Effect of Formulation and Manufacturing Parameters on Process Cheese Food Functionality—I. Trisodium Citrate

S. K. Garimella Purna, A. Pollard, and L. E. Metzger¹

Minnesota–South Dakota Dairy Foods Research Center, Department of Food Science and Nutrition, University of Minnesota, St. Paul 55108

ABSTRACT

The objective of this research was to use a Rapid Visco Analyzer to study the effect of natural cheese age, trisodium citrate (TSC) concentration, and mixing speed on process cheese food (PCF) functionality. In this study 3 replicates of natural cheese were manufactured, and a portion of each cheese was subjected to 6 different PCF manufacturing treatments at 2, 4, 6, 12, and 18 wk of ripening. These treatments were factorial combinations of 3 levels of TSC (i.e., 2.0, 2.5, and 3.0%) and 2 mixing speeds during manufacture (450 and 1,050 rpm). Functional properties of the PCF evaluated included manufacturing properties [apparent viscosity after manufacture (VAM)], unmelted textural properties (firmness), melted cheese flow properties [hot apparent viscosity (HAV)], and cheese thickening during cooling [time at 5000 cP (T5)]. All 4 parameters (VAM, firmness, HAV, and T5) were significantly affected by natural cheese age and mixing speed, whereas VAM, HAV, and T5 were also significantly influenced by the amount of TSC. The VAM and firmness decreased as cheese age increased, whereas T5 values increased as cheese age increased. Similarly, VAM, HAV, and firmness values increased because of the increased mixing speed, whereas T5 values decreased. The age × mixing speed interaction was significant for VAM and firmness. The age × concentration of the TSC interaction term was significant for VAM, whereas the age × age × TSC concentration term was significant for HAV. The results demonstrate that natural cheese age, mixing speed during manufacture, and concentration of TSC have a significant impact on process cheese functionality.

Key words: process cheese, functionality, unmelted texture, emulsifying salts

INTRODUCTION

Process cheese is used as an ingredient in many applications, such as a slice on a burger or a dip for snacks.

Consequently, it is important to control process cheese functional properties (i.e., texture, melt, and flow) that are inherent to its proper functioning as an ingredient. The functional properties of process cheese are determined by the ingredients used in the formulation (i.e., type of natural cheese, age of natural cheese, amount of natural cheese, type and amount of emulsifying salt) as well as processing conditions (i.e., cooking temperature, cooking time, and mixing speed during manufacture).

The effect of natural cheese characteristics on process cheese functionality is largely attributed to the level of intact casein present in the natural cheese (Berger et al., 1998). Intact casein is the amount of casein that has not been hydrolyzed by enzymes during ripening. It is generally expressed in terms of relative casein content, a ratio of the amount of unhydrolyzed casein nitrogen to the total nitrogen in the cheese (Berger et al., 1998). During ripening, the protein present in natural cheese is progressively hydrolyzed, thereby decreasing the intact casein content of the natural cheese. Intact casein forms the structural network of process cheese, and a high level of intact casein indicates the potential for extensive protein–protein and protein–fat interactions (Berger et al., 1998). Consequently, process cheese made from excessively ripened natural cheese (i.e., with lower intact casein levels) is soft, whereas process cheese made from unripened cheese (i.e., with a higher level of intact casein) is very firm (Templeton and Sommer, 1930).

In addition to natural cheese age, other important formulation parameters that influence process cheese functionality include the type and amount of emulsifying salt (Thomas, 1973). The purpose of the emulsifying salt is to chelate calcium from the natural cheese paracasein network, thereby solubilizing the protein. Subsequently, solubilized caseins are free to interact with the lipid and water phase, which results in the formation of an emulsion (Caric et al., 1985). Emulsifying salts have different calcium-chelating properties that influence the final emulsion properties (Gupta et al., 1984). The Code of Federal Regulations (U.S. Department of Health and Human Services, 2003) identifies 13 emulsifying salts that can be used in making process cheese

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¹Corresponding author: lmetzger@umn.edu

products. However, most process cheese products are manufactured with trisodium citrate (**TSC**), disodium phosphate (**DSP**), or a combination of both. Disodium phosphate is primarily used in loaf applications that require storage at room temperature, whereas TSC is primarily used in slice applications in which the process cheese is cooled with a chill belt. Numerous studies have been conducted to compare and contrast the effects of TSC and DSP on process cheese texture and melt properties (Caric et al., 1985; Cavalier-Salou and Chef-tel, 1991; Sutherawattananonda and Bastian, 1998). In general, process cheese made with DSP is characterized by larger fat pockets, a grainy texture, and a weaker body, as compared with process cheese made with TSC (Templeton and Sommer, 1936; Rayan et al., 1980). Additionally, the amount of emulsifying salt has been reported to influence process cheese flow properties (Templeton and Sommer, 1936). However, more recent experiments have reported that no significant changes in the body and textural properties result from an increase in TSC concentration from 2 to 4% (Thomas et al., 1980).

