Influence of Calcium and Phosphorus, Lactose, and Salt-to-Moisture Ratio on Cheddar Cheese Quality: Manufacture and Composition

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ABSTRACT

Eight Cheddar cheeses with 2 levels of calcium (Ca) and phosphorus (P), residual lactose, and salt-to-moisture ratio (S/M) were manufactured. All cheeses were made using a stirred-curd procedure and were replicated 3 times. Treatments with a high level of Ca and P were produced by setting the milk and drawing the whey at a higher pH (6.6 and 6.3, respectively) compared with the treatments with a low level of Ca and P (pH of 6.2 and 5.7, respectively). The lactose content in the cheeses was varied by adding lactose (2.5% by weight of milk) to the milk for high lactose cheeses, and washing the curd for low lactose cheeses. The difference in S/M was obtained by dividing the curds into halves, weighing each half, and salting at 3.5 and 2.25% of the weight of the curd for high and low S/M, respectively. All cheeses were salted at a pH of 5.4. Modifications in cheese-making protocols produced cheeses with desired differences in Ca and P, residual lactose, and S/M. Average Ca and P in the high Ca and P cheeses was 0.68 and 0.48%, respectively, vs. 0.53 and 0.41% for the low Ca and P cheeses. Average lactose content of the high lactose treatments at d 1 was 1.48% compared with 0.30% for the low lactose treatments. The S/M for the high and low S/M cheeses was 6.68 and 4.77%, respectively. Mean moisture, fat, and protein content of the cheeses ranged from 32.07 to 37.57%, 33.32 to 35.93%, and 24.46 to 26.40%, respectively. The moisture content differed among the treatments, whereas fat and protein content on dry basis was similar.

Key words: calcium, phosphorus, residual lactose, salt

INTRODUCTION

Cheese makers often face challenges in producing Cheddar cheeses with consistent flavor, texture, and appearance characteristics (Lochry et al., 1951; Lawrence et al., 1984). The characteristics of Cheddar

cheese result from a variety of physicochemical changes that occur during ripening. These changes include lactose breakdown (glycolysis), proteolysis, lipolysis (to a certain extent), shifts in the calcium-phosphate equilibria, and changes in pH (Lawrence et al., 1984; Fox et al., 1990). Although these physicochemical properties are important for a good quality Cheddar cheese, they can also lead to various defects (bitterness, poor meltability, surface crystal formation, liquid expulsion). Therefore, proper control of the physicochemical properties during ripening is essential. Approaches to control these changes during ripening should include control over critical compositional factors in the fresh, unripened cheese before it is stored for ripening. We hypothesize that calcium (Ca) and phosphorus (P), residual lactose, and salt-to-moisture ratio (S/M) are 3 critical compositional factors that impact the quality of cheese, provided the same starter culture is used for cheese manufacture.

Calcium and P participate in the protein network that forms the structural matrix of cheese, and hence, directly affect cheese texture. Thus, cheeses with a high concentration of total Ca tend to be firmer and less meltable than cheeses with low Ca concentration (Lucey and Fox, 1993; Metzger et al., 2001; Guinee et al., 2002; Joshi et al., 2004). Moreover, cheeses with low Ca content tend to have a higher rate of proteolysis than cheeses with high Ca content (Fox, 1970; Lucey and Fox, 1993; Joshi et al., 2004). This is attributed to the increased susceptibility of Ca-depleted caseins to proteolytic enzymes (Fox, 1970; Fox et al., 1990). In addition, Ca and P contribute to the buffering properties of cheese and therefore influence changes in pH during ripening (Lucey and Fox, 1993). The concentration of Ca, P, and lactic acid in the serum phase, in excess to the solubility of calcium phosphate or calcium lactate (or both) can lead to crystallization of these salts on the cheese surface.

Although lactose is water-soluble and most of it is lost in the whey during cheese manufacture, a portion of it remains trapped in the serum phase of cheese (Huffman and Kristofferson, 1984; Shakeel-Ur-Rehman et al., 2004). During ripening, the residual lactose is converted to different glycolytic products (sugars and

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organic acids), which can affect the flavor and pH of the cheese (Huffman and Kristofferson, 1984; Shakeel-Ur-Rehman et al., 2004). The rate at which glycolysis occurs depends on the S/M of cheese (Fox et al., 1990; Thomas and Pearce, 1981). The recommended S/M for a premium quality Cheddar cheese is 4.0 to 6.0% (Lawrence et al., 1984), and a S/M of more than 6.0% has been shown to decrease the rate of lactose fermentation (Thomas and Pearce, 1981; Lawrence et al., 1984). Salt reduces water activity of cheese, and thereby influences the growth of starter and nonstarter lactic acid bacteria, the activity of bacterial, indigenous milk enzymes, and residual chymosin (Thomas and Pearce, 1981; Fox et al., 1990). Additionally, the S/M affects the hydration of proteins, and hence, affects cheese texture (Pastorino et al., 2003a).

