Effect of different curd-washing methods on the insoluble Ca content and rheological properties of Colby cheese during ripening

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ABSTRACT

A curd-washing step is used in the manufacture of Colby cheese to decrease the residual lactose content and, thereby, decrease the potential formation of excessive levels of lactic acid. The objective of this study was to investigate the effect of different washing methods on the Ca equilibrium and rheological properties of Colby cheese. Four different methods of curd-washing were performed. One method was batch washing (BW), where cold water (10°C) was added to the vat, with and without stirring, where curds were in contact with cold water for 5 min. The other method used was continuous washing (CW), with or without stirring, where curds were rinsed with continuously running cold water for approximately 7 min and water was allowed to drain immediately. Both methods used a similar volume of water. The manufacturing pH values were similar in all 4 treatments. The insoluble (INSOL) Ca content of cheese was measured by juice and acid-base titration methods and the rheological properties were measured by small amplitude oscillatory rheology. The levels of lactose in cheese at 1 d were significantly higher in CW cheese (0.06–0.11%) than in BW cheeses (~0.02%). The levels of lactic acid at 2 and 12 wk were significantly higher in CW cheese than in BW cheeses. No differences in the total Ca content of cheeses were found. Cheese pH increased during ripening from approximately 5.1 to approximately 5.4. A decrease in INSOL Ca content of all cheeses during ripening occurred, although a steady increase in pH took place. The initial INSOL Ca content as a percent of total Ca in cheese ranged from 75 to 78% in all cheeses. The INSOL Ca content of cheese was significantly affected by washing method. Stirring during manufacturing did not have a significant effect on the INSOL Ca content of cheese during ripening. Batch-washed cheeses had significantly higher INSOL Ca contents than did CW cheeses during the first 4 wk of ripening. The maximum loss tangent val-

ues (meltability index) of CW cheese at 1 d and 1 wk were significantly higher compared with those of BW cheeses. In conclusion, different curd washing methods have a significant effect on the levels of lactose, lactic acid, meltability, and INSOL Ca content of Colby cheese during ripening.

Key words: insoluble Ca, curd washing, cheese rheology, meltability

INTRODUCTION

Curd washing or dilution of whey with water is used in the manufacture of Colby, Monterey, and Gouda-type cheeses (Fox and McSweeney, 2004). In Colby cheese making, curd washing was originally introduced to help produce high moisture (39–40%), reduced acid, and open-textured cheese (Wilson and Reinbold, 1965). During Colby cheese making, cold water is added after whey drainage and the curd is held for 5 to 20 min in the water/whey mixture with continuous agitation to help achieve a uniform temperature (Van Slyke and Price, 1936).

Various types of curd-washing approaches are performed in the cheese industry, depending on the targeted properties of cheese, and these include varying the time that the curd is being held in wash water, the amount of water added and the temperature of wash water.

Including a washing step during cheese manufacturing can affect the yield (higher moisture) as well as the chemical composition, such as residual lactose, lactic acid, and pH (Van den Berg and De Vries, 1974; Lolkema, 1994; Walstra et al., 1999; Fox and McSweeney, 2004). In Gouda-type cheese making, after approximately 25 to 45% of the whey is drained, warm water (35°C) is added. Warm water enhances syneresis and some lactose is removed. After the wash water is added, the lactose content in curd decreases with only a slight increase in the lactic acid of the curd (Fox and McSweeney, 2004). Lolkema (1994) pointed out that to decrease the risk of producing an acid Gouda cheese, the use of correct quantities of wash water was critical; he suggested that the required amount of wash water

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should be selected based on the composition of the cheese milk. He also suggested that the pH, composition (e.g., moisture and lactic acid), and yield of cheese can be determined by the quantity of wash water used. He demonstrated that as the amount of wash water increased from 0 to 70% (as a percentage of curd after the first drain), the yield of cheese decreased from 11.2 to 10.7 kg of cheese/100 kg of milk. Also, the moisture content of Gouda cheese varied from 34.7 to 50% when the amount of wash water (as a percentage of curd after the first drain) was increased from 0 to approximately 76%; the amount of lactose and protein in second whey of Gouda cheese decreased from 5.1 to 2.8% and 0.8 to 0.4%, respectively, as the amount of wash water increased from 0 to 70% (Lolkema, 1994).