In addition to formulation parameters, the temperature and mixing conditions used during manufacture also influence the functional properties of process cheese. Previous researchers have demonstrated that fat particle size distribution is affected by the mixing speed and temperature during manufacture (Rayan et al., 1980; Glenn et al., 2003; Lee et al., 2003). Under a constant cooking time and temperature, a higher mixing speed results in a larger number of small, evenly distributed fat globules, as compared with a low mixing speed (Lee et al., 2003). It is theorized that a large number of small, evenly distributed fat globules enhance fat-protein and protein-water interactions and thus facilitate the formation of a stronger network (Drake, 1973; Lee et al., 2003). However, increasing the mixing speed beyond certain limits will enhance fat-protein and protein-protein interactions to an extent that the casein molecules coagulate into a "pudding-like" structure. This phenomenon is generally referred to as "overcreaming" and is indicated by an increase in viscosity and reduction in meltability (Drake, 1973; Berger et al., 1998; Glenn et al., 2003).

Although many studies have been conducted on the effects of formulation and processing parameters on process cheese quality, these studies have been limited and have typically evaluated the effect of 1 or 2 parameters owing to limitations imposed by manufacturing techniques, raw material requirements, or time constraints. Moreover, it is difficult to choose a particular cooker design to simulate the effect of all the potential manufacturing parameters (Berger et al., 1998). Consequently, a small-scale manufacturing system, with the

ability to vary the manufacturing profile, would be desirable for studying the effect of various parameters on process cheese functionality.

Recently, an instrument called the Rapid Visco Analyzer (**RVA**; Newport Scientific Pvt. Ltd., Warriewood, Australia) has been used to manufacture process cheese on a small scale (Metzger et al., 2002; Kapoor et al., 2004; Kapoor and Metzger, 2005). The RVA is a computer-integrated manufacturing instrument that can be used to determine the apparent viscosity of food products during manufacture using a sample size of approximately 25 g. Its versatility lies in its ability to vary cooking time, cooking temperature, and mixing speed. In a study by Kapoor et al. (2004), significant differences in textural and melt properties were reported between process cheese samples manufactured on a pilot plant scale and the RVA. The differences between the samples were attributed to manufacturing and sampling techniques. Appropriate modifications in the sampling and manufacturing techniques were reported in a subsequent study (Kapoor and Metzger, 2005). Furthermore, Lai et al. (2000) have suggested that the RVA could be used to characterize the rheological properties of non-Newtonian fluids. Subsequently, researchers (Rosenberg and Metzger, 2003; Biswas et al., 2005; Prow and Metzger, 2005) have demonstrated that the RVA can be used to manufacture process cheese and subsequently analyze its melt properties.

The objective of this study was to determine the effect of the age of natural cheese, the amount of emulsifying salt, and the mixing speed during manufacture on process cheese food (**PCF**) functionality.

MATERIALS AND METHODS

Experimental Design and Statistical Analysis

A split-plot design was used to study the effect of natural cheese age, concentration of TSC, and mixing speed during manufacture on PCF functionality. Three replicates of natural cheese were manufactured, and a portion of each cheese was subjected to 6 different treatments at 2, 4, 6, 12, and 18 wk of ripening. These treatments were factorial combinations of 3 levels of TSC (2.0, 2.5, and 3.0%) and 2 mixing speeds during manufacture (450 and 1,050 rpm). Five replicates of PCF were manufactured for each treatment. Statistical analysis was performed by treating natural Cheddar cheese replicates as blocks, age as the whole-plot factor, and 6 different treatments as split-plot factors.

Age of natural cheese was considered a continuous variable and was analyzed for linear effects, quadratic effects, and deviations from the quadratic effects. Multicollinearity of the age terms (continuous variables) was reduced by using "mean-centered" data. Mean-center-

Table 1. Blend formulations used to manufacture process cheese food

Ingredient	Replicate 1	Replicate 2	Replicate 3
Cheddar cheese, %	78.99	79.09	75.51
Water, %	14.40	14.40	15.05
Skim milk powder (nonfat dry milk), %	5.39	5.39	8.70
Salt, %	0.82	0.77	0.74
Butter oil, %	0.40	0.35	0.00

ing of continuous variables involves subtracting the mean from each of the individual time points during ripening (Glantz and Slinker, 2001). Furthermore, separate pairwise comparison tests were performed, using least significance difference (**LSD**), for each functional property at each time point across all treatments, and also for each treatment across all time points. Macanova 4.12 (School of Statistics, University of Minnesota, Minneapolis) was used to perform all statistical analyses. The level of significance was $P < 0.05$ throughout the paper.

PCF Formulations

A blend was prepared by mixing all the ingredients, except TSC, in a Blentech twin-screw process cheese cooker (Blentech Corporation, Rohnert Park, CA) at 120 rpm. The blend formulation developed with each of the 3 natural cheese replicates is shown in Table 1. Each blend formulation was developed using Techwizard, an Excel-based formulation program provided by OWL Software (Lancaster, PA). The formulation program was used to balance the salt, moisture, and fat for each replicate to 1.8, 44.0, and 25.0%, respectively. The 3 replicates of natural cheese used for this study were manufactured at the University of Minnesota pilot plant facility and were analyzed for fat using the Mojonnier method (Atherton and Newlander, 1977), for moisture content using a forced-draft oven method (Bradley and Vanderwarn, 2001), for salt content using a Corning 926 chloride analyzer (Corning Glass Works, Medfield, MA), and for pH using a Corning pH/ion meter model 450 (Corning Glass Works) fitted with a Thermo Orion combination pH probe (Thermo Electron Corporation, Louisville, CO). Additionally, the intact casein of the natural cheese at 2, 4, 6, 12, and 18 wk of ripening was determined by measuring the amount of pH 4.6 soluble nitrogen and subtracting it from the total nitrogen (Bynum and Barbano, 1985). Other ingredients in the formulation were nonfat dry milk (low heat; Dairy America, Fresno, CA), sodium chloride salt (Cargill Inc., MN), anhydrous butter oil (MidAmerica Farms, Springfield, MO), trisodium citrate duohydrate (Archer Daniels Midland Company, Decatur, IL), and water.

