Production of fresh Cheddar cheese curds with controlled postacidification and enhanced flavor

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ABSTRACT

Cheddar cheese in curd form is very popular in eastern Canada. It is retailed immediately after cheese manufacturing and can be maintained at room temperature for 24 h to provide better texture and mouthfeel. Subsequently, the cheese curds must be stored at 4°C. The shelf life is generally 3 d. In this study, Cheddar cheese curds were produced by adding a high diacetyl flavor-producing strain (*Lactococcus diacetylactis*) to a thermophilic-based starter. The objective was to achieve both postacidification stability to increase the shelf life and enhanced flavor. The addition of *L. diacetylactis* increased processing time but did not affect cheese composition or the evolution of proteolysis and texture. During cheese manufacturing, streptococci became the dominant microflora in all cheeses, whereas populations of *Lactococcus cremoris* and *L. diacetylactis* decreased. During cheese storage, viable counts of *L. diacetylactis* and *Streptococcus thermophilus* increased but the counts of *L. cremoris* decreased. During cheese manufacturing and storage, the concentrations of lactic acid and diacetyl increased rapidly in cheeses produced with *L. diacetylactis*. Citric acid and galactose contents remained high in cheese made without *L. diacetylactis*. Sensory evaluation indicated that cheeses containing the *L. diacetylactis* strain were more flavorful and also had less sourness and could be stored at 4°C for up to 7 d.

Key words: fresh cheese curd, aromatic starter strain, thermophilic starter

INTRODUCTION

Cheddar cheese in curd form (fresh Cheddar cheese curds) is very popular in eastern Canada, especially in the province of Quebec. This unripened cheese is retailed immediately after cheese manufacturing and can be maintained at room temperature to provide better texture and mouthfeel. Canadian legislation authorizes the retail sale of fresh Cheddar cheese curds made from

pasteurized milk and kept at room temperature until 24 h after manufacturing. After that, the cheese curds must be stored at 4°C. The shelf life is generally 3 d.

The quality and the typical properties of unripened cheeses strongly depend on the lactic acid bacteria cultures that are inoculated into the milk (Law, 1984). Fresh Cheddar cheese curds are produced by using a mesophilic starter generally composed of *Lactococcus lactis* ssp. *lactis* and *Lactococcus lactis* ssp. *cremoris* strains. However, the time period during which the cheese curds are kept at room temperature and then at 4°C is sufficient to result in overacidification and syneresis, which is detrimental to the flavor and texture of the cheese and reduces its shelf life. Therefore, the fresh cheese curd market is restricted to areas close to the cheese factories, and means of preventing postacidification are of interest to the industry.

To limit postmanufacture acidification and to extend the shelf life of the product, it is possible to use a thermophilic culture of *Streptococcus thermophilus* with the conventional mesophilic starter (Tison et al., 1982; Garrault et al., 2006). Because *S. thermophilus* is unable to grow rapidly at room temperature or at 4°C, postacidification defects in Cheddar cheese curds are reduced by this method (Oberg and Broadbent, 1993). During cheese manufacturing, the combined use of a thermophilic culture and a mesophilic starter offers 2 other advantages. The starter blend is less sensitive to bacteriophages (Oberg and Broadbent, 1993) and the manufacturing time for Cheddar cheese is decreased (Tison et al., 1982). Unfortunately, the use of a thermophilic strain does not contribute to the typical flavor of fresh cheese curds (G. Arora, Abiasa Inc., St-Hyacinthe, Quebec, Canada; personal communication) because *S. thermophilus* is unable to transform citrate into diacetyl, a key aromatic compound in fresh cheese curds. Among lactococci strains, *Lactococcus diacetylactis* is able to transform citrate into diacetyl (McSweeney, 2004). Therefore, using a *Lactococcus* strain that produces a high yield of diacetyl in conjunction with a thermophilic-based starter could allow postacidification stability and enhanced flavor.

