Effect of Natural Cheese Characteristics on Process Cheese Properties

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ABSTRACT

Natural cheese is the major ingredient utilized to manufacture process cheese. The objective of the present study was to evaluate the effect of natural cheese characteristics on the chemical and functional properties of process cheese. Three replicates of 8 natural (Cheddar) cheeses with 2 levels of calcium and phosphorus, residual lactose, and salt-to-moisture ratio (S/M) were manufactured. After 2 mo of ripening, each of the 8 natural cheeses was converted to 8 process cheese foods that were balanced for their composition, including moisture, fat, salt, and total protein. In addition to the standard compositional analysis (moisture, fat, salt, and total protein), the chemical properties (pH, total Ca, total P, and intact casein) and the functional properties [texture profile analysis (TPA), modified Schreiber melt test, dynamic stress rheometry, and rapid visco analysis] of the process cheese foods were determined. Natural cheese Ca and P, as well as S/M, significantly increased total Ca and P, pH, and intact casein in the process cheese food. Natural cheese Ca and P and S/M also significantly affected the final functional properties of the process cheese food. With the increase in natural cheese Ca and P and S/M, there was a significant increase in the TPA-hardness and the viscous properties of process cheese food, whereas the meltability of the process cheese food significantly decreased. Consequently, natural cheese characteristics such as Ca and P and S/M have a significant influence on the chemical and the final functional properties of process cheese.

Key words: natural cheese, calcium, intact casein, process cheese

INTRODUCTION

Process cheese is obtained by mixing natural cheese and other ingredients, along with emulsifying salts, and using heat and agitation to produce a homoge-

neous product that is used in a variety of forms such as slices, blocks, shreds, and sauces. Natural cheese (mainly Cheddar cheese in the United States) is one of the most important ingredients in process cheese. Depending on the type of process cheese manufactured, the amount of natural cheese in a process cheese formula varies from 51 to >80% of the final process cheese (FDA, 2006). Consequently, a substantial portion of Cheddar cheese produced in the United States is used as an ingredient in process cheese manufacture.

The characteristics of natural cheese utilized to manufacture process cheese have a major influence on process cheese characteristics. Numerous researchers have highlighted the importance of natural cheese characteristics on functional properties such as unmelted texture and meltability of process cheese (Barker, 1947; Meyer, 1973; Thomas, 1973; Caric et al., 1985; Shimp, 1985; Zehren and Nusbaum, 2000). Natural cheese made from concentrated milk has also been found to influence the chemical as well as functional properties of process cheese (Acharya and Mishra, 2005). Appropriate selection of natural cheese is important to achieve a process cheese with the desired chemical and functional characteristics. Researchers have highlighted some of the important physicochemical characteristics of a natural cheese that influence the functional properties of process cheese. These include pH, Ca content, and age or amount of intact CN present in the natural cheese (Templeton and Sommer, 1930; Barker, 1947; Olson et al., 1958; Vakaleris et al., 1962; Meyer, 1973; Thomas, 1973; Zehren and Nusbaum, 2000).

The importance of natural cheese pH on process cheese properties has been highlighted in a study performed by Olson et al. (1958), in which they manufactured Cheddar cheeses with a modified manufacturing protocol so as to produce 2 Cheddar cheese treatments with different final pH levels. The 2 Cheddar cheeses were then used to manufacture process cheeses (at 10, 30, 60, 90, and 150 d of ripening), which were analyzed for unmelted texture using penetrometry and meltability using the tube melt test. Their results indicated that even after the final pH of the process cheese was adjusted to 5.4 to 5.5, the process cheese made using

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Cheddar cheese with the higher pH was harder and less meltable at all stages of ripening when compared with the process cheese made using Cheddar cheese with the normal pH. There have been no direct studies related to the effect of the level and the state of Ca of natural cheese on process cheese properties. However, researchers have discussed its importance on process cheese properties (Olson et al., 1958; Zehren and Nusbaum, 2000).

The intact CN content of natural cheese is inversely related to the age of the natural cheese. As a natural cheese is ripened, its intact CN content decreases (Garimella Purna et al., 2006). This occurs as the natural cheese ages because the enzymes and residual starter or nonstarter lactic acid bacteria present in the cheese hydrolyze the proteins into peptides, thereby reducing the amount of CN that is still present in an intact (unhydrolyzed) form. Researchers have described the effect of the age of natural cheese on the functional properties of process cheese (Templeton and Sommer, 1930; Arnott et al., 1957; Olson et al., 1958; Vakaleris et al., 1962; Piska and Štětina, 2003; Garimella Purna et al., 2006). All the studies consistently indicate that as the age of natural cheese used in process cheese manufacture increased, the unmelted firmness of the resulting process cheese decreased (Templeton and Sommer, 1930; Olson et al., 1958; Vakaleris et al., 1962; Piska and Štětina, 2003; Garimella Purna et al., 2006), and the meltability of the resulting process cheese increased (Olson et al., 1958; Vakaleris et al., 1962; Garimella Purna et al., 2006).

Previous research has shown that changes in the manufacturing protocols during natural cheese manufacture such as set and drain pH, and level of salting can significantly change the Ca and P content, the salt-to-moisture ratio percentage (S/M), and the amount of residual lactose in the natural cheese (Dolby et al., 1937; Czulak et al., 1969; Thomas and Pearce, 1981; Upreti and Metzger, 2006a). Changes in natural cheese Ca and P, S/M, and the amount of residual lactose have been found to affect the physicochemical properties of the natural cheese such as the pH, the state and amount of Ca, as well as the rate and extent of protein hydrolysis (the amount of intact CN present) in the natural cheese (Czulak et al., 1969; Upreti and Metzger, 2006a).

