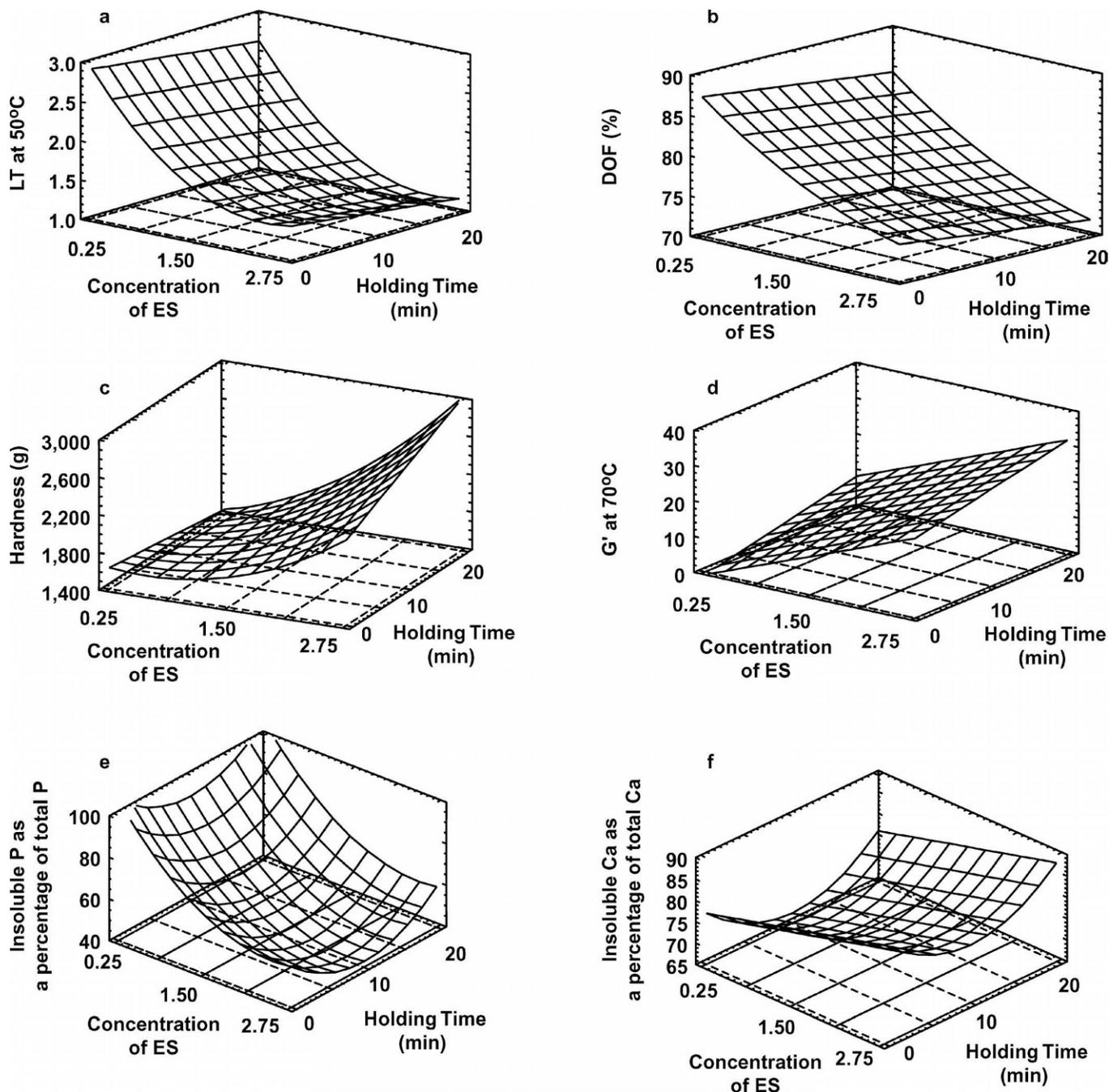


Figure 2. Response surface plots for the effect of concentrations of emulsifying salts (ES) and holding times on loss tangent (LT) at 50°C (a), degree of flow (DOF) at 60°C from the University of Wisconsin Meltprofiler (b), hardness (c), storage modulus (G') at 70°C (d), insoluble P as a percentage of total P in process cheese (e), and insoluble Ca as a percentage of total Ca (f).

