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Enhanced Lactose Cheese Milk does not Guarantee Calcium Lactate Crystals in Finished Cheddar Cheese

A. J. Blake, J. R. Powers, L. O. Luedecke, and S. Clark
Department of Food Science and Human Nutrition, Washington State University, Pullman 99164-6376

ABSTRACT

Three experimental batches of Cheddar cheese were manufactured in duplicate, with standardization of the initial cheese-milk lactose content to high (5.24%), normal (4.72%, control), and low lactose (3.81%). After 35 d of aging at 4.4°C, the cheeses were subjected to temperature abuse (24 h at 21°C, unopened) and contamination (24 h at 21°C, packages opened and cheeses contaminated with crystal-containing cheese). After aging for 167 d, residual cheese lactose (0.08 to 0.43%) and L(+)-lactate concentrations (1.37 to 1.60%) were high and D(−)-lactate concentrations were low (<0.03%) for all cheeses. No significant differences in lactose concentrations were attributable to temperature abuse or contamination. No significant differences in L(+)- or D(−)-lactate concentrations were attributable to temperature abuse. However, concentrations of L(+)-lactate were significantly lower and D(−)-lactate were significantly higher in contaminated cheeses than in control cheeses, indicating inoculation (at d 35) with heterofermentative nonstarter lactic acid bacteria able to racemize L(+)-lactate to D(−)-lactate. The fact that none of the cheeses exhibited crystals after 167 d demonstrates that high cheese milk or residual lactose concentrations do not guarantee crystal formation. Contamination with nonstarter lactic acid bacteria can significantly contribute to D(−)-lactate accumulation in cheese.
(**Key words:** lactose, calcium lactate crystal, Cheddar cheese, lactic acid)

Abbreviation key: CLC = calcium lactate crystals, **HL** = high lactose (5.24%), **LL** = low lactose (3.81%), **NL** = normal lactose (4.72%), **NSLAB** = nonstarter lactic acid bacteria, **S/M** = salt in moisture, **TA** = titratable acidity, **TACS** = temperature abuse closed system, **TAOS** = temperature abuse open system.

INTRODUCTION

A periodic problem for cheese manufacturers is the appearance of white specks on the surface of hard cheese types, such as Cheddar, as early as 3 to 6 mo into aging (Dybing et al., 1988; Chou et al., 2003). Although these nonmold specks pose no health hazards, consumers do not readily accept a product exhibiting them, and marketability of the cheese is consequently reduced (Bodyfelt et al., 1988).

Van Slyke and Publow (1910) suggested that the specks might be calcium soaps created when free fatty acids from the cheese fat form complexes with available calcium. However, more recently, the deposits have been identified as crystals of calcium lactate (Tuckey et al., 1938; McDowall and McDowell, 1939; Harper et al., 1953; Conochie et al., 1960; Pearce et al., 1973; Washam et al., 1985; Severn et al., 1986), a racemic mixture of L(+)- and D(−)-calcium lactate (Severn et al., 1986), calcium phosphate (Conochie and Sutherland, 1965), tyrosine (Dorn and Dahlberg, 1942; Harper et al., 1953; Conochie et al., 1960), or mixtures of amino acids (Harper et al., 1953; Bianchi et al., 1974). The formation of calcium lactate crystals (**CLC**) in young Cheddar cheese is a complex interaction among factors including cheese-milk composition, cheese making procedures, packaging conditions, ripening temperature, and cheese microflora (Dybing et al., 1988). In contrast, crystals found in aged cheeses are commonly the amino acid of low solubility, tyrosine, a product of proteolysis by nonstarter bacteria (Pearce et al., 1973).

The 2 most important substrates for the formation of CLC are lactic acid and calcium. Early in fermentation, homofermentative starter lactic acid bacteria generate nearly all of the L(+)-lactic acid found in fresh curd (Thomas and Crow, 1983; Dybing et al., 1988). McSweeney et al. (1998) demonstrated that numbers of viable starter cells decrease to around 1% of their maximum within the first month of ripening. As cheese ages, starter lactic acid bacteria lyse, and are succeeded by nonstarter lactic acid bacteria (**NSLAB**). Nonstarter lactic acid bacteria begin at low levels (10^3 cfu/g), increase to 10^5 cfu/g in about 7 d, then to 10^7 cfu/g at 28 d, and remain at this level through aging (Thomas and Crow, 1983; Crow et al., 1995). In the presence of adventitious NSLAB, residual lactose and galactose may be metabolized to L(+)-lactic acid, D(−)-lactic acid, or a racemic mixture of D(−)/L(+)-lactic acid (Dybing et al., 1988;

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Corresponding author: Stephanie Clark; e-mail: stephclark@wsu.edu.

Fox et al., 2000). The growth and metabolism of NSLAB at elevated aging temperatures contributes to an increased probability that L(+)-lactate will be racemized to D(−)-lactate (Turner and Thomas, 1980). If D(−)/L(+)-lactate remain soluble in cheese, crystals are not a problem. However, D(−)/L(+)-lactate can precipitate with calcium ions, leading to the appearance of CLC in cheese (Thomas and Crow, 1983). The calcium salt of D(−)-lactate is less soluble than the calcium salt of L(+)-lactate (Cao et al., 2001). Complicating the issue is the fact that temperatures used to store cheese reduce solubility of calcium lactate (Cao et al., 2001). The solubility of calcium lactate in 100 g of water is 3.1 g at 0°C, 5.4 g at 15°C, and 7.9 g at 30°C (Linke, 1958). Johnson et al. (1990a,b) reported earlier and more abundant CLC formation in cheeses aged at 3.3 and 4.4°C than at 7.2°C. Chou et al. (2003) confirmed that combining accelerated ripening with subsequent cooling of cheese might contribute to CLC. The earliest and most abundant CLC were observed in cheeses first aged at 13°C then transferred to 4°C. Several days later, CLC appeared on cheeses continuously aged at 4°C. Calcium lactate crystal development was slowest and lightest in cheeses aged at 4°C then transferred to 13°C or aged continuously at 13°C.

