Gas-Flushed Packaging Contributes to Calcium Lactate Crystals in Cheddar Cheese

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ABSTRACT

Gas-flushed packaging is commonly used for cheese shreds and cubes to prevent aggregation and loss of individual identity. Appearance of a white haze on cubed cheese is unappealing to consumers, who may refrain from buying, resulting in lost revenue to manufacturers. The objective of this study was to determine whether gas flushing of Cheddar cheese contributes to the occurrence of calcium lactate crystals (CLC). Cheddar cheese was manufactured using standard methods, with addition of starter culture, annatto, and chymosin. Two different cheese milk compositions were used: standard (lactose:protein = 1.47, protein:fat = 0.90, lactose = 4.8%) and ultrafiltered (UF; lactose:protein = 1.23, protein:fat = 0.84, lactose = 4.8%), with or without adjunct *Lactobacillus curvatus*. Curds were milled when whey reached 0.45% titratable acidity, and pressed for 16 h. After aging at 7.2°C for 6 mo, cheeses were cubed (1 × 1 × 4 cm) and either vacuum-packaged or gas-flushed with carbon dioxide, nitrogen, or a 50:50 mixture of carbon dioxide and nitrogen, then aged for an additional 3 mo. Heavy crystals were observed on surfaces of all cubed cheeses that were gas-flushed, but not on cheeses that were vacuum-packaged. Cheeses without *Lb. curvatus* exhibited L(+)-CLC on surfaces, whereas cheeses with *Lb. curvatus* exhibited racemic mixtures of L(+)/D(-)-CLC throughout the cheese matrices. The results show that gas flushing (regardless of gas composition), milk composition, and presence of nonstarter lactic acid bacteria, can contribute to the development of CLC on cheese surfaces. These findings stress the importance of packaging to cheese quality. (**Key words:** gas flush, calcium lactate crystals, Cheddar cheese, *Lactobacillus curvatus*)

Abbreviation key: CLC = calcium lactate crystals, NSLAB = nonstarter lactic acid bacteria.

INTRODUCTION

Quality and appearance defects in Cheddar cheeses discourage repeat purchases by consumers, and thus, white crystals on the surface of Cheddar cheese detrimentally affect sales. Unattractive crystals on Cheddar cheese have been documented since the 1930s (McDowall and McDowell, 1939), and yet the problem remains a challenge and expense to cheese manufacturers (Chou et al., 2003). A large number of Cheddar cheeses manufactured in the United States have the problem of calcium lactate crystals (CLC; Johnson, 2004). According to Johnson (2004), almost all cheese plants experience CLC problems in mild and medium Cheddar cheese, which previously was a common problem found only in aged cheeses.

With the large-scale production of rindless cheese, packaging plays an important role in maintaining the quality of cheese. Various flexible and barrier films are used to package cheese for aging. The 2 main packaging methods used by cheese manufacturers are vacuum packaging and gas-flushed packaging. Gas flushing and heat sealing are used to package cheese shreds and cubed cheeses to prevent aggregation and loss of individual identity of cheese particles. The gases generally used to flush out air are carbon dioxide, nitrogen, or a combination of the two. Johnson et al. (1990a) observed faster and greater crystal formation on cheeses that were gas-flushed using CO₂ than on cheeses that were vacuum-packaged. A small number of crystals were seen on vacuum-packed cheese after 5 mo of aging compared with a large number of crystals in gas-flushed packaged cheese. Dybing et al. (1988) hypothesized that free ionic calcium combines with lactate through a mechanism involving carbonic acid. According to Dybing et al. (1988), packaged cheese flushed with CO₂ absorbs CO₂. As CO₂ is absorbed, the pH of the serum phase is reduced. It is hypothesized that low pH in cheese shifts colloidal calcium to soluble calcium (Hassan et al., 2004), and increased serum calcium concentration facilitates CLC formation.