PCF Manufacture

The PCF were manufactured with an RVA as described by Kapoor and Metzger (2005). Samples were prepared in RVA canisters according to the blend formulations in Table 1, and TSC was added at rates of 0.5, 0.625, and 0.75 g into 24.5, 24.325, and 24.25 g of process cheese blend, respectively. This corresponded to 2.0, 2.5, and 3.0% TSC, respectively. An additional 0.5 g of water was added to each RVA canister to compensate for moisture loss during manufacture. The RVA canister was held in a water bath at 40°C for 7 min prior to manufacture. The manufacturing profile included holding the RVA canister at 85°C during the entire period of manufacture and subjecting the contents to 1 of 2 mixing speeds (450 rpm for low and 1,050 rpm for high) for 6 min, and then a mixing speed of 160 rpm for 1 min. The 450 and 1,050 rpm mixing speeds used during the first 6 min of manufacture were chosen based on previous research, which concluded that a mixing speed of 450 rpm in the RVA produced process cheese that was similar to process cheese produced in a twin-screw pilot-scale process cheese cooker operating at 140 rpm (Kapoor and Metzger, 2005). Consequently, the 450 rpm mixing speed represents a typical mixing speed used to manufacture process cheese, whereas 1,050 rpm represents a higher than normal mixing speed. Each mixing speed and TSC combination was manufactured 5 times. Immediately after manufacture, the PCF samples were transferred to electroplated copper cylinders (20 mm in diameter and 30 mm in height). The cylinders were wrapped in Reynolds food service film (Nogg Chemicals and Paper, Omaha, NE) and were stored in a refrigerator (4°C) for 18 h prior to further analysis. A preliminary study (data not shown) was conducted to ensure that there was no difference in the moisture content of the PCF manufactured using the various mixing speeds and TSC concentrations utilized in this study. The pH of each process cheese was determined 24 h after manufacture and was between 5.65 and 5.75 for all formulations at all ripening times (data not shown).

PCF Functionality Analysis

The functional properties evaluated included manufacturing properties [apparent viscosity after manufac-

ture (VAM)], unmelted textural properties (firmness), melted cheese flow properties [hot apparent viscosity (HAV)], and cheese thickening during cooling [time at 5,000 cP (T5)].

Apparent viscosity after manufacture values for all PCF samples were determined during manufacture in the RVA as described by Kapoor and Metzger (2005). These values are a measure of process cheese apparent viscosity immediately after manufacture and were determined at 160 rpm, which corresponds to a shear rate of 53.6 s^{-1} . For unmelted texture analysis, the PCF samples were removed from the electroplated copper cylinders (20 mm diameter) and cut to a height of 20 mm, and texture profile analysis was performed at 4°C . The texture profile analysis was performed using a TA.XT2 texture analyzer (Texture Technologies Corp., Scarsdale, NY/Stable Microsystems, Godalming, UK). A uniaxial single-bite compression was performed with a 50-mm cylindrical flat probe (TA-25) at 80% compression with a cross-head speed of 0.8 mm/s. For the HAV and T5 analyses, the PCF samples were ground to a particle size of 2 to 3 mm and analyzed with the RVA melt test, as described by Kapoor and Metzger (2005). In the RVA melt test, a 14-g sample of the ground process cheese was weighed into an RVA canister, along with 1 g of propylene glycol. Subsequently, the RVA melt test was performed by raising the temperature of the canister from 25°C to a peak temperature of 85°C in 5 min, holding for 3 min at the peak temperature, and finally cooling to 25°C in 6 min. The stirring speed was held at 0 rpm for 30 s, 20 rpm for 30 s, 100 rpm for 1 min, and 300 rpm for the remainder of the test. The minimum apparent viscosity during the holding period and the time required to reach an apparent viscosity of 5,000 cP during the cooling period were collected from the apparent viscosity vs. time curve. These data points collected during the RVA melt analysis are referred to as HAV and T5, respectively, and were determined at a mixing speed of 300 rpm, which corresponds to a shear rate of 100.5 s^{-1} .

RESULTS AND DISCUSSION

Natural Cheese Intact Casein

Changes in the level of intact casein during ripening, for each replicate of natural cheese, are shown in Figure 1. The intact casein content in the natural cheese decreased with an increase in age for all replicates of process cheese that were manufactured. A large decrease in intact casein was observed between 2 and 12 wk of ripening, but remained stable between 12 and 18 wk of ripening. Intact casein is predominantly (80%)

Figure 1. Intact casein values for the 3 replicates (replicate 1: ◆; replicate 2: ■; replicate 3: ▲) of natural Cheddar cheese during ripening.

made up of α_{s1} and β fractions of casein (1:1 ratio). During ripening, the casein fractions are hydrolyzed, with a half-lives of 2 and 37 wk, respectively (De Jong, 1976; Basch et al., 1989). Because α_{s1} has a half-life of 2 wk, most of this fraction is hydrolyzed within the first 6 to 12 wk of ripening, and its hydrolysis is associated with the large decrease in the intact casein content of the natural cheese during early ripening. Although the intact casein content continues to decrease in later ripening, the decrease is small compared with the first several weeks.