From the above summary, it follows that cut and wrap facility practices may contribute to CLC formation in Cheddar cheese. Many cheese manufacturing plants make, package, and then sell 18-kg blocks of Cheddar cheese to vendors or cut and wrap facilities for repackaging and distribution. Typically, the large blocks are vacuum packaged in long-hold ethyl vinyl alcohol barrier material immediately after pressing, placed in cardboard boxes, and held for several days at 4.4°C before selling to vendors. Vendors routinely cut and package the blocks into smaller consumer-sized portions. The cheeses may receive little or significant additional aging at 4.4°C after repackaging. Occasionally, vendors complain of a white haze of crystals along the cutting surfaces, after only 60 to 90 d of aging. This practice will be elaborated upon below.

Pearce et al. (1973) recommended that CLC incidence could be inhibited by reducing lactose and lactate in cheese. Although it is logical that cheese-milk lactose may play a role in the formation of CLC, no studies like the one described here have been reported. The primary objective of this study was to determine the effect of cheese-milk lactose concentration on CLC formation in Cheddar cheese. Three experimental batches of cheese were manufactured with standardization of the initial lactose content of the cheese-milk to high, normal (control), and low lactose levels. An experiment to investigate the effect of cut and wrap facility practices on CLC formation, involving temperature abuse and cheese contamination with NSLAB, was also conducted.

MATERIALS AND METHODS

Milk Standardization

Three 68-kg batches of cheese milk, each in duplicate, were standardized to control the initial lactose content of the cheese milk to high lactose (**HL**, 5.24%), normal lactose (**NL**, 4.72%, control), and low lactose (**LL**, 3.81%), and maintain protein and fat levels at 3.5% and 3.6%, respectively.

For each 68-kg batch of cheese milk, UF was used to remove lactose, some minerals, and water from the cheese-milk and increase the total milk solids. Ultrafiltration was conducted on 136 kg of pasteurized (73.3°C for 16 s) fresh skim milk (Vitamilk, Dairy Inc., Seattle, WA) using a Romicon model HF-Lab-5 Ultrafiltration System (Woburn, MA) containing a hollow fiber membrane cartridge (Koch Membrane Systems, Pleasanton, CA) with a 10,000-Da molecular weight cut-off pore size. Cleaning, sanitizing, conditioning, and general operating instructions were followed as outlined in the Romicon Ultrafiltration System manual (Section 1-3/85). All cheese-milk was ultrafiltered at 40.6°C with an inlet and outlet pressure of 172 and 103 kPa, respectively. Flow was reversed at 30-min intervals throughout the process operation.

A trial UF run was used to determine the appropriate filtration time to reach the desired protein and lactose content for each cheese milk. The HL milk was ultrafiltered for 105 min. After UF, 104.3 kg of the HL retentate was standardized to 5.24% lactose, 3.5% protein, and 3.6% fat by the addition of 12.9 kg of cream (Vitamilk, Dairy Inc.), 12.5 kg of skim milk, 5.2 kg of water, and 1.76 kg of D-lactose monohydrate (Sigma Chemical Co., St. Louis, MO). The NL (control) milk was ultrafiltered for 120 min. After UF, 111.1 kg of NL retentate was standardized to 4.72% lactose, 3.6% protein, and 3.7% fat by the addition of 12.9 kg of cream, 12.3 kg of skim milk, and 0.64 kg of D-lactose monohydrate. After UF for 90 min, 6.8 kg of water was added to the LL retentate, for diafiltration, and it was filtered for an additional 90 min. The 90.7 kg of LL retentate was standardized to 3.81% lactose, 3.5% protein, and 3.6% fat by the addition of 12.9 kg of cream, 10.3 kg of skim milk, 21.4 kg of water, and 1.06 kg of D-lactose monohydrate.

Cheese Making

A starter culture containing *Lactococcus lactis* spp. *cremoris* (#355M) was purchased from Gist-Brocades (Millville, UT) as a frozen concentrate. The culture was

grown in sterilized fresh skim milk at 21°C. Before inoculation into cheese milk, the titratable acidity (**TA**) of the starter culture was 0.77% (as lactic acid). Because fresh skim milk was used (8.5 to 9.0% total solids, 0.12% TA), a TA of 0.65 to 0.85% indicates full incubation by starters (Lewis, 1987).

Three batches of Cheddar cheese were manufactured using HL, LL, and NL (control) cheese milks. Duplicate batches were manufactured on the following day. Cheeses were made in 189-L jacketed stainless steel open cheese vats with hot water heating (Grand Metal Products, Portland, OR). Clean vats were sanitized with chlorine (200 ppm) before adding 68 kg of cheese milk. The milk temperature was adjusted to 32°C and the starter culture was added at 1.5%. After ripening for 30 min, 6.75 mL of double strength Chy-Max coagulator (Chr. Hansen Laboratories, Milwaukee, WI), diluted in 270 mL of deionized water, was mixed into the milk. The milk was allowed to coagulate for 30 min at 32°C.