Variation in Ca and P, residual lactose, and S/M in cheese can arise either from the milk that is used for cheese manufacture, or from the alterations in cheese-making protocols. Variation in the concentration and distribution of Ca and P in milk with season or stage of lactation has been reported (Rook and Campling, 1965). A high concentration of total Ca, total P, and colloidal calcium phosphate was observed in early or late lactation milk (Kamal et al., 1961). Variation in the lactose content of milk (as wide as 1%) with stage of lactation was observed, with a decrease in lactose concentration toward the end of lactation (Rook and Campling, 1965). These seasonal differences in milk composition are more significant in countries such as Ireland or New Zealand, where cattle are pasture-fed, and are comparatively minor in countries like the United States. However, the use of concentrated milk (membrane-filtered or vacuum-condensed) for cheese manufacture is gaining popularity in dairies throughout the world. The type of concentration technique and the extent to which milk has been concentrated will influence the Ca and P, lactose, and S/M of the cheese produced (Sutherland and Jameson, 1981; Anderson et al., 1993; Acharya and Mistry, 2004; Nair et al., 2004).

In addition, variation in numerous cheese-manufacturing parameters can have a significant impact on the Ca and P, residual lactose, and S/M of cheese. For example, low set and drain pH result in lower Ca and P in cheese (Dolby et al., 1937; Metzger et al., 2001; Joshi et al., 2004). Use of a faster acid-producing starter or higher rate of starter addition can lead to a larger drop in pH before rennet is added. Holding curd in the whey for a longer period increases the residual lactose content of the cheese (Czulak et al., 1969), whereas washing the curd decreases the residual lactose content of the cheese (Shakeel-Ur-Rehman, 2004). Additionally, variation in the moisture content of curds before salting, acidity of milled curd, rate or quantity of salt deliv-

ered by salting equipment, and dimensions of the milled curd can all result in considerable variation in salt uptake and the S/M of the cheese (Fox et al., 1990). A recent study (Nair et al., 2004) has shown that use of homogenized cream for standardizing unconcentrated or concentrated milk to obtain cheese milk can also lead to an increase in salt retention.

Realizing the importance of Ca and P, residual lactose, and S/M on cheese quality, several researchers have investigated the influence of these individual factors on different aspects of cheese (Czulak et al., 1969; Thomas and Pearce, 1981; Huffman and Kristofferson, 1984; Lawrence et al., 1984; Schroeder et al., 1988; Lucey and Fox, 1993; Guinee et al., 2002; Pastorino et al., 2003a,b; Joshi et al., 2004; Shakeel-Ur-Rehman et al., 2004). No study has investigated the effects of all 3 factors in combination on cheese quality. The objective of this research was to examine the influence of modifications in cheese-making protocols on the composition of cheese.

MATERIALS AND METHODS

Experimental Design

The manufactured Cheddar cheeses constituted 3 factors (Ca and P, residual lactose, and S/M) each at 2 levels for a total of 8 different treatments. The 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**).

Cheese Manufacture

The 8 cheeses for each replicate were manufactured on 2 separate days. The treatments manufactured on each day were randomized. The cheese-manufacturing protocols used are summarized in Table 1. On each day of cheese manufacture, 1,450 kg of raw whole milk was pasteurized at 73°C for 16 s and then cooled to 30°C in a plate-heat exchanger (Super Plate, Cherry-Burrell Corporation, Chicago, IL), and 725 kg of the pasteurized milk was transferred to each of two 908-kg cheese vats (Kusel Equipment Co., Watertown, WI). Color (AFC-WS-1X, Chr. Hansen, Inc., Milwaukee, WI) was added to all treatments at a rate of 6.61 mL/100 kg of milk (30 mL/454 kg of milk). To get high residual lactose in the high lactose treatments, lactose (edible lactose, fine grind, Davisco Foods International, Inc., Eden Prairie, MN) was added at a rate of 2.5 kg/100 kg of milk, whereas no additional lactose was added for low lactose