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Lacroix (2008) has studied the characteristics of 13 old-style cheese starters collected in 1968 from various Canadian cheese factories. Approximately 360 lactococci strains were isolated from these old-style starters. Among these strains, 30 demonstrated the ability to produce high amounts of diacetyl in Cheddar cheese manufacturing conditions (Gagnon, 2006). The strain *Lactococcus lactis* ssp. *lactis* biovar. *diacetylactis* ULAAC I11 was selected to be combined with a thermophilic-based starter for the production of fresh Cheddar cheese curds.

The objective of this study was to evaluate the concept of enriching a thermophilic-based starter with a high flavor-producing strain to achieve postacidification stability and enhanced flavor in fresh Cheddar cheese curds. Another aim was to determine the impact on cheese manufacturing, cheese composition, and cheese storage.

MATERIALS AND METHODS

Preparation of the Starters

Three lactic starters were prepared to produce fresh Cheddar cheese curds. The starters contained different ratios of *Streptococcus thermophilus* R0083 (*S. thermophilus*) obtained from Institut Rosell Inc. (Montreal, Canada), *Lactococcus lactis* ssp. *cremoris* W62 (*L. cremoris*) obtained from Wisby (Danlac, Airdrie, Alberta, Canada), and a proteinase-negative strain of *Lactococcus lactis* ssp. *lactis* biovar. *diacetylactis* ULAAC I11 (*L. diacetylactis*; Laval University-Agriculture and Agri-Food Canada lactic acid bacteria collection). The latter strain was isolated from old-style cheese starters collected from Canadian cheese factories in 1968 (Lacroix, 2008); it was selected for its capacity to produce a high concentration of diacetyl in fermented milk (Gagnon, 2006) as well as its capacity to grow in the presence of *S. thermophilus* and *L. cremoris*.

Each lactic acid strain was kept at -20°C in reconstituted skim milk (20% of DM) containing 5% sucrose and 0.35% ascorbic acid. Working cultures were obtained after 2 transfers into autoclaved (110°C , 10 min), reconstituted skim milk (12% of DM). For the first transfer of *S. thermophilus* (inoculum size 1.0%), the incubation was performed for 4 h at 40°C , whereas the incubation for the second transfer (inoculum size 1.5%) was performed for 3.5 h at 40°C . For both mesophilic strains, inoculum size was 1.0% and the incubation was performed for 16 h at 21°C for the first transfer, whereas for the second transfer, the inoculum size was 1.5% and the incubation was performed for 15 h at 21°C . In the case of *L. diacetylactis*, the reconstituted skim milk was supplemented with 0.2% yeast extract (Difco Laboratories, Detroit, MI).

Cheddar Cheese Manufacturing

Four repetitions of Cheddar cheese curd making were performed. Three cheeses were made on each occasion. For each cheese-making trial, 800 kg of raw milk obtained from Agropur (Granby, Quebec, Canada) was pasteurized at 76°C for 16 s, cooled to 30°C , and distributed in three 250-kg cheese vats (200 kg of milk per vat). Total protein, fat, and DM concentrations of pasteurized milk were $3.25 \pm 0.02\%$, $3.72 \pm 0.02\%$, and $12.42 \pm 0.06\%$, respectively. Pasteurized milks were inoculated with the 3 lactic acid bacteria to obtain a total initial bacterial population of 1.0×10^7 cfu/mL. In the first cheese vat (**D0**), the initial population consisted of 30% *S. thermophilus* (3.0×10^6 cfu/mL), 70% *L. cremoris* (7.0×10^6 cfu/mL), and 0% *L. diacetylactis*. In the second cheese vat (**D30**), the initial population consisted of 30% *S. thermophilus* (3.0×10^6 cfu/mL), 40% *L. cremoris* (4.0×10^6 cfu/mL), and 30% *L. diacetylactis* (3.0×10^6 cfu/mL). In the third cheese vat (**D50**), the initial population consisted of 10% *S. thermophilus* (1.0×10^6 cfu/mL), 40% *L. cremoris* (4.0×10^6 cfu/mL), and 50% *L. diacetylactis* (5.0×10^6 cfu/mL).