as a function of total Ca content increased. Holding time exhibited a more complex trend, i.e., the value decreased during short holding times but increased at long holding times. The response surface plot for the percentage of insoluble P as a function of total P is shown in Figure 2e. The insoluble P content decreased

as ESC increased. The effect of holding time on insoluble P content exhibited a similar trend to insoluble Ca. These results suggest that TSC dispersed the residual colloidal Ca phosphate (CCP) and released phosphate into the serum phase of cheese. The increase of insoluble Ca content with increasing ESC may be due to the

Table 4. Second-order polynomial models describing loss tangent (LT) at 50°C, degree of flow (DOF) at 60°C from University of Wisconsin-Meltprofiler, hardness, storage modulus (G') at 70°C, insoluble P as a percentage of total P, insoluble Ca as a percentage of total Ca, and Schreiber melt area. ESC = emulsifying salt

Dependent variable	Independent variable	Coefficient	R ² (adjusted) ¹	P
LT at 50°C	Constant	1.532	0.98	<0.001
	ESC***	-0.5296		
	Holding time**	-0.09558		
	ESC ² ***	0.2592		
DOF	Constant	77.81	0.94	<0.001
	ESC***	-4.446		
	Holding time*	-0.8927		
	ESC ² †	0.8539		
Hardness	Constant	1850	0.95	<0.001
	ESC***	401.5		
	Holding time*	95.02		
	ESC × holding time*	114.2		
	ESC ² , *	128.7		
Chewiness	Constant	1204	0.97	<0.001
	ESC***	287.5		
	Holding time*	56.55		
	ESC × holding time*	80.94		
G' at 70°C	Constant	15.59	0.80	<0.001
	ESC***	8.370		
	Holding time*	3.574		
	ESC ² *	7.877		
Insoluble P as a percentage of total P	Constant	51.00	0.82	<0.001
	ESC***	-16.88		
	ESC ² *	7.877		
	Holding time ² *	8.572		
Insoluble Ca as a percentage of total Ca	Constant	73.04	0.86	<0.001
	ESC**	4.150		
	Holding time*	1.785		
	Holding time ² ***	4.564		
Schreiber melt area	Constant	26.81	0.66	<0.005
	ESC**	-3.897		
	Holding time*	-2.054		

¹R² values were adjusted for the degree of freedom.

†P < 0.1, *P < 0.05, **P < 0.01, and ***P < 0.001.

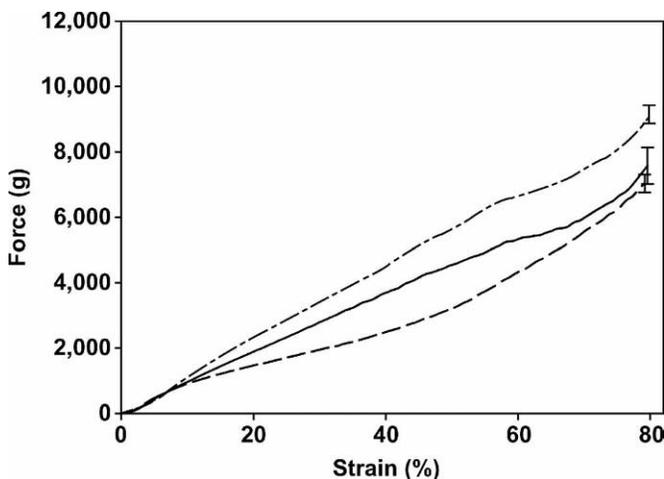


Figure 3. Force-strain curves during uniaxial compression of process cheeses to 20% of the original height. Cheeses were made with various concentrations of trisodium citrate: 0.25% (—●—), 1.50% (—▲—), and 2.75% (—■—). Holding time was 10 min for all process cheese samples. Data represent means (n = 5); error bars represent standard deviations.