Table 1. Modifications in cheese-making protocols to obtain Cheddar cheeses with high and low levels of Ca and P, residual lactose, and salt-to-moisture ratio (S/M)

	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 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	6.2	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 (37°C)	—	Replace half of the whey with pH-adjusted wash water (37°C)
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
Pressing	69 kPa	69 kPa	138 kPa	138 kPa
Salting	Salted at 2 levels (2.25 and 3.5% of curd weight respectively for low and high S/M)			

treatments. The same direct vat-set, frozen, concentrated starter culture (Marschall Superstart concentrated cultures, strain M62, Rhodia, Inc., Madison, WI) was used for all treatments. Starter was added at a rate of 27 mL/100 kg of milk for low Ca and P treatments and at a rate of 54 mL/100 kg of milk for high Ca and P treatments. For high Ca and P treatments, preliminary cheese-making trials had shown that it was difficult to drop the pH of cheese curds to 5.4 during the cheddaring stage. In the present study, we wanted all the cheeses to be salted at pH 5.4, so starter was added at a rate of 54 mL per 100 kg of milk for high Ca and P treatments and 27 mL per 100 kg of milk for low Ca and P treatments.

Calcium chloride (45% wt/vol, Cal-Sol, Chr. Hansen, Inc.) was added at a rate of 19.8 mL/100 kg (90 mL/454 kg) of milk to the high Ca and P treatments to increase the level of ionic calcium in the milk and increase the coagulum firmness. However, no calcium chloride was added for the low Ca and P treatments because these treatments were set at a lower pH. Rennet (Chy-max, Chr. Hansen, Inc.) was added at a rate of 9.9 mL/100 kg (45 mL/454 kg) of milk for all treatments. In the case of high Ca and P treatments, starter and rennet were added simultaneously to the milk. However, for low Ca and P treatments, starter was added to the milk and rennet was subsequently added when the milk reached pH 6.2. The rennet was diluted with 20 parts deionized water at 22 to 24°C to 1 part coagulant, immediately before adding to the milk. The coagulant was gently stirred into the milk, agitation was subsequently stopped, and the vats were allowed to set quiescently. The cut time was determined by subjective assessment of the firmness of the coagulum. The time of coagulation ranged from 20 to 30 min, with low Ca and P treatments having a shorter coagulation time compared with the high Ca and P treatments. The

coagulum was cut with 0.63-cm knives and allowed to heal for 5 min before initiating the cooking procedure.

The protocol for cooking differed in cheeses with high or low Ca and P, and high or low lactose. In treatments with high Ca and P and high lactose, the curd-whey temperature was raised to 37°C in 30 min and then maintained at 37°C for another 30 min with continuous stirring. For high Ca and P and low lactose treatments, the curd-whey temperature was raised to 37°C in 30 min. However, upon reaching 37°C, half of the whey was drained and replaced with a pH-adjusted (pH 6.4 using 85% lactic acid, Fisher Scientific, Fairlawn, NJ) wash solution containing dairy minerals (Fieldgate natural dairy calcium, First District Association, Litchfield, MN) at 37°C. This solution had a Ca and P concentration of 0.02 and 0.05%, respectively. This concentration was determined based on the Ca and P concentration obtained in the drained whey during preliminary trials. After replacing half of the whey with the wash solution, cooking was continued at 37°C for an additional 30 min. This washing technique was used to reduce the lactose content of the cheese curd without affecting the Ca and P content of the cheese curd. For the low Ca and P and high lactose treatments, the curd-whey temperature was raised to 38°C in 30 min, and then maintained at 38°C for an additional 30 min with continuous stirring. For low Ca and P and low lactose, the curd-whey temperature was raised to 38°C in 30 min. On reaching 38°C, half of the whey was drained and replaced with potable water (pH 6.1, adjusted with lactic acid) at 38°C. After replacing the whey with wash water, cooking was continued at 38°C for an additional 30 min. The final temperature of cooking for high Ca and P treatments was lower than low Ca and P treatments to decrease syneresis in the curds for high Ca and P treatments.