For each cheese vat, when the pH of milk had decreased by approximately 0.20 unit, a double-strength rennet (10 mL/100 kg; Maxiren, Gist Brocades, France) and a solution of calcium chloride (26 mL/100 kg; Danisco USA Inc., New Century, KS) were added. Once a proper coagulum had formed (approximately 30 min), the curd was cut. The cut curd was allowed to settle for 10 min, and then cooking under slow agitation was started. The curd temperature was gradually raised from 30 to 38°C in 30 min. The cheese was further cooked for 30 min at 38°C . After cooking, the temperature was reduced and maintained at 35°C . The whey was drained at pH 6.00. The curd was piled and cheddared until pH 5.10 was reached. The curds were milled and salted (2.0% wt/wt) and then stored at 4°C in plastic bags for 22 d. Cheese yield, expressed as kilograms of cheese per 100 kg of milk, was determined by weighing the amount of milk and the amount of cheese after salting.

Compositional Analysis

The composition of the Cheddar cheeses was determined in triplicate. Dry matter was determined by air-drying at 100°C for 16 h. Fat content was evaluated by the Mojonnier extraction procedure and ash was evaluated by heating samples at 550°C overnight in a muffle furnace. Total protein was measured using the macro-Kjeldahl method (AOAC, 1995). A nitrogen-to-protein conversion factor of 6.38 was applied. Salt content of cheese was determined with a Corning chloride

Table 1. Cheddar cheese composition and cheese yields (%)

Item	Type of cheese ¹			SEM
	D0	D30	D50	
Moisture	44.6 ^a	44.3 ^a	44.0 ^a	0.3
Total proteins	21.5 ^a	21.8 ^a	21.9 ^a	0.3
Fat	27.3 ^a	27.4 ^a	28.0 ^a	0.3
Ash	3.4 ^a	3.5 ^a	3.5 ^a	0.05
Salt	1.8 ^a	1.8 ^a	1.8 ^a	0.07
Yield	11.4 ^a	11.3 ^a	11.1 ^a	0.1

^aMeans within a row with the same superscripts do not differ ($P > 0.05$).

¹D0 = fresh Cheddar cheese curds made with 0% *Lactococcus diacetylactis* in thermophilic-based starter; D30 = fresh Cheddar cheese curds made with 30% *L. diacetylactis* in thermophilic-based starter; D50 = fresh Cheddar cheese curds made with 50% *L. diacetylactis* in thermophilic-based starter.

analyzer, model 926 (Nelson-Jameson Inc., Marshfield, WI).

The concentrations of lactic acid, lactose, galactose, and citric acid in the cheeses were determined after 1, 3, 7, 14, and 22 d by HPLC (St-Gelais et al., 1991), whereas the diacetyl content was determined by GC (Ulberth, 1991).

Microbiological Analysis

The populations of *L. cremoris* and *L. diacetylactis* in cheeses were determined after 1, 3, 7, 14, and 22 d on Kempler and McKay agar medium (KMK; Kempler and McKay, 1980), which made it possible to distinguish between citrate-fermenting and non-citrate-fermenting lactic acid bacteria. The KMK agar medium was incubated anaerobically at 30°C for 72 h. Blue colonies were identified as *L. diacetylactis* and white colonies were identified as *L. cremoris*. *Streptococcus thermophilus* cell counts were determined on M17 agar plates incubated anaerobically at 45°C for 48 h.

Cheese samples (10 g) were diluted in 100 mL of 0.1% peptone water and homogenized using a Stomacher (Model 400, Seward Medical, London, UK). To allow disintegration of long chains of lactococci, 3 g of 4-mm solid glass beads were used in all subsequent dilution bottles, which were vigorously shaken 40 times before inoculation on M17 or KMK agar plates (St-Gelais et al., 1992).

Monitoring of Proteolysis

The evolution of proteolysis in cheeses was followed after 1, 3, 7, 14, and 22 d by determination of total nitrogen (TN), water-soluble nitrogen (WSN), TCA-soluble nitrogen (TCASN), and phosphotungstic acid-soluble nitrogen (PTASN) according to methods described by Christensen et al. (1991). Results were

expressed as the ratio of the different soluble nitrogen fractions to TN as percentage values of TN.

