Differences Between Cheddar Cheese Manufactured by the Milled-Curd and Stirred-Curd Methods Using Different Commercial Starters

Shakeel-ur-Rehman,* M. A. Drake,† and N. Y. Farkye*¹

*Dairy Products Technology Center, California Polytechnic State University, San Luis Obispo 93407

†Southeast Dairy Foods Research Center, North Carolina State University, Raleigh 27695

ABSTRACT

Traditionally, Cheddar cheese is made by the milled-curd method. However, because of the mechanization of cheese making and time constraints, the stirred-curd method is more commonly used by many large-scale commercial manufacturers. This study was undertaken to evaluate quality differences during ripening (at 2 and 8°C) of Cheddar cheese made by the milled-curd and stirred-curd methods, using 4 different commercial starters. Twenty-four vats (4 starters × 2 methods × 3 replicates) were made, with ~625 kg of pasteurized (72°C × 16 s) whole milk in each vat. Fat, protein, and salt contents of the cheeses were not affected by the starter. Starter cell densities in cheese were not affected by the method of manufacture. Non-starter lactic acid bacteria counts at 90, 180, and 270 d were influenced by the manufacturing method, with a higher trend in milled-curd cheeses. Proteolysis in cheese (percentage of water-soluble N) was influenced by the starter and manufacturing method (270 d). Sensory analysis by a trained descriptive panel (n = 8) revealed differences in cooked, whey, sulfur, brothy, milk fat, umami, and bitter attributes caused by the starter, whereas only brothy flavor was influenced by storage temperature. The method of manufacture influenced diacetyl, sour, and salty flavors.

Key words: stirred curd, milled curd, Cheddar cheese, proteolysis

INTRODUCTION

Cheddar cheese making involves 2 phases—the conversion of milk to curd in the first phase, which is generally accomplished in 24 h, and the transformation of the young “green” cheese into a mature cheese in the second phase. Traditionally, the milled-curd (MC) method is used for Cheddar cheese manufacture. The

MC method involves the process of “cheddaring” (which involves turning and flipping loaves of warm curd at regular intervals for 1 to 2 h for the development of acid, leading to a fibrous, “chicken breast-like” structure), followed by milling, hooping, and pressing. In the stirred-curd (SC) method of manufacture, the curds are continuously stirred after whey drainage, hence eliminating the traditional cheddaring and milling process. The SC method is the method of choice in highly mechanized cheese plants because of its relatively short manufacturing time.

Acidification is one of the most important operations in the manufacture of Cheddar cheese. Starters are added to cheese milk to achieve a uniform and predictable rate of acid production during manufacture. The postcoagulation steps for MC Cheddar are different from those for SC Cheddar; therefore, the development of acidity and the growth rate of starter bacteria may vary. Starter bacteria also contribute to proteolysis during Cheddar cheese ripening through the action of their cell envelope-associated proteinase and intracellular peptidases (Law and Haandrikman, 1997; Singh et al., 2003).

The process of converting young Cheddar cheese into mature Cheddar is governed by the ripening conditions, such as temperature. Cheese-ripening temperature is a major factor controlling bacterial growth and the activity of enzymes in the cheese. Although SC Cheddar is similar to MC Cheddar in composition, it is thought to exhibit a curdy and open texture and is less elastic than MC Cheddar; the characteristic “chicken breast” texture does not develop because of elimination of the cheddaring step. Little or no information is available in the literature that quantitatively compares the ripening of Cheddar cheese made by the MC and SC methods. The objectives of this study were to determine the effect of the method of manufacture on the microbiological, biochemical, and sensory changes during ripening of Cheddar cheese made with different starters, and to determine the influence of ripening temperature on those changes.

Received January 2, 2007.

Accepted September 10, 2007.

