Flavor Enhancement of Reduced Fat Cheddar Cheese Using an Integrated Culturing System

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Mild cheese flavor in reduced fat Cheddar cheese was enhanced by using an integrated starter culture system. Three cultures, *Lactococcus lactis* subsp. *cremoris* SK11, *L. lactis* subsp. *lactis* biovar. *diacetylactis* JVI, and *Lactobacillus casei* 7A, were carefully selected to obtain a nonbitter, mildly acid, buttery flavored cheese. Cheeses were produced from all possible combinations of these cultures with the constraint that *L. lactis* subsp. *cremoris* SK11 was used as the primary acid-producing culture. Cheeses made with SK11 were compared to cheeses produced using an *L. lactis* subsp. *cremoris* commercial starter culture. Cheeses were ripened for 150 days and periodically sampled for chemical, microbiological, and sensory analysis. Cheeses produced with *L. lactis* subsp. *cremoris* SK11 had substantially lower bitterness intensity than the cheeses produced with commercial starter culture. *L. lactis* subsp. *lactis* biovar. *diacetylactis* JVI significantly increased diacetylacetoin and acetate concentrations. Sensory results indicate that these cheeses had increased buttery (diacetyl) flavor.

Keywords: *Mild cheese flavor; diacetyl; acetoin; acetate; lactate; cheese cultures; starter system; reduced fat cheese; Cheddar*

INTRODUCTION

Reduced fat cheese has been characterized as being bland, lacking Cheddar flavor, and containing off-flavors such as bitterness, meaty, brothy, and unclean. Some researchers claim that the lack of flavor development is directly a result of the fat reduction with decreased levels of free fatty acids (Banks et al., 1989). This view is likely overly simplistic because altering the fat concentration changes the product microenvironment, affecting bacterial growth patterns, enzyme activity (Mistry, 1995), and flavor retention and perception (Law, 1984). The solvent function of fat likely plays a more significant role in the flavor of reduced fat Cheddar cheese than the contribution of fatty acids. Compounds that are believed to contribute to Cheddar cheese flavor, such as thiols, hydrogen sulfide, esters, aldehydes, alcohols, and ketones, have been isolated from the lipid phase of ripened cheese (Olson, 1980). Reduced fat levels diminish the protective environment for these potential critical flavor compounds.

The perception of off-flavors, particularly bitter compounds, has been noted to be more prevalent in reduced fat cheese systems. Bitterness is due to the production of bitter peptides by rennet and starter bacteria, with these peptides containing predominantly hydrophobic amino acid residues (Cogan and Hill, 1993). Rank (1986) studied flavor development in low fat and whole milk Colby and Cheddar-like cheeses and found that off-flavor compounds, especially bitter ones, are likely to absorb on fat interfaces. Adsorption on the fat phase reduces the off-flavor concentration in the aqueous phase, thereby reducing their perception level, suggesting that fat masks particular flavors. With fewer fat globules in reduced fat cheese, there is less lipid to harbor these compounds and therefore a higher concentration of readily perceptible off-flavors in the aqueous phase.

Many methods have been developed to enhance the flavor of reduced fat Cheddar cheese. Most published research has focused on attaining a flavor comparable to that of full-fat, mature Cheddar cheese. Our approach was different.

By examining consumer liking of Cheddar cheese, Roberts and Vickers (1994) found that consumers in the United States preferred clean acid, nonbitter, milky, and buttery notes, characteristics of young Cheddar cheese. Therefore, our goal was to produce a mild cheese that consumers would find desirable.

Using available metabolic information about specific starter cultures, we carefully selected a blend of cultures to produce cheese with consumer-preferred characteristics after 2−3 months of ripening. The lactic acid bacterial strains selected to produce these characteristics were *Lactococcus lactis* ssp. *cremoris* SK11(S), *L. lactis* subsp. *lactis* biovar. *diacetylactis* JVI, (J) and *Lactobacillus casei* 7A (L).

MATERIALS AND METHODS

Starter Cultures. Thermolytic strain S was obtained from the University of Minnesota stock culture collection. A defined culture consisting of a mixture of three *L. lactis* ssp. *cremoris* strains, SCO 112, 230, and 233 (C) was obtained from Chr. Hansen's Laboratory, Inc. (Milwaukee, WI). Strains 112 and 230 were reported to have fast activity and strain 233 slow activity. Culture J was obtained from Rhone-Poulenc, Inc.