Czulak et al. (1969) highlighted the effect of drain pH of Cheddar cheese on the Ca content as well as the final pH of the cheese. They found that as the pH of the curd during whey drainage was decreased from 6.14 to 5.75, there was a 27% reduction in the total Ca content in the cheese curd at the time of whey separation. They also found that with the decrease in the drain pH (as indicated above) there was a decrease

in the pH of the cheese from 5.32 to 5.12 at 9 wk of ripening. The level of salting in natural cheese and natural cheese S/M have been found to have an effect on the amount of residual lactose, cheese pH, and rate and extent of protein hydrolysis in cheese. Thomas and Pearce (1981) salted Cheddar cheeses at different rates in order to achieve different S/M. They found that, in the cheeses with lower S/M (4%), lactose was completely utilized in approximately 1 to 2 wk and the pH of the cheese at 2 wk was 5.08. In cheeses with 6% S/M, there was 0.31% residual lactose even after approximately 12 wk of ripening and the pH of the cheese at 2 wk was 5.31. Moreover, Thomas and Pearce (1981) found that, at 4 wk of ripening, approximately 72.5% of the major caseins were hydrolyzed in Cheddar cheese with 4% S/M compared with only approximately 45% that were hydrolyzed in Cheddar cheese with 6% S/M.

Presently, another major thrust in the natural cheese industry is the utilization of concentrated milk to manufacture natural cheeses to increase the throughput of cheese plants. The type of concentration technique and the extent to which milk has been concentrated also influences the Ca and P, S/M, and residual lactose content of the cheese produced (Sutherland and Jameson, 1981; Anderson et al., 1993; Acharya and Mistry, 2004; Nair et al., 2004). Acharya and Mistry (2004) manufactured Cheddar cheeses with milk concentrated using vacuum condensing and ultrafiltration to concentration factors of 1.5× and 2.0×, respectively. They found that, as the concentration factor of the milk utilized to manufacture Cheddar cheese was increased to 1.5, the Ca content of the Cheddar cheese manufactured increased by 10% when the milk was ultrafiltered and by 4% when the milk was vacuum-condensed. Moreover, when the concentration factor of the milk utilized to manufacture Cheddar cheese was increased to 2.0, the calcium content of the Cheddar cheese manufactured increased by 18% when the milk was ultrafiltered and by 13% when the milk was vacuum-condensed. Anderson et al. (1993) manufactured reduced-fat Cheddar cheese using condensed milk. They found that as the concentration factor of the milk was increased, the lactose content and S/M of the resulting cheese increased. The lactose content (at 5 d of ripening) of their cheeses increased from 1.05 to 2.26%, and the S/M increased from 2.88 to 3.74% in the cheese made using the same cheese milk when concentrated to 2.2×.

The literature cited above indicates that day-to-day variations in natural cheese manufacturing protocols, as well as utilization of concentrated milk to manufacture natural cheese, causes changes in the Ca and P, residual lactose, and S/M. These changes in natural

Table 1. Cheese manufacturing protocol used to manufacture the 8 natural cheese treatments

	High Ca and P, High lactose	High Ca and P, Low lactose	Low Ca and P, High lactose	Low Ca and P, Low lactose
Lactose addition	2.5 kg/100 kg of milk	—	2.5 kg/100 kg of milk	—
Starter addition	54 mL/100 kg of milk	54 mL/100 kg of milk	27 mL/100 kg of milk	27 mL/100 kg of milk
CaCl ₂ addition	19.8 mL/100 kg of milk	19.8 mL/100 kg of milk	—	—
Set pH	6.6	6.6	6.21	6.2
Cooking	37°C in 30 min and at 37°C for another 30 min	37°C in 30 min → washing → at 37°C until 1 h of cooking	38°C in 30 min and at 38°C for another 30 min	38°C in 30 min → washing → at 38°C until 1 h of cooking
Washing	—	Replace half of the whey with Ca, P, and pH-adjusted wash solution	—	Replace half of the whey with pH-adjusted wash water
Drain pH	6.3–6.4	6.3–6.4	5.7	5.7
pH at salting	5.4	5.4	5.4	5.4
Salting	Salted at 2 levels (2.25 and 3.5% of curd weight respectively for low and high S/M)			

cheese Ca and P, residual lactose, and S/M not only influence its chemical properties such as the pH and intact CN content, but may also have an effect on the functional properties of the process cheese manufactured from it. Consequently, even if process cheese manufacturers consistently utilize natural cheese from a particular manufacturing facility, they may struggle to produce process cheese with consistent product characteristics. In a previous study performed in our laboratory, we manufactured Cheddar cheese with modified manufacturing protocols to produce Cheddar cheeses with different levels of Ca and P, residual lactose, and S/M (Upreti and Metzger, 2006a). The compositional differences of the natural cheeses resulted in differences in their physicochemical properties such as total Ca, total P, pH, and the rate of protein hydrolysis (amount of intact CN). The objective of the present study was to utilize the natural cheeses (manufactured in the above indicated study) as an ingredient in process cheese and thereby evaluate the influence of natural cheese Ca and P, residual lactose, and S/M on the chemical as well as the functional properties of process cheese.

MATERIALS AND METHODS

Experimental Design

The 3 replicates of 8 different natural (Cheddar) cheeses were manufactured. Each replicate of natural cheese manufacture consisted of 3 factors (Ca and P, residual lactose, and S/M) at 2 levels for a total of 8 different natural cheese treatments. The 8 treatments were high Ca and P–high lactose–high S/M (**HHH**); high Ca and P–high lactose–low S/M (**HHL**); high Ca and P–low lactose–high S/M (**HLH**); high Ca and P–low lactose–low S/M (**HLL**); low Ca and P–high lactose–high S/M (**LHH**); low Ca and P–high lactose–

low S/M (**LHL**); low Ca and P–low lactose–high S/M (**LLH**); and low Ca and P–low lactose–low S/M (**LLL**).