After coagulating, the mixture was cut into 0.6-cm cubes, which were cooked by raising the temperature to 38°C over 30 min with slow stirring. After stirring at 38°C for an additional 45 min, the curd was allowed to settle (~15 min), and the whey was drained. The loaves were cut, then cheddared until a TA of 0.54 to 0.56% (as lactic acid, pH 5.15 to 5.18) was reached. The curd was milled into 1.3-cm cubes and salted (0.30% salt based on weight of milk), then placed in stainless steel cylinders (14.5 cm in diameter), and pressed for 20 h at 2.8×10^5 Pa. After pressing, the cheese was cut into approximately 140-g pieces, vacuum-sealed in 3-mil high barrier nylon/ethyl vinyl alcohol barrier/polyethylene vacuum pouches (Koch Supplies, Inc., Kansas City, MO), and stored at 4.4°C.

Temperature Abuse and Contamination Treatments

After 35 d of storage, cheese batches were split into thirds for temperature abuse and contamination treatments. To create the temperature abuse closed system (**TACS**) cheese, one-third of the HL, NL, and LL cheese packages were moved to room temperature (21°C) then returned to 4.4°C after 24 h. The cheeses remained in their original (unopened) packages throughout the procedure.

To create the temperature abuse open system (**TAOS**) cheese, one-third of the HL, NL, and LL cheeses were removed from their original packages after 35 d of storage. The opened cheeses were then cut in half. The utensil caused cross-contamination, modeled by first passing the cutting blade through a cheese aged 12 mo covered with a film of CLC, immediately before cutting through the TAOS experimental cheese. The contaminated knife blade was also pressed against all surfaces of the experimental cheese immediately after cutting, before vacuum packaging in 3-mil high barrier nylon/ethyl vinyl alcohol barrier/polyethylene vacuum pouches. The purpose of cutting the experimental cheese with a contaminated knife blade was to inoculate experimental cheese with bacteria present in the commercial cheese, which was shown to contain heterofermentative NSLAB (Chou, 2000). The repackaged cheese was then moved to 21°C. After 24 h at 21°C, the TAOS cheese was returned to 4.4°C. A final one-third portion of each cheese was not abused.

Analysis of Cheese

For all analyses, 140-g pieces of cheese were aseptically ground with a cheese grater.

Proximate Analysis of Cheese

Moisture, fat, salt, and pH analyses of the experimental cheese were conducted in duplicate after 10 d of storage, according to standard procedures (Marshall et al., 1992). Moisture and pH were also determined after 6 mo of aging. Moisture content was determined using a forced-draft oven at 100°C for 16 h. The Babcock method was used to determine fat content (Marshall et al., 1992). The pH was determined using a pH meter (Corning pH meter 125, Corning, NY) with a flat surface electrode. A dye binding colorimeter protein analyzer (UDY Corp., Fort Collins, CO) and appropriate reagents were used to determine protein content. The assay for salt content was modified according to the method of the chloride electrode manufacturer (Corning, Medfield, MA).

Lactose

On d 0, 4, 9, 16, 23, 35, 71, 109, and 167, lactose concentration in the experimental cheeses was determined. Approximately 3 g of grated cheese was placed in a Stomacher (Seward model 400, London, UK) bag with 70 mL of redistilled water, and stomached for 2 min. The mixture was added to a 100-mL volumetric flask and incubated for 15 min at 70°C. After cooling to room temperature, 2 mL of 3 *M* trichloroacetic acid was added and stirred. After 5 min, the mixture was neutralized with 6 mL of 1 *M* NaOH. The volume was brought up to 100 mL with water and the solution was placed at 7.2°C for 20 min to separate the fat. The aqueous phase was filtered through #42 Whatman filter paper. The filtrate was then assayed for lactose.

Lactose concentration in standards and samples was determined using the method of Boehringer Mannheim following detailed test kit inserts (lactose/D-galactose

test combination, cat no. 986 119, Boehringer Mannheim Co., Indianapolis, IN). The sample solution (0.1 mL) was mixed with 0.2 mL of solution 1 (citrate buffer, pH 6.6; NAD, 5 mg/mL; magnesium sulfate) and 0.05 mL of solution 2 (β-galactosidase suspension), and incubated at room temperature (21°C). After 20 min, 1.0 mL of solution 3 (potassium diphosphate buffer, pH 8.6) and 1.9 mL of redistilled water were added and the absorbance (A_1) was measured at 340 nm using an Ultrospec 4000 spectrophotometer (Pharmacia Biotech Co., San Francisco, CA). A galactose dehydrogenase suspension (0.5 mL) (solution 4) was added to start the reaction.

Redistilled water (0.1 mL) was substituted for samples in blank solutions. The reaction mixture was held at 21°C for 15 min or until a constant absorbance was reached. Because the time required to complete the assay varied with the amount of lactose present, preliminary analysis of standard lactose solutions at approximately 20, 40, and 60 mg/100 mL was conducted to determine the optimum incubation period. Absorbance of the reaction mixtures was read at 340 nm, and the concentration of anhydrous lactose (g/L) was calculated according to detailed inserts. Results were converted from grams of lactose per liter to percentage lactose in experimental cheese.