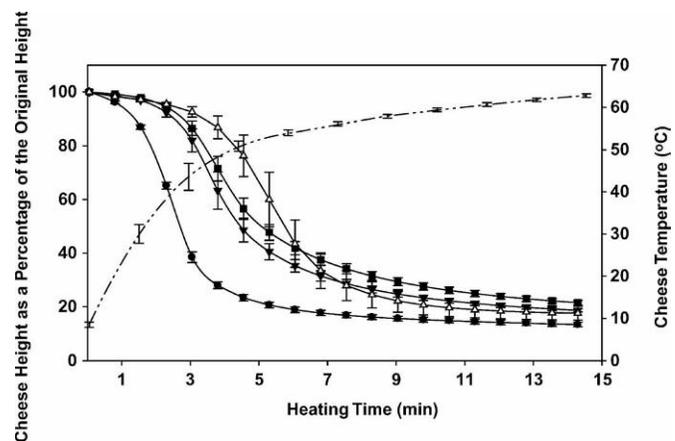


Figure 4. Changes in cheese height as a percentage of the original height from the University of Wisconsin Meltprofiler for process cheese made with various concentrations of trisodium citrate and natural Cheddar cheese used as an ingredient (Δ). Trisodium citrate concentrations: 0.25% (●), 1.50% (▼), and 2.75% (■). Cheese temperature is represented by —●—, data represent means (n = 3); error bars represent standard deviations.