Calcium lactate crystals have also been observed on vacuum-packaged cheeses in which the package has lost integrity (Johnson et al., 1990a). Because cheese serum tends to move to the surface of the cheese or to

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cracks and crevices inside the cheese during aging, lactic acid concentration in those spaces increases. In loosely packaged cheese, the surface of the cheese dries due to evaporation, forming nucleation sites that accelerate crystal formation.

Residual calcium and lactose, after pressing, also contribute to CLC. Lactose is metabolized to lactic acid and can react with calcium in cheese to form CLC (Pearce et al., 1973; Sutherland and Jameson, 1981). The calcium content in Cheddar cheese is approximately 7.6 g/kg (Fox et al., 2000) but cheese processing conditions affect the distribution of calcium between soluble and insoluble compartments. During Cheddar cheese manufacture and within the first few weeks of aging, most of the lactose is converted to lactic acid (Huffman and Kristoffersen, 1984) by starter bacteria or nonstarter lactic acid bacteria (NSLAB; Thomas and Crow, 1983; Dybing et al., 1988; Fox et al., 2000). Elevated lactose (more than 4.8%) in cheese milk yields increased lactose in cheese, which can be used by starter bacteria or NSLAB to produce lactate in cheese and potentially increase calcium lactate concentrations (Pearce et al., 1973; Dybing et al., 1988). However, Blake et al. (2005) showed that high lactose in cheese milk does not guarantee CLC.

Another major contributor to CLC in Cheddar cheese is NSLAB. Most facultative heterofermentative NSLAB produce a racemic mixture of D(-)/L(+)-lactate (Turner and Thomas, 1980; Johnson et al., 1990b; Chou et al., 2003). Facultative heterofermentative NSLAB also tend to produce lactic acid from other substrates present in cheese such as galactose and citrate, contributing to increased concentration of lactic acid in cheeses with NSLAB (Sharma, 2003). Galactose and citrate are starter metabolites not utilized by starter bacteria. Moreover, the presence of facultative heterofermentative NSLAB such as *Lactobacillus curvatus*, which produces a racemic mixture of L(+) and D(-)-lactic acid in cheese, contributes to CLC (Johnson et al., 1990b; Somers et al., 2001; Chou et al., 2003). The calcium salt of D(-)-lactate is less soluble than the calcium salt of L(+)-lactate (Cao et al., 2001; Kubantseva et al., 2004), so the presence of D(-)-lactate often results in crystallization of D(-)-calcium lactate on cheese surfaces.

Visible CLC, especially on cubed or shredded cheese surfaces, have troubled the cheese industry for some time. Recently, CLC have been observed on cubed and shredded cheeses with predominantly L(+)-lactic acid and no or low D(-)-lactic acid. A desire to understand the formation of L(+)-lactate crystals on cheese surfaces led us to investigate the effect of gas flushing on the formation of CLC. The objective of this study was to determine effects of flushing CO₂, N₂, and CO₂:N₂ (50:50) on formation of CLC in Cheddar cheese made

from 2 different milk compositions, in the absence and presence of the NSLAB *Lb. curvatus*.

MATERIALS AND METHODS

Cheese Manufacture and Packaging

Two different batches of milk were standardized to make cheeses, based on selected cheese-milk formulations used in industry. Cheeses were manufactured in duplicate, with each replicate made from 90.8 kg of milk for a total of 8 cheeses. Standard milk (lactose:protein = 1.47, protein:fat = 0.90, lactose = 4.8%) was used to make standard milk plus starter culture (batch 1), and standard milk plus starter culture plus *Lb. curvatus* adjunct culture (batch 2). Two duplicate batches of cheese were made from UF milk (lactose:protein = 1.23, protein:fat = 0.84, lactose = 4.8%); UF milk plus starter culture (batch 3), and UF milk plus starter culture plus *Lb. curvatus* adjunct culture (batch 4). Cheeses were made using standard procedures followed at the Washington State University Creamery (Pullman, WA). The standardized and pasteurized cheese milk was added to a hot-water-jacketed cheese vat. Starter culture *Lactococcus lactis* ssp. *cremoris* #98 (Chr. Hansen, Milwaukee, WI), was grown to a cell density of 10⁸ cfu/mL in sterilized internal pH controlled buffer media (Vivolac, Indianapolis, IN), and inoculated into standardized milk at a rate of 1% (wt/wt) at 32°C. *Lactobacillus curvatus* were grown to a cell density of 10⁸ cfu/mL in lactobacillus de Man, Rogosa, and Sharpe broth (Becton Dickinson and Co., Sparks, MD) and added to milk to achieve initial populations of 500 to 700 cfu/mL in the cheese milk to mimic the low initial NSLAB counts typically observed in pasteurized cheese milk (Johnson et al., 1990b). Double-strength coagulator (Chy-Max, Chr. Hansen Laboratories), diluted 1:40 with tap water, was used to assist coagulation of the milk. At the time of cutting with 6-mm grid cheese knives, titratable acidity (as % lactic acid) of cheese whey was 0.12%. Curds were cooked by increasing the temperature from 31 to 38°C at a rate of 1°C every 5 min over a 30-min period. Curds and whey were stirred at 38°C for 45 min before draining, at whey titratable acidity of 0.15%, and cheddaring.