After manufacture, each of the 8 natural cheeses from all 3 replicates were ripened for 2 mo and subsequently utilized as an ingredient to manufacture 8 different process cheese foods (**PC**) thereby producing 3 replicates of 8 PC treatments. The 8 PC treatments were process cheese–high Ca and P–high lactose–high S/M (**PC-HHH**); process cheese–high Ca and P–high lactose–low S/M (**PC-HHL**); process cheese–high Ca and P–low lactose–high S/M (**PC-HLH**); process cheese–high Ca and P–low lactose–low S/M (**PC-HLL**); process cheese–low Ca and P–high lactose–high S/M (**PC-LHH**); process cheese–low Ca and P–high lactose–low S/M (**PC-LHL**); process cheese–low Ca and P–low lactose–high S/M (**PC-LLH**); and process cheese–low Ca and P–low lactose–low S/M (**PC-LLL**).

Natural Cheese Manufacture

Each of the 3 replicates of the 8 natural cheeses was manufactured using a variety of protocols that resulted in a range of Ca and P, residual lactose, and S/M levels. A detailed description of the natural cheese manufacturing protocols followed to produce the above treatments is discussed in a previous paper (Upreti and Metzger, 2006a). Important modifications in the natural cheese manufacture that were utilized to produce the above 8 treatments are summarized in Table 1 (adapted from Upreti and Metzger, 2006a). The compositional differences of the natural cheeses resulted in differences in their physicochemical properties including total Ca, total P, pH, and rate of protein hydrolysis (amount of intact CN). The mean composition (at 2 mo of ripening) of the 8 natural cheeses manufactured including moisture, fat, protein, salt, S/M, lactose, total Ca, total P, pH, and intact CN content is listed in Table 2.

Table 2. Average chemical composition of the natural cheeses (mean of 3 replicates)

Chemical property	Natural cheese treatments ¹							
	HHH	HHL	HLH	HLL	LHH	LHL	LLH	LLL
Moisture, %	32.1 ^a	33.8 ^{bc}	33.1 ^{ab}	35.2 ^{de}	34.1 ^{bcd}	35.9 ^e	34.4 ^{cd}	37.6 ^f
Fat, %	35.9 ^a	35.0 ^{ab}	35.7 ^{ab}	34.8 ^{abc}	34.5 ^{bcd}	33.6 ^{cd}	34.7 ^{abc}	33.3 ^d
Protein, %	26.4 ^a	25.6 ^{abc}	26.0 ^{ab}	25.3 ^{bcd}	25.2 ^{cd}	24.8 ^{cd}	25.3 ^{bcd}	24.5 ^d
Salt, %	2.0 ^{bc}	1.7 ^d	2.1 ^{ab}	1.7 ^{cd}	2.3 ^{ab}	1.6 ^d	2.5 ^a	1.8 ^{cd}
S/M, ² %	6.4 ^a	5.0 ^b	6.5 ^a	4.9 ^b	6.7 ^a	4.5 ^b	7.2 ^a	4.7 ^b
Lactose (d 1), %	1.52 ^a	1.35 ^c	0.32 ^{de}	0.11 ^e	1.64 ^{ab}	1.41 ^{bc}	0.49 ^d	0.27 ^e
Total Ca, %	0.69 ^a	0.68 ^a	0.67 ^a	0.66 ^a	0.55 ^{ab}	0.54 ^b	0.55 ^b	0.51 ^b
Total P, %	0.48 ^a	0.48 ^a	0.48 ^a	0.47 ^a	0.42 ^a	0.42 ^b	0.41 ^b	0.40 ^b
pH (2 mo)	5.35 ^{ab}	5.16 ^{bc}	5.37 ^a	5.27 ^{ab}	5.28 ^{ab}	5.07 ^c	5.27 ^{ab}	5.05 ^c
Intact CN (2 mo), %	23.6 ^a	22.0 ^{bc}	23.0 ^{ab}	21.31 ^{cd}	21.1 ^{cd}	20.0 ^e	20.8 ^{de}	19.4 ^e

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

¹Natural cheese treatments: HHH = high Ca and P, high lactose, and high salt-to-moisture (S/M); HHL = high Ca and P, high lactose, and low S/M; HLH = high Ca and P, low lactose, and high S/M; HLL = high Ca and P, low lactose, and low S/M; LHH = low Ca and P, high lactose, and high S/M; LHL = low Ca and P, high lactose, and low S/M; LLH = low Ca and P, low lactose, and high S/M; LLL = low Ca and P, low lactose, and low S/M.

²S/M = salt-to-moisture ratio.

PC Formulation and Manufacture

Each of the 8 natural cheeses, from each replicate, were ripened for 2 mo and then utilized as an ingredient to manufacture 3 replicates of 8 different PC treatments. The emulsifying salt used was trisodium citrate (duohydrate; Archer Daniels Midland Company, Decatur, IL). Other ingredients were NDM (low heat; Dairy America, Fresno, CA), anhydrous butter oil (MidAmerica Farms, Springfield, MO), salt, and water. The source of emulsifying salt and other ingredients used to manufacture the 8 PC in each of the 3 replicates was the same. All 8 PC formulations for each of the 3 replicates were developed using Techwizard, an Excel-based formulation software program (Metzger, 2003) provided by Owl Software (Columbia, MO). The detailed ingredient blend and formulations (mean values of the 3 replicates) of the 8 process cheese food treatments are shown in Table 3. The formulation software

was used to balance the moisture, fat, salt, and total protein of the resulting 8 PC to 43.0, 25.0, 2.0, and 21.0%, respectively.