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(Madison, WI). All *Lactococcus* cultures were grown in 10% wt/vol. solids of reconstituted nonfat dry milk (steamed at 100 °C for 60 min). Culture L was obtained from Dr. R. T. Marshall's laboratory at the University of Missouri—Columbia. L was grown in autoclaved MRS broth (Difco, Detroit, MI) and centrifuged (5520*g* for 15 min) to remove the broth and obtain the pellet containing the cells.

Experimental Design. The experiment was three replicates of a full 2^3 factorial design in four blocks of size 2. The three experimental factors were starter culture type (S or C), J (presence or absence), and L (presence or absence). A total of $8 \times 3 = 24$ vats of cheese were produced. Capacity constraints allowed only two lots of cheeses to be produced at the same time. Therefore the blocks were arranged in two dimensions, day of production (day 1 or day 2) and time of day (AM or PM). Thus 8 vats of cheese were made in a 2-day period from the same milk. The cheese making schedule was arranged by constrained randomization of pairs of factor combinations. The experiment was replicated three times (October 1994, March 1995, June 1995).

Four of the eight cheeses were produced using S as the primary starter, and the other four cheeses were made using C. Cheeses of each starter culture were produced such that one vat of cheese contained the starter alone (S or C), a second vat contained the starter and J (SJ or CJ), third vat contained the starter and L (SL or CL), and the fourth vat contained the starter with both adjuncts as the complete integrated culturing system (SJL or CJL).

Cheese Manufacture. Reduced fat Cheddar cheeses were made in the University of Minnesota Department of Food Science and Nutrition pilot plant from whole milk obtained from Mid-America Dairymen (Roseville, MN), standardized to approximately 1.25% fat, and pasteurized at 85 °C for 16 sec. The cheeses were made according to a standard Cheddar cheese making procedure (Price and Calbert, 1953) using approximately 635 kg of milk per vat of cheese. Each vat was inoculated with 2% concentration of the respective primary starter culture (S or C). Cheese milks containing J were inoculated at a concentration of 0.2%. Cheese milks containing L were inoculated such that cell population as colony forming units (cfu) averaged 2.5×10^6 cfu/mL of milk. All cheeses were ripened at 4 °C and sampled at 1, 14, 30, 90, and 150 days. They were sampled by taking a cross-section from the middle of a 9 kg block, trimming the edges and cutting the slab into cubes. Cubes for blending were taken from the center middle and sides of the cross section.

Microbiological Analysis. A 10 g sample of cheese was aseptically removed from the center of the cheese cross-section with a sterile cheese sampler and placed in a sterile blender jar. A 90 mL solution of 2% sodium citrate (Sigma Chemical Co., St. Louis, MO) was added to the jar and the mixture was blended for 2 min in an Osterizer Serial dilutions were prepared with 9 mL blanks of 2% sodium citrate, and 0.1 mL aliquots were spread plated in duplicate onto each agar medium. Plates were incubated anaerobically at 30 °C for 48 h and enumerated.

Colony-forming units per gram of cheese at 1, 14, 30, 90, and 150 days were determined for the various cultures using the following: Reddy's differential agar medium (Reddy et al., 1972) for S and C, Kempler—McKay medium (Kempler and McKay, 1980) for J and Rogosa acid acetate agar for the enumeration of Lactobacillus species (Sandine et al., 1976) for L.

The agar medium for differential enumeration of *Lactococci* results in colonies of *L. lactis* subsp. *cremoris* being yellow, whereas colonies of *L. lactis* subsp *lactis* are white.

In the Kempler—McKay medium for determining the numbers of citrate fermenting *L. lactis* subsp. *lactis* biovar. *diacetylactis* these colonies are blue whereas colonies unable to utilize citrate remain white, thus the counts are valid for *L. lactis* subsp. *lactis* biovar. *diacetylactis*.

Chemical Analysis. Cheese pH, moisture, salt, fat and protein content were measured after 14 days of ripening. Cheese pH also was determined at 30, 90 and 150 days. Cheese block cross-section samples were ground and used for composition analyses. The pH was measured in a cheese slurry (2 parts cheese: 1 part water) using a calibrated pH meter (model 420A; Orion Research Inc., Boston, MA). Moisture was determined using a Moisture/Solids Analyzer AVC-80 (CEM Corp., Matthews, NC). Salt content was measured using a Corning Chloride Analyzer 926 (Nelson-Jameson, Inc., Marshfield, WI). Fat and protein were determined by AOAC (1990) methods.