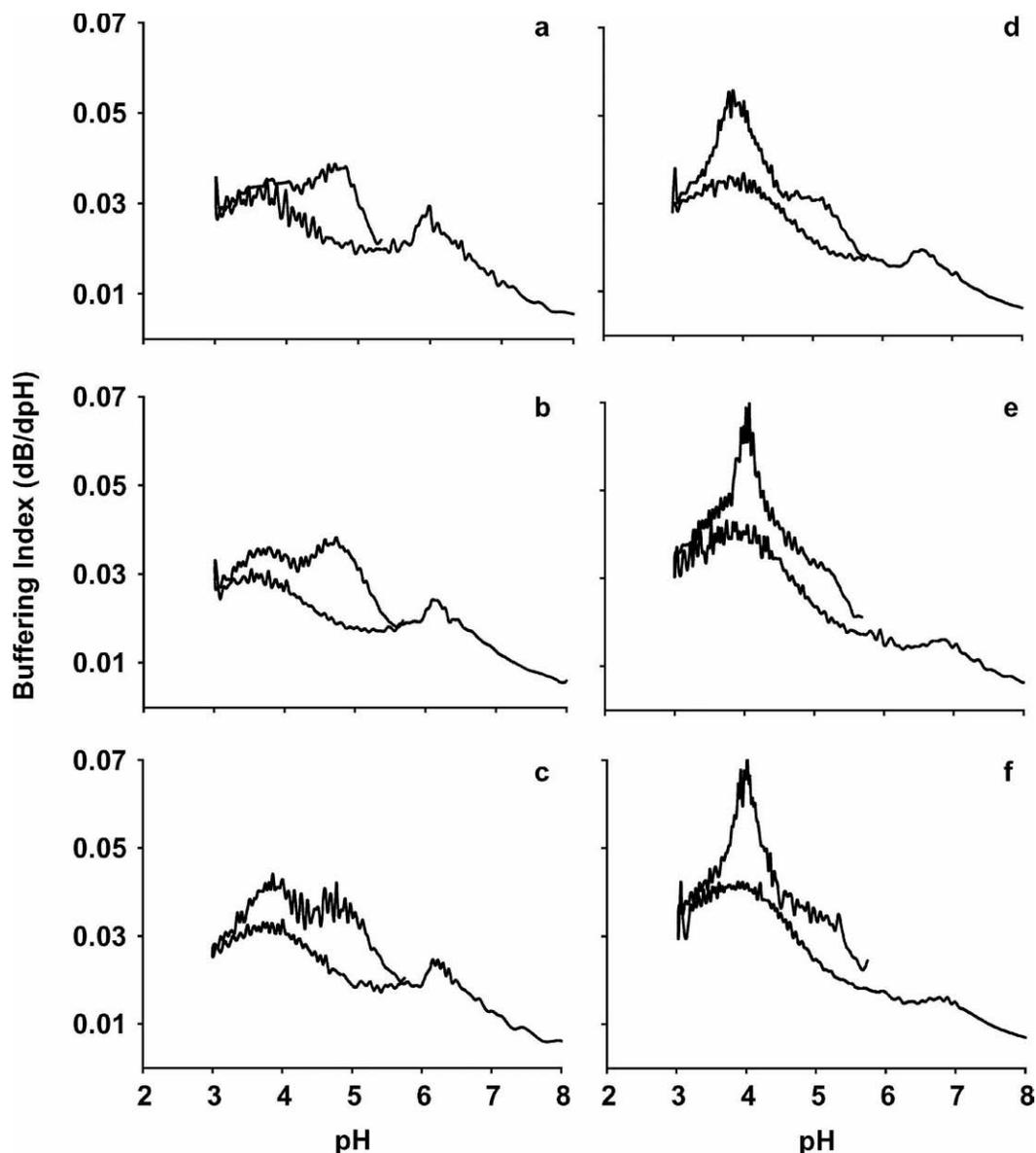


Figure 5. Acid-base buffering curves for natural Cheddar cheese as the cheese-making ingredient (a) and process cheeses made with various trisodium citrate concentrations: 0.25% (b), 0.62% (c), 1.50% (d), 2.38% (e), and 2.75% (f).

formation of some type of insoluble Ca citrate. A highly significant ($R^2 = 0.82$) prediction model was obtained for insoluble P, and ESC and the quadratic terms for ESC and holding time were significant terms (Table 4).

Acid-Base Buffering Curves

Changes in the acid-base buffering curves were observed for process cheese made with different concentrations of TSC (Figure 5). No significant differences were observed for cheeses made with various holding times (results not shown). The buffering curve of natu-

ral cheese is shown in Figure 5a. In natural cheese, a peak was observed at pH ~4.8, which was due to the solubilization of residual CCP (Lucey and Fox, 1993; Hassan et al., 2004). During back-titration with base, the peak of pH ~4.8 was absent, and the buffering peak appeared at pH ~6.0, which was due to the formation of insoluble Ca phosphate (Lucey et al., 1993). At the lowest concentration of TSC, the area of peak at pH ~4.8 was slightly higher than that of natural cheese (Figure 5b). The buffering peak of pH 4.8, observed during acid titration, decreased with increasing ESC, and a new peak at pH ~4.0 concomitantly appeared.