On completion of cooking, a stirred-curd procedure for Cheddar cheese making was used. In the case of low Ca and P treatments, all of the whey was quickly drawn at the end of cooking, and curd was continuously stirred. For the high Ca and P cheeses, a small portion of the whey was left with the curds, and curds were periodically stirred to prevent matting. Upon reaching pH 5.4, the cheese curds from each treatment were weighed and divided into 2 portions. Salt was added at a rate of 2.25 and 3.5% to the low and high salt treatments respectively. The salt was added in 3 installments, with 10 min between each salt application. The approximate time for cheese making from starter addition to salting for high Ca and P cheeses was from 3.5 to 4 h and for low Ca and P cheeses was from 4.5 to 5 h. Following salting, the salted curds were hooped and pressed at 69 kPa for high Ca and P treatments, and at 138 kPa for low Ca and P treatments. The cheeses were pressed at different pressures as an additional effort to obtain similar moisture in all the cheeses. The cheeses were removed from the press after 5 h, vacuum-sealed in polyethylene bags (3 mil nylon/PE pouches, Prime Source vacuum pouches, Koch Supplies LLC, North Kansas City, MO), and transferred to a ripening room at 6 to 8°C.

Cheese Sampling

For each treatment, three 9.1-kg blocks of cheese were obtained. The 3 blocks were randomly designated for different studies: one block was assigned for compositional and chemical/microbiological/spectroscopic analysis during ripening. Another block was used for process cheese manufacture, and the third block was used for monitoring textural changes and appearance (surface crystal formation and liquid expulsion) during ripening. The first block was cut into several portions and different pieces were randomly allocated to different time points. To prepare cheeses for chemical/microbiological analysis, samples were ground in a blender (Osterizer Galaxie, Sunbeam Products, Inc., Boca Raton, FL) to a particle size of 2 to 3 mm. The ground cheese particles were packed into 50-mL plastic snap-lid vials (leaving no head space) and stored at 4°C until analyzed.

Compositional Analysis

The pH of the milk, whey, and cheese curds during manufacturing, and cheese after manufacturing, was measured using a combination glass electrode (Orion 91-02 pH electrode, Thermo Electron Corporation, Beverly, MA) and pH meter (Corning pH/ion meter 450, Corning, Inc., Corning, NY). The milk samples were

also analyzed for fat, protein, lactose, and total solids using infrared milk analysis (AOAC, 1995). Compositional analysis was performed on cheeses after 1 wk of ripening. The moisture content of the cheeses was analyzed gravimetrically, by drying 1.5 g of cheese at 100°C in a forced draft oven (Lindberg/Blue M, Asheville, NC) for 24 h. Fat content was determined by using the Mojonnier ether extraction method (Atherton and Newlander, 1977). Total protein in cheeses was determined by measuring total nitrogen 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. Salt content in cheeses was determined using a Corning Chloride Analyzer 926 (Ciba Corning Diagnostics, Medfield, MA), based on the Volhard test (Marshall, 1992). Total Ca in cheeses was measured using an atomic absorption spectroscopy procedure adapted from Brooks et al. (1970). Total P in cheeses was determined colorimetrically (AOAC, 1995; method 991.25). Lactose and lactic acid were determined at d 1 using an HPLC-based method developed by Zeppa et al. (2001), with suitable modifications.

Statistical Analyses

A $2 \times 2 \times 2$ factorial design with 3 replications was used for statistical analysis (Table 2). For each replication, the 8 treatments (HHH, HHL, HLH, HLL, LHH, LHL, LLH, LLL) were made from the same pasteurized milk. The PROC GLM procedure of SAS, which involved 3 factors (Ca and P, residual lactose, and S/M) as class variables, was used for all data analyses (SAS Institute, 1990). If the *F*-test for the factors was significant ($P < 0.05$), treatment means were compared using least significant difference test.

RESULTS AND DISCUSSION

Milk Composition

Average fat, protein, and lactose content of milk that was used to manufacture the low lactose treatments was 3.72, 3.02, and 4.61% (wt/wt) respectively, whereas milk that was used for manufacturing the high lactose cheeses had an average fat, protein, and lactose content of 3.56, 2.97, and 7.09% (wt/wt), respectively. The lower values for fat and protein in the high lactose milk is an artifact of additional lactose in milk that decreased the level of other constituents on a percentage basis.