When the titratable acidity of the whey reached 0.45 to 0.47%, the loaves were milled and curds were salted (0.3% wt/wt of milk). After overnight pressing at 275 MPa, cheeses were cut into wedges (7.5 × 5.5 × 4 cm) weighing approximately 150 g, and vacuum-packaged (Model X180, Koch Supplies Inc., Kansas City, MO) in 15 × 20 cm, 3-mil high-barrier nylon/EVOH/PE vacuum pouches (Koch Supplies Inc.). Finished cheeses were then aged at 7.2°C for 6 mo.

Table 1. Proximate analysis of experimental Cheddar cheeses at d 2 compared with typical Cheddar cheese.

Treatments	Composition ¹					
	Fat (% wwb)	Protein (% wwb)	Moisture (% wwb)	Salt (% wwb)	Salt in moisture (% wwb)	Lactose (g/100 g of cheese)
Standard cheese without adjunct	34.5 ^a	24.0 ^a	35.1 ^a	1.66 ^a	4.73 ^a	0.44 ^a
Standard cheese + 1 adjunct (<i>Lactobacillus curvatus</i>)	33.8 ^a	24.6 ^a	36.2 ^{ab}	1.22 ^b	3.36 ^b	0.36 ^{ab}
Ultrafiltered cheese without adjunct	34.0 ^a	25.5 ^b	36.4 ^{ab}	1.61 ^a	4.44 ^a	0.41 ^a
Ultrafiltered cheese + 1 adjunct (<i>Lactobacillus curvatus</i>)	34.5 ^a	24.7 ^{ab}	36.9 ^b	1.16 ^b	3.15 ^b	0.47 ^a
Typical Cheddar cheese ²	32.0	25.0	37.0	1.50	4.05	NA ³

^{a,b}Means within the same category (fat, protein, moisture, salt, salt in moisture, pH, and lactose) with different superscripts differ significantly ($P < 0.05$).

¹Fat, protein, moisture, salt, and salt in moisture (% wwb = wet weight basis) were analyzed on d 2 after cheese manufacture; lactose (g/100 g of cheese) was analyzed on d 1 after cheese manufacture.

²Kosikowski and Mistry (1997).

³NA = Not available.

After 6 mo of aging, the cheeses were cut and repackaged as in a cut-and-wrap facility. The cheeses from individual treatments were cut into $1 \times 1 \times 4$ cm pieces with a french fry cutter (Shaver Specialty Co., Los Angeles, CA). Randomly, 10 pieces of cut cheese were either vacuum packaged or gas-flushed packaged (Model X180, Koch Supplies Inc.) in 15×20 cm, 3-mil high-barrier nylon/EVOH/PE vacuum pouches (Koch Supplies Inc.) with either CO₂, N₂, or a 50:50 mixture of both gases (CO₂:N₂).