VAM

The change in VAM as a function of ripening time is shown in Figure 2. Apparent viscosity after manufacture was significantly ($P < 0.05$) influenced by age, mixing speed, TSC concentration, and the interaction terms age $\times$ age, age $\times$ mixing speed, and age $\times$ TSC concentration (Table 2). The mean values and LSD for each treatment, at each ripening point, are shown in Table 3.

Figure 2. Effect of mixing speed (low: —; high: ---) and trisodium citrate concentration (2.0%: ●; 2.5%: ■; 3.0%: ▲) on process cheese food viscosity after manufacture (VAM) during ripening of natural cheese.

Table 2. Mean squares and *P* values (in parentheses) of melted and unmelted functional properties for process cheese food¹

Component	df	VAM	Firmness	HAV	T5
Whole plot					
Replicate (Rep)	2	9.8 × 10 ⁵ (0.131)	910.27 (0.24)	29,113* (0.02)	0.010 (0.80)
Age (A)	1	3.42 × 10 ⁷ * (<0.01)	31,122* (<0.01)	57,341* (<0.01)	2.40* (<0.01)
Age × age (A ²)	1	3.34 × 10 ⁶ * (0.02)	983.71 (0.21)	105,490* (<0.01)	0.31* (0.02)
Deviations from quadratic (Ads)	2	2.46 × 10 ⁵ (0.54)	81.54 (0.85)	3,663.9 (0.45)	0.018 (0.67)
Whole-plot error	8	3.74 × 10 ⁵	530.24	4,117.1	0.042
Split plot					
Mixing speed (MS)	1	8.65 × 10 ⁶ * (<0.01)	15,830* (<0.01)	91,278* (<0.01)	0.894* (<0.01)
A × MS	1	2.91 × 10 ⁵ * (0.01)	1,426.5* (<0.01)	256.07 (0.61)	0.005 (0.30)
A ² × MS	1	19,110 (0.52)	59.40 (0.11)	292.19 (0.59)	0.002 (0.54)
Ads × MS	2	1.65 × 10 ⁵ * (0.03)	4.167 (0.83)	733.32 (0.48)	0.001 (0.82)
TSC concentration (C)	2	6.51 × 10 ⁵ * (<0.01)	12.47 (0.58)	60,706* (<0.01)	0.065* (<0.01)
A × C	2	4.08 × 10 ⁵ * (<0.01)	13.455 (0.56)	1,671.9 (0.19)	0.001 (0.71)
A ² × C	2	68,359 (0.23)	20.126 (0.42)	3,359.8* (0.04)	0.007 (0.19)
Ads × C	4	17,233 (0.82)	10.753 (0.76)	146.58 (0.96)	0.001 (0.90)
MS × C	2	8,136.3 (0.83)	75.57* (0.04)	584.9 (0.56)	0.002 (0.61)
Ads × MS × C	8	32,526 (0.671)	13.77 (0.77)	548.59 (0.80)	0.002 (0.92)
Error	50	45,084	22.72	985.9	0.004

¹Model equation: $Y = \text{Rep} + A + A^2 + \text{Ads} + E(\text{Rep, Ads}) + R + A \times R + A^2 \times R + \text{Ads} \times R + C + A \times C + A^2 \times C + \text{Ads} \times C + R \times C + \text{Ads} \times R \times C$. VAM = viscosity after manufacture; HAV = hot apparent viscosity; T5 = time at 5,000 cP; TSC = trisodium citrate.

*Statistically significant ($P < 0.05$).

The significance ($P < 0.05$) of the age term indicates that there was a change in VAM as a function of the ripening period, whereas the significance ($P < 0.05$) of the interaction term age × age indicates that these changes were not linear. As shown in Figure 2, all treatments had a substantial decrease in VAM during the first 12 wk of ripening; however, there were no significant ($P > 0.05$) differences between the mean values for each treatment at 12 and 18 wk of ripening (Table 3). The trends in VAM (Figure 2) are similar to trends in intact casein values (Figure 1), which indicate that changes in VAM may be related to intact casein.

Previous researchers have demonstrated that the viscosity of process cheese, at the end of manufacture, is largely determined by protein–protein interactions and protein–fat interactions (Lee et al., 2003). However, the extent of protein–protein and protein–fat interactions in process cheese is dependent on the characteristics of the protein (mainly casein) that forms the structure and framework of process cheese (Guinee et al., 2004). The presence of intact (unhydrolyzed) casein results in

extensive protein–protein and protein–fat interactions and a fibrous casein network, whereas the presence of hydrolyzed casein results in weaker protein–protein and protein–fat interactions and a nonfibrous casein network (Taneya et al., 1980). Because young natural cheese has a larger amount of intact casein as compared with a mature natural cheese, process cheese manufactured with young natural cheese should have more extensive protein–protein and protein–fat interactions, and consequently a higher viscosity at the end of manufacture as compared with process cheese manufactured with a mature natural cheese.