Hardness Measurement

Cheeses were assessed at 1, 3, 7, 14, and 22 d for hardness (MPa) by using a TA-XT2 texture analyzer (Mono Research Laboratories Ltd., Brampton, Ontario, Canada) and a texture profile analysis. Cheese samples of 1.0 cm³ were bored and placed into Petri dishes for 2 h at 22°C before compression tests. For each cheese, 10 cubes were individually compressed to a deformation of 50% using a 2.5-cm cylinder attached to the 5-kg cell. The deformation speed of the sample was 0.4 mm/s.

Sensory Evaluation

Sensory evaluation of all cheeses was performed after 1 and 5 d of storage. A group of 18 trained panelists (3 working sessions) were asked to compare 2 sensory cheese attributes (sourness and diacetyl) by using the quantitative descriptive analysis methodology (Meilgaard et al., 1999). Both these attributes were recorded using a 7-point hedonic scale ranging from nothing (score 0) to extreme (score 7). The sensory analysis was repeated 4 times. Analysis of variance with the Fizz software (Sensory Analysis and Consumer Test Management Software, version 2.10a, Biosystemes, Couternon, France) was performed on each attribute and a Duncan test was used to compare means at $P < 0.1$.

Statistical Methods

Analysis of variance was performed according to a factorial design to determine the effects of the type of starter (D0, D30, and D50) used on cheese composition and cheese yield. A split-plot design was applied to determine the effect of the starter type and refrigerated storage time on the evolution of firmness, populations of lactococci and streptococci, proteolysis (WSN, TCASN, and PTASN), organic acids, sugars, and volatile compounds. Starters and replicates were the main plots and refrigerated storage time (d) was the subplot. The experiment was replicated 4 times, and significant differences were tested at $P < 0.05$. Statistical analyses were carried out with the general linear models procedure of SAS (1999).

RESULTS AND DISCUSSION

The processing steps for the manufacture of Cheddar cheese were carried out at identical pH values (Figure 1), which meant that the time required for those steps could vary as a function of the acidification rate of the

starter. The processing time for the different steps involved in the production of fresh Cheddar cheese curds is presented in Figure 1. Manufacture of the cheeses was affected ($P < 0.05$) by the composition of the starter used to produce the cheeses, especially after the cutting step. Total processing time for the production of D0, D30, and D50 Cheddar cheese curds was 317, 342, and 362 min, respectively. Therefore, processing time increased with the proportion of *L. diacetylactis* present in the lactic acid bacteria starter. However, this did not modify cheese composition or cheese yields, which were statistically similar for all cheeses (Table 1). The highest *L. diacetylactis* content was also accompanied by a lower *S. thermophilus* content. The shortest fermentation time observed with a higher *S. thermophilus* content is in line with reports in the literature. For instance, Michel and Martley (2001) showed that the presence of less than 0.1% *S. thermophilus* in the starter resulted in increased rates of acid production. Data from the present study supported the Cheddar manufacturing literature by showing that a shift from 30 to 10% in *S. thermophilus* content also affected processing time.

The evolution of cell populations of *S. thermophilus*, *L. cremoris*, and *L. diacetylactis* in cheeses during storage is presented in Figures 2A, 2B and 2C, respectively. It was observed that *S. thermophilus* was affected ($P < 0.05$) by starter composition and storage time. No significant interaction was observed between type of starter and storage time. The viable counts of *S. thermophilus* in all cheeses increased during the first 7 d and stabilized thereafter (Figure 2A). After cheese manufacturing (d 1), the number of *S. thermophilus* was higher in D30 cheeses than in other cheeses, and it remained higher during cheese storage. No difference was observed between D0 and D50 cheeses during the first 14 d. However, after 22 d the number of *S. ther-*

Figure 1. Evolution of pH (○) and processing time during production of Cheddar cheese curds made with 0 (◆), 30 (■), and 50% (▲) *Lactococcus diacetylactis* in thermophilic-based starter. Error bars represent the standard errors of the means.

mophilus was lower ($P < 0.05$) in D50 cheese. Although the proportion of thermophilic bacteria in D0 and D30 starters was the same (30%), the *S. thermophilus* population was larger in D30 cheese. A previous study showed that the growth of *S. thermophilus* R0083 was

Figure 2. Evolution of cell populations of *Streptococcus thermophilus* (a), *Lactococcus cremoris* (b), and *Lactococcus diacetylactis* (c) during storage of Cheddar cheese curds made with 0 (◆), 30 (■), and 50% (▲) *L. diacetylactis* in thermophilic-based starter. Error bars represent the standard errors of the means.