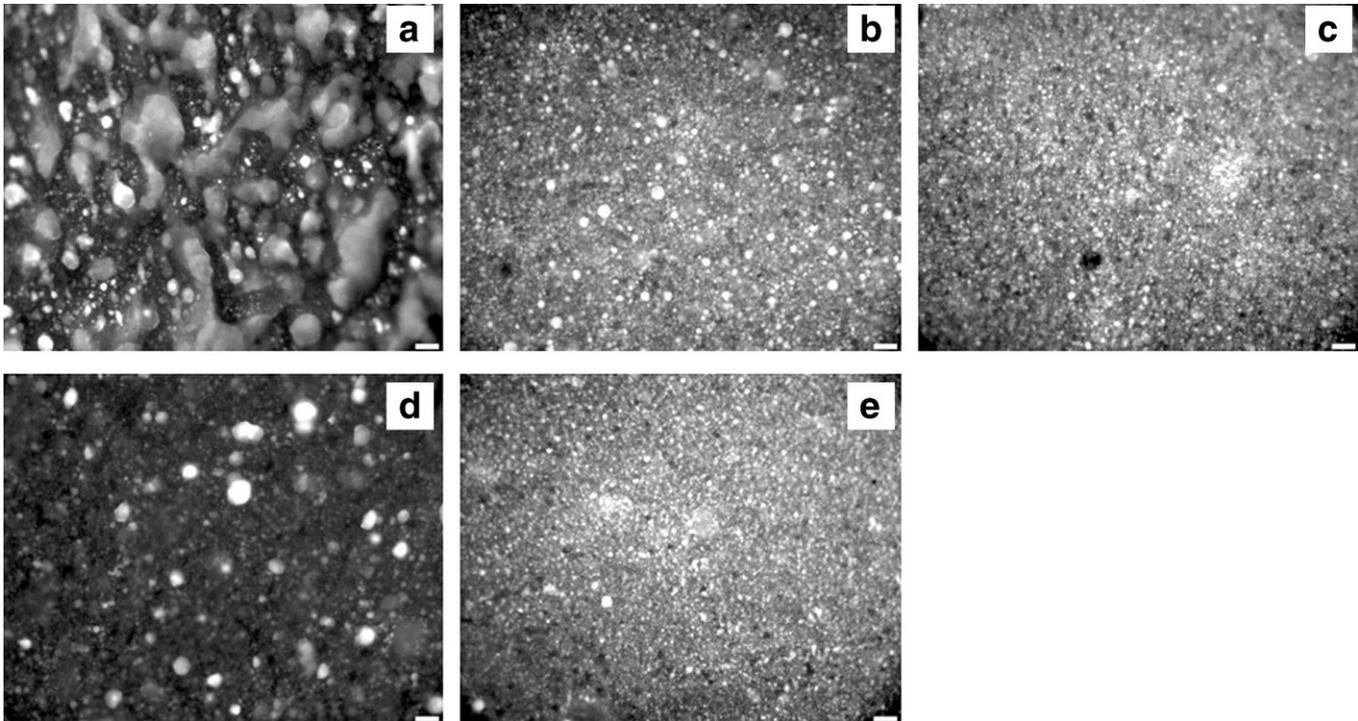


Figure 6. Fluorescence micrographs showing fat droplets as light areas against a dark background (protein) for process cheese made with various concentrations of trisodium citrate (TSC) and with the same holding time (10 min). TSC concentrations: 0.25% (a), 1.50% (b), and 2.75% (c) or cooked for various holding times and the same TSC concentration (1.50%). Holding times: 0 min (d), 10 min (b), and 20 min (e). Scale bar = 50 μ m.

Cheese made with $\geq 2.38\%$ TSC did not have any peak at pH 4.8, which suggested that CCP was no longer present (i.e., it was solubilized; Figure 5e, f). The absence of the peak at pH 4.8 was probably due to the chelation of Ca from CCP by TSC and the formation of Ca citrate, which probably resulted in the appearance of the new peak at pH ~ 3.8 to 4.0, as was described recently by Mizuno and Lucey (2005).

Fluorescence Microscopy

Micrographs of the fat globules (white droplets) in process cheese made with various concentrations of TSC and holding times are shown in Figure 6. Large, coalesced pools of fat were observed at the lowest concentration TSC (Figure 6a). With the addition of 2.75% ES, the fat appeared to be very well emulsified and formed small, round droplets (Figure 6c). In process cheese made with 1.50% ES and no holding time (i.e., removed from cooker when cheese reached 80°C), large fat droplets were observed (Figure 6d). When cheese made with 1.5% TSC was given a longer holding time, the fat droplets became very small fine droplets (Figure 6e), which is in agreement with the results reported by Sutheerawattananonda et al. (1997).