Lactic Acid

On d 0, 4, 9, 16, 23, 35, 71, 109, and 167, L(+)-lactate, and D(−)-lactate concentrations in the experimental cheeses were determined. Grated cheese (10 g) was added to 100 mL of 2% sodium citrate and the mixture was blended for 2 min. The mixture was added to a 100-mL volumetric flask and boiled for 5 min to dissolve the sample. After cooling to room temperature (21°C), redistilled water was added to bring the volume to 100 mL. Three milliliters of the sample solution was mixed with 6 mL of 10% TCA. The mixture was placed in an ice bath for 5 min and filtered through #42 Whatman filter paper. The filtrate was assayed for lactic acid.

Lactic acid concentration in standards and samples was determined enzymatically based on the Sigma diagnostic procedure (Sigma pyruvate/lactate kit). The reaction mixture for L(+)-lactic acid determination contained 0.92 mL of hydrazine/glycine buffer (0.6 *M* glycine and 0.5 *M* hydrazine, pH 9.2), 1.93 mL of redistilled water, 0.05 mL of L(+)-lactate dehydrogenase (LDH) approximately 476 U/mg of protein (10 mg/mL) from rabbit muscle (Fluka Co., Milwaukee, WI), 10 mg of NAD (Sigma), 0.05 mL of 10% TCA, and 0.05 mL of sample.

The reaction mixture for D(−)-lactic acid determination contained 0.92 mL of hydrazine/glycine buffer (0.6 *M* glycine and 0.5 *M* hydrazine, pH 9.2), 1.88 mL of redistilled water, 0.1 mL of D(−)-lactate dehydrogenase (LDH) approximately 258 U/mg (4.0 mg/mL) from *Lactobacillus leichmannii* (Fluka Co.), 10 mg of NAD (Sigma), and 0.1 mL of sample. Reaction mixtures were made immediately before the addition of sample. Trichloroacetic acid (10%) was substituted for samples in blank solutions. After addition of both sample and enzyme, the reaction mixture was held at room temperature (21°C) until constant absorbance was reached. Because the time required to complete the assay varied with the amount of lactic acid present, preliminary analysis of 20, 40, 60, 80, and 120 mg/100 mL standard lactic acid solutions (Sigma) was conducted to determine the optimum incubation period. Absorbance of the reaction mixtures was read at 340 nm using an Ultrospec 4000 spectrophotometer (Pharmacia Biotech Co.) according to detailed inserts (Boehringer Mannheim Co.).

Statistical Analyses

The statistical design (ANOVA) was based on a split-plot experimental design (Kuehl, 2000). The whole-plot design structure was completely randomized with 2 replicates (batches) per lactose treatment (high, normal, and low). The subplot experimental design structure was a randomized complete block design with a 2-way treatment structure. The 2-way treatment structure consisted of a temperature abuse treatment with 3 levels (control, open system, closed system) and days of storage with levels ranging from 0 to 167 d.

For the ANOVA, *F*-statistics were computed using the GLM procedure of SAS (SAS Institute, 1989). Means of significant main effects (treatment, temperature abuse, and storage) were statistically separated using the least square means option of PROC GLM (α = 0.05; SAS Institute, 1989). Assessing differences between means in the presence of 2 or more interacting main effects was accomplished by analyzing the data at each level of one of the interaction components.

RESULTS AND DISCUSSION

The temperature abuse experiments (TACS and TAOS) were conducted to test the hypothesis that increasing cheese temperature promotes the fermentation of lactose to lactic acid by NSLAB, thereby increasing the likelihood of CLC formation. The purpose of cutting the experimental cheese with a contaminated knife blade (TAOS) was to simulate cut and wrap facility practices. Isolates from cheeses used to contaminate TAOS cheeses included the facultatively heterofermentative (Somers et al., 2001) racemase-positive NSLAB

Table 1. Proximate analysis of experimental and commercial Cheddar cheeses. Data are the average analysis of duplicate cheeses (except pH).

Cheese[4]	Moisture[1]	Salt[2]	S/M[3]	Protein[2]	Fat[2]	Lactose (d 1)	Lactic acid (d 1)	pH[1]
	%							
HL	39.7	1.5	3.7	23.8	32.2	0.80	1.29	4.9
HL (TACS)	39.4	1.5	3.7	23.8	32.2	0.80	1.29	4.9
HL (TAOS)	38.9	1.5	3.8	23.8	32.2	0.80	1.29	4.9
NL	39.7	1.6	3.9	23.3	31.6	0.31	1.41	4.8
NL (TACS)	40.4	1.6	3.9	23.3	31.6	0.31	1.41	4.8
NL (TAOS)	39.7	1.6	3.9	23.3	31.6	0.31	1.41	4.8
LL	39.2	1.5	3.9	23.1	32.7	0.26	1.18	4.9
LL (TACS)	38.7	1.5	4.0	23.1	32.7	0.26	1.18	4.9
LL (TAOS)	38.9	1.5	4.0	23.1	32.7	0.26	1.18	4.9
Cheddar[5]	36.0	1.5	4.3	25.0	35.0	—	—	5.0

[1]After 6 mo aging.

[2]After 10 d aging.

[3]S/M = Salt-in-moisture.

[4]HL = High lactose cheese; NL = normal lactose cheese; LL = low lactose cheese; TACS = temperature abuse closed system; TAOS = temperature abuse open system.

[5]Commercial Cheddar cheese made at WSU Creamery (2000).