All of the PC treatments were manufactured in 4.5-kg batches using a Blentech twin-screw pilot-scale cooker blender (Blentech Corporation, Rohnert Park, CA). During PC manufacture, a preblend was prepared by mixing all the ingredients (indicated above) including natural cheese (4.5-kg batch total) in the Blentech twin-screw cooker at 50 rpm for 30 min at room temperature to achieve a homogeneous paste. This was followed by increasing the temperature of the preblend to 80°C in approximately 5 min and holding for an additional 5 min. The auger speed throughout the heating and holding stages was 140 rpm. Three copper cylinders (20 mm diameter and 30 mm height) were filled with each of the cooked PC treatments for texture profile analysis (TPA). The cylinders were sealed with

Table 3. Ingredient blend and formulations utilized to manufacture the 8 process cheese treatments (mean of 3 replicates)

Ingredients, %	Process cheese treatments ¹							
	PC-HHH	PC-HHL	PC-HLH	PC-HLL	PC-LHH	PC-LHL	PC-LLH	PC-LLL
Natural cheese (2 mo)	69.3	71.6	70.2	72.3	72.1	72.6	72.3	74.9
NDM	6.8	6.6	7.2	7.2	6.7	6.8	7.3	7.3
Butter oil	0.1	0.1	0.0	0.0	0.2	0.5	0.2	0.0
Salt	0.3	0.6	0.3	0.5	0.1	0.6	0.2	0.5
Trisodium citrate (duohydrate)	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Water	21.0	18.6	19.8	17.5	18.4	17.0	17.5	14.8
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

¹Process cheese treatments: PC-HHH = process cheese, high Ca and P, high lactose, and high salt-to-moisture (S/M); PC-HHL = process cheese, high Ca and P, high lactose, and low S/M; PC-HLH = process cheese, high Ca and P, low lactose, and high S/M; PC-HLL = process cheese, high Ca and P, low lactose, and low S/M; PC-LHH = process cheese, low Ca and P, high lactose, and high S/M; PC-LHL = process cheese, low Ca and P, high lactose, and low S/M; PC-LLH = process cheese, low Ca and P, low lactose, and high S/M; PC-LLL = process cheese, low Ca and P, low lactose, and low S/M.

Saran wrap and transferred to the cold room (4°C) after 15 min. The remainder of each PC was placed in 1-kg boxes and transferred to a cold room (4°C) after 15 min. All the cooked PC treatments were stored at 4°C until further analysis was completed.

Compositional and Chemical Analyses

The moisture content of the PC produced was analyzed using a vacuum oven as described by Bradley and Vanderwarn (2001). Fat content of the PC was determined using the Mojonnier method (Atherton and Newlander, 1977). Salt content was measured using a Corning Chloride Analyzer 926 (Ciba Corning Diagnostics, Medfield, MA), based on the Volhard test (Marshall, 1992), and pH was measured with a Corning pH/ion meter model 450 (Corning Glass Works, Medfield, MA) with a glass electrode. Total protein in the PC was determined by measuring total N in the cheeses using the Dumas combustion method (Leco Tru Spec N analyzer, Leco, St. Joseph, MI; Wiles et al., 1998), and converting it to protein using a multiplication factor of 6.38. Total Ca in the PC was measured using an atomic absorption spectroscopy procedure adapted from Brooks et al. (1970). Total P was determined colorimetrically (AOAC, 1995; method number 991.25). The total intact CN in each of the 8 PC was calculated by taking into account the amount of intact CN provided by each ingredient utilized in that PC formula; that is, intact CN of the natural cheese used in the formula (Table 2) and the intact CN from the NDM utilized in the formula. For NDM, the value of intact CN (28.9%) was calculated from the total protein present in the NDM. The formula utilized to calculate the intact CN in process cheese is indicated below.

$$\text{Percentage calculated intact CN} = [(\% \text{ IC cheese}) \times (\% \text{ cheese})/100] + [(28.9\%) \times (\% \text{ NDM})/100]$$

where % IC cheese = percentage of intact CN present in the natural cheese (Table 2); % cheese = percentage of natural cheese used in the PC formula (Table 3); % NDM = percentage NDM used in the PC formula (Table 3).

Because the intact CN of the final process cheese food was not experimentally determined but mathematically calculated, it is referred to as calculated intact CN (CIC) in the rest of the paper.

Functional Analyses (Unmelted Textural Properties)

TPA-Hardness. For TPA analysis, the cylinders of process cheese food (20 mm × 30 mm) that were filled

during manufacture were removed from the copper molds and cut to a height of 20 mm. The TPA analysis was performed using a TA.XT2 Texture Analyzer (Texture Technologies Corp., Scarsdale, NY/Stable Microsystems, Godalming, Surrey, UK) as described by Drake et al. (1999). The test conditions were uniaxial two-bite compression; 50-mm diameter cylindrical flat probe (TA-25); compression, 80%; and crosshead speed, 0.8 mm/s. Process cheese was analyzed for TPA-hardness as described by Breene (1975). Breene (1975) defines TPA-hardness as a measure of unmelted texture of a cheese that describes the firmness of the cheese.

Functional Analysis (Melted Textural Properties)

Modified Schreiber Melt Test. Meltability of each process cheese food sample was measured using the modified Schreiber test as described by Muthukumarappan et al. (1999). Each process cheese food sample was cut into discs of 28.5 mm diameter and 7 mm height. Three discs of equal weights (5 g) were randomly selected and kept in covered Petri plates at 20°C for 30 min. The discs were then placed on 0.95-mm thick aluminum plates (100 mm × 100 mm), which were immediately transferred to an air convection oven (Gallenkamp Plus Oven, Loughborough, UK) at 90°C. After 5 min, the plates with the melted cheese discs were cooled to room temperature. Area of the melted cheese was measured using image-processing software (HL Image++98, Western Vision Software, Salt Lake City, UT). The meltability of process cheese was reported as the area of the melted cheese in millimeters squared.