The significance ($P < 0.05$) of the mixing speed term indicates that there were differences in VAM between high and low mixing speed treatments; however, the significance ($P < 0.05$) of the age × mixing speed interaction term indicates that the differences in VAM between high and low mixing speeds were not constant across all ripening periods (Figure 2). As shown in Table 3, the high mixing speed treatment had significantly ($P < 0.05$) higher VAM values at each TSC level, as com-

Table 3. Mean (n = 3) melted and unmelted functional properties of process cheese food manufactured at 3 levels (2.0, 2.5, and 3.0%) of trisodium citrate (TSC) and 2 mixing speeds¹

Ripening point	Low mixing speed			High mixing speed		
	2.0%	2.5%	3.0%	2.0%	2.5%	3.0%
(VAM, cP)						
Week 2	5,127 ^{A,ab}	4,758 ^{A,b}	4,549 ^{A,b}	5,805 ^{A,a}	5,119 ^{A,ab}	4,986 ^{A,ab}
Week 4	4,144 ^{B,ab}	3,690 ^{B,b}	3,681 ^{B,b}	4,544 ^{B,a}	4,490 ^{B,a}	4,239 ^{B,ab}
Week 6	3,774 ^{B,ab}	3,472 ^{BC,b}	3,553 ^{B,b}	4,151 ^{BC,a}	3,929 ^{C,ab}	3,927 ^{B,ab}
Week 12	2,933 ^{C,b}	2,878 ^{C,b}	2,877 ^{C,b}	3,718 ^{C,a}	3,747 ^{C,a}	3,810 ^{B,a}
Week 18	2,997 ^{C,b}	2,928 ^{C,b}	2,774 ^{C,b}	3,667 ^{C,a}	3,624 ^{C,a}	3,679 ^{B,a}
(Firmness, N)						
Week 2	95 ^{A,b}	102 ^{A,b}	104 ^{A,b}	141 ^{A,a}	135 ^{A,a}	142 ^{A,a}
Week 4	86 ^{A,b}	88 ^{AB,b}	89 ^{AB,b}	123 ^{A,a}	119 ^{AB,a}	117 ^{B,a}
Week 6	70 ^{B,b}	74 ^{BC,b}	73 ^{BC,b}	98 ^{B,a}	97 ^{BC,a}	95 ^{C,a}
Week 12	63 ^{B,b}	68 ^{C,ab}	67 ^{C,b}	85 ^{BC,a}	85 ^{C,a}	85 ^{C,a}
Week 18	60 ^{B,a}	61 ^{C,a}	58 ^{C,a}	76 ^{C,a}	77 ^{C,a}	78 ^{C,a}
(HAV, cP)						
Week 2	608 ^{A,b}	657 ^{A,bc}	715 ^{A,ab}	685 ^{A,bc}	703 ^{A,ab}	781 ^{A,a}
Week 4	542 ^{B,c}	572 ^{AB,bc}	592 ^{B,bc}	589 ^{B,bc}	624 ^{AB,ab}	661 ^{BC,a}
Week 6	508 ^{BC,a}	559 ^{B,ab}	579 ^{B,ab}	577 ^{B,ab}	634 ^{AB,a}	650 ^{BC,a}
Week 12	474 ^{C,b}	553 ^{B,ab}	594 ^{B,a}	563 ^{B,a}	594 ^{B,a}	619 ^{C,a}
Week 18	513 ^{BC,d}	595 ^{AB,c}	630 ^{AB,bc}	590 ^{B,b}	653 ^{AB,b}	721 ^{AB,a}
(T5, min)						
Week 2	11.26 ^{B,a}	11.25 ^{C,a}	11.12 ^{C,ab}	11.05 ^{D,bc}	11.02 ^{D,bc}	10.92 ^{D,c}
Week 4	11.51 ^{AB,a}	11.49 ^{B,ab}	11.44 ^{B,abc}	11.31 ^{C,bc}	11.25 ^{C,c}	11.26 ^{C,c}
Week 6	11.64 ^{A,a}	11.55 ^{AB,b}	11.54 ^{AB,b}	11.40 ^{BC,c}	11.38 ^{B,c}	11.35 ^{BC,c}
Week 12	11.74 ^{A,a}	11.66 ^{A,ab}	11.59 ^{AB,abc}	11.48 ^{AB,bc}	11.44 ^{AB,c}	11.43 ^{AB,c}
Week 18	11.71 ^{A,a}	11.68 ^{A,a}	11.63 ^{A,ab}	11.56 ^{A,ab}	11.52 ^{AB,ab}	11.45 ^{A,b}

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

^{A-D}Means within the same column (separate for each functional property) not sharing a common superscript are different ($P < 0.05$).

¹VAM = viscosity after manufacture; HAV = hot apparent viscosity; T5 = time at 5,000 cP.

pared with the low mixing speed treatment at 12 and 18 wk of ripening. However, the differences were not significant ($P > 0.05$) at 2, 4, and 6 wk of ripening. These results indicate that a high mixing speed influences VAM when matured natural cheese (>6 wk) is used to manufacture PCF, but is less critical when young natural cheese is used.