Lactobacillus curvatus, which has been shown to induce CLC (Chou et al., 2003). Facilities often cut and repackage cheeses of various ages and sources into smaller packages for resale. It is suspected that when companies cut aged cheese followed by younger cheese, without cleaning and sanitizing the cutting blades or surfaces, the younger cheese is contaminated with NSLAB from the older cheese. The NSLAB are then thought to use the available lactose and other substrates in the younger cheese and produce additional lactic acid, thereby increasing the likelihood of crystal formation. Increasing the temperature to 21°C for 24 h would enhance NSLAB metabolism of available lactose in the cheese (Turner and Thomas, 1980; Thomas and Crow, 1983). Subsequent cooling could increase CLC due to decreased solubility at low temperatures (Linke, 1958; Cao et al., 2001).

Proximate Analysis of Cheese

In an effort to help understand the factors that govern crystal growth in Cheddar cheese, the chemical composition of the experimental cheeses was examined. The composition of the experimental cheeses was similar to that of typical commercial Cheddar cheese (Table 1). However, the pH values of the experimental cheeses (4.81 to 4.89) were lower than in commercial Cheddar cheese (5.0 to 5.5). The protein levels were lower and the moisture levels were higher than in typical Cheddar cheese. The difference in pH can be explained by a combination of high milling acidities and fast acid production by the *Lactococcus lactis* starter culture used in this study, which is known to rapidly produce acid in cheese curd (D. Anker, WestFarm Foods, Inc., Sunnyside, WA; personal communication, 1999). The high moisture in the experimental cheese (1 to 4% higher than commercial cheese) may be explained by the different pressing apparatus used in the current work, compared with commercial operations. The high moisture yielded lower salt-in-moisture (**S/M**) values in experimental cheeses compared with commercial Cheddar. It should be noted that the S/M levels of these cheeses should not have inhibited most starter microorganisms (Turner and Thomas, 1980) and thus should not have inhibited lactic acid production by starter cultures. However, the low pH and low aging temperature likely inhibited the starters. Additionally, Turner and Thomas (1980) demonstrated no effect of sodium chloride on the racemizing activity of pediococci and lactobacilli NSLAB until concentrations exceeded 5 and 2%, respectively.

Lactose

Lactose, the primary energy source for lactic acid bacteria in cheese, is metabolized to lactic acid via pyruvic acid (Scott et al., 1998). Pyruvic acid is also an important precursor to other compounds in cheese. The average percentage lactose in cow milk is 4.8% (Holsinger, 1988). In this experiment, cheeses were manufactured from cheese milk containing lactose concentrations of 5.24, 4.72, and 3.81%. It was reasoned that increasing the lactose content of the cheese milk would lead to high residual lactose levels in the cheese curd, providing high levels of substrate for CLC formation. Turner and Thomas (1980) demonstrated that if the

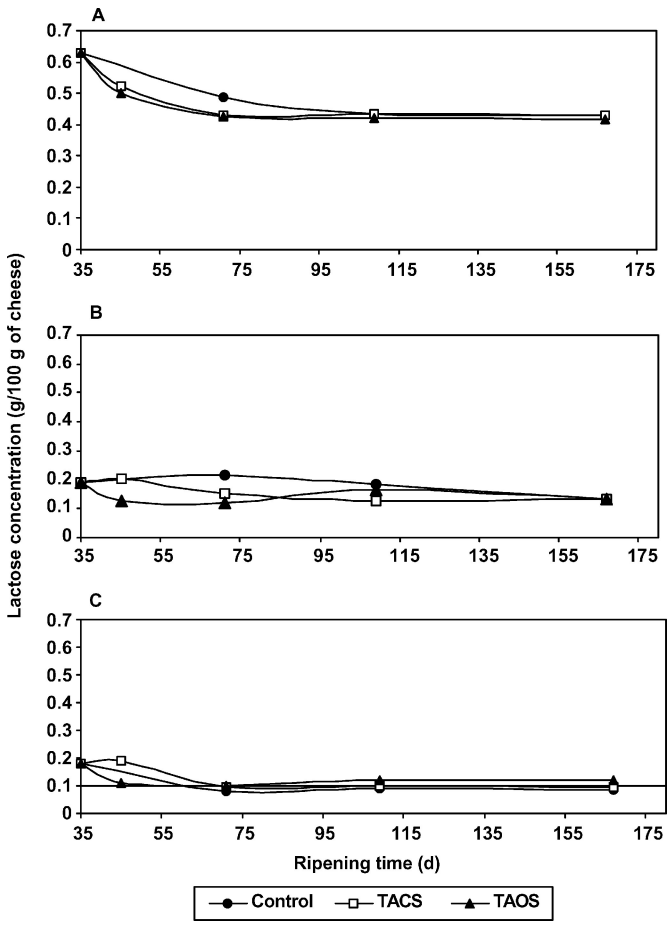

Figure 1. Lactose concentration of cheeses [temperature abuse closed system (TACS), temperature abuse open system (TAOS), and control] made with high (5.24%; A), normal (4.72%; B), and low (3.81%; C) lactose. Temperature abuse was conducted at 21°C for 24 h on d 35.

lactose concentration in the cheese curd is high when starter fermentation is inhibited, high levels of heterolactic products including L(+)- and D(−)-lactic acid might be produced in the cheese by NSLAB. At the start of aging, lactose concentrations in the experimental cheeses were 0.80, 0.31, and 0.26% in the HL, NL, and LL cheeses, respectively (Table 1). These values are similar to those reported by Turner and Thomas (1980), where initial lactose levels ranged from 0.5 to 0.9%.