Dynamic Rheological Analysis. Dynamic rheological analysis of each process cheese food sample was performed using a modified method as described by Suthewattananonda and Bastian (1998) using a rheometer (ATS Rheosystems, Rheologica Instruments Inc., Bordentown, NJ) with parallel plate geometry. Modifications of the method included the use of fine sandpaper (400 grit), which was glued to the upper plate of the rheometer to prevent sample slippage. Process cheese food samples (slice of ~2.0 mm) were prepared using a wire cutter.

Cylindrical cheese samples of 28.3 mm diameter were then cut using a cork borer. Before analysis, the PC samples were tempered to room temperature for 15 min. During loading, the sample was placed on the lower plate and the upper plate was brought in contact with it. The exposed edge of the sample was coated with vegetable oil (Midwest Country Fare, Des Moines, IA) to minimize drying during measurement. Dynamic rheological properties (G' , elastic modulus, and G'' ,

viscous modulus) were analyzed using a dynamic temperature ramp test from 30 to 90°C with a heating rate of 4°C/min. Frequency was maintained at 1 Hz with 0.5% strain and 750-Pa stress. The gap maintained between the parallel plates was 2 mm. Transition temperature (melting point) was defined as the temperature at which $\tan \delta = 1$ (G''/G') and was recorded as the dynamic stress rheometer (**DSR**) – melt temperature. The G'' values at 85°C were used to evaluate viscous properties at elevated temperature.

Rapid Visco Analyzer-Hot Apparent Viscosity. The Rapid Visco Analyzer (**RVA**; RVA-4, Newport Scientific Pty. Ltd., Warriewood, Australia) was used to measure the apparent viscosity of all the process cheese food samples. The RVA melt test continuously measures the apparent viscosity during a heating, holding, and cooling profile as described by Prow (2004). For the RVA melt test, a representative sample of PC was cut from the 1-kg block and was ground using an Osterizer blender (model 6641, Jarden Corp., Rye, NY). Fourteen grams of the ground PC was weighed into an RVA canister along with 1 g of propylene glycol. The RVA melt test utilizes a heating, holding, and cooling temperature profile where the temperature of the canister was raised from 25 to 85°C in 5 min, held for 3 min at 85°C, and then cooled to 25°C in 6 min. During this temperature profile, 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 RVA melt test was performed in duplicate on all PC samples. The minimum apparent viscosity (in cP) during the holding period was collected from the apparent viscosity vs. time curve and is referred to as hot apparent viscosity (Prow, 2004). The RVA-hot apparent viscosity is a measure of how well the cheese flows when heated to a specific temperature.

Statistical Analysis

A $2 \times 2 \times 2$ factorial design with 3 replications was used for statistical analysis to study the effect of natural cheese Ca and P, residual lactose, and S/M ratio on PC chemical and functional properties. Each replicate of the 8 PC was treated as the blocks of the design. An ANOVA was performed to obtain the mean squares and P -values using Macanova 4.12 software (School of Statistics, University of Minnesota, Minneapolis). The comparisons were made at the 0.05 level of significance; the results were considered significant at $P < 0.05$. If the F -test for the factors was significant ($P < 0.05$), the treatment means were compared using least significant difference test.

RESULTS AND DISCUSSION

PC Composition and Chemical Properties

Mean values of PC composition and chemical properties including moisture, fat, salt, total protein, total Ca, total P, pH, and CIC of the 8 PC are listed in Table 4. The mean square values and the P -values for the PC composition and chemical properties are indicated in Table 5. There was a significant replicate effect in all the PC compositional and chemical properties ($P < 0.05$) (except total protein). None of the 3 factors (Ca and P, residual lactose, or S/M) and their interactions had a significant effect ($P > 0.05$) on moisture, fat, salt, and total protein contents of the manufactured PC. These results are as expected because all the PC were balanced for moisture, fat, salt, and protein (as indicated earlier). There was a significant effect ($P < 0.05$) of natural cheese Ca and P and natural cheese S/M on total Ca, total P, and CIC of the PC. Moreover, the pH of the PC was significantly affected ($P < 0.05$) by natural cheese Ca and P, residual lactose, and S/M.

Effect of Natural Cheese Ca and P. As indicated above, there was a significant effect of natural cheese Ca and P on PC total Ca and total P content (Table 4). As expected, natural cheeses with higher Ca and P content produced PC with higher Ca and P content (PC-HHH, PC-HHL, PC-HLH, and PC-HLL; Tables 3 and 4). The pH of the PC was also significantly affected by the natural cheese Ca and P (Table 4). Higher Ca and P natural cheese treatments produced PC (PC-HHH, PC-HHL, PC-HLH, and PC-HLL) with higher pH compared with when lower Ca and P natural cheese treatments were used to manufacture PC (PC-LHH, PC-LHL, PC-LLH, and PC-LLL; Table 4). High Ca and P contents in a natural cheese are typically associated with a higher pH of that natural cheese. This is due to the increased buffering capacity of the cheese with a high Ca and P (mineral) content (Dolby et al., 1937; Upreti and Metzger, 2007). Similar effects can be expected in process cheese. In the study by Acharya and Mistry (2004, 2005), in which they manufactured process cheeses with Cheddar cheeses manufactured using milks concentrated to different levels (either by ultrafiltration or vacuum condensing), they found that as the level of concentration of milk to make the Cheddar cheese was increased, the total Ca content as well as the pH of the Cheddar cheese increased (Acharya and Mistry, 2004). When these Cheddar cheeses were used to manufacture process cheese, the pH of manufactured process cheeses was also found to be higher (Acharya and Mistry, 2005).