No previous research describes the effect of mixing speed on process cheese viscosity immediately after manufacture. However, previous researchers have used empirical and rheological melt tests to describe the effect of mixing speed on process cheese flow characteristics during melting (Taneya et al., 1980; Glenn et al., 2003; Lee et al., 2003). It is plausible that the results for flow characteristics during melting may be related to VAM; therefore, we believe the underlying intermolecular interactions governing these functional properties to be similar. Moreover, a study by Kapoor and Metzger (2005) demonstrated a correlation ($R^2 = 0.89$) between the apparent viscosity of process cheese immediately after manufacture and the apparent viscosity of process cheese during melting; both were measured using the RVA. Therefore, the results found by previous researchers for the flow characteristics during melting

may be relevant to viscosity immediately after manufacture and will be included in the discussion.

A study by Glenn et al. (2003) demonstrated that during manufacture, an increase in mixing speed from 50 to 150 rpm, at a constant temperature, reduces the melt characteristics of the process cheese. The process cheese formulation in their study used 75% young Cheddar cheese and 14% medium Cheddar cheese; the manufacturing conditions included 3 different temperatures (74, 80, and 86°C) and 3 mixing speeds (50, 100, and 150 rpm). However, the exact age of the young and medium Cheddar, and their intact casein contents, were not reported. These results are similar to the results obtained in our study for process cheese manufactured using mature natural cheese (>6 wk), in which a significant ($P < 0.05$) difference was observed between the high and low mixing speeds. Previous researchers have demonstrated that in some process cheese formulations the protein structure is deformable when a high mixing speed is used during manufacture (Taneya et al., 1980). The deformation at high mixing speeds leads to a decrease in fat globule size and a more homogeneous fat globule distribution within the protein structural network (Lee et al., 2003). The reduction in fat

globule size and increase in homogeneity of fat globule distribution is thought to increase the viscosity of process cheese at the end of manufacture for 2 reasons: 1) It allows more proteins to be absorbed at the fat–water interface, and 2) it strengthens the protein matrix by including fat globules in the protein matrix (Glenn et al., 2003; Lee et al., 2003).

The significance ($P < 0.05$) of the TSC concentration term indicates that there were differences in VAM between various levels of TSC, and the significance ($P < 0.05$) of the age $\times$ TSC concentration term indicates that these differences were not constant across all periods of ripening (Figure 2). Additionally, the differences between each TSC level, at each ripening point and mixing speed, were not significant ($P > 0.05$; Table 3). However, at 2, 4, and 6 wk of ripening, the lowest level of TSC (within each ripening point and mixing speed) had the highest VAM values. These results indicate that the level of TSC may have some effect on VAM when young natural cheese (<12 wk) is used in a formulation.

The function of TSC in a process cheese formulation is to chelate calcium from the natural cheese paracaseinate complex by disrupting the calcium bridges that facilitate solubilization and dispersion of the casein (Meyer, 1973; Guinee et al., 2004). A high level of TSC in a formulation may lead to an extensive disintegration of the paracaseinate complex (Meyer, 1973). Conversely, a lower level of TSC in a formulation may leave a portion of the paracasein complex aggregated, which, upon heating and mixing, may result in extensive protein–protein interactions and a fibrous protein network, leading to a more viscous product. Previous researchers have reported that the amount of calcium bound to the proteins in natural cheese decreases significantly after the first 4 wk of ripening (Hassan et al., 2004). It is plausible that after 4 wk of ripening, even the lowest level of TSC may cause complete disintegration of the natural cheese paracaseinate complex, whereas at less than 4 wk of ripening, the lowest level of TSC may not cause complete disintegration of the paracaseinate complex. Consequently, TSC concentration may play an important role in controlling the VAM when young natural cheese (<4 wk) is used in a process cheese formulation.

Firmness

The change in firmness as a function of ripening time is shown in Figure 3. The mean values and LSD for each treatment, at each ripening point, are shown in Table 3. Firmness was significantly ($P < 0.05$) influenced by the age of natural cheese, mixing speed during manufacture, and the interaction terms age $\times$ mixing speed and mixing speed $\times$ TSC concentration.

Figure 3. Effect of mixing speed (low: —; high: ---) and trisodium citrate concentration (2.0%: ●; 2.5%: ■; 3.0%: ▲) on process cheese food firmness during ripening of natural cheese.

As shown in Figure 3, all treatments exhibited a substantial decrease in firmness during the first 6 wk of ripening. Overall, there were no significant ($P > 0.05$) differences in firmness among the mean values for each treatment at 6, 12, and 18 wk of ripening; however, the PCF samples manufactured with a combination of high mixing speed and 2% TSC showed significant differences in firmness between 6 and 18 wk of ripening (Table 3).

A decrease in process cheese firmness, owing to an increase in the age of the natural cheese used to manufacture process cheese, has been associated with the decreasing level of intact casein in natural cheese during ripening (Templeton and Sommer, 1930; Vakaleris et al., 1962; Piska and Stetina, 2003). Young natural cheese with a high level of intact casein is typically used to produce process cheese when a firm body is desired (Templeton and Sommer, 1930; Palmer and Sly, 1943; Berger et al., 1998). In addition to our study, other researchers (Vakaleris et al., 1962) have shown a substantial decrease in process cheese firmness (measured with a penetrometer) as the age of the natural cheese increases. This decrease in firmness may not be related solely to intact casein, but could be linked to the rapid hydrolysis of α_{s1} -casein during early ripening. Previous researchers have observed a decrease in the firmness of process cheese with the hydrolysis of α_{s1} -casein in natural cheese (Tamime et al., 1990; Acharya and Mistry, 2005).