Van den Berg and De Vries (1974) also showed that to make Gouda cheeses with similar pH and moisture content, only 20 kg of water per 100 kg of whey + curd mixture was needed when the lactose content of cheese milk was 4.3%, whereas 33 kg of water per 100 kg of whey + curd mixture was required when the lactose content of cheese milk was 4.7%. They also proposed that the wash effect depends on the timespan between the addition of curd wash water and the holding time of curd in wash water.

Several studies have been done to determine changes in the chemical properties of cheese during ripening when curd washing or whey replacement was used (Huffman and Kristoffersen, 1984; Shakeel-Ur-Rehman et al., 2004). Huffman and Kristoffersen (1984) varied the lactose content in curd by the addition of lactose to the whey or replacement of one-half of the whey with buffer during cheese making. Huffman and Kristoffersen (1984) varied the lactose content in cheese at 1 d from 0.1 to 0.4%; after 9 mo, lactose in cheese ranged from 0 to 0.2%. Shakeel-Ur-Rehman et al. (2004) varied the residual lactose content in cheese by replacing some part of whey with warm water as well as by the addition of lactose powder. The lactose content of cheese at 1 d ranged from 0.3 to 2.2% and the high residual lactose cheese exhibited a significant decrease in cheese pH (from 5.3 to 4.8) during the 9-mo ripening period.

Lee et al. (2010) investigated the effect of different manufacturing conditions (i.e., varying the lactose content of the milk and pH values at critical steps in the cheesemaking process) on the insoluble (**INSOL**) Ca content and rheological properties of Colby cheese during ripening. The washing method used by Lee et al. (2010) was batch washing [**BW**; i.e., water added after the whey was completely drained (in most of the studies mentioned above, part of the whey was replaced with water or buffer)]. Lee et al. (2010) reported that curd washing significantly decreased the levels of lactic acid produced during ripening and resulted in signifi-

cantly higher **INSOL** Ca content compared with cheese made without curd washing.

Although several previous studies have been done on curd washing, none of the studies previously mentioned investigated the effect of using different washing methods on cheese properties. Two approaches to washing, or soaking, are used by Colby cheese manufacturers: **BW** and continuous washing (**CW**). During **CW**, the curd is stirred as water is added and allowed to drain immediately. In **BW**, water is added to the vat and the curd remains in contact with the water. We predicted that the different washing methods could alter the amount of lactose, lactic acid, and soluble Ca remaining in the curd after washing. In Gouda cheese making, about 98% of the lactose is washed out or lost in the whey, but that is dependent on the duration of washing (contact time), the size of curd grains, and the intensity of stirring (Walstra et al., 1999). The objectives of this study were to investigate the effects of different curd washing methods on the Ca equilibrium and the functional properties of Colby cheese.

MATERIALS AND METHODS

Cheese Manufacturing

Four types of full-fat, washed-curd Colby cheese were manufactured, described as **BW** with no stirring (**BWNS**), **BW** with stirring (**BWWS**), **CW** with no stirring (**CWNS**), and **CW** with stirring (**CWWS**). Cheeses were made on 3 occasions by licensed cheese makers at the University of Wisconsin–Madison Dairy Plant. The detailed make-procedure for these cheeses is shown in the Table 1. Three cheese trials were performed over a 4-mo period. A mixed strain starter culture consisting of *Lactococcus lactis* ssp. *cremoris* and *Lactococcus lactis* ssp. *lactis* was inoculated into the milk at the rate of 1.490 g/226 kg of milk (the amount of milk used in the vat was 226 kg). This bulk culture had been grown up in skim milk overnight. Double-strength chymosin (Chymax Extra, Chr. Hansen, Inc., Milwaukee, WI) was added at the rate of 17 mL/226 kg of milk at 32°C. The coagulum was cut with a 0.63-cm knife and the curd was given a 5-min heating time before gentle agitation for 10 to 15 min. The curd-whey mixture was heated slowly from 32 to 39°C and continuously stirred at 39°C until the pH of curd reached approximately 6.2. For the **BW** cheeses, after the first draining, approximately 18 kg of cold water (~10°C) was added immediately (within a 2-min period) to the cheese curd. After addition of the cold water, the curd was held (soaked) in cold water for 5 min with or without manual stirring. For the **CW** cheeses, a similar quantity of cold water was added