Cheese Composition

Average composition of the cheeses is shown in Table 3. The results indicate that the cheese-making proce-

Table 2. Statistical model used for the data analysis of cheese composition attributes, and mean squares of the respective attributes (attribute with DB indicates calculations on dry basis)

Compositional attributes													
Sources of variation	df	Moisture	Fat	Fat (DB)	Protein	Protein (DB)	Salt	S/M	Lactose ¹	Lactose (DB) ¹	Lactic acid ¹	Ca	P
Ca and P	1	23.01* (<0.01)	10.18* (<0.01)	0.98 (0.34)	4.80* (<0.01)	0.25 (0.57)	0.10 (0.12)	0.04 (0.77)	0.098* (<0.01)	0.33* (<0.01)	0.00 (0.89)	0.125* (<0.01)	0.0267* (<0.01)
Lactose	1	7.09* (<0.01)	0.13 (0.65)	2.61 (0.13)	0.25 (0.33)	0.66 (0.36)	0.08 (0.17)	0.15 (0.54)	8.319* (<0.01)	18.99* (<0.01)	0.02 (0.21)	0.005 (0.06)	0.0011 (0.11)
Salt-to-moisture (S/M)	1	29.84* (<0.01)	6.29* (<0.01)	0.38 (0.55)	2.91* (<0.01)	0.40 (0.47)	1.72* (<0.01)	21.97* (<0.01)	0.254* (<0.01)	0.46* (<0.01)	0.49* (<0.01)	0.001 (0.46)	0.0003 (0.41)
(Ca and P) $\times$ lactose	1	0.08 (0.69)	0.05 (0.78)	0.01 (0.91)	0.08 (0.58)	0.08 (0.75)	0.01 (0.65)	0.11 (0.61)	0.009 (0.20)	0.006 (0.46)	0.00 (0.57)	0.001 (0.53)	0.0002 (0.54)
(Ca and P) $\times$ S/M	1	0.52 (0.33)	0.04 (0.79)	0.07 (0.80)	0.05 (0.66)	0.62 (0.37)	0.14 (0.07)	1.16 (0.10)	0.002 (0.59)	0.002 (0.69)	0.02 (0.18)	0.000 (0.86)	0.0000 (0.84)
Lactose $\times$ S/M	1	1.11 (0.16)	0.06 (0.75)	0.25 (0.62)	0.04 (0.69)	0.11 (0.71)	0.01 (0.71)	0.10 (0.62)	0.000 (0.80)	0.007 (0.43)	0.00 (0.80)	0.000 (0.95)	0.0000 (0.84)
(Ca and P) $\times$ lactose $\times$ S/M	1	0.31 (0.44)	0.12 (0.66)	0.00 (0.95)	0.12 (0.50)	0.03 (0.83)	0.00 (0.98)	0.01 (0.88)	0.001 (0.67)	0.003 (0.63)	0.01 (0.38)	0.000 (0.86)	0.0000 (1.00)
Error	16	0.51	0.59	1.00	0.25	0.73	0.04	0.39	0.005	0.01	0.01	0.001	0.0004

¹Concentration at d 1.*Statistically significant ($P < 0.05$).

dures used in this study produced cheeses with significantly ($P < 0.05$) different (high and low) levels of Ca and P, residual lactose, and S/M (Table 2).

Moisture Content. The moisture content of the cheeses was significantly ($P < 0.05$) affected by the cheese-making parameters that also affected Ca and P, residual lactose, and S/M (Table 2). Cheeses with low Ca and P (LHH, LHL, LLH, LLL) had higher moisture compared with those with high Ca and P (HHH, HHL, HLH, HLL). In spite of the higher cooking temperature, lower drain pH, and higher pressing pressure to decrease the moisture in low Ca and P cheeses, treatments with low Ca and P had higher moisture than the high Ca and P treatments. The lower moisture content of the high Ca and P treatments may have been caused by the longer dry stirring time for these treatments, which would cause increased syneresis. A similar influence of Ca content on cheese moisture was observed by other researchers; however they attributed the lower moisture in high Ca cheese to reduced hydration of the cheese para-casein matrix compared with low Ca cheese (Guinee et al., 2002; Pastorino et al., 2003b; Joshi et al., 2004).

In addition, the moisture content of the low lactose treatments (HLH, HLL, LLH, LLL) was higher than that of the high lactose treatments (HHH, HHL, LHH, LHL). These differences may be attributed to the higher solids content of the milk used to manufacture the high lactose treatments compared with that used to manufacture the low lactose treatments. Similar differences in the moisture content of cheeses were observed when cheeses were made from vacuum-condensed milk and ultrafiltered milk that had similar fat and protein content but different lactose content (Acharya and Mistry, 2004). A lower solids (or higher moisture) content in the low lactose treatments may have also been caused by the washing of curds used in these treatments.