All cheeses were analyzed for total diacetylacetoin content, acetate, and lactate at 14, 30, 90, and 150 days during ripening. Tests for flavor compounds were run in duplicate.

Total diacetyl-acetoin was measured by the colorimetric method of Westerfeld (1945). Samples were prepared by blending 10 g of ground cheese with 80 mL of 0.1 M sodium citrate solution for 2 min and adjusting the final volume to 100 mL. The resulting mixtures were incubated at 40 °C for 120 min and centrifuged at 3840*g* for 15 min (Beckman JA-21 centrifuge, rotor type JA-14) to separate the fat. Samples were held overnight at 4 °C and analyzed the following day. Standards were made by adding acetoin to 11% wt/vol reconstituted NDM (steamed at 100 °C for 60 min pH 7.0) to obtain a range of 0—150 μg of acetoin/mL reconstituted milk. Standards and cheese sample preparations (1 mL) were mixed with 2 mL of distilled water, 1 mL of 10% sodium tungstate solution, and 1 mL of 0.66 N H_2SO_4 solution and filtered through Whatman No. 4 filter paper. One milliliter of the filtrate was then mixed with 4 mL of distilled water, 1 mL of 0.5% creatine solution, and 1 mL of freshly prepared alkaline α-naphthol (0.5 g of α-naphthol in 10 mL of 2.5 NaOH solution). The mixtures were incubated at 25 °C for 60 min and the absorbance was measured at 540 nm using a spectrophotometer (model DU-650; Beckman Instruments, Inc., Fullerton, CA).

Both acetate and lactate were measured by enzyme assays (kits 148261 and 1112821, Boehringer Mannheim, Mannheim, Germany).

Sensory Analysis. A trained sensory panel (9 panelists) evaluated all cheeses at 30, 90, and 150 days of ripening for the following attributes: overall flavor intensity, salty, bitter, sour (acid), diacetyl, milky, brothy, sulfury, firmness, corky, pasty and chewy. To train the panel for various characteristics, the following references were used; for overall flavor intensity, a sharp, full-fat Cheddar cheese was used for training. The panel was trained for salt using 0.25, 0.5, and 0.75% w/w NaCl solutions. To train for bitterness, 0.06% w/w solutions of caffeine were used. For sourness, 0.05% solutions of lactic acid were used. Diacetyl (0.55 mL) was added to deodorized mineral oil (100 mL) and then tasted. For milky flavor, whole milk was used; for brothy flavor, 2 g monosodium glutamate in 118 mL water; for sulfury flavor, New York Cracker Barrel Cheddar cheese (Kraft Foods Inc., Glenview, IL); for fruity flavor, a sharp Cheddar cheese sample that had previously been determined to be fruity was used. For firmness, a low fat cheese was used and panelists were trained to determine the amount of force required to bite once through the sample. For pasty, a pre-selected cheese that was determined to be pasty was used. Panelists were trained for chewiness by determining how much work was required to chew a sample of low-fat cheese.

To test the sensory characteristics of experimental cheeses, panelists were asked to place a mark on a 130 mm line that was labeled from very low on the left end to very high on the right end. Data were digitized to a 100 category scale 1 = low, 100 = very high. This was done for each attribute. Cheese samples were cut, wrapped, and held at 22 °C for 1 h before sensory evaluation.

Statistical Analysis. Analysis of variance (ANOVA) was run on pH, diacetylacetoin, acetate, lactate and all sensory characteristics data using SAS statistical software. The experimental design involved partial confounding of the main effects and two-factor interactions with the blocking effects of day of production and time of day. The three-factor interaction was completely confounded with the day of production blocking effect; To determine the significance of the blocking effects (day of production and time of day), the data were analyzed in subsets: each experimental replicate alone, two experimental replicates in combination, and the complete model of all three

Table 1. Mean Composition of Reduced Fat Cheddar Cheeses at 14 Days Postmanufacture ($n = 24$)

variable	mean ± SD (%)	variable	mean ± SD (%)
FDB[a]	27.49 ± 1.00	MNFS[b]	51.96 ± 0.84
protein	33.48 ± 1.02	S/M[c]	3.54 + 0.39
moisture	43.95 ± 0.80		