Table 1. Cheesemaking protocols used for Colby cheese manufactured using different washing methods (n = 3)¹

Item	Without stirring		With stirring	
	Total elapsed time (min)	pH	Total elapsed time (min)	pH
Batch washing				
Initial milk	0	6.59 ± 0.01	0	6.59 ± 0.02
Add starter	0	6.59 ± 0.01	0	6.59 ± 0.02
Add coagulant	60 ± 0	6.51 ± 0.01	60 ± 0	6.51 ± 0.01
Cut	80 ± 1	6.50 ± 0.00	80 ± 1	6.49 ± 0.01
First drain	160 ± 0	6.05 ± 0.06	162 ± 3	6.00 ± 0.00
Add water	203 ± 10	5.70 ± 0.00	201 ± 10	5.70 ± 0.00
Second drain	210 ± 10	5.65 ± 0.04	208 ± 10	5.62 ± 0.00
Add salt	223 ± 10	5.59 ± 0.07	222 ± 12	5.56 ± 0.04
Continuous washing				
Initial milk	0	6.58 ± 0.01	0	6.58 ± 0.01
Add starter	0	6.58 ± 0.01	0	6.58 ± 0.01
Add coagulant	60 ± 0	6.51 ± 0.01	60 ± 0	6.45 ± 0.13
Cut	80 ± 1	6.50 ± 0.03	81 ± 1	6.48 ± 0.02
First drain	160 ± 0	5.98 ± 0.06	160 ± 0	5.92 ± 0.06
Add water	201 ± 10	5.70 ± 0.00	198 ± 16	5.69 ± 0.04
Second drain	208 ± 10	5.69 ± 0.00	207 ± 14	5.69 ± 0.00
Add salt	222 ± 12	5.51 ± 0.07	222 ± 16	5.53 ± 0.02

¹Values ± SD.

(sprinkled/sprayed) to the cheese curd with continuous draining (from the vat) for 7 min with and without manual stirring. After the second drain, curd was salted at the rate of 0.72 kg/226 kg of milk. Salted curd was packed in 9-kg Wilson-style hoops (Koss Industrial, Inc., Green Bay, WI) and pressed at 276 kPa for 3 h at ambient temperature. Cheese was kept overnight at ambient temperature before vacuum packaging. After vacuum packaging, the cheese was stored at 6°C for further analysis. Two 9-kg blocks of cheese were made from each treatment in each trial.

Compositional Analysis

All compositional tests were done at least in triplicate. Milk was analyzed for total solids, CN, fat, and protein content (Marshall, 1992). Rennet whey was made from cheese milk on the same cheesemaking day (Lee et al., 2010) and the total Ca content of milk and rennet whey was measured by inductively-coupled argon plasma emission spectroscopy (Park, 2000). Cheese was analyzed for moisture, fat, protein (Marshall, 1992), and salt by a Corning (Medfield, MA) salt analyzer (Marshall, 1992). Lactose and lactic acid (both D- and L-lactate) of milk and cheese were determined by enzymatic methods (Severn et al., 1986; IDF, 1993). During cheese ripening, cheese pH using a pH electrode (Sam Gray gold electrode; Nelson-Jameson, Marshfield, WI; Marshall, 1992), pH 4.6-soluble nitrogen (Kuchroo and Fox, 1982), and the INSOL Ca contents of cheese were analyzed (juice and titration methods; Hassan et al.,

2004) at 1 d, 1 wk, 1 mo, and 3 mo. The buffering capacity of cheese was determined by acid-base titration (Hassan et al., 2004) and the buffering capacity was reported as volume of 0.5 N HCl needed to decrease the pH of cheese dispersions by 1.0 pH unit from the initial pH of cheese dispersions (Lee et al., 2010).

Dynamic Small Amplitude Oscillatory Rheometry

The viscoelastic properties of cheese were determined by a Paar Physica universal dynamic spectrometer (UDS 200; Physica Messtechnik, Stuttgart, Germany) using dynamic small amplitude oscillatory rheology (SAOR). The procedure described by Lucey et al. (2005) was used. The viscoelastic properties were measured at an applied strain of 0.2%. Cheeses were heated from 5 to 80°C at the rate of 1°C/min. The rheological parameters, including storage modulus (G'), or stiffness; loss modulus (G''); and loss tangent (LT), which is the ratio between the viscous and the elastic properties of the material ($LT = G''/G'$), were determined from SAOR tests. At least 3 replicates were measured for each cheese sample at each ripening time point.