The concentrations of lactose between d 35 and 167 of ripening in HL, NL (control), and low LL Cheddar cheeses are shown in Figure 1. Throughout the fermentation, lactose concentrations in HL cheeses were significantly ($P < 0.05$) higher than in the NL and LL cheeses. At 35 d of aging, the lactose concentration in the HL cheese (0.63%) was more than 3 times higher than in the NL and LL cheeses (0.19 and 0.18%, respectively). At this stage of fermentation, lactose levels were considerably higher than levels reported by Turner and Thomas (1980), who made Cheddar cheese using milk with normal lactose levels. Turner and Thomas (1980) reported that lactose use in Cheddar cheese with S/M levels of 4.5% was rapid during the first 4 d of fermentation and that lactose was completely used in about 8 d. The fact that the cheese was aged at a higher temperature (12°C) than in the present study (4.4°C) probably explains the differences found, because high temperatures increase the rate of lactose fermentation in cheeses (Turner and Thomas, 1980).

Cheese lactose declined and reached concentrations of 0.43% (HL), 0.14% (NL), and 0.08% (LL) after 167 d of aging, demonstrating high levels of residual lactose in the cheeses compared with previous research (Turner and Thomas, 1980). Only cheeses made with high salt (6% S/M) by Turner and Thomas (1980) had residual lactose over 0.4% (pH > 5.2) after 16 d of aging. On the other hand, cheeses with low S/M (4.5%) exhibited almost no lactose after 8 d of aging and pH of < 5.1 at 16 d of aging (Turner and Thomas, 1980). High residual lactose levels were expected in the HL cheese, due to the high lactose levels in the cheese-milk, but not in the other cheeses because pH and salt were low. No significant differences in lactose concentration were observed between NL and LL cheeses throughout the fermentation.

The cheeses discussed above underwent no temperature abuse. High temperatures during aging typically speed up both bacterial growth and biochemical reactions (Turner and Thomas, 1980; Scott et al., 1998) and the inverse is true. Thus, it was reasoned that increasing cheese environment temperature from 4.4 to 21°C for 24 h would cause lactose concentrations to decrease in the experimental cheeses. However, concentrations of lactose in the TACS, TAOS, and control cheeses did not significantly differ throughout the aging period (Figure 1). It is unlikely that the S/M levels or pH in the cheeses inhibited starter microorganism metabolism. Turner and Thomas (1980) demonstrated that inhibition typically occurs at greater than 5% S/M for certain strains. Additionally, lactic acid bacteria survive below pH 5.0 in numerous cheeses (e.g., cottage, cream, Chevre, Mizithra, Quarg) (Fox et al., 2000). The lack of temperature effect could be because the temperature abuse was not implemented until d 35 of aging. Day 35 was selected based upon the assumption that cut and wrap facilities may not subdivide blocks of cheese until such a time. It is also possible that only low levels of NSLAB were present in the TAOS cheeses after intentional contamination, and that the duration of the temperature abuse (24 h) and subsequent aging period was not long enough for the injured bacteria to recover and induce significant changes in lactose concentration.

Lactic Acid

Most starter bacteria are homofermentative and primarily convert lactose to the L(+)-isomer of lactic acid (Dybing et al., 1988). During the first 9 d of fermentation, L(+)-lactate concentrations increased in all cheeses that were not exposed to temperature abuse, reaching maximum concentrations of 1.64, 1.78, and 1.61% for the HL, NL, and LL cheeses, respectively. The higher L(+)-lactate concentration in NL cheeses compared with HL and LL cheeses can be explained by the fact that, during cheese making, fermentation proceeded further in the NL cheeses than in the HL and LL cheeses (Table 1), demonstrated by the higher initial lactic acid of NL cheese (1.41%) compared with HL (1.29%) and LL cheeses (1.18%). Beyond d 9, L(+)-lactate concentrations declined and remained between 1.32 and 1.66% throughout the remainder of the fermentation. Levels of L(+)-lactate were expected to fluctuate because lactate may participate in numerous reactions (e.g., fermentation to acetate) during cheese aging (Fox et al., 2000).

The concentrations of L(+)-lactate at various stages of cheese ripening (d 35 through 167), in HL, NL (control), and LL experimental Cheddar cheeses are shown in Figure 2. The L(+)-lactate concentrations on d 167 were 1.43, 1.60, and 1.37% for the HL, NL, and LL cheeses that were not abused, respectively. L(+)-Lactate concentrations in the LL cheese were significantly ($P < 0.05$) lower than in the NL cheese, but L(+)-lactate concentrations in the HL cheese were not significantly different from the NL or LL cheeses. In previous studies (Turner and Thomas, 1980; Thomas and Crow, 1983), L(+)-lactate concentrations reached levels as high as 1.6% over the first 2 wk of the fermentation and then declined, reaching levels between 0.4 and 0.8% after several months. The decrease in L(+)-lactate in the previous studies coincided with an increase in D(−)-lactate, concluded to be caused by heterofermentative NSLAB that contained L(+)- and D(−)-lactate dehydrogenase enzymes able to racemize L(+)-lactate to D(−)-lactate. In the present study, continuously high levels of L(+)-lactate throughout the fermentation, in noncontaminated cheeses, suggested that active heterofermentative NSLAB were not present at high enough concentrations to depress L(+)-lactate levels in the TACS and control cheeses. Further evidence that heterofermentative NSLAB were not present or active in the noncontaminated cheeses is the absent or minimal amount of D(−)-lactate production throughout the fermentation of the same cheeses (Figure 3).

When NSLAB were intentionally introduced to cheese (TAOS) at d 35, significant ($P < 0.05$) differences in L(+)-lactate concentrations, attributable to contamination, were found after 167 d of aging (Figure 2).