Analyses

Proximate analysis of cheese (Table 1) was conducted using standard procedures (Marshall, 1992). The volume of gas inside each gas-flushed package was determined by measuring the volume of water (at 21°C) displaced by submerging the cheese packages in a graduated cylinder. The volume of gas in each flushed package was measured after wk 1, 2, 4, 8, and 12 of gas flushing. The pH of the cheeses was measured after 1, 2, 4, 8, 12 wk of packaging using a pH electrode (Orion 915500, Beverly, MA) calibrated with buffer at pH 4 and 7 (Fisher Scientific, Fair Lawn, NJ) before each use. Total counts and lactobacilli were measured in cheeses at the end of 12 wk of aging after gas flushing. Cheese microflora were enumerated by emulsifying 11 g of aseptically obtained cheese samples in 2% (wt/vol) trisodium citrate buffer at 45°C (pH 8.75), serially diluting with 0.2% (wt/vol) peptone, and plating on 4 sets of Petri dishes. M17 agar (Difco, Detroit, MI) containing 0.5% (wt/vol) lactose (LM17) plates were selected to determine total counts of starter culture and NSLAB. Rogosa SL agar (Difco) plates, selective for enumeration of lactobacillus species, were used to enumerate *Lb.*

curvatus contaminants, and incubated at 32°C for 5 d under anaerobic conditions.

Crystal Development

The cheeses were observed for the development of crystals after wk 1, 2, 4, 8, and 12. All pieces of cheese were thoroughly examined for occurrence of crystals and graded on a 1-to-10 scale of crystal intensity (Figure 1). The location, size, and intensity of crystals were recorded, along with the pH, and headspace volume of cheese packages after 1, 2, 4, 8, and 12 wk. The observed crystals, at the end of 12 wk of gas flushed storage, were assayed for the presence of L(+) and D(−) lactic acid using D-lactic acid/L-lactic acid enzyme test kits, according to detailed inserts (Boehringer Mannheim, Indianapolis, IN). In the presence of the D-lactate dehydrogenase, D-lactic acid is oxidized to pyruvate by nicotinamide adenine dinucleotide. The oxidation of L-lactic acid requires the presence of enzyme L-lactate dehydrogenase. The amount of NADH formed in the reaction is stoichiometric to the amount of L(+) and D(−) lactic acid. The increase in NADH was determined by measuring its absorbance at 340 nm using an Ultraspec 4000 spectrophotometer (Pharmacia Biotech Inc., San Francisco, CA). Data obtained were analyzed with LSD using SAS Proc GLM (SAS Institute, 1989).

RESULTS AND DISCUSSION

Gas Flushing and Effect on Volume

In standard cheeses, the control (no NSLAB) cheeses flushed with CO₂ and CO₂:N₂ (50:50) had 6.7 and 3.8% reductions in volume, respectively, during 2 wk of storage (Figure 2A). Similarly, for standard cheeses inocu-

Figure 1. Chart developed for grading intensity of calcium lactate crystals in cheeses during storage.

lated with NSLAB, in packages flushed with CO_2 and $\text{CO}_2:\text{N}_2$, 8.0 and 5.6% reductions in volume were observed during 2 wk of storage, respectively (Figure 2A). The percentage reduction in headspace volume remained stable throughout the 12-wk storage period. Little change was observed in the headspace volume of packages flushed with N_2 throughout the 12 wk of storage in both standard cheeses without and with NSLAB, respectively (Figure 2A). In UF milk cheeses, control (no NSLAB adjunct) cheeses flushed with CO_2 and $\text{CO}_2:\text{N}_2$ had 4.7 and 3.6% reductions in volume during 2 wk of storage, respectively (Figure 2B). The UF cheeses

inoculated with NSLAB and flushed with CO_2 and $\text{CO}_2:\text{N}_2$ had 8.3 and 7.6% reductions in volume during 2 wk of storage, respectively (Figure 2B). As with the standard cheeses, no appreciable change was observed in the volume of packages flushed with N_2 gas.