Statistical Analysis

Data was analyzed using the statistical analysis system, version 8.02 (SAS Institute Inc., Cary, NC). Experimental effects of different curd washing method (BWNS, BWWS, CWNS, and CWWS) and week (ag-

Table 2. Composition of milk and Colby cheeses made with different methods of washing (means $\pm$ SD)

Item	Composition	Batch washing		Continuous washing	
		No stirring	With stirring	No stirring	With stirring
Milk					
Total solids (%)	12.0 ± 0.2				
Casein (%)	2.5 ± 0.1				
Lactose (%)	4.5 ± 0.1				
Fat (%)	3.5 ± 0.1				
Total Ca (mg/100 g)	105 ± 4				
Soluble Ca (mg/100 g)	37 ± 2				
Cheese					
Moisture (%)		38.5 ± 0.2 ^a	38.4 ± 0.5 ^a	38.4 ± 0.7 ^a	38.6 ± 0.8 ^a
Fat (%)		32.9 ± 0.7 ^a	33.0 ± 0.7 ^a	33.0 ± 0.8 ^a	32.9 ± 0.7 ^a
Salt (%)		1.3 ± 0.2 ^a	1.4 ± 0.2 ^a	1.3 ± 0.0 ^a	1.4 ± 0.2 ^a
Protein (%)		23.9 ± 0.8 ^a	23.9 ± 0.1 ^a	24.0 ± 0.3 ^a	24.0 ± 0.4 ^a
Salt-in-moisture (%)		3.4 ± 0.4 ^a	3.6 ± 0.1 ^a	3.3 ± 0.0 ^a	3.6 ± 0.5 ^a
Lactose at 1 d (%)		0.02 ± 0.0 ^b	0.02 ± 0.0 ^b	0.06 ± 0.0 ^a	0.11 ± 0.0 ^a
Lactic acid at 2 wk (%)		1.17 ± 0.1 ^c	1.05 ± 0.1 ^d	1.31 ± 0.0 ^a	1.23 ± 0.0 ^b
Lactic acid at 12 wk (%)		1.11 ± 0.0 ^b	1.12 ± 0.0 ^b	1.21 ± 0.1 ^a	1.20 ± 0.0 ^a
Buffering capacity at 1 d ¹ (mL)		3.4 ± 0.0 ^a	3.4 ± 0.0 ^a	3.1 ± 0.0 ^b	3.1 ± 0.0 ^b
Buffering capacity at 1 mo ¹ (mL)		3.1 ± 0.0 ^a	3.1 ± 0.0 ^a	2.8 ± 0.0 ^b	2.9 ± 0.0 ^b
Total Ca (mg/100 g)		704 ± 32 ^a	706 ± 25 ^a	704 ± 18 ^a	704 ± 25 ^a
Insoluble Ca at 1 d ²		22.6 ± 0.6 ^a	22.7 ± 1.1 ^a	21.1 ± 1.7 ^b	21.7 ± 0.1 ^b
Insoluble Ca at 1 wk ²		22.1 ± 0.7 ^a	22.7 ± 0.3 ^a	20.8 ± 2.1 ^b	21.1 ± 1.5 ^b
Insoluble Ca at 1 mo ²		20.3 ± 0.0 ^a	21.0 ± 0.8 ^a	18.2 ± 1.6 ^b	19.1 ± 1.9 ^b
Insoluble Ca at 3 mo ²		18.9 ± 2.4 ^a	18.6 ± 1.7 ^a	18.2 ± 3.2 ^a	19.1 ± 0.9 ^a
LT _{max} value at 1 d ³		0.82 ± 0.0 ^b	0.85 ± 0.1 ^b	1.06 ± 0.1 ^a	1.05 ± 0.2 ^a
LT _{max} value at 1 wk ³		1.74 ± 0.2 ^b	1.79 ± 0.0 ^b	1.89 ± 0.0 ^a	2.13 ± 0.0 ^a
LT _{max} value at 1 mo ³		2.53 ± 0.1 ^a	2.38 ± 0.2 ^a	2.52 ± 0.1 ^a	2.51 ± 0.1 ^a
LT _{max} value at 3 mo ³		2.58 ± 0.1 ^a	2.44 ± 0.2 ^a	2.47 ± 0.1 ^a	2.42 ± 0.1 ^a

^{a-d}Means with different letters in the same row indicate that values were significantly different ($P < 0.05$).