DISCUSSION

Increasing the concentration of TSC used in process cheese resulted in an increase in hardness, a decrease in G' at 70°C , and lower meltability (Figure 2). Gupta and Reuter (1993) reported that the use of higher concentrations of TSC for process cheese food resulted in higher firmness and lower meltability of the product (pH not reported). Gupta et al. (1984) reported that the firmness of process Cheddar cheese increased with an increase in the concentration of TSC (1.2 to 2.1%; pH ~ 5.8). In contrast, Cavalier-Salou and Cheftel (1991) reported that the firmness of a process cheese analog made with TSC decreased with increasing concentrations of TSC (0 to 3%). These analog cheeses were made from sodium caseinate and butter oil, and the pH varied considerably (pH 6.1 to 6.8). pH values were not similar to pasteurized process Cheddar cheese. Sutheerawattananonda et al. (1997) reported that process cheese made with 2% NaCl (without TSC as an ES) had a firm texture. In our study, process cheese made with the lowest concentration (0.25%) of TSC was soft. Cheese with only 2% NaCl (Sutheerawattananonda et al., 1997) probably had a low pH (close to the natural cheese pH). It is well known that pH has a major impact on process cheese

texture (Marchesseau et al., 1997; Lee and Klostermeyer, 2001); therefore, this differences in pH is one reason for some of the conflicting results from previous studies.

There are 2 possible mechanisms that could explain why high concentrations of TSC resulted in process cheese with higher hardness and lower meltability compared with cheese made with low concentrations of TSC. The 2 mechanisms are improved fat emulsification and greater dispersion of CN (although these are related, as this process cheese system contains fat and CN dispersion facilitates fat emulsification).

Poorly emulsified fat globules were obvious at very low ESC (0.25%; Figure 6a). During the heating or cooking of natural cheese, fat droplets are released from the cheese matrix and coalesce with each other (Paquet and Kaláb, 1988; Guinee et al., 2000). In process cheese, soluble CN probably cover these fat globules, and emulsification is aided by the mechanical shearing during cooking. Emulsified fat covered with a CN interface may behave like large particles of protein (pseudo-protein particles), which are actively incorporated into CN networks (Walstra and Jenness, 1984). Emulsified fat globules in process cheese are generally thermo-stable as reflected by their low tendency to coalesce on reheating (Guinee et al., 2000). The poor emulsification observed for the lowest ESC (Figure 6a) resulted in cheese that had less active structural reinforcement from the protein-stabilized fat globules. This could have contributed to this cheese having the lowest hardness and highest meltability (Figure 2). This phenomenon is similar to the increased stiffness and viscosity of whole milk yogurt gels with increasing homogenization pressure; the greatly increased membrane area of CN forming the new artificial fat globule membrane actively reinforces the CN gel network (Tamime and Robinson, 1999).

Casein dispersion, caused by the mechanical and thermal energies applied to the cheese mass, is another important event during process cheese manufacture (Glenn et al., 2003). During the cooking stage, there are several different physicochemical reactions probably occurring in the CN matrix that aid dispersion including reduced contact between CN molecules with the increase in hydrophobic interactions and the weakened strength of certain types of interactions (e.g., hydrogen bonding; Lucey et al., 2003). Paquet and Kaláb (1988) reported that shrinkage of protein network was observed in heated Mozzarella cheese using scanning electron microscopy; something similar may occur in process cheese during cooking. The use of ES greatly aids in the solubilization of CN; process cheese has a higher soluble CN content than the natural Cheddar cheese used in its manufacture (Templeton and Sommer, 1936). In the presence of TSC, CN are solubilized and