One apparent reason for the larger percentage change in headspace volume in packages of standard and UF cheeses inoculated with *Lb. curvatus* compared with control cheeses could be pH (Table 2). Lower pH values were observed in standard and UF cheeses (4.90 to 4.94) inoculated with *Lb. curvatus* compared with pH of standard and UF control cheeses (5.05 to 5.12)

Figure 2. Percentage change in initial gas volume of packages after gas flushing of A) standard cheese, B) UF cheese (control and cheese inoculated with *Lactobacillus curvatus*) during 12 wk of storage at 7.2°C.

at the end of 6 mo of aging. At low pH, the water-holding capacity of casein decreases (Fox et al., 2000) and leads to increased expulsion of serum onto the cheese surface (Walstra, 1999). Increased serum on the cheese surface leads to increased absorption of CO₂ (Fava and Piergiovanni, 1992; Sivertsvik et al., 2004).

Gas Flushing and Effect on pH

Lactic acid produced by cheese microflora (starter and NSLAB) is the major source of H⁺ ions in cheese (Cogan and Hill, 1999), whereas another source of H⁺ ions can be carbonic acid formed when CO₂ is absorbed

Table 2. The pH¹ of standard and UF control cheeses and standard and UF cheeses with added *Lactobacillus curvatus* throughout 12 wk of storage after gas flushing at 7.2°C.

	Standard control cheese				Standard cheese with <i>Lb. curvatus</i>				UF control cheese				UF cheese with <i>Lb. curvatus</i>			
	Vacuum	N ₂	CO ₂	CO ₂ + N ₂	Vacuum	N ₂	CO ₂	CO ₂ + N ₂	Vacuum	N ₂	CO ₂	CO ₂ + N ₂	Vacuum	N ₂	CO ₂	CO ₂ + N ₂
Initial ²	5.12 ^a	5.12 ^a	5.12 ^a	5.12 ^a	5.06 ^c	5.06 ^c	5.06 ^c	5.06 ^c	5.06 ^a	5.06 ^a	5.06 ^a	5.06 ^a	5.03 ^b	5.03 ^b	5.03 ^b	5.03 ^b
Wk 1	5.10 ^a	5.14 ^a	5.05 ^b	5.08 ^b	5.05 ^c	5.06 ^c	5.03 ^c	5.04 ^c	5.04 ^a	5.05 ^a	5.04 ^a	5.02 ^a	5.01 ^b	5.00 ^b	4.99 ^b	5.00 ^b
Wk 2	5.08 ^a	5.10 ^a	5.01 ^b	5.03 ^b	5.04 ^c	5.05 ^c	5.01 ^c	5.02 ^c	5.02 ^a	5.01 ^a	5.00 ^a	4.99 ^a	4.98 ^b	4.97 ^b	4.96 ^b	4.98 ^b
Wk 4	5.07 ^a	5.08 ^a	5.00 ^b	5.03 ^b	5.04 ^c	5.04 ^c	4.99 ^d	5.02 ^c	4.99 ^a	4.98 ^a	4.99 ^a	4.97 ^a	4.96 ^b	4.96 ^b	4.95 ^b	4.96 ^b
Wk 8	5.07 ^a	5.08 ^a	5.02 ^b	5.02 ^b	5.03 ^c	5.04 ^c	4.98 ^d	5.01 ^c	4.97 ^a	4.99 ^a	4.97 ^a	4.97 ^a	4.94 ^b	4.97 ^b	4.96 ^b	4.95 ^b
Wk 12	5.08 ^a	5.09 ^a	5.01 ^b	5.02 ^b	5.04 ^c	5.04 ^c	4.98 ^d	5.01 ^c	4.97 ^a	4.99 ^a	4.97 ^a	4.98 ^a	4.95 ^b	4.97 ^b	4.95 ^b	4.96 ^b

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

¹pH expressed as the mean of 3 readings from vacuum-packaged or gas-flushed cheese in each category.