Table 2. Proportion (%) of *Streptococcus thermophilus*, *Lactococcus cremoris*, and *Lactococcus diacetylactis* before and after cheese manufacturing and after 22 d of cheese storage

Type of starter ¹	Bacterium	Cheese manufacturing		Cheese storage for 22 d	SEM
		Before	After		
D0	<i>S. thermophilus</i>	30.2 ^c	82.7 ^b	93.4 ^a	3.4
	<i>L. cremoris</i>	69.7 ^a	17.3 ^b	6.6 ^c	2.6
	<i>L. diacetylactis</i>	0 ^a	0 ^a	0 ^a	2.3
D30	<i>S. thermophilus</i>	30.7 ^b	84.8 ^a	84.6 ^a	3.4
	<i>L. cremoris</i>	41.9 ^a	10.9 ^b	8.0 ^b	2.6
	<i>L. diacetylactis</i>	27.4 ^a	4.3 ^b	7.4 ^b	2.3
D50	<i>S. thermophilus</i>	11.6 ^b	69.3 ^a	62.2 ^a	3.4
	<i>L. cremoris</i>	37.3 ^a	18.1 ^b	16.0 ^b	2.6
	<i>L. diacetylactis</i>	51.2 ^a	12.6 ^c	21.8 ^b	2.3

^{a-c}Means within a row with different superscripts differ ($P < 0.05$).

¹D0 = starter consisting of 0% *L. diacetylactis*, 30% *S. thermophilus*, and 70% *L. cremoris*; D30 = starter consisting of 30% *L. diacetylactis*, 30% *S. thermophilus*, and 40% *L. cremoris*; D50 = starter consisting of 50% *L. diacetylactis*, 10% *S. thermophilus*, and 40% *L. cremoris*.

stimulated by the presence of *L. diacetylactis* ULAAC I11 (Gagnon, 2006), which could explain the higher populations of *S. thermophilus* in D30 cheese products. In addition, after cheese manufacturing, the population of *S. thermophilus* in D50 and D0 cheeses was relatively similar even though the proportions of *S. thermophilus* in D50 starter (10%) and D0 starter (30%) were different. This suggests that the presence of *L. diacetylactis* also stimulated the growth of the thermophilic strain in D50 cheese manufacture. Very little is known about interactions between *S. thermophilus* and lactococci in Cheddar starters. Some lactococci produce bacteriocins that can affect the streptococci. Therefore, in the preparation of starters containing such bacteriocin-producing strains, it is essential to use resistant *S. thermophilus* cultures (Morgan et al., 2002). Data from this study showed that strains selected for compatibility (Gagnon, 2006) could indeed result in growth stimulations.

Storage time was the only variable that had an effect ($P < 0.05$) on *L. cremoris* cell counts in cheeses. The populations of *L. cremoris* increased during the first few days and decreased thereafter (Figure 2B). Although the number of *L. cremoris* was higher in D0 starter (70%) compared with D30 and D50 starters (40%), no differences were observed after cheese manufacturing, probably because of the greater cell growth of *S. thermophilus* during the cooking step at 38°C, which was near the optimal growth temperature for thermophilic lactic acid bacteria (Oberg and Broadbent, 1993).