Natural cheese Ca and P content also significantly affected the CIC of the resulting PC. Higher Ca and P natural cheese treatments produced PC (PC-HHH,

Table 4. Mean values (n = 3) of the composition and chemical properties of the 8 process cheese treatments

	Process cheese treatments ¹							
	PC-HHH	PC-HHL	PC-HLH	PC-HLL	PC-LHH	PC-LHL	PC-LLH	PC-LLL
Moisture, ² %	42.9	43.1	43.0	43.6	42.9	43.2	43.0	43.0
Fat, ² %	25.6	25.5	25.5	25.4	24.9	25.3	25.6	25.2
Salt, ² %	2.0	2.0	2.1	2.0	2.0	1.9	2.1	2.1
Protein, ² %	21.8	20.9	21.2	21.1	20.8	21.0	21.0	21.1
S/M, ² %	4.7	4.6	4.9	4.6	4.7	4.4	4.9	4.9
Total Ca, %	0.39 ^a	0.38 ^a	0.39 ^a	0.39 ^a	0.35 ^b	0.35 ^b	0.34 ^b	0.33 ^b
Total P, %	0.56 ^a	0.55 ^{ab}	0.56 ^a	0.56 ^a	0.48 ^c	0.48 ^{bc}	0.46 ^c	0.46 ^c
pH	6.10 ^{ab}	5.76 ^{cd}	6.16 ^a	5.89 ^{bc}	5.96 ^{abc}	5.65 ^d	5.99 ^{ab}	5.77 ^{cd}
CIC, ³ %	18.3 ^a	17.7 ^{bc}	18.0 ^{ab}	17.5 ^{bc}	17.1 ^{cd}	16.5 ^d	17.1 ^{cd}	16.6 ^d

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

¹Process cheese treatments: PC-HHH = process cheese, high Ca and P, high lactose, and high salt-to-moisture (S/M); PC-HHL = process cheese, high Ca and P, high lactose, and low S/M; PC-HLH = process cheese, high Ca and P, low lactose, and high S/M; PC-HLL = process cheese, high Ca and P, low lactose, and low S/M; PC-LHH = process cheese, low Ca and P, high lactose, and high S/M; PC-LHL = process cheese, low Ca and P, high lactose, and low S/M; PC-LLH = process cheese, low Ca and P, low lactose, and high S/M; PC-LLL = process cheese, low Ca and P, low lactose, and low S/M.

²Moisture, fat, salt, protein, and salt-to-moisture ratio (S/M) of the 8 process cheeses were not significantly different.

³CIC = calculated intact CN.

PC-HHL, PC-HLH, and PC-HLL) with higher CIC compared with when lower Ca and P natural cheese treatments were used (PC-LHH, PC-LHL, PC-LLH, and PC-LLL; Table 4). Calcium acts as a cross-linking agent within the CN molecules thereby limiting their flexibility. Researchers have found that, as the level of Ca is reduced in a model CN system, the solubility of the CN molecules increases (Sood et al., 1979; Cavalier-Salou and Cheftel, 1991). This increase in solubility of CN molecules may result in an increase in the availability of the caseins for hydrolysis during ripen-

ing. Consequently, natural cheeses with lower Ca and P levels should have a higher level of protein hydrolysis during ripening and less intact CN. We reported this in a related study in which the cheeses with high Ca and P (HHH, HHL, HLH, and HLL) had a lower level of proteolysis at 2 mo of ripening compared with low Ca and P natural cheeses (Upreti and Metzger, 2006b) and therefore had a higher intact CN level (Table 2). These differences in the natural cheese intact CN content resulted in the observed differences in the intact CN content of the process cheeses.

Table 5. Mean squares and *P*-values (in parentheses) of the process cheese composition and chemical properties

Sources of variation ¹	Process cheese composition and chemical properties								
	df	Moisture	Fat	Salt	Protein	pH	Calculated intact CN	Total Ca	Total P
Replicate	2	1.15*	1.46*	0.12*	0.33	0.10*	0.05*	0.01*	0.002*
		(0.02)	(<0.01)	(0.02)	(0.11)	(<0.01)	(0.02)	(<0.01)	(<0.01)
Ca and P	1	0.07	0.33	0.01	0.45	0.11*	6.10*	0.05*	0.01*
		(0.58)	(0.21)	(0.59)	(0.08)	(<0.01)	(<0.01)	(<0.01)	(<0.01)
Lactose	1	0.05	0.04	0.06	0.0004	0.04*	0.07	0.0002	0.0001
		(0.64)	(0.64)	(0.12)	(0.96)	(<0.01)	(0.40)	(0.53)	(0.35)
S/M	1	0.45	0.03	0.06	0.18	0.47*	1.98*	0.0001	0.0001
		(0.18)	(0.72)	(0.12)	(0.35)	(<0.01)	(<0.01)	(0.65)	(0.35)
(Ca and P) × lactose	1	0.18	0.24	0.02	0.12	0.0007	0.15	0.0007	0.0007*
		(0.38)	(0.28)	(0.42)	(0.25)	(0.64)	(0.22)	(0.25)	(0.03)
(Ca and P) × S/M	1	0.05	0.02	0.002	0.70*	0.0018	0.004	4.2 × 10 ⁻⁶	4.2 × 10 ⁻⁶
		(0.64)	(0.78)	(0.79)	(0.03)	(0.45)	(0.84)	(0.93)	(0.85)
Lactose × S/M	1	0.01	0.24	0.002	0.18	0.01	0.02	4.2 × 10 ⁻⁶	3.8 × 10 ⁻⁵
		(0.83)	(0.28)	(0.79)	(0.25)	(0.08)	(0.65)	(0.93)	(0.57)
(Ca and P) × lactose × S/M	1	0.22	0.38	0.03	0.35	0.0001	0.0004	3.8 × 10 ⁻⁵	0.0002
		(0.33)	(0.18)	(0.29)	(0.12)	(0.86)	(0.95)	(0.79)	(0.20)
Error	14	0.22	0.19	0.02	0.13	0.003	0.09	0.0005	0.0001

¹Ca and P = calcium and phosphorus content; S/M = salt-to-moisture ratio.