The significance ($P < 0.05$) of the mixing speed term indicates differences in firmness between the high and low mixing speed treatments; however, the significance ($P < 0.05$) of the age $\times$ mixing speed interaction term indicates that these differences in firmness (between the high and low mixing speeds) were not constant across all ripening periods (Figure 2). Moreover, the significance ($P < 0.05$) of the mixing speed $\times$ TSC concentration interaction term suggests that the differences

in firmness, as a result of varying concentrations of TSC, were influenced by mixing speed. As shown in Table 3, the high mixing speed treatments had significantly ($P < 0.05$) higher firmness values at each TSC level, as compared with the low mixing speed treatments at 2, 4, 6, and 12 wk of ripening. However, at 18 wk of ripening the differences were not significant ($P > 0.05$), even though the PCF manufactured with high mixing speeds were substantially firmer than the PCF manufactured at the low mixing speeds. Previous researchers have reported similar effects of mixing speed on imitation process cheese, where an increase in firmness was observed with an increase in the mixing speed (Norohna et al., 2005).

The effect of mixing speed on firmness could be due to the impact of mixing speed on fat globule size and distribution in the protein matrix, as well as to protein-protein interactions (Aguilera and Kessler, 1989; Lee et al., 2003). As discussed in the VAM section, using a high mixing speed during manufacture results in small homogeneous fat globules within the protein matrix. Upon cooling of process cheese after manufacture, these small and homogeneous fat globules further strengthen the protein matrix by acting as fillers (Aguilera and Kessler, 1989; Fox et al., 2000). These fat globules will also crystallize within the protein matrix during cooling and may contribute to process cheese firmness (Fox et al., 2000). Moreover, a higher mixing speed could result in a more ordered protein network with an increased level of protein-protein interactions, and upon cooling, may result in a firmer product (Aguilera and Kinsella, 1991; Zhong et al., 2004).

As shown in Table 2, the mixing speed $\times$ TSC concentration interaction term was significant ($P < 0.05$), indicating that changes in firmness with respect to TSC concentration for both mixing speeds were dissimilar, although the differences among the various TSC concentrations were not significantly different ($P > 0.05$).

HAV

Hot apparent viscosity is a measure of the apparent viscosity of melted process cheese. The change in HAV as a function of ripening time is shown in Figure 4. The mean values and LSD for each treatment, at each ripening point, are shown in Table 3. Hot apparent viscosity was significantly ($P < 0.05$) influenced by age, mixing speed, TSC concentration, and the interaction terms age $\times$ age, age $\times$ mixing speed, and age $\times$ age $\times$ TSC concentration (Table 2).

The significance ($P < 0.05$) of the age term indicates that there was a change in HAV as a function of the ripening period, whereas the significance ($P < 0.05$) of the interaction term age $\times$ age indicates that these

Figure 4. Effect of mixing speed (low: —; high: ---) and trisodium citrate concentration (2.0%: ●; 2.5%: ■; 3.0%: ▲) on process cheese food hot apparent viscosity (HAV) during ripening of natural cheese.

changes were not linear. As shown in Figure 2, all treatments had a substantial decrease in HAV during the first 12 wk of ripening. The trends in HAV between 2 and 12 wk of ripening (Figure 4) are similar to trends in VAM (Figure 2). As discussed in the VAM section, the decrease in HAV during ripening could be due to the hydrolysis of the intact casein in the natural cheese during ripening, which in turn results in process cheese with weaker protein-protein and protein-fat interactions, and lower melted viscosity.

It is interesting to note the substantial increase in HAV from 12 to 18 wk of ripening (Table 3), although the differences between each treatment at 12 and 18 wk of ripening were not significantly different ($P > 0.05$), except for PCF manufactured with a combination of 3.0% TSC and a high mixing speed. The increase in viscosity from 12 to 18 wk of ripening may be due to the phenomenon of overcreaming. Overcreaming occurs when process cheese develops into a thick custard-like texture, and is a consequence of the increased solubility and water-binding capacity of protein (Berger et al., 1998; Glenn et al., 2003). However, our results demonstrate that the phenomenon of overcreaming could also be influenced by mixing speed and TSC concentration.

The significance ($P < 0.05$) of the mixing speed term indicates that there were differences in HAV between the high and low mixing speed treatments; however, the significance ($P < 0.05$) of the age $\times$ mixing speed interaction term indicates that the differences in HAV between the high and low mixing speeds were not constant across all ripening periods (Figure 2). As shown in Table 3, the high mixing speed treatments had substantially higher HAV values than the low mixing speed treatments at each TSC level and ripening period. Between 2 and 12 wk of ripening, the difference in HAV between the low and high mixing speed was not significant ($P > 0.05$). However, at 18 wk the HAV with the

high mixing speed was significantly ($P < 0.05$) higher than that at the low mixing speed for all TSC concentrations. Again, these results indicate that the effect of mixing speed is magnified in process cheese made from aged (>18 wk) natural cheese. Previous researchers have reported a similar effect of mixing speed on imitation process cheese, in which a decrease in meltability was observed with an increase in the mixing speed during manufacture (Norohna et al., 2005). As mentioned previously, a high HAV is an indication of decreased meltability. As discussed in the VAM and firmness sections, the effect of mixing speed on HAV could be due to the effect of mixing speed on fat globule size and distribution within the protein matrix, as well as to protein–protein and protein–fat interactions.