²Mean of 3 measurements after cheese was aged for 6 mo at 7.2°C.

by cheese serum (Fava and Piergiovanni, 1992). The pH of a cheese is not affected by NSLAB alone, but also by the CO₂ used to flush the package. It was observed that pH of the standard cheeses in packages flushed with CO₂ (5.01) or CO₂:N₂ (5.02) were significantly different ($P < 0.05$) from cheeses that were either vacuum-packaged or flushed with N₂ (5.08 to 5.09) after 12 wk of storage (Table 2). No significant differences were observed in the pH of standard control cheeses that were either vacuum-packaged (5.08) or flushed with N₂ (5.09), after 12 wk of storage (Table 2). Similarly, there were no significant differences in cheeses that were flushed with CO₂ (5.01) or CO₂:N₂ (5.02) after 12 wk of storage (Table 2). However, in UF cheeses, no significant differences in pH were observed in cheeses vacuum-packaged or gas-flushed with N₂, CO₂, or CO₂:N₂, after 12 wk of storage (Table 2). One possible reason for lack of pH differences in UF cheeses could be the increased quantity of calcium para-caseinate in the pH range of 4.8 to 5.2 (Fox et al., 2000). Higher levels of calcium para-caseinate provide increased buffering capacity, minimizing differences in pH of cheese. The pH of all standard and UF cheeses were in the range of 4.90 to 5.20 (Table 2).

Gas Flushing and CLC

After storing gas-flushed standard control cheese packages for 4 wk, small, light patches of CLC (crystal intensity 7) were observed in cheeses flushed with CO₂ and N₂, whereas no crystals were observed in vacuum-packaged cheeses or cheeses flushed with the CO₂:N₂ mixture. After 8 wk of storage, standard control cheeses flushed with CO₂ developed a medium haze all over the cheese surfaces, whereas only a light haze was observed in cheeses flushed with N₂. Standard control cheeses flushed with CO₂:N₂ developed small light patches (crystal intensity 7) at the end of 8 wk of storage after

gas flushing. After 12 wk of storage, standard control cheeses flushed with either N₂ or CO₂ developed heavy crystals (crystal intensity 9), whereas standard control cheeses flushed with CO₂:N₂ developed medium intensity crystals (crystal intensity 8). No crystals were observed on vacuum-packaged cheeses after 12 wk of storage (Figure 3A).

In standard cheese inoculated with *Lb. curvatus*, needlepoint-size CLC were observed throughout the cheese matrix (inside the cheese and on the surface), even before gas flushing. The crystal intensity in standard cheeses inoculated with *Lb. curvatus* (Figure 3B) and gas flushed with N₂, CO₂, or CO₂:N₂, increased from the size of tiny needle points to the size of pin heads and the crystal intensity increased from 2 to 6 during 12 wk of storage after gas flushing. No differences in the size and intensity of crystals were observed in standard cheeses inoculated with *Lb. curvatus* that were flushed with different gases N₂, CO₂, or CO₂:N₂.

The size and intensity of crystals in standard control cheeses (without NSLAB) and standard cheeses with *Lb. curvatus* were different throughout aging. The CLC observed on standard control cheeses were surface phenomena, with crystals tending to develop on edges and in depressions of cheese surfaces. A CLC intensity of 8 was observed in standard control cheeses after 12 wk of storage after gas flushing (Table 3). In the case of standard cheese with *Lb. curvatus*, CLC were observed both on surfaces and inside the cheeses and the CLC observed were pin-head size, with a CLC intensity of 3 or 4 after 12 wk of storage after gas flushing, explaining the lower intensity of CLC observed on the surface. Therefore, after 12 wk of storage (Table 3), fewer crystals were observed on the surfaces of standard cheeses with *Lb. curvatus* compared with control cheeses that were gas flushed.

After storing gas-flushed UF control cheese packages for 4 wk, small light patches of CLC (crystal intensity

Figure 3. Comparison of calcium lactate crystals observed in standard cheese that was gas flushed or vacuum packaged after 12 wk of aging at 7.2°C. A) Control cheese (no adjunct culture), and B) cheese inoculated with *Lactobacillus curvatus*.

Table 3. Summary of results and implications of calcium lactate crystals research.