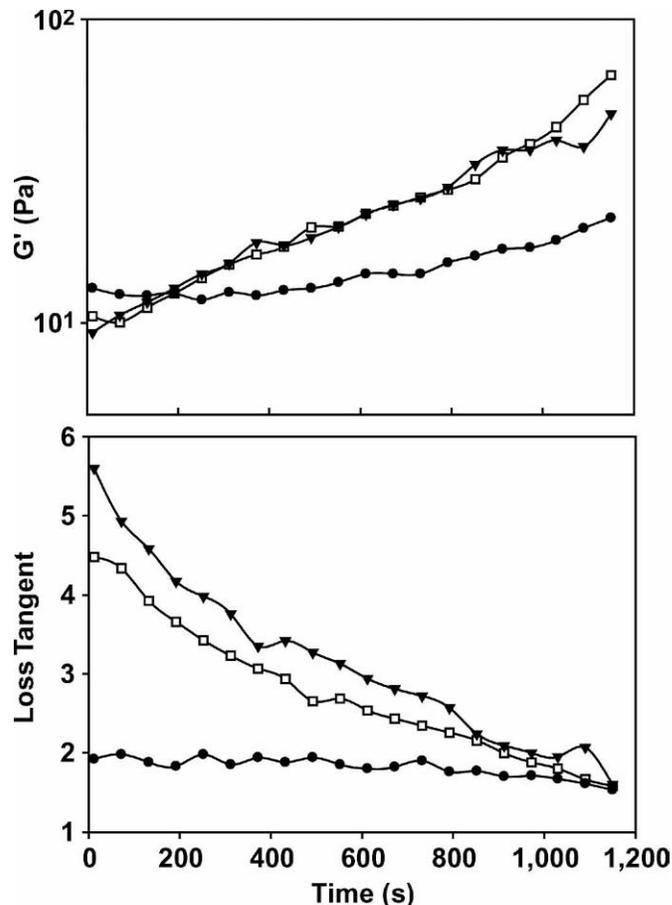


Figure 7. Storage modulus, G' (a), and loss tangent (b) for melted process cheeses made with various concentrations of trisodium citrate (TSC): 0.25% (\bullet), 1.50% (\square), and 2.75% (\blacktriangledown) during holding at 80°C. The data for 1.50% TSC level represent means ($n = 4$). Other curves are single tests. Rheological measurements were started 4 min after process cheese was removed from a cooker where it was heated at 80°C for 10 min.

dispersed (Berger et al., 1998) probably because of the loss of Ca from the CCP cross-links caused by chelation of Ca by TSC and enhanced electrostatic repulsion between the polyvalent anions of the ES and CN. Evidence supporting an increase in CN dispersion during process cheese manufacture with increasing TSC concentration is shown in Figure 7. The rheological properties of hot process cheese (80°C) were tested after holding at 80°C for 14 min. Very high values for the LT were observed in cheeses with high levels of TSC (Figure 7), indicating greater fluidity probably because of greater CN dispersion.

However, some other types of protein-protein interactions may occur in process cheese during cooking and during cooling. Lee et al. (2003) suggested that, during cooking, protein-protein associations occur via their ex-

posed nonpolar groups. In the process cheese system, the charged groups on CN molecules are probably exposed because the strong polyvalent anions from the ES break intramolecular interactions, such as hydrogen bonds, with mechanical and heat energies. This process may create the potential for new bonds or interactions between CN molecules. Caseins reassociate or repolymerize during cooling and form a new network between CN via hydrophobic, electrostatic interactions, hydrogen bonds, or some other types of interactions. It is possible that greater dispersion or exposure of groups on CN helps in the creation of a stiffer, less meltable matrix once cooled. Some supporting evidence for this suggestion can be seen in Figure 7, in which the use of high TSC levels resulted in process cheese with a higher rate of increase in G' compared with cheese made with low TSC concentration.