The number of *L. diacetylactis* was affected ($P < 0.05$) by the type of starter used to produce cheeses and by storage time. No significant interaction was observed between these 2 parameters. The *L. diacetylactis* population increased during the first 7 d and was relatively constant thereafter. The number of *L. diacetylactis* was greater in D50 cheese than in D30 cheese at the start of and throughout cheese storage. As a result, the

original proportion of each lactic acid bacterium that existed in each starter before cheese making changed during cheese manufacturing and during storage (Table 2). The same observation was reported by Michel and Martley (2001). During cheese production, the proportion of *S. thermophilus* increased in all cheeses, whereas the proportions of *L. cremoris* and *L. diacetylactis* decreased. During cheese storage, the proportion of *S. thermophilus* increased slowly in D0 and D30 cheeses and remained constant in D50 cheeses. *Lactococcus cremoris* cell counts declined in all cheeses over time, but counts of *L. diacetylactis* increased, especially in D50 cheeses (Table 2).

The evolution of lactic acid, lactose, and galactose contents in cheeses is presented, respectively, in Figures 3A, 3B, and 3C. The concentrations of lactic acid and lactose were affected ($P < 0.05$) by starter composition and by storage time. No significant interaction was observed. After cheese manufacturing (d 1), the concentration of lactic acid was lower, whereas that of residual lactose was higher in D0 cheeses than in other cheeses. The lactose concentration decreased quickly during the first 3 d and decreased slowly after that. The concentration of lactose remained high in D0 cheeses (without *L. diacetylactis*). During cheese storage, the concentration of lactic acid increased rapidly during the first 3 d and increased slowly thereafter. This was mainly attributable to lactose consumption, rather than galactose consumption (Figure 3). The concentration of lactic acid was slightly higher in D50 cheeses after 14 and 22 d.

The concentration of galactose was affected ($P < 0.05$) by the type of starter used to make the cheese. The concentration of galactose in cheeses seemed to be related mainly to the number of *S. thermophilus* cells. Galactose concentration was high in D30 cheeses, intermediate in D0 cheeses, and low in D50 cheeses. Most *S. thermophilus* strains cannot use galactose, whereas the

Figure 3. Evolution of lactic acid (a), lactose (b), and galactose (c) concentrations during storage of Cheddar cheese curds made with 0 (♦), 30 (■), and 50% (▲) *Lactococcus diacetylactis* in thermophilic-based starter. Error bars represent the standard errors of the means.

majority of mesophilic strains are able to metabolize it (Thomas et al., 1980; Poolman, 1993; Michel and Martley, 2001). Michel and Martley (2001) showed that the galactose concentration increased in cheese when a thermophilic strain was used in conjunction with a mesophilic starter. The results obtained in the present study confirmed that the galactose concentration in

Figure 4. Evolution of citric acid (a) and diacetyl (b) concentrations during storage of Cheddar cheese curds made with 0 (♦), 30 (■), and 50% (▲) *Lactococcus diacetylactis* in thermophilic-based starter. Error bars represent the standard errors of the means.

cheese was related to the *S. thermophilus* cell concentration. After cheese manufacturing, the *S. thermophilus* population was high in D30 cheeses but low in D50 cheeses, and the concentration of galactose also was high in D30 cheeses and low in D50 cheeses. Because the number of *L. cremoris* was statistically similar in all cheeses, its effect on sugars and lactic acid concentrations was similar in all cheeses.

The evolution of the citric acid and diacetyl concentrations in the cheeses is presented in Figures 4A and 4B, respectively. Citric acid and diacetyl concentrations were affected ($P < 0.05$) by the type of starter used and by storage time. No significant interaction was observed. After cheese production (d 1), the citric acid concentrations in D30 and D50 cheeses containing *L. diacetylactis* were very low (approximately 0.02%), compared with the concentration in D0 cheeses (0.18%) produced using a starter without the *L. diacetylactis* strain. Therefore, during the production of D30 and D50 cheeses, starters containing *L. diacetylactis* produced diacetyl, presumably from citrate (Figure 4B; Boumerdassi et al., 1997; McSweeney, 2004). The concentrations of diacetyl and

Figure 5. Evolution of hardness (a) and pH value (b) during storage of Cheddar cheese curds made with 0 (◆), 30 (■), and 50% (▲) *Lactococcus diacetylactis* in thermophilic-based starter. Error bars represent the standard errors of the means.

citric acid were similar in D30 and D50 cheeses after manufacturing and after 22 d, even though the number of *L. diacetylactis* was higher in D50 cheeses. The production of diacetyl during storage was probably limited by the amount of citrate available, as evidenced by the very low residual concentration (0.02%) in both cheeses after processing. No diacetyl was detected in D0 cheeses immediately after processing. During cheese storage, a small amount of diacetyl was observed after 3 d, but the diacetyl concentration remained low compared with that in D30 and D50 cheeses.