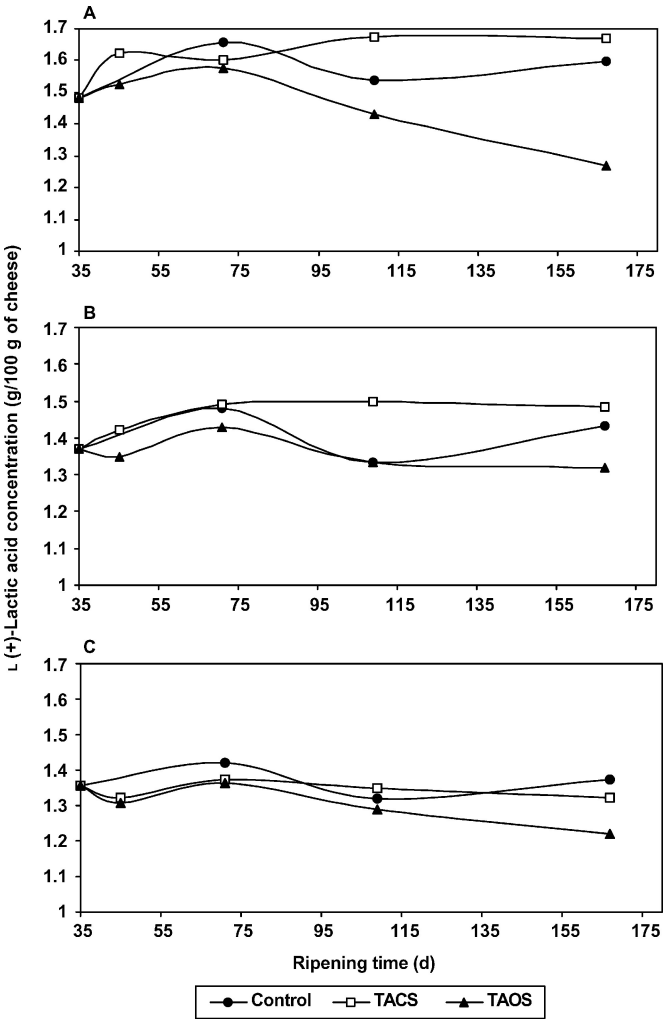

Figure 2. L(+)-Lactic acid concentration of cheeses [temperature abuse closed system (TACS), temperature abuse open system (TAOS), and control] made with high (5.24%; A), normal (4.72%; B), and low (3.81%; C) lactose. Temperature abuse was conducted at 21°C for 24 h on d 35.

L(+)-Lactate concentrations were significantly ($P < 0.05$) lower in all TAOS cheeses than in their respective controls, whereas there were no significant differences in L(+)-lactate concentrations between the noncontaminated TACS and control cheeses. The lower levels of L(+)-lactate in the TAOS cheeses compared with the unopened cheeses demonstrated that the TAOS cheeses were contaminated with heterofermentative NSLAB that were able to use L(+)-lactate as a substrate. Further evidence of heterofermentative NSLAB activity in the TAOS cheese is the increase in D(−)-lactate, corresponding with the decrease in L(+)-lactate (Figure 3).

Turner and Thomas (1980) suggested that D(−)-lactate concentrations in Cheddar cheese aged 48 d normally range between 0.2 and 0.6%. Control cheeses and

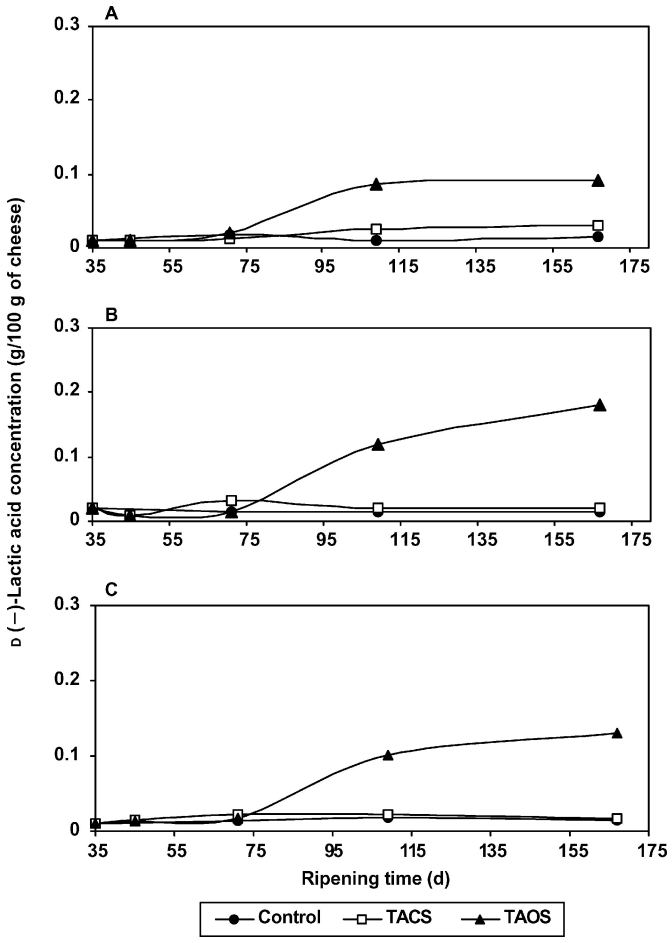

Figure 3. D(−)-Lactic acid concentration of cheeses [temperature abuse closed system (TACS), temperature abuse open system (TAOS), and control] made with high (5.24%; A), normal (4.72%; B), and low (3.81%; C) lactose. Temperature abuse was conducted at 21°C for 24 h on d 35.