		Packaging type			
		Vacuum	N ₂	CO ₂	CO ₂ :N ₂ (50:50)
Standard cheese	Control	No crystals observed	Heavy crystal cover found all over cheese. Crystal intensity 9		Medium crystal cover found on the edges of cheese. Crystal intensity 8
	<i>Lactobacillus curvatus</i>	Small needlepoint crystals barely visible appearing all over cheese. Crystal intensity 2	Pinhead size crystals visible all over cheese. Crystal intensity 4		Pinhead size crystals visible all over cheese. Crystal intensity 3
Implications		Avoid contamination of cheeses with nonstarter lactic acid bacteria (NSLAB) and gas flushing of cheeses having low pH (pH ≤ 5.1). Use vacuum for packaging cheese with pH less than 5.1. Avoid gas flushing of cheeses having NSLAB.			
UF cheese	Control	No crystals observed	Heavy crystal cover found all over cheese. Crystal intensity 10		
	<i>Lactobacillus curvatus</i>	Small needlepoint crystals barely visible appearing all over cheese. Crystal intensity 2	Large pinhead size crystals visible all over cheese. Crystal intensity 6		
Implications		Avoid contamination of cheeses with NSLAB. Use vacuum to package cheeses made from concentrated milk, and having low pH (pH ≤ 5.1). Avoid gas flushing of cheeses having NSLAB.			

7) were observed in cheeses flushed with N₂, CO₂, and CO₂:N₂, whereas no crystals were observed in vacuum-packaged cheeses. The CLC intensity in UF control cheeses increased from small light patches to medium haze (crystal intensity 8) in cheese flushed with CO₂, N₂, and CO₂:N₂ at the end of 8 wk. At the end of 12 wk of storage, heavy crystals (crystal intensity 10) were observed on all gas-flushed cheeses (CO₂, N₂, or CO₂:N₂). No crystals were observed on vacuum-packaged cheeses after 12 wk of storage (Figure 4A). The CLC observed in UF control cheeses gas flushed with N₂, CO₂, or CO₂:N₂ were surface phenomena; crystals were not observed inside the cheese matrix.

In UF cheeses inoculated with *Lb. curvatus*, small needlepoint-size (crystal intensity 2) CLC were observed throughout the cheese matrix, before gas flushing. The crystal intensity in UF cheeses, inoculated with *Lb. curvatus* and gas flushed increased from size of light needle point-size (crystal intensity 2) to size of large pinheads (crystal intensity 6) during 12 wk of storage (Figure 4B). No differences in the size and intensity of crystals were observed in cheeses that were flushed with CO₂, N₂, or CO₂:N₂. The low surface intensity of CLC in UF cheeses inoculated with *Lb. curvatus* can be explained by the fact that CLC were distributed both inside and on the surfaces of cheeses.

The size and intensity of CLC in vacuum-packaged standard cheese with *Lb. curvatus* remained the same (crystal intensity 2) throughout 12 wk of storage. Ultrafiltered control cheeses and UF cheeses inoculated with *Lb. curvatus* that were gas flushed had higher

crystal intensity than standard control cheeses and standard cheeses inoculated with *Lb. curvatus*. A CLC intensity of 10 was observed in UF control cheese compared with a CLC intensity of 9 in standard control cheese (Figures 3A and 4A), whereas CLC intensity of 6 was observed in UF cheeses with *Lb. curvatus* compared with a CLC intensity of 4 in standard cheese with *Lb. curvatus* (Figures 3B and 4B). The increased occurrence of CLC in UF cheeses, when compared with standard cheeses, was consistent throughout all packages that were flushed with CO₂, N₂, or CO₂:N₂, suggesting that concentrated cheese milk leads to increased intensity of crystal formation compared with standard cheese milk. In UF milk, even though lactose was maintained at 4.8%, increased intensity of CLC was seen, substantiating the important role of calcium in formation of CLC. Concentration of milk concentrates protein and results in higher bound calcium in cheese that may contribute to greater intensity of CLC formation.