[a] Fat on a dry basis. [b] Moisture in nonfat substance. [c] Salt (NaCl) in moisture.

experimental replicates. As expected, the blocking effects were found to be insignificant, therefore an ANOVA ignoring blocking effects of the complete 2^3 factorial model of all three experimental replicates was used. The three factor interactions (day of production × time of day × design factors) were used as the experimental error term. Significance of the changes in the concentration data over the ripening period were analyzed using the split plot error term. Least squares means were calculated by SAS for those factors found to be significant by ANOVA.

RESULTS AND DISCUSSION

Starter Culture Selection. S culture was chosen for its ability to consistently produce a clean acid Cheddar cheese free of bitterness (Lawrence and Gilles, 1969; Lowrie et al., 1972). The development of nonbitter cheese may be attributed to the presence of thermoinducible prophage and subsequent lysis of some S cells upon exposure to typical cooking temperatures used in Cheddar cheese manufacture allowing the release of intracellular enzymes which may have a role in degrading bitter peptides (Feirtag and McKay, 1987). This strain also has cell envelope proteinase conducive to production of nonbitter cheese (Exterkate, 1995).

J culture was selected to produce buttery notes and acetate. Diacetyl along with acetoin are the main flavor compounds that result from citrate fermentation by *L. lactis* ssp. *lactis* biovar. *diacetylactis* in cheese. Diacetyl is thought to be an essential component of Cheddar cheese flavor (buttery) and may improve flavor quality at elevated concentrations (Calbert and Price, 1949; Keen and Walker, 1974; Manning, 1979; McDonald, 1992). A similar flavor compound is acetoin. It has a mild creamy, butter-like flavor, slightly sweet like mild cheese and tends to reduce the harshness of diacetyl. Diacetyl combined with acetoin gives mild, pleasant, buttery notes (Arctander, 1969; Burdock, 1994). Acetate is a primary odorant in mild full-fat and low-fat Cheddar cheese (Milo and Reineccius, 1997). Acetate is produced from citrate fermentation by *L. lactis* ssp. lactis biovar *diacetylactis*.

To further enhance flavor, L was selected. This strain of *Lb. casei* was isolated and investigated by (Peterson, 1988) who found that it significantly reduced bitterness and increased characteristic Cheddar cheese flavor. Similar results, in a study of reduced-fat Cheddar cheese, were reported by Tempas (1991).

Composition. Within each experimental, replicate, all cheeses were manufactured from the same lot of standardized milk therefore chemical composition was fairly constant. Mean chemical composition of all cheeses for the three experimental replicates measured 14 days postmanufacture are presented in Table 1. On average, the experimental cheeses had a fat on dry basis (FDB) of 27.49% which is a 45% reduction from standard full fat (50% FDB) Cheddar cheese.

Microbiological Analysis. Cell populations of both the S starter culture (Figure 1) and the C starter culture (Figure 2) in the cheeses were determined over a 150 days ripening period. Both cultures decreased rapidly in numbers during the ripening period. Others have also shown that starter culture cell populations are at a maximum during or shortly after manufacture and quickly decline thereafter (Dawson and Feagan, 1957; Martley and Lawrence, 1972). At 1 day postmanufacture, cheeses produced with S generally had lower cell populations than the cheeses manufactured with the commercial culture. Both cultures were added at a 2% inoculum, but since S exhibits a significant thermolytic response to the cooking temperature used during cheese manufacture, the resulting cell populations were lower (Feirtag and McKay, 1987).

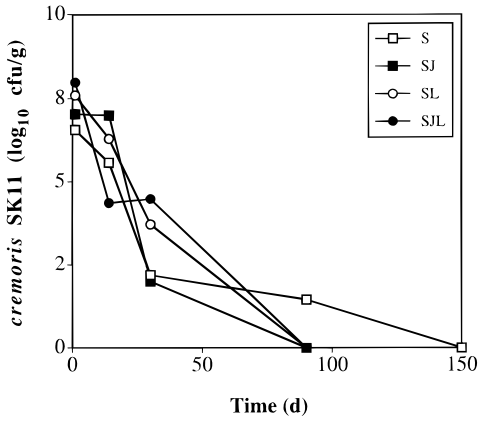

Figure 1. Changes in cfu of the *L. lactis* subsp. *cremoris* SK11 starter culture in reduced fat cheeses inoculated with SK11 as the primary culture (S) alone or with adjunct *L. lactis* subsp. *lactis* biovar. *diacetylactis* JVI (SJ) or *Lb. casei* (SL) or both (SJL) during ripening. Data points are means from three trials. All counts were made on Reddy's differential agar.