The evolution of hardness during cheese storage is presented in Figure 5A. Hardness decreased significantly with time ($P < 0.05$) but was statistically similar for all cheeses (Figure 5A). Proteolysis (data not shown) increased significantly ($P < 0.05$) over time for WSN (1.51 to $3.24 \pm 0.05\%$), TCASN (0.64 to $1.18 \pm 0.04\%$), and PTASN (0.13 to $0.17 \pm 0.01\%$), but it was also statistically similar for all cheeses. The evolution of pH, as shown in Figure 5B, was affected ($P < 0.05$) by the

Figure 6. Diacetyl (a) and sourness (b) flavor scores obtained during sensory evaluation of Cheddar cheese curds after 1 d (■) and 5 d (□) of cheese storage. Error bars represent the standard errors of the means.

type of starter used and by cheese storage time. After cheese manufacturing (d 1), the pH value in all cheeses was statistically similar. The pH decreased slowly in D0 cheese produced without *L. diacetylactis*. By contrast, in D30 and D50 cheeses made with the *L. diacetylactis* strain, the pH increased slowly during cheese storage. Although no explanation was found for this phenomenon, it is noteworthy that the cheeses that showed pH increases had low citrate levels.

A sensory evaluation of the cheeses was carried out after 1 and 5 d of storage. The diacetyl and sourness flavor scores are presented in Figures 6A and 6B, respectively. All cheeses obtained moderate diacetyl and sourness flavor scores. The diacetyl flavor score was higher ($P < 0.1$) for D30 and D50 cheeses. During cheese storage, diacetyl flavor scores remained relatively constant in D0 cheeses but increased in D30 cheeses and especially in D50 cheeses (Figure 6A). These results are correlated with the diacetyl concentrations, which were higher in D30 and D50 cheeses than in D0 cheeses. A 3-strain starter incorporating *S. thermophilus* has been suggested as a way to control flavor in Cheddar (Morgan et al., 2002), but it was based on bacteriocin-producing cultures and targeted flavors in

ripened cheeses. The present study is innovative in the use of such a streptococci-lactococci blend for enhanced diacetyl production.

The sourness flavor score after 1 d was similar in all cheeses (Figure 6B). However, after 5 d, the sourness score was higher ($P < 0.1$) in D0 cheese than in the other cheeses. For D30 and D50 cheeses, sourness did not increase during storage. Hence, D30 and D50 cheeses were judged to be less sour than D0 cheeses, based on the increase in the pH value during the storage of the cheeses. However, these results seem to contradict the higher lactic acid production found in D30 and D50 cheeses relative to D0 cheeses (Figure 3A). Hartwig and McDaniel (1995) showed that the degree of sourness of citric acid was higher than that of lactic acid and was more pronounced as the pH decreased. Citric acid concentration was very high in D0 cheeses compared with D30 and D50 cheeses. This could explain the higher sourness flavor scores assigned to D0 cheeses compared with D30 and D50 cheeses.

CONCLUSIONS

Blending streptococci and lactococci in Cheddar cheese starters was previously suggested as a way to control bacteriophages (Stokes et al., 2001) or ripened cheese flavor (Morgan et al., 2002). In the present study, that approach was used for the control of sourness and enhanced flavor in fresh cheeses.

The addition of an aromatic strain (*L. diacetylactis*) to a starter containing a thermophilic strain increased processing time but did not have an effect on cheese composition or on cheese proteolysis and texture. As postulated in the hypothesis, this type of starter permitted the production of fresh Cheddar cheese curds with reduced sourness and increased diacetyl flavor. Moreover, postacidification during storage (as evidenced by pH) was controlled.

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