¹The amount of 0.5 N HCl solution required to titrate cheese homogenate (8 g of cheese/40 g of water) from its starting pH to 1.0 pH unit lower than the initial pH.

²Milligrams of insoluble Ca/g of protein in cheese.

³LT_{max} is the maximum loss tangent during heating from small amplitude oscillatory rheology (SAOR) test.

ing time) on INSOL Ca content, rheological properties and pH 4.6-soluble nitrogen were evaluated using the Proc MIXED procedure for repeated measurement of the SAS software package. Effects included method, week, and method $\times$ week interactions. The least mean squares for cheese, nested within method, were used as random error terms in the test method. The Fisher protected least significant difference test was used to compare means and differences between means were considered to be significant at $P < 0.05$. Pearson correlation coefficients were estimated between the various responses (i.e., INSOL Ca, pH 4.6-soluble nitrogen, rheological, and parameters).

RESULTS AND DISCUSSION

Cheesemaking

Similar ($P > 0.05$) first draining pH values of approximately 6.0 to 6.05 and 5.92 to 5.98 were obtained for BW and CW cheese, respectively (Table 1). The

key manufacturing pH values were kept constant when the washing process was varied (Table 1). The second draining pH was also similar in all cheeses. The similar manufacturing pH values at the critical points during cheese making resulted in cheese having similar total Ca contents (Table 2). The amount of Ca retained after cheese making is very dependent on the pH at whey draining (Lucey and Fox, 1993).

Chemical Composition of Cheese

The moisture content of all cheeses (Table 2) was approximately 38 to 39%, which is typical of Colby cheese (Fox and McSweeney, 2004). The moisture, fat, and protein contents of cheese were not significantly different (Table 2).

The residual (1-d) lactose contents of BW cheese (0.02%) were significantly lower (Table 2) than those of CW cheeses (0.06 to 0.11%). The lactose level of all of these cheeses was much lower compared with the typical residual (1-d) lactose content (0.4–0.8%) of

Cheddar cheese (Fox and McSweeney, 2004). The use of higher salt levels in Cheddar cheese also slows the fermentation of lactose (Swearingen et al., 2004).

The lactic acid content of cheeses at 2 wk of ripening ranged from 1.05 to 1.31% (Table 2) and decreased in the following order; CWNS > CWWS > BWNS > BWWS. The higher lactic acid levels in CW cheeses (at 2 and 12 wk) were probably due to their higher initial residual lactose contents (Table 2). Fermentation of the residual lactose in cheese during ripening increases the lactic acid levels (Huffman and Kristoffersen, 1984; Shakeel-Ur-Rehman et al., 2004).

Cheese pH

The pH of all cheeses at 1 d was significantly lower ($P < 0.05$) compared with the cheese pH values at 3 mo (Figure 1). The pH values at 1 d for all cheeses were not significantly different. Batch washing with stirring cheese had a significantly higher cheese pH compared with BWNS cheese at the 1-wk and 1-mo ripening time (Figure 1). An increase in cheese pH is due to the reduction of INSOL Ca. When Ca is solubilized, phosphate groups are released from colloidal Ca phosphate and this phosphate combines with hydrogen ions decreasing the concentration of free hydrogen ions in cheese, which results in an increase in cheese pH (Hassan et al., 2004). An increase in cheese pH during ripening in cheeses with a curd washing or whey dilution step has been reported in other studies (Huffman and Kristoffersen, 1984; Shakeel-Ur-Rehman et al., 2004).

A higher buffering capacity was observed in BW cheeses compared with CW cheeses at both 1 d and 1 mo of ripening (Table 2) and this corresponded with higher levels of INSOL Ca in the BW cheeses.