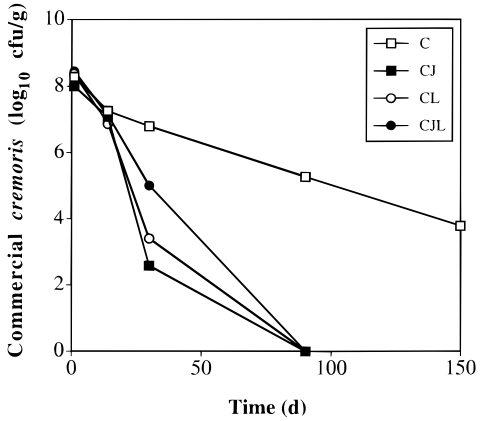

Figure 2. Changes in cfu of *L. lactis* subsp. *cremoris* defined-strains commercial starter culture in reduced fat cheeses inoculated with the primary commercial culture (C) alone or with adjunct *L. lactis* subsp. *lactis* biovar. *diacetylactis* JVI (CJ) or *Lb. casei* (CL) or both (CJL) during ripening. Data points are means from three trials. All counts were made on Reddy's differential agar.

Comparison among cheeses produced with the same starter culture provides insight to the influence of the other cultures on starter cell populations. The cheese containing the commercial culture alone (C, Figure 2) showed greater survival during ripening compared with those cheeses containing the adjuncts. In adjunct cheeses, the cremoris culture died off within 90 days. A similar survival pattern was shown in cheeses containing S (Figure 1).

J cell populations changed with time in cheeses containing this culture (Figure 3.) Except for SJL

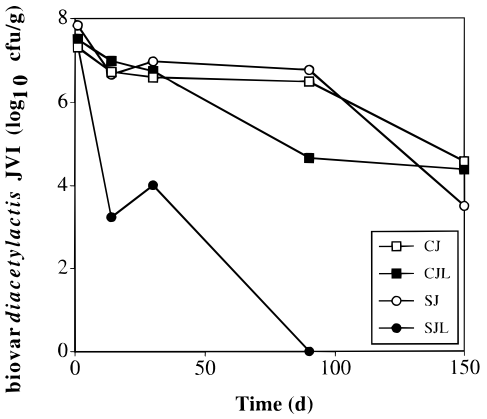

Figure 3. Changes in cfu of *L. lactis* subsp. *lactis* biovar. *diacetylactis* JVI in reduced fat cheeses inoculated with this adjunct (CJ, CJL, SJ, SJL) during ripening. (S = *L. lactis* subsp. *cremoris* SK11; C = *L. lactis* subsp. *cremoris* defined-strains commercial culture; J = *L. lactis* subsp. *lactis* biovar. *diacetylactis* JVI; L = *Lb. casei* 7A.) Data points are means from three trials. Counts were made on Kempler−McKay agar.

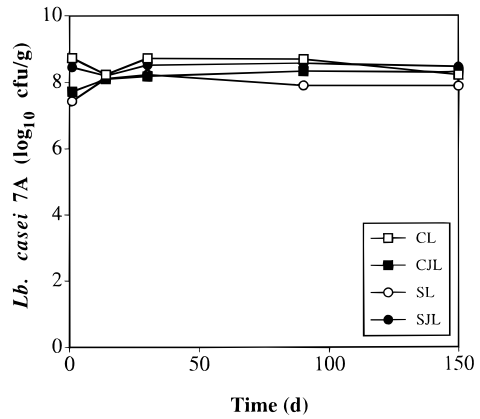

Figure 4. Changes in cfu of *Lb. casei* 7A in reduced fat cheeses inoculated with this adjunct (CL, CJL, SL, SJL) during ripening. (S = *L. lactis* subsp. *cremoris* SK11; C = *L. lactis* subsp. *cremoris* defined strains commercial culture; J = *L. lactis* subsp. *lactis* biovar. *diacetylactis* JVI; L = *Lb. casei* 7A.) Data points are means from three trials. Counts were made on Rogosa acid acetate agar for the enumeration of *Lactobacillus* species.