*Statistically significant ($P < 0.05$).

Effect of Natural Cheese Lactose Content. The natural cheese residual lactose content (at d 1 of ripening) significantly affected the pH of the resulting PC (Table 5). The natural cheese treatments with higher residual lactose produced PC with lower pH (PC-HHH, PC-HHL, PC-LHH, and PC-LHL) compared with the PC manufactured using the natural cheeses with lower residual lactose level (PC-LH, PC-HLL, PC-LLH, and PC-LLL). This can be attributed to the fact that the residual lactose in natural cheese had an effect on the final pH (at 2 mo of ripening) of that cheese (Table 2; Upreti and Metzger, 2007) and this effect was carried onto the corresponding PC treatments.

Effect of Natural Cheese S/M. The natural cheese S/M had a significant effect on the pH as well as the CIC of the resulting PC (Table 5). We previously reported that the S/M in natural cheese influenced its final pH, which was attributed to the fact that the S/M of natural cheese has an effect on the growth and activity of starter and nonstarter lactic acid bacteria; thereby, influencing the rate and amount of conversion of residual lactose to lactic acid and other organic acids (Upreti and Metzger, 2007). This effect on the natural cheese pH was carried through to the PC; the PC manufactured using natural cheeses with high S/M (PC-HHH, PC-HLH, PC-LHH, and PC-LLH) showed a higher pH compared with those manufactured using the natural cheeses with low S/M (PC-HHL, PC-HLL, PC-LHL, and PC-LLL). Moreover, as indicated above, PC manufactured using the natural cheeses with high S/M also had higher CIC when compared with PC manufactured using the natural cheeses with low S/M. This can again be attributed to the fact that the natural cheeses with higher S/M had a lower level of proteolysis (Upreti and Metzger, 2006b) and therefore higher intact casein at 2 mo of ripening (Table 2), the time when they were used to make the PC.

PC Functional Properties

Mean values of the functional properties (TPA-hardness, melt area, DSR-melt temperature, G'' at 85°C, and RVA-hot apparent viscosity) of the 8 PC are indicated in Table 6. The mean square values and the P -values for the PC functional properties are indicated in Table 7. There was a significant replicate effect in all PC functional properties ($P < 0.05$) except TPA-hardness. There was a significant effect of natural cheese Ca and P and natural cheese S/M on all the functional properties of the manufactured PC (Table 7). However, natural cheese residual lactose content did not have an effect on the functional properties of the PC.

Effect of Natural Cheese Ca and P. Natural cheese Ca and P content significantly affected the functional properties of the resulting PC. The TPA-hardness, melt area, and DSR-melt temperature values of the process cheeses indicate that the PC manufactured using the natural cheeses with high Ca and P (PC-HHH, PC-HHL, PC-HLH, and PC-HLL) were firmer and less meltable than those manufactured using natural cheeses with low Ca and P (PC-LHH, PC-LHL, PC-LLH, and PC-LLL; Table 6). Moreover, the PC manufactured using natural cheeses with high Ca and P were more viscous at high temperature (85°C) than those manufactured using natural cheeses with low Ca and P, as indicated by higher G'' at 85°C and RVA-hot apparent viscosity.

Effect of Natural Cheese S/M. Natural cheese S/M level also significantly affected the functional properties of the resulting process cheeses. The TPA-hardness, melt area, and the DSR-melt temperature values of the PC indicate that the PC manufactured using the natural cheeses with high S/M (PC-HHH, PC-HLH, PC-LHH, and PC-LLH) were firmer and less meltable than those manufactured using natural cheeses with low S/M (PC-HHL, PC-HLL, PC-LHL, and PC-LLL; Table 6). Moreover, the PC manufactured using natural cheeses with high S/M were more viscous at high temperature (85°C) than those manufactured using natural cheeses with low S/M, as indicated by higher G'' at 85°C and RVA-hot apparent viscosity.

Relationship Between PC Chemical and Functional Properties

Tables 5 and 7 indicate that natural cheese Ca and P and S/M significantly affected the pH, total Ca content, total P content, and the CIC of the resulting PC. Tables 6 and 7 indicate a significant influence of natural cheese Ca and P and S/M on the functional properties of the resulting PC. When the chemical properties (Table 4) of PC-LHL and PC-LLL are compared with PC-HHH and PC-HLH, total Ca content; total P content; pH; and CIC were 0.35% and 0.33%; 0.48% and 0.46%; 5.65 and 5.77; and 16.5% and 16.6% for PC-LHL and PC-LLL respectively, compared with 0.39% and 0.39%; 0.56% and 0.56%; 6.10 and 6.16; and 18.3% and 18.0% for PC-HHH and PC-HLH, respectively. Similarly, comparing the functional properties (Table 6) of PC-LHL and PC-LLL with PC-HHH and PC-HLH, TPA-hardness; melt area; DSR-melt temperature; G'' at 85°C; and RVA-hot apparent viscosity were 61 N and 67 N; 1,545 mm² and 1,463 mm²; 70.6°C and 69.4°C; 212 Pa and 352 Pa; and 465 cP and 570 cP for PC-LHL and PC-LLL respectively, and 130 N and 148 N; 920 mm² and 885 mm²; 76.8°C and 76.3°C; 1,163 Pa