Ca Equilibrium in Cheese

Total Ca content of cheese was about 704 mg/100 g of cheese, which is typical for Colby cheese (Johnson and Lucey, 2006). The total Ca contents (Table 2) of all of the cheeses were not significantly different, presumably due to use of similar draining pH values in all treatments. The initial (1-d) INSOL Ca content was significantly higher in BW cheeses than in CW cheeses (Table 2; Figure 2). It should be noted that this indicated that it was possible to vary the initial INSOL Ca content of cheese by adopting different washing methods, without changing the total Ca content of cheese (Table 2). After 1 mo of ripening, no significant difference was observed in the INSOL Ca content of all cheeses (Table 2).

A decrease in INSOL Ca content of all cheeses during ripening was observed, as measured by both acid-base titration and cheese juice methods (Figure 2). A sig-

nificant decrease in the INSOL Ca content of Cheddar (Hassan et al., 2004; Lee et al., 2005; Lucey et al., 2005) and Colby (Lee et al., 2010) cheese during ripening has been reported.

The extent of the decrease in the INSOL Ca content (mg/100 g of protein in cheese) of the various types of cheeses during the first month of ripening was in the following order: CWNS > CWWS > BWNS > BWWS (Table 2). After 1 mo, the CW cheese did not show any further decrease in INSOL Ca content (Table 2), whereas BW cheeses continued to exhibit a decrease in the INSOL Ca content (1.4 and 2.4 mg/100 g of protein in cheese for BWNS and BWWS, respectively). This trend was observed with both juice and acid-base titration methods (Figure 2).

The significantly higher initial lactose content in CW cheeses and its subsequent fermentation to lactic acid during the first month of ripening (Table 2) contributed to its greater loss of INSOL Ca compared with BW cheeses (Lee et al., 2005). After 1 mo, a stable pseudo-equilibrium was probably reached for the CW cheese (Lee et al., 2005; O'Mahony et al., 2006) as little further decrease in INSOL Ca content took place. The initial decrease in the INSOL Ca content in BW cheese was slower than in CW cheese probably due to the low levels of lactic acid (Table 2), but the decrease in INSOL Ca content in BW cheeses continued during the 3 mo of ripening. The BW cheeses appeared to have a lower concentration of soluble components, as indicated by the lower initial lactose content of the curd, probably due to a more effective washing process compared

Figure 1. Changes in cheese pH for batch washing with no stirring (BWNS; ●), batch washing with stirring (BWWS; ○), continuous washing with no stirring (CWNS; ▲), and continuous washing with stirring (CWWS; △) as a function of ripening time. The data represent the means ($n = 3$), whereas the error bars represent the SD for each time point.

Table 3. Pearson correlation coefficients between different ripening parameters¹ of cheese made with the different washing methods

Item	Cheese pH	LT _{max}	Temperature at LT _{max}	G' value at 40°C
INSOL Ca	−0.56****	−0.76****	0.62****	0.67****
Cheese pH		0.60****	−0.60****	−0.51****
LT _{max}			−0.44****	−0.79****

¹INSOL Ca = percentage of insoluble Ca as a percentage of total Ca; LT_{max} = maximum loss tangent; G' = storage modulus.

**** $P < 0.0001$.

with that for the CW cheeses. A lower initial soluble Ca concentration in cheese sera made from BW could explain why we observed the slow ongoing solubilization of INSOL Ca during ripening for this type of cheese.

Significant correlations were observed between ripening parameters, although the strength of these relationships was not very high ($r < 0.80$). Cheese pH values and INSOL Ca contents of cheese during aging were significantly negatively correlated ($P < 0.0001$, $r =$

−0.56; Table 3). Lower pH values (higher acid) probably help to solubilize the INSOL Ca in cheese.

The INSOL Ca content of cheese during ripening was significantly affected ($P < 0.001$) by washing method (i.e., BW or CW) and ripening time (Table 4). The stirring process did not significantly affect the INSOL Ca content of cheese. For both the batch and the CW approaches, only minor differences existed in the lactic acid/lactose or INSOL Ca contents of cheeses made with stirring or without stirring (Table 2).