cheeses, these bacteria remained viable over 150 days of ripening. The results shown in Figures 1 and 2 compared with Figure 3 are consistent with the results of others who found that *L. lactis* subsp. *lactis* biovar. *diacetylactis* declined at a slower rate in Cheddar cheese than did *L. lactis* subsp. *cremoris* (Dawson and Feagan, 1957; Fryer et al., 1970). The reason for the early reduction in cell numbers of J in the SJL cheese is unknown.

Lactobacilli populations generally ranged between 10^7 and 10^8 cfu/g cheese at 14 days postmanufacture and remained at this level throughout the ripening period (Figure 4).

There was concern that nonstarter lactic acid bacteria may have been present as well as *Lb. casei.* Inoculated milks and all cheese samples were plated on the acid acetate agar. In all three trials samples of the milks from the six vats of cheese not inoculated with *Lb. casei* and cheese made from these milks were also plated on this agar at the various times during ripening. No growth was obtained on any of these plates. This shows that there were no other lactobacilli present. The high heat treatment (85 °C for 20 s) would kill any nonstarter lactobacilli and lactococci that might have been present in the milk and strict cleaning and sanitizing procedures that were used would eliminate possible other contaminants. The counts accurately reflect the numbers of *Lb. casei*.

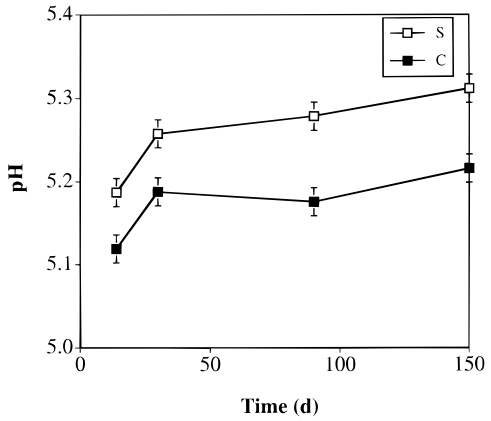

Figure 5. Changes in pH during ripening of reduced fat Cheddar cheese inoculated with *L. lactis* subsp. *cremoris* SK11 (S) and *L. lactis* subsp. *cremoris* defined-strains commercial culture (C) as primary starters. Data points are least-squares means ± standard error (n = 12).

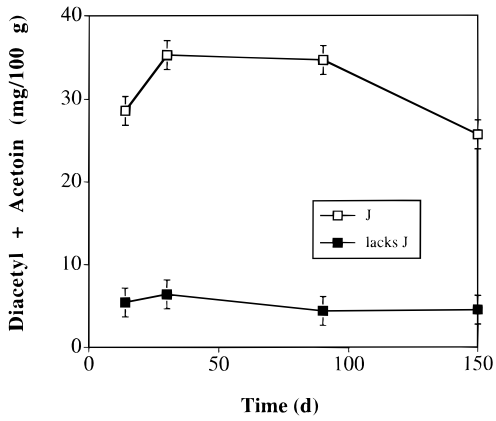

Figure 6. Comparison of the amount of diacetyl-acetoin produced during ripening in cheeses made with and without added *L. lactis* subsp. *lactis* biovar. *diacetylactis* JVI (J). Data points are least-squares means ± standard error (n = 12).

Our findings agree with the population trends reported by Tempas (1991) who, in adding *L. casei* 7A as an adjunct to reduced fat Cheddar cheese, also found lactobacilli-levels of 10^7 to 10^8 cfu/g cheese after 14 d of ripening and the populations remained fairly constant during a 6 month ripening period.

pH. The cheeses made using S culture consistently had a higher pH than those made using C starter cheeses during ripening (Figure 5). Since S is a slow acid producing culture and the commercial starter contained a fast acid producing strain, it was expected that the S-containing cheeses would have a higher pH.