cheeses that were subjected to temperature abuse (no contamination), contained levels of D(−)-lactate less than 0.03% throughout fermentation. The very low levels of D(−)-lactate produced in the control and TACS cheeses suggest the absence of active heterofermentative NSLAB. Cheeses subjected to temperature abuse and contamination (TAOS) produced significantly ($P < 0.05$) higher levels of D(−)-lactate than control cheeses, suggesting that significant numbers of active heterofermentative NSLAB were present (introduced at d 35) to produce the D(−)-lactate. The TAOS cheeses slowly accumulated D(−)-lactate, reaching final concentrations (d 167) of 0.09, 0.18, and 0.13% for the HL, NL, and LL cheeses, respectively.

Even though the TAOS cheeses had significantly higher levels of D(−)-lactate than the other cheeses, crystal formation was not observed in any of the experimental cheeses within 167 d of aging. The absence of crystals is not surprising, because crystal growth usually occurs when a racemic mixture of L(+)- and D(−)-lactate is present (Turner and Thomas, 1980; Thomas and Crow, 1983; Johnson et al., 1990a,b; Chou et al., 2003). Johnson et al. (1990b) never observed crystals in cheeses with less than 20% of the lactic acid in the D(−) form. Chou et al. (2003) did not observe crystals until 50% of the lactic acid was present in the D(−) form. In this experiment, D(−)-lactate as a percentage of total lactic acid reached maximum levels of 6.4, 12.4, and 9.7%, for the HL, NL, and LL TAOS cheeses, respectively. It is possible that contamination was introduced too late in the aging (35 d) or at too low of a temperature (4.4°C) for significant levels of D(−)-lactate to accumulate for CLC to form in 167 d.

Dybing et al. (1988) reported that fast acid development and high milling acidity are associated with reduced occurrence of CLC in cheese. As curd pH decreases with formation of lactic acid during cheese making, serum calcium increases at the expense of casein-bound calcium. The result is that calcium is lost in whey; and propensity for CLC formation declines because less calcium is available to bind with lactate. High levels of residual lactose in all of the experimental cheeses in the present study suggest that sufficient lactose was available for microorganisms to use. However, calcium levels in cheeses may have been insufficient for CLC formation, due to the high milling acidities (0.54 to 0.56) resulting from fast acid production.

After 167 d of aging, none of the experimental cheeses exhibited crystal formation either on the surface, or throughout the matrix. Therefore, although lactose is a critical substrate for CLC formation, elevated cheese-milk lactose concentration alone does not guarantee crystal formation. Even at low pH, low temperature, and high residual lactose and high lactic acid levels, CLC may not form. The results found in this experiment appear to conflict with those found by Swearingen et al. (2004). Their Cheddar cheeses, of similar composition to our cheeses at the end of shelf life (S/M 4.19 to 4.55, pH 5.00 to 5.05; lactic acid levels 1.35 to 1.52, and D(−)-lactic acid less than 0.04%), exhibited CLC. In both studies, cheeses were also milled between 0.54 and 0.56% TA (pH 5.37 to 5.41). The high milling acidity was selected for the present study because high milling acidities are not associated with CLC (Dybing et al., 1988). Swearingen et al. (2004) suggested that post-manufacture fermentation contributes to release of moisture in packages, leading to CLC. However, although the pH of the cheeses in both studies declined significantly, the primary difference between the 2 studies is the packaging used. The cheeses in the present study were vacuum packaged and stored at 4.4°C, whereas those of Swearingen et al. (2004) were sealed

in plastic bags, placed in 18.14-kg corrugated, lined cheese boxes, and cured at 7°C. These differences are significant because packaging (Johnson et al., 1990a; S. Agarwal, unpublished data, 2005) and temperature (Pearce et al., 1973; Turner and Thomas, 1980; Dybing et al., 1988; Johnson et al., 1990b; Chou et al., 2003) have been demonstrated to affect the appearance of CLC.

Heterofermentative NSLAB, the timing of contamination with such microorganisms, and temperature fluctuations play important roles in the formation of CLC in Cheddar cheese (Chou et al., 2003). Processors are encouraged to limit residual lactose and calcium levels by draining and milling at low pH (6.0 and 5.3, respectively) so that much of the lactic acid is removed during whey drainage (Johnson, 2000). Minimization of NSLAB contamination at any point in processing and handling through strict sanitation measures would be of additional importance in reducing the occurrence of CLC.

CONCLUSIONS

Manufacturing Cheddar cheese with abnormally high lactose milk does not guarantee that CLC will form, even if residual lactose in the cheese is high and the cheese is subjected to temperature abuse. Furthermore, high L(+)-lactic acid in cheese does not guarantee that D(−)-lactic acid or CLC will be produced during the fermentation. Tempering cheese for 24 h will not necessarily cause an increase of D(−)-lactic acid. However, if the cheese is contaminated with NSLAB, D(−)-lactic acid levels will likely increase, due to conversion of L(+)-lactic acid to D(−)-lactic acid by heterofermentative NSLAB. Although Cheddar cheeses that contain low levels of D(−)-lactic acid will not likely form CLC, in time, under appropriate conditions of concentration and temperature, D(−)-lactic acid may increase and CLC may form. Strict sanitation is critical to minimize the occurrence of CLC in the industry.

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