Trisodium citrate is well known as an effective Ca chelating agent, and its addition to milk results in disruption of the CN micelles (e.g., Walstra and Jenness, 1984). Mizuno and Lucey (2005) demonstrated that CN-bound Ca and CN-bound inorganic P decreased with increasing levels of TSC added to a milk protein concentrate solution. They concluded that TSC is a chelator of Ca and that the added citrate was not associated with CN. Moreover, Udayarajan et al. (2005) suggested that the increase in G' value of natural Cheddar cheese at high temperature (Figure 1a) could be due to heat-induced formation of additional Ca phosphate cross-links between CN. We did not observe any increase in the G' values of TSC cheese (Figure 1a). It is possible that the added citrate chelated the available Ca and hindered the formation of heat-induced Ca phosphate. In process cheese, it is also likely that TSC disrupted the residual CCP as was demonstrated by the decrease in insoluble P with increasing TSC concentration (Figure 2e). The solubilized Ca that was released from the residual CCP appeared to have associated with the added citrate, resulting in the formation of a new buffering peak at ~ 4.0 (Figure 5) and an increase in the insoluble Ca content as a function of total Ca (Figure 2f). Calcium citrate has a low solubility in water (Chatterjee and Dhar, 1924), and it is possible that the solubility for Ca citrate in the serum phase of process cheese may have been exceeded. Pastorino et al. (2003) attempted to measure the soluble Ca and P content in natural cheese to which a TSC buffer solution was injected using high pressure. They reported that the bound P content of cheese decreased with an increase in the number of injections. However, soluble Ca content decreased with an increased number of injections. Pastorino et al. (2003) suggested that this was probably due to the formation of some type of Ca crystal in the cheese. It has been reported that the use high levels of TSC and

high pH values may cause a "bloom" of Ca citrate crystals in process cheese (Scharpf and Kichline, 1969). However, we did not observe any "bloom," which is a growth of crystals on the surface during storage, even at the highest ESC. There have been reports of $\sim 10\text{-}\mu\text{m}$ needle-like Ca citrate crystals in process cheese made with TSC (Rayan et al., 1980; Caric et al., 1985). Although further study is needed relative to the state of citrate in process cheese, it is possible that Ca citrate might have been trapped within the matrix as very small crystals that were not visible without some specific microscopic analysis.

In the University of Wisconsin Meltprofiler test, the natural cheese started to flow at a higher temperature compared with the process cheeses (Figure 4). This result was probably because natural Cheddar cheese has a stronger protein matrix than process cheese (as also indicated by its higher hardness values). Natural cheese still has its indigenous CCP cross-linking materials, whereas process cheeses made with TSC have lost most of their native CCP cross-links and the CN were dispersed during cooking. The degree of flow of process cheese decreased with increasing holding time (Figure 2), and smaller fat droplets were also observed with longer holding times (Figure 6). Holding time enhanced fat emulsification and CN dispersion, both of which could potentially contribute to reduced meltability.

The effect of holding time on insoluble Ca content (Figure 2f) is difficult to explain. The 10-min holding time resulted in the lowest insoluble Ca content. It is possible that at very short holding times, there was insufficient shear and time to adequately mix the cheese mass and TSC; thus, TSC did not chelate all of the Ca from CCP, which it could do with a 10-min holding time. As a result, the insoluble Ca content remained high at very short holding times. Very efficient mixing occurred by ~ 10 min, and Ca ions might dissolve in the serum phase, decreasing the insoluble Ca content; further holding time might have allowed sufficient time for the formation of Ca citrate complexes, which could reduce the serum Ca concentration.

CONCLUSIONS

The concentration of TSC used as an ES in pasteurized process Cheddar cheese manufacturing greatly affected the textural and melting properties, even when these process cheeses had a similar pH value. The added TSC appeared to dissolve the residual CCP present in cheese and helped to disperse the CN network during cooking. A small quantity of TSC (0.25%) was not enough to efficiently disperse the CN network, even with long holding times during cooking; consequently, fat was poorly emulsified, and the process cheese did not

form a firm protein network. Holding times increased hardness but needed a certain minimum TSC concentration to alter hardness. Response surface plots facilitate the visualization of how TSC concentrations of TSC and cooking times altered the functionality of pasteurized process Cheddar cheese. The results of this study will assist process cheese manufacturers in understanding the role of TSC as an ES and demonstrates the impact of ESC and holding time on process cheese functionality.

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