Diacetyl-acetoin and Acetate. The addition of J to experimental cheeses significantly increased concentrations of diacetyl-acetoin (Figure 6) and acetate (Figure 7). The cheeses made with J had a concentration of diacetyl-acetoin approximately 6 times that of the cheeses without this culture. The highest concentration (35.3 mg/100g) occurred at 30d and gradually declined to 26 mg/100 g cheese at 150 days. Without J, cheeses had a low level of diacetyl-acetoin concentration (4.4−

Table 2. Mean Sensory Scores by Attribute and Factor[a]

	age (month)			starter		adjunct J		adjunct L		time of year		
attribute	1	3	5	C	S	no	yes	no	yes	fall	spring	summer
flavor intensity	42^a	51^b	58^c	50	51	48^a	53^b	50	51	52^b	48^a	52^b
salty	41	43	41	41	42	41	42	41	42	40^a	42^b	43^b
sour	44	44	44	45	43	42^a	47^b	45	44	36^a	48^b	49^b
bitter	8^a	22^b	31^c	30^b	1^a	20	21	19^a	23^b	23^b	19^a	20^a
brothy	3	12^b	12^b	9	11	9^a	11^b	10	10	13^b	8^a	8^a
bite	2	2	1	2	2	2	2	2	2	3^b	1^a	1^a
diacetyl	37^c	31^b	24^a	30	32	26^a	35^b	28^a	34^b	34^b	32^b	27^a
milky	11^c	7^b	5^a	4^a	8^b	8	7	8	8	9^b	5^a	9^b
sulfury	6^a	14^b	18^c	10^a	15^b	12	14	14^b	11^a	18^c	13^b	8^a
burnt	2	5	3	3	4	5^b	1^a	4	2		5	2
fruity	7	9	9	6^a	11^b	8	9	8	9		10	11
unclean			5	8^b	2^a	4^a	7^b	8^b	3^a			5
firm	67^c	54^b	52^a	54^a	61^b	60^b	56^a	60^b	56^a	52^a	60^b	61^b
chewy	57^c	43^b	40^a	43^a	50^b	48	46	46	47	46^b	50^c	44^a
corky	53^c	34^b	29^a	34^a	43^b	41^b	36^a	36^a	41^b	36^a	43^b	37^a

[a] Samples within an attribute and factor group having no letter superscripts or letter superscripts in common do not differ significantly ($p > 0.05$). Blank cells indicate that the attribute was not measured in those cheeses.

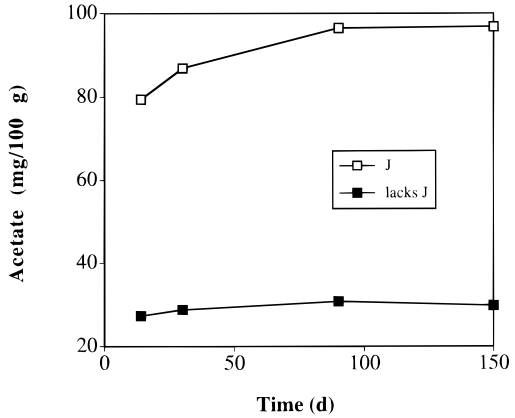

Figure 7. Comparison of the amount of acetate produced during ripening in cheeses made with and without added *L. lactis* subsp. *lactis* biovar. *diacetylactis* JVI (J). The least-squares mean standard error of 1.5 mg/100 g is too small to be displayed on the graph.

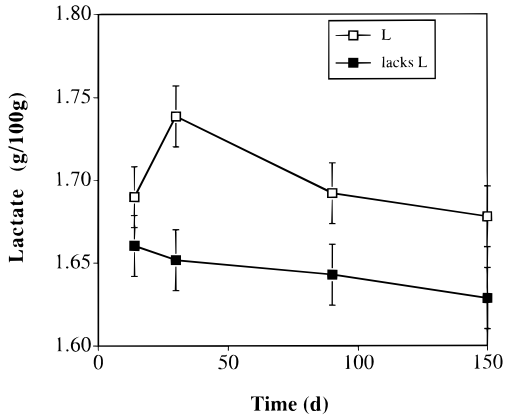

Figure 8. Comparison of the amount of lactate produced during ripening in cheeses made with and without added *Lb. casei* 7A (L). Data points are least-squares means ± standard error ($n = 12$).

6.4 mg/100 g). The sensory panel perceived a more intense diacetyl (buttery) flavor in cheeses containing J substantiating the chemical data (compare Figure 6 with Table 2).