Rheological Properties of Cheese

The G' values of cheese at 5°C during ripening were considerably higher than the values at 40°C, which were higher than those at 80°C (Figure 3). A similar trend was reported by Lucey et al. (2005) for Cheddar cheese. The initial G' of cheeses at 5°C at 1 d was in the range of 260 to 350 kPa (Figure 3a). The G' values at 5°C for the CWNS cheese was lower compared with the other cheeses (Figure 3a). The G' values at 1 d and 1 wk for BW cheeses measured at 80°C tended to be higher than those for the CW cheeses (Figure 3c). It should be noted that BW cheese had a significantly higher INSOL Ca content at 1 d and 1 wk compared with CW cheese (Table 2). The INSOL Ca content has been related to the G' value of cheese measured at high temperatures because INSOL Ca interactions are heat stable, whereas most other interactions (e.g., hydrogen bonds) may weaken with increasing temperature (O'Mahony et al., 2006). At ripening periods longer than 1 wk, the G' values at 5, 40, and 80°C were similar between treatments (Figure 3). The G' values at 40°C and INSOL Ca contents were significantly positively correlated ($P < 0.0001$; Table 3). A significant negative correlation ($P < 0.0001$) existed between the G' values at 40°C and pH values, probably due to effect of pH on the INSOL Ca content.

The initial (1-d) maximum LT (LT_{max}) values were in the range of 0.8 to 1.1 and an increase in the LT_{max} values occurred in all cheeses during the first 4 wk (Table 2). During ripening, an increase in the LT_{max} value of cheese with pH values >5.0 has been reported in other

Figure 2. Changes in the percentage of insoluble Ca content (percentage of total Ca in cheese) for batch washing with no stirring (BWNS; ●), batch washing with stirring (BWWS; ○), continuous washing with no stirring (CWNS; ▲), and continuous washing with stirring (CWWS; △) as a function of ripening time by cheese juice (a) and by acid-base titration (b) methods. The data represent the means ($n = 3$), whereas the error bars represent the SD for each time point.

Table 4. Mean squares, probabilities, and df for factors that may influence the insoluble (INSOL) Ca content¹ of Colby cheese

Factor	df	Mean squares	<i>P</i> -value
Washing method ²	1	70.84	0.0008*
Stirring method ³	1	10.11	0.0690
Error	7	2.19	
Week ⁴	3	257.60	<0.0001*
Washing method × week	3	11.23	0.04*
Stirring method × week	3	0.46	0.93
Washing method × week × stirring method	3	0.50	0.93
Error	19	3.34	
R ²	0.95		

¹Insoluble Ca content as a percentage of total Ca in cheese determined by the cheese juice method.

²Washing methods used were batch washing and continuous washing.

³Stirring methods during cheese manufacture were stirring or no stirring.

⁴Cheese-ripening time.

**P* < 0.05.

studies (Lee et al., 2005; Lucey et al., 2005). During ripening at the 1-d and 1-wk time points, significantly higher LT_{max} values were observed for CW cheeses compared with BW cheese (Table 2). No differences existed in LT_{max} values between cheeses manufactured with or without stirring (Table 2). Cheese pH was significantly positively correlated with LT_{max} value ($P < 0.0001$; Table 3). Significantly negative correlations were observed between the INSOL Ca content of cheese and the LT_{max} values of cheeses ($P < 0.0001$, $r = -0.76$). Higher LT_{max} values with lower INSOL Ca contents of Cheddar cheese have been previously reported (Lucey et al., 2005; O'Mahony et al., 2006), suggesting that the loss of INSOL Ca facilitates more melttable cheese (Lucey et al., 2003).

The temperature of the LT_{max} generally decreased during ripening for all cheeses, apart from a slight increase between 1 d and 1 wk (Figure 4); the small increase in the temperature of the LT_{max} value (i.e., more thermal energy required for cheese to melt/flow) during the first week may have been due to a combination of an increase in cheese pH and small increase in the G' values at 5°C in this period. A decrease in the temperature of the LT_{max} has been observed in other cheeses during ripening (Lee et al., 2005; Lucey et al., 2005). The decrease in the temperature of the LT_{max} during most of the ripening period could be related to the combined effect of the ongoing loss of INSOL Ca cross-linking between CN and proteolysis, which makes it easier for cheese to melt when heated.