Table 6. Mean values (n = 3) of the functional properties of the 8 process cheese treatments

Functional property ²	Process cheese treatments ¹							
	PC-HHH	PC-HHL	PC-HLH	PC-HLL	PC-LHH	PC-LHL	PC-LLH	PC-LLL
TPA-hardness, N	130 ^a	82 ^b	148 ^a	86 ^b	94 ^b	61 ^b	79 ^b	67 ^b
Melt area, mm ²	920 ^{ab}	1,346 ^{cd}	885 ^a	1,354 ^{cd}	1,210 ^{bc}	1,545 ^d	1,247 ^{cd}	1,463 ^{cd}
DSR-melt temperature, °C	76.8 ^a	70.4 ^b	76.3 ^a	72.6 ^{ab}	72.0 ^{ab}	70.6 ^b	73.1 ^{ab}	69.4 ^b
G'' at 85 °C, Pa	1,163 ^a	678 ^b	1,179 ^a	571 ^{bc}	729 ^{ab}	212 ^c	744 ^{ab}	352 ^{bc}
RVA-hot apparent viscosity, cP	747 ^a	542 ^b	742 ^a	522 ^b	603 ^{ab}	465 ^b	588 ^{ab}	570 ^{ab}

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

¹Process cheese treatments: PC-HHH = process cheese, high Ca and P, high lactose, and high salt-to-moisture (S/M); PC-HHL = process cheese, high Ca and P, high lactose, and low S/M; PC-HLH = process cheese, high Ca and P, low lactose, and high S/M; PC-HLL = process cheese, high Ca and P, low lactose, and low S/M; PC-LHH = process cheese, low Ca and P, high lactose, and high S/M; PC-LHL = process cheese, low Ca and P, high lactose, and low S/M; PC-LLH = process cheese, low Ca and P, low lactose, and high S/M; PC-LLL = process cheese, low Ca and P, low lactose, and low S/M.

²TPA = texture profile analysis; DSR = dynamic stress rheometers; RVA = rapid visco analyzer.

and 1,179 Pa; and 747 cP and 742 cP for PC-HHH and PC-HLH, respectively. It is obvious that PC with lower total Ca content, lower total P content, lower pH, and lower CIC (PC-LHL and PC-LLL) were less firm, more meltable, and less viscous at high temperature than the PC with higher total Ca content, higher pH, and higher CIC (PC-HHH and PC-HLH). Because the other compositional properties (moisture, fat, salt, and total protein) were not significantly different among PC, there is a relationship between PC total Ca and total P content, final pH, and intact CN content and the functional properties of process cheese. However, the relative effect of total Ca content, total P content, pH, and intact CN content on PC functional properties

could not be determined from this study and could be the subject of future research.

CONCLUSIONS

Natural cheese Ca and P and natural cheese S/M were found to significantly affect PC functional properties (unmelted texture, as well as melt properties of PC). The results also indicated that natural cheese Ca and P, residual lactose, and S/M had significant effects on the chemical properties such as the pH, total Ca content, total P content, and the CIC of the resulting PC. Consequently, it is not only important to balance the moisture, fat, salt, and total protein of a PC but

Table 7. Mean squares and *P*-values (in parentheses) of the process cheese functional properties

Source of variation ¹	Process cheese functional properties ²					
	df	TPA-hardness	Melt area	DSR-melt temperature	G'' at 85°C	RVA-hot apparent viscosity
Replicate	2	555.5 (0.22)	117,050.0* (0.02)	32.4* (0.03)	437,550.0* (0.03)	35,835.0* (0.02)
Ca and P	1	7,993.5* (<0.01)	345,360.0* (<0.01)	45.4* (0.02)	905,590.0* (<0.01)	40,344.0* (0.03)
Lactose	1	66.7 (0.66)	1,962.0 (0.77)	2.0 (0.59)	1,472.7 (0.86)	1,536.0 (0.65)
S/M	1	8,893.5* (<0.01)	783,730.0* (<0.01)	92.0* (<0.01)	1,502,000.0* (<0.01)	126,730.0* (<0.01)
(Ca and P) $\times$ lactose	1	400.2 (0.29)	117.0 (0.94)	1.0 (0.70)	22,571.0 (0.51)	4,873.5 (0.43)
(Ca and P) $\times$ S/M	1	1,600.7 (0.05)	44,290.0 (0.18)	12.0 (0.20)	12,881.0 (0.61)	27,068.0 (0.08)
Lactose $\times$ S/M	1	13.5 (0.84)	2,223.4 (0.76)	4.1×10^{-2} (0.94)	4.2 (0.99)	4,213.5 (0.46)
(Ca and P) $\times$ lactose $\times$ S/M	1	450.7 (0.26)	9,882.0 (0.52)	15.0 (0.16)	23,188.0 (0.50)	6,800.7 (0.35)
Error	14	333.5	22,747.0	6.8	48,490.0	7,321.2

¹Ca and P = calcium and phosphorus content; S/M = salt-to-moisture ratio.

²TPA = texture profile analysis; DSR = dynamic stress rheometer; RVA = rapid visco analyzer.

*Statistically significant ($P < 0.05$).

also to control the final Ca, P, pH, and intact CN to produce a process cheese with targeted functional properties. Future work should involve evaluation of the individual influence of the total Ca, total P, pH, and intact CN of PC on the PC functional properties when all other chemical properties of the PC such as moisture, fat, salt, and total protein are constant.

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