As shown in Figure 7, cheeses containing J had acetate amounts of 79−97 mg/100 g cheese, >3 times the amount of acetate in cheeses without J. However, the only impacts of J noted by our panelists were an increase in diacetyl, a slight increase in bitterness, and a slight decrease in unclean flavor. The impact of the increased level of acetate is unclear. Law et al. (1976) noted that over a broad range of acetate concentrations, only small flavor differences were detected. At excessively high concentrations, however, acetate may cause vinegary off-flavors (Manning and Nursten, 1985). Also, Thomas (1987) indicated that 70 mg acetate/100 g cheese could cause off-flavor in young cheese. Recently Milo and Reineccius (1997), in a study of odorants in regular-fat and low-fat mild Cheddar cheese, concluded that acetic acid along with diacetyl, butyric acid, methional and homofuraneol are primary contributors to the pleasant mild aroma of Cheddar cheese.

Lactate. The L culture had a significant effect on lactate concentration ($P = 0.0024$). Cheeses containing L consistently had a higher concentration of lactate than those without L (Figure 8). The mean lactate content of all cheeses for all sampling periods was 1.67 ± 0.08 g/100 g of cheese. This agrees with the results of Johnson et al. (1995) showing total lactate to be 1.7 g/100 g cheese in a reduced fat Cheddar cheese system, although the typical reported concentration of total lactate in full fat Cheddar cheese is 1.5 g/100 g of cheese (Cogan and Hill, 1993).

Sensory. Because U.S. consumers prefer buttery, clean acid, nonbitter cheese, these traits were included in our sensory evaluation. Diacetyl (buttery) flavor perception was highest in cheese that contained culture J and/or L (Table 2), confirming the difference in diacetyl-acetoin concentration that was determined analytically (Figure 6).

Bitterness increased with increasing age (Table 2). Culture C produced higher levels of bitterness than culture S (Table 2); the magnitude of this difference in bitterness increased with increasing age (F age X starter = 41.2; $P = 0.0001$). At 150 days of ripening the mean bitterness scores for C and S were 46 and 16. This large difference in bitterness suggests that extreme care must be taken in selecting primary acidifying cultures for manufacture of reduced and low-fat cheese. Culture selection for production of nonbitter cheese should be partially based on the specificity of the cell envelope proteinase (CEP). The specificity of CEP in lactic acid bacteria towards α_{s1}-casein fragment 1−23 has been elucidated (Exterkate, 1995). Because hydrolysis of this fragment (fragment 1−23) can lead to formation of

several bitter peptides in Cheddar cheese (Lemieux and Simard, 1992), strains that do not release such bitter peptides need to be selected. Unfortunately, many strains of lactic acid bacteria, that have been selected for fast acid production in commercial Cheddar cheese manufacture in the United States, also have a propensity to release bitter peptides from α_{s1}-casein fragment 1−23. Peptides from β-casein also are involved in contributing to Cheddar cheese bitterness. In our study, we looked at β-casein hydrolysis and found that the rate of hydrolysis was faster in C cheese than in S cheese (data not shown).

The panel also found that addition of culture J produced more intense sourness; brothiness was very slightly increased in cheeses containing culture J; sulfury flavor was increased in cheese containing culture S; overall intensity was higher in older cheeses and in cheeses containing culture J; and like full-fat cheese, reduced-fat cheese became less firm corky and chewey during the ripening period.

CONCLUSIONS

The integrated starter culture system of *L. lactis* subsp. *cremoris* SK11, *L. lactis* subsp. *lactis* biovar. *diacetylactis* JVI, and *L. casei* 7A enhanced the flavor of reduced-fat Cheddar cheese. The addition of *L. lactis* subsp. *lactis* biovar. *diacetylactis* JVI significantly increased total diacetylacetoin and acetate concentrations. Sensory results indicated that the increased diacetyl concentration was readily perceptible, contributing to the buttery flavor of the cheese. *Lb. casei* 7A had significant effect on the lactate concentration. Cheeses made with the *L. lactis* subsp. *cremoris* SK11 starter culture were less bitter than cheeses made using the commercial starter culture.

ABBREVIATIONS USED

S, *Lactococcus lactis* subsp. *cremoris* SK11; C, commercial blend *Lactococcus lactis* subsp. *cremoris*; J, *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis* JVI; L, *Lactobacillus casei* 7A; cfu, colony-forming units.

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