Proteolytic Activity (Level of pH 4.6-Soluble Nitrogen)

A continuous increase occurred in the level of pH 4.6-soluble nitrogen with age in all cheeses (Figure 5); no significant differences were observed in the levels of

pH 4.6-soluble nitrogen in cheeses at 1 d, 1 wk, and 3 mo. This suggests that different curd-washing methods did not affect proteolysis during cheese ripening. Thus, pH 4.6-soluble nitrogen was not influenced by the differences in the INSOL Ca content of our experimental cheeses, which is in contrast to other studies that observed higher levels of proteolysis in cheeses that had greater acid development (Watkinson et al., 2001; Lee et al., 2005). The increase in pH observed in Colby cheese was in contrast to these previous studies and higher pH values may have favored plasmin activity but decreased residual rennet activity.

O'Mahony et al. (2005) reported that the decrease in hardness of Cheddar cheese during first 3 wk of ripening was more highly correlated with the level of INSOL Ca than the level of pH-4.6 soluble nitrogen in cheese where residual coagulant activity was inhibited by the addition of pepstatin (a potent competitive inhibitor of aspartyl proteases). O'Mahony et al. (2005) proposed that the initial softening of texture in Cheddar cheese was largely due to the solubilization of INSOL Ca.

Therefore, the initial differences in melting (e.g., higher LT_{max} values) properties of cheese from the different washing techniques was likely due to the differences in the levels of INSOL Ca content in these cheeses (Table 2), rather than the levels of pH 4.6-soluble nitrogen because no significant differences in proteolysis were observed between treatments (Figure 5). Insoluble Ca is a key cross-linking material between CN (Horne, 1998) and a lower INSOL Ca content of cheese can give softer and more melttable cheese (Lucey and Fox, 1993) unless the pH of cheese is very low (pH <4.9; Lee et al., 2005). The higher initial INSOL Ca level in BW cheese compared with CW cheese may result in greater protein-Ca interactions compared with those in CW cheese and limit the ability of CN-CN interactions to relax at high temperatures and, thus, retard melt (Lu-

Figure 3. Changes in storage modulus (G') at 5 (a), 40 (b), and 80°C (c) as a function of ripening time for cheese tested at 1 d, 1 wk, and 3 mo using the small amplitude oscillatory rheology method. The data represent the means ($n = 3$), whereas the error bars represent the SD for each time point. BWNS = batch washing with no stirring; BWWS = batch washing with stirring; CWNS = continuous washing with no stirring; CWWS = continuous washing with stirring.

Figure 4. Changes in the temperature at maximum loss tangent from the small amplitude oscillatory rheology test for batch washing with no stirring (BWNS; ●), batch washing with stirring (BWWS; ○), continuous washing with no stirring (CWNS; ▲), and continuous washing with stirring (CWWS; △) as a function of ripening time. The data represent the means ($n = 3$), whereas the error bars represent the SD for each time point.

cey et al., 2005). The G' value at 40°C was significantly correlated with INSOL Ca content of cheese, indicating that cheese stiffness (G') was related to INSOL Ca cross-linking.

CONCLUSIONS

Different curd-washing methods altered the levels of residual lactose, lactic acid, and INSOL Ca in Colby cheese. Cheeses made with the CW method had higher residual lactose and lactic acid contents compared with cheeses made with BW. The total Ca contents of

Figure 5. Changes in the pH 4.6-soluble nitrogen as percentage of total nitrogen for batch washing with no stirring (BWNS; ●), batch washing with stirring (BWWS; ○), continuous washing with no stirring (CWNS; ▲), and continuous washing with stirring (CWWS; △) as a function of ripening time. The data represent means ($n = 3$), whereas the error bars represent the SD for each time point.

cheeses made with different washing methods were not significantly different; the total Ca content of cheese is largely dictated by the pH values at renneting and at whey drainage and in our cheesemaking experiments, all of the key manufacturing pH values were similar. The INSOL Ca content of cheese was influenced by the lactic acid levels in cheese, which were altered by the method of curd washing. The pH of all cheeses increased during ripening, probably due to the ongoing solubilization of INSOL Ca. No significant differences in overall proteolytic activity during ripening were observed in cheeses made from the different washing methods. When heated, cheeses made with the CW method were softer and more meltable, presumably due to their lower INSOL Ca content, compared with cheeses made with BW. In conclusion, the method used to wash curd can be used to modify the chemical and rheological properties of cheese. Washing method is another factor for Colby cheese makers to consider if they wish to alter the textural properties of their cheese.

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