Moisture was higher in low S/M cheeses (HHL, HLL, LHL, LLL) compared with high S/M cheeses (HHH, HLH, LHH, LLH). It is well established that higher rates of salting increase syneresis and decrease cheese moisture (Thomas and Pearce, 1981; Pastorino et al., 2003a). Pairwise comparison of average moisture of all the treatments indicate that the treatment with high levels of all 3 factors differed the most compared with the treatment with low levels of the three factors (i.e., HHH vs. LLL; Table 3). The average moisture content of remaining treatments ranged from 34 to 36% (wt/wt; Table 3).

Fat and Protein Content. The fat and protein content of the cheeses was significantly ($P < 0.05$) affected by the cheese-making parameters that also affected Ca and P, and S/M. Average fat and protein content of cheeses ranged from 33 to 36% (wt/wt) and 24.5 to 26.5%

Table 3. Average chemical composition of cheeses expressed as percentage by weight of cheese (mean of 3 replicates)

	Treatment ¹							
	HHH	HHL	HLH	HLL	LHH	LHL	LLH	LLL
Moisture	32.07 ^a	33.80 ^{bc}	33.07 ^{ab}	35.21 ^{de}	34.08 ^{bcd}	35.94 ^e	34.39 ^{cd}	37.57 ^f
Fat	35.93 ^a	34.95 ^{ab}	35.66 ^{ab}	34.75 ^{abc}	34.48 ^{bcd}	33.62 ^{cd}	34.67 ^{abc}	33.32 ^d
Fat (DB) ²	52.89	52.80	53.27	53.64	52.30	52.48	52.83	53.37
Protein ³	26.40 ^a	25.55 ^{abc}	26.02 ^{ab}	25.29 ^{bcd}	25.15 ^{cd}	24.77 ^{cd}	25.29 ^{bcd}	24.46 ^d
Protein (DB)	38.87	38.60	38.88	39.02	38.16	38.67	38.54	39.18
Salt	2.04 ^{bc}	1.68 ^d	2.14 ^{ab}	1.73 ^{cd}	2.28 ^{ab}	1.63 ^d	2.47 ^a	1.75 ^{cd}
S/M	6.37 ^a	4.98 ^b	6.48 ^a	4.92 ^b	6.71 ^a	4.53 ^b	7.17 ^a	4.65 ^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
Lactose (d 1, DB)	2.24 ^b	2.04 ^c	0.48 ^e	0.17 ^f	2.49 ^a	2.20 ^{bc}	0.75 ^d	0.43 ^e
Lactic acid (d 1)	0.87 ^a	1.07 ^b	0.81 ^a	1.06 ^b	0.79 ^a	1.18 ^b	0.76 ^a	1.05 ^b
Total Ca	0.69 ^a	0.68 ^a	0.67 ^a	0.66 ^a	0.55 ^b	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 ^b	0.42 ^b	0.41 ^b	0.40 ^b

^{a-f}Means in a row with common superscripts do not differ ($P \geq 0.05$).

¹Treatments: 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.

²DB or % dry basis = [attribute ÷ (100 – moisture)] × 100.

³Protein = total nitrogen × 6.38.

(wt/wt), respectively (Table 3). Differences in fat and protein content of the cheeses with different levels of Ca and P, and S/M were attributed to the observed differences in their moisture content. This is supported by the fact that the fat and protein content on a dry basis was not affected ($P \geq 0.05$) by Ca and P, lactose, or S/M (Table 2).

Ca and P Content. As expected, the Ca and P content of the cheese was significantly ($P < 0.05$) affected by the modifications in set and drain pH used in this study (Table 2). The average Ca and P content of the high Ca and P treatments (HHH, HHL, HLH, HLL) was 0.68 and 0.48% (wt/wt) compared with 0.53 and 0.41% (wt/wt) for the low Ca and P treatments (LHH, LHL, LLH, LLL; Table 3). In the high Ca and P treatments, the milk was set and whey was drained at a higher pH (6.6 and 6.4, respectively) compared with the low Ca and P treatments (6.2 and 5.7, respectively). A similar approach for altering the level of Ca in cheese has been used by other researchers (Dolby et al., 1937; Guinee et al., 2002; Joshi et al., 2004). A decrease in the pH of milk causes solubilization of protein-bound Ca and P that is subsequently lost in the whey during cheese manufacture, leading to a lower Ca and P content in the final cheese produced. However, this approach to alter the concentration of Ca and P in cheese may have some secondary effects on cheese characteristics. A decrease in set or drain pH not only causes solubilization of Ca and P, but can also influence protein-protein interactions due to a decrease in the net charge of caseins as the pH is lowered toward the iso-

electric point. Therefore, any observed differences in cheese characteristics may not be solely due to differences in Ca and P content, but may be related to other confounding factors related to protein characteristics. Regardless of the potential confounding factors, a reduced set and drain pH was used because it is the only practical approach that cheese makers use to obtain cheeses of different levels of Ca and P.