Cheese Microbiology and CLC

High NSLAB counts were observed in standard cheese (5.98 log cfu/g) and UF cheese (5.38 log cfu/g) inoculated with *Lb. curvatus* compared to control standard (1.97 log cfu/g) and control UF (1.86 log cfu/g) cheeses after 9 mo of storage (Figure 5). The CLC present in standard and UF cheeses inoculated with *Lb. curvatus* were identified as 50:50 racemic mixtures of D(-)/L(+)-lactate crystals. High NSLAB counts (Fig-

Figure 4. Comparison of calcium lactate crystals observed in UF cheese that was gas flushed or vacuum packaged after 12 wk of aging at 7.2°C. A) Control cheese (no adjunct culture), and B) cheese inoculated with *Lactobacillus curvatus*.

Figure 5. Microbial counts observed in standard and UF cheeses flushed with CO₂ and stored for 12 wk at 7.2°C.

ure 5) accounted for racemization of L(+) lactic acid to D(−) lactic acid. Crystals observed on control standard and UF cheeses were identified as L(+)–lactate crystals, irrespective of CO₂, N₂, or CO₂:N₂ gas used to flush the cheese packages. The NSLAB population (e.g., *Lactobacillus* and *Pediococcus* spp.) are capable of racemizing L(+)–lactate to D(−)–lactate in cheese during aging (Thomas and Crow, 1983). Populations of NSLAB as high as 4.0 log cfu/g are necessary in cheese to induce any appreciable increase in D(−)–lactate (Johnson et al., 1990b; Somers et al., 2001; Chou et al., 2003). Again, the calcium salt of D(−)–lactic acid has lower solubility than L(+)–lactic acid (Cao et al., 2001; Kubantseva et al., 2004). However, the presence of L(+)–lactate crystals in control standard and UF cheeses that were gas flushed with either CO₂, N₂, or CO₂:N₂, coupled with low NSLAB counts in control standard and UF cheeses, suggest that NSLAB are not always involved in CLC formation on cheeses. This result consolidates the fact that appearance of CLC on control standard and UF cheeses was more due to a concentration of calcium and lactate ions on the cheese surface than the presence of NSLAB.

A summary of the results and the implications of this research are compiled in Table 3. There are 3 possible reasons why CLC developed in gas flushed packages of standard and UF cheeses. First, increased surface area may have led to a loss of moisture to the surrounding environment. Second, movement of free moisture from inside of cheese to surfaces may have led to increased concentration of calcium and lactate ions, and third, concentration of calcium lactate on the surface of cheese because of loss of moisture from surface may have initiated crystallization of calcium lactate.

In this research, CLC were observed in gas-flushed cheeses after 1 mo of storage. Thus, cheese manufactur-

ers should plan to sell cubed and shredded cheese well within this period instead of stocking shelves with cubed cheeses. Alternatively, cheeses destined for gas flushing should not have high total solids (calcium) or low pH, as these contribute to CLC formation. Research is currently underway to study migration dynamics of calcium and lactate ions in cubed cheeses at different storage conditions. Flushing cubed cheeses with gases high in relative humidity, or reducing headspace volume are practices that should be researched further.

CONCLUSIONS

Calcium lactate crystals were observed in control cheeses that were gas flushed, but not in control cheeses that were vacuum packaged. L(+)–Lactate crystals were observed in both standard and UF cheeses (control), and a 50:50 racemic mixture of D(−)/L(+)–lactate was observed in cheeses with NSLAB, indicating that NSLAB are not always necessary for CLC, but influence the form and severity of CLC. Increased CLC intensity was observed in UF cheeses compared with standard cheeses, demonstrating the importance of available calcium to CLC formation. Gas flushing of cheese packages with different gases had a significant effect on pH in standard cheeses but not in UF cheeses. Loss of headspace gas volume was observed in both standard and UF cheeses flushed with either CO₂ or CO₂:N₂ (50:50) when compared with cheese packages that were only flushed with N₂ gas, indicating that some of the CO₂ in the package dissolved readily in cheese serum. The lower pH observed in cheese packages flushed with either CO₂ or CO₂:N₂ compared with cheese packages that were flushed with N₂ gas or were vacuum packaged is consistent with the formation of carbonic acid.

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