The significance ($P < 0.05$) of the TSC concentration term indicates that there were differences in the HAV between various levels of TSC, and the significance ($P < 0.05$) of the age $\times$ age $\times$ TSC concentration term indicates that these differences were not constant across all periods of ripening (Figure 2). However, the mean HAV at each TSC level, at each ripening point and mixing speed, were not significantly different ($P > 0.05$; Table 3). It is interesting to note that the lowest level of TSC had the lowest HAV values within each mixing speed at all ripening times. Previous researchers have demonstrated similar results, in which a decrease in the meltability was observed with an increase in TSC concentration (Templeton and Sommer, 1932; Cavalier-Salou and Cheftel, 1991).

The trends with TSC concentration for HAV are the opposite of the trends reported for VAM. The VAM is a measure of the apparent viscosity of process cheese immediately after manufacture, whereas HAV measures the apparent viscosity of the cheese after it has cooled and solidified and then been melted in the RVA melt test. The observed differences between VAM and HAV may be due to the effect of cooling on the microstructure of process cheese. During cooling, the structure of process cheese is further modified and strengthened by protein–protein and protein–fat interactions (Fox et al., 2000; Zhong et al., 2004).

T5

Typically, it is desirable to have process cheese that takes a longer time to thicken during cooling when it is used as a dip for snacks, and a shorter thickening time during cooling when it is used as a slice on burgers. Consequently, postmelt characteristics (e.g., thickening during cooling), as measured using T5 values, can be an important assessment of the functional properties of process cheese. The T5 values measured during the RVA melt test measure the time required for a melted

Figure 5. Effect of mixing speed (low: —; high: ---) and trisodium citrate concentration (2.0%: ●; 2.5%: ■; 3.0%: ▲) on process cheese food time at 5,000 cP (T5) during ripening of natural cheese.

process cheese to thicken to an apparent viscosity of 5,000 cP during cooling. The change in T5 values as a function of ripening time is shown in Figure 5. The mean values and LSD for each treatment, at each ripening point, are shown in Table 3. The T5 values were significantly ($P < 0.05$) influenced by age, mixing speed, TSC concentration, and the interaction term age $\times$ age (Table 2).

The significance ($P < 0.05$) of the age term indicates that there was a change in the T5 values as a function of the ripening period, whereas the significance ($P < 0.05$) of the interaction term age $\times$ age indicates that these changes were not linear. As shown in Figure 5, all treatments had a substantial increase in T5 values during the first 6 wk of ripening. This indicates that the process cheese required a longer time to thicken during cooling, which is related to the age of the natural cheese that was used to manufacture the process cheese. However, there were no significant ($P > 0.05$) differences between the mean values for each treatment at 12 and 18 wk of ripening (Table 3).

The increasing trend in T5 values with age could again be due to the hydrolysis of casein in natural cheese during ripening. When process cheese is manufactured with young cheese, it is more likely to have longer protein strands and more protein–protein interactions than process cheese manufactured with ripened cheese (Berger et al., 1998). An increase in protein–protein interactions should result in a cheese that thickens faster during cooling. This may explain why process cheese manufactured with a young cheese thickens faster during cooling as compared with process cheese made with ripened cheese. However, the increase in T5 values was not linear. The T5 values increased faster during the first 6 wk of ripening as compared with 6 to 18 wk of ripening. The trends in T5 are similar to firmness, in which a large decrease was observed during the first 6 wk of ripening. A larger increase in T5 values

during initial ripening may again be related to degradation of certain casein fractions, especially α_{s1} -casein, during early ripening.

The significance ($P < 0.05$) of the mixing speed term indicates that there were differences in T5 values between high and low mixing speed treatments, where increasing the mixing speed significantly ($P < 0.05$) lowered T5 values. Moreover, the amount of emulsifying salt also had a significant ($P < 0.05$) impact on T5 values, with higher TSC concentrations resulting in lower T5 values. The reasons for these differences could be the same as explained in the VAM and firmness section, in which process cheese manufactured with high mixing speeds or high emulsifying salt concentrations is characterized by small and homogeneous fat globules and a more ordered protein network (Lee et al., 2003; Zhong et al., 2004). Upon melting, these small and homogeneous fat globules may aid in faster thickening of the protein matrix during cooling as compared with the large and heterogeneous fat globules observed when a low mixing speed or low concentration of emulsifying salt is used.

CONCLUSIONS

This study demonstrated that the flow properties of process cheese immediately after manufacture, unmelted textural properties, flow properties of process cheese during melting, and postmelt characteristics are all influenced by the formulation and processing parameters used during process cheese manufacture. A change in the age of natural cheese used to manufacture process cheese, and a change in mixing speed during manufacture resulted in a change in all the above-mentioned functional properties of process cheese. Additionally, a change in the TSC concentration influenced only the melt and flow characteristics, but not the unmelted texture properties. Consequently, it is important to control these formulation and processing parameters simultaneously to obtain a product with the desired texture and melt properties.

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