It is interesting to note, however, that there was a larger difference among the treatments in the Ca content (~0.14%) compared with the P content (~0.07%). A similar result was observed by Sutherland and Jameson (1981), who varied Ca and P content of their cheeses by changing the pH of milk, and then concentrating it by ultrafiltration with or without diafiltration. In their study, they also obtained cheeses with a wider range in Ca content (~0.50%) compared with P content (~0.14%). This difference can be explained by differences in the relative solubilization of Ca and P with a change in pH. Existence of Ca and P in cheese can be broadly divided into 2 forms: free (associated with the serum phase of cheese), and bound (associated with the milk protein) (Holt, 1992). Phosphorus associated with milk proteins (i.e., bound P) can be further divided into 2 forms: organic P (phosphorus that is covalently linked to phosphoserine residues of milk proteins), and bound-inorganic P (phosphorus that is electrostatically linked with bound Ca, and is often referred to as colloidal calcium phosphate). A decrease in the pH of milk solubilizes colloidal calcium phosphate (bound Ca and bound-inorganic P), which subsequently is lost during whey drain-

age. However, it is impossible to solubilize organic P with a change in pH, because it is covalently linked to the protein (i.e., phosphoserine). Therefore, for any milk system (concentrated or unconcentrated), Ca can be solubilized to a greater extent than P, and hence, a wider range in Ca content can be achieved compared with P content.

S/M. The S/M of the cheeses was significantly ($P < 0.05$) higher for the high S/M treatments compared with the low S/M treatments (Table 2). The average S/M in the high S/M cheeses (HHH, HLH, LHH, LLH) was 6.68% (wt/wt) compared with 4.77% (wt/wt) for low S/M cheeses (HHL, HLL, LHL, LLL) (Table 3). Differences in S/M in routine cheese manufacturing can result from pretreatment of milk (adjustment of pH or concentration) or cream (homogenization of cream) before its use in cheese manufacture (Sutherland and Jameson, 1981; Fox et al., 1990; Nair et al., 2004), differences in cheese curd characteristics before salting (Fox et al., 1990), or from use of different salting rates (Thomas and Pearce, 1981; Schroeder et al., 1988).

It should be noted that the S/M that we refer to as low S/M in this study is indeed in the recommended range (4.0 to 6.0%) for a premium quality Cheddar cheese (Lawrence et al., 1984). As shown by earlier researchers (Thomas and Pearce, 1981; Schroeder et al., 1988; Pastorino et al., 2003a), a small change in S/M can lead to significant differences in pH, lactose fermentation, protein hydration, and proteolysis. Therefore, when interpreting our results for influence of S/M on cheese characteristics, readers should be cautious that the inferences should be based not only on relative values of S/M (say 4.77% for low and 6.68% for high), but also on their absolute values. Extrapolation of our results to other values of S/M needs to be done with caution.

Lactose Content. The lactose content of cheeses at d 1 was significantly ($P < 0.05$) influenced by the cheese-making parameters that also affected Ca and P, residual lactose, and S/M (Table 2). The average lactose content of the high lactose treatments (HHH, HHL, LHH, LHL) at d 1 was 1.48% (wt/wt) compared with 0.30% (wt/wt) for low lactose treatments (HLH, HLL, LLH, LLL) (Table 3). Although lactose is water-soluble, the additional lactose in the milk led to a concomitant change in the level of residual lactose in the cheese. Similar results have been observed by other researchers (Dolby et al., 1937; Huffman and Kristofferson, 1984; Anderson et al., 1993; Acharya and Mistry, 2004; Shakeel-Ur-Rehman et al., 2004).

In our study, the milks for high lactose treatments (HHH, HHL, LHH, LHL) were supplemented with 2.5% lactose, and the results would be applicable to situations where 1.5 $\times$ concentrated (vacuum-condensed or

reverse osmosis) milk is used for cheese manufacture. In the treatments with a low lactose content (HLH, HLL, LLH, LLL), the curd was washed during the cooking stage of cheese manufacture. This is analogous to the manufacture of some cheese varieties (e.g., Edam, Gouda, or Colby) where a portion of whey is removed and replaced with water. The stage at which water is added, the temperature of water, and the extent of washing are varied according to different cheese varieties. In our study, we replaced half of the whey with warm water (at 37 to 38°C) in the middle of cooking stage to efficiently remove lactose from the porous cheese curds, with a minimal influence on cheese moisture. A similar approach for lowering lactose in cheese has been used by other researchers (Dolby et al., 1937; Huffman and Kristofferson, 1984; Shakeel-Ur-Rehman et al., 2004).

Additionally, the treatments with a high S/M (HHH, HLH, LHH, LLH) had a significantly ($P < 0.05$) higher lactose content compared with cheeses with a low S/M (HHL, HLL, LHL, LLL). A higher level of lactose in the high S/M treatments may be due to less fermentation of lactose in these cheeses compared with the low S/M treatments. This hypothesis is supported by the higher levels of lactic acid at d 1 in the low S/M treatments compared with high S/M treatments (Table 3). Moreover, cheeses with low Ca and P (LHH, LHL, LLH, LLL) had a higher lactose content compared with cheeses with high Ca and P (HHH, HHL, HLH, HLL). This difference could not be explained by differences in moisture content between high and low Ca and P treatments, as determined statistically using the values for lactose on a dry basis (Table 2). It can be related to the differences in the levels of starter inoculation and buffering capacities of the cheese curds for high and low Ca and P treatments. High Ca and P treatments were inoculated with higher levels of starter bacteria, and fermented more lactose than did the low Ca and P treatments. In addition, even though all the treatments were salted at pH 5.4, more lactic acid was needed to obtain a pH of 5.4 for high Ca and P curds because of their higher buffering capacity. This caused additional fermentation of lactose to lactic acid in the high Ca and P treatments compared with the low Ca and P treatments, resulting in high residual lactose in low Ca and P treatments.

pH Changes During Cheese Manufacture

The modifications used in this study for manufacturing cheese also influenced the rate of acid production (or changes in pH) during cheese manufacture. The observed changes in pH during manufacture are shown

Figure 1. pH changes during manufacture of 3 replications of cheese with high Ca and P, high lactose (●); high Ca and P, low lactose (○); low Ca and P, high lactose (▲); and low Ca and P, low lactose (△). Zero time corresponds to addition of starter bacteria to cheese milk. The time at which cheese milk was set, whey was drained, and curds were salted are represented by the letters A, B, and C (high Ca and P with high or low lactose) and A', B', and C' (for low Ca and P with high or low lactose), respectively.

in Figure 1. The rate of acid production was higher and the total cheese-making time was shorter in high Ca and P treatments compared with low Ca and P treatments. These differences were due to the fact that the amount of starter culture used in the high Ca and P treatments was higher compared with that used in the low Ca and P treatments. Our results are in agreement with the conclusions of Van den Berg and de Vries (1974), who saw a rapid decrease in pH when a higher rate of starter was added during cheese manufacture. However, there is a minimal difference in the rate of acid development in the case of milk with different levels of lactose (represented by open vs. solid symbols in Figure 1). This indicates that lactose was not a limiting factor for acid production in milk. In other words, there was enough lactose in the milk to decrease pH, and the starter bacteria did not consume substrate (lactose) at a faster rate when more substrate was present.

The overall trend for changes in pH during cheese manufacture is comparable with previous studies (Van den Berg and de Vries, 1974; Pandey et al., 2003). Pandey et al. (2003) studied the kinetics of pH change at various stages of cheese manufacture and found that the pH decreased linearly with time during ripening and cooking/holding, whereas it decreased logarithmically with time in the cheddaring stage. Our observations of change in pH are in agreement with the conclusions of Pandey et al. (2003). Figure 1 indicates that the rate at which the pH decreased in the milk after the starter culture has been added (milk ripening) is

slower than the rate at which the pH decreased in the cheese curds after the whey was drained.

CONCLUSIONS

The modifications in cheese-making protocols used in this study produced Cheddar cheeses with the desired differences in Ca and P, residual lactose, and S/M. All the modifications used in this study for varying the levels of experimental factors were chosen with an anticipation that cheese makers could follow these modifications in cheese-making protocols to use the conclusions of this study. Although the cheeses manufactured in our study differed in moisture content, the observed differences in Ca and P, residual lactose, and S/M on cheese characteristics are still applicable. The manufactured cheeses will subsequently be used to study changes in various cheese characteristics during ripening (lactose metabolism, proteolysis, pH of cheese), and the influence of natural cheese characteristics on process cheese characteristics.

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