¹Corresponding author: nfarkye@calpoly.edu

MATERIALS AND METHODS

Cheese Making

Three experimental cheese-making trials were conducted. Four frozen direct vat set starter types (a, b, c, d), each consisting primarily of mesophilic strains of *Lactococcus lactis* ssp. *cremoris* or *Lactococcus lactis* ssp. *lactis*, were obtained from independent starter culture companies (Chr. Hansen Inc., Milwaukee, WI; Rhodia, Madison, WI; Degussa Bioactives, Waukesha, WI; and DSM Food Specialties, Parsippany, NJ). Each starter was used for the manufacture of Cheddar cheese in parallel vats, with the SC method in one vat and the MC method in the second vat. Each starter was used for triplicate cheese-making trials. Each cheese-making trial consisted of 8 vats (4 starters $\times$ 2 methods), and each trial was completed over 4 d (one vat milled and one vat stirred with one culture type each day). The order of starter used was randomized. Each vat contained approximately 625 kg (605 L) of pasteurized (72°C $\times$ 16 s) whole milk. The milk supply was from the California Polytechnic State University dairy herd, and milk composition was consistent over the cheese-making period. The basic cheese-making protocols for the SC and MC methods are outlined in Figure 1. Chymax (Chr. Hansen, Milwaukee, WI) was used as a coagulant. After salting, the curds were hooped into 18.2-kg Wilson hoops and pressed (0.275 MPa) in a vacuum chamber overnight with the application of 24-mmHg vacuum for the first 60 min. After removal from the press, the cheeses were vacuum-packaged in Cryovac (Sealed Air Corp., Duncan, SC) bags. Three 18.2-kg blocks were obtained from each vat. Two blocks from each vat were ripened at 8°C for 9 mo, and one block was ripened at 2°C.

Compositional Analyses

Total solids in cheese were determined by the microwave oven method (CEM AVC 80 microwave oven, CEM Corporation, Matthew, NC; Marshall, 1992). Protein (total N $\times$ 6.38) was determined by the Kjeldahl method and salt by the titrimetric method with a Corning 926 chloride analyzer (Corning, Medfield, MA). Cheese pH was determined with a glass electrode on a slurry prepared by thoroughly blending 10 g of grated cheese with 10 mL of deionized water with a mortar and pestle. Cheeses were analyzed 7 d postmanufacture. All analyses were conducted in duplicate.

Microbiological Analysis

Starter bacteria were enumerated in cheese 1 d postmanufacture and in 90-d-old cheeses on LM-17 agar

(Difco Laboratories, Detroit, MI) after incubation for 3 d at 31°C. Starter bacteria were not enumerated on cheeses beyond 90 d of age because nonstarter lactic acid bacteria (**NSLAB**) also grow on LM-17 agar, making starter counts inaccurate (Cogan and Beresford, 2002). The NSLAB in the cheeses (1, 90, 180, and 270 d) were enumerated on Rogosa agar (Difco Laboratories) after incubation for 5 d at 31°C. Cheeses (10 g) were blended with warm 2% sodium citrate, followed by dilution in 0.1% peptone water before pour-plating in duplicate on the respective agars.

Proteolysis

The cheeses were sampled after 7, 90, and 270 d of ripening and frozen at -20°C until analyzed for proteolysis. A water-soluble N (**WSN**) extract of the cheeses was prepared according to the method of Kuchroo and Fox (1982), and the extract was analyzed for total N by the Kjeldahl method to assess primary proteolysis. Reversed-phase (**RP**) HPLC of the WSN extract of 90, 180, and 270-d-old cheeses was performed in duplicate by the method of Farkye et al. (1995) to determine the peptide profiles.

Sensory Analysis

Flavor attributes of 90, 180, and 270-d-old cheeses were evaluated by a trained 8-member descriptive sensory panel, using 17 terms for Cheddar flavor previously identified by Drake et al. (2001). Definitions and references for the Cheddar flavor sensory descriptors have been published previously (Drake et al., 2001). Cheeses (1.5 kg) cut from 18.2-kg blocks were shipped to North Carolina State University by overnight carrier on blue ice gel packs.

Each sensory panelist (6 females, 2 males; ages 22 to 47 yr) had more than 80 h of training on descriptive sensory analysis of Cheddar cheese. Panelists evaluated and scored the descriptors using a 10-point universal intensity scale consistent with the Spectrum descriptive analysis technique (Meilgaard et al., 1999; Drake and Civille, 2003). Prior to sensory analysis, the outer edges (~1 cm) of each block were carefully trimmed and discarded to minimize variability and flavor transfer from packaging. Cheeses were prepared by slicing into 4 $\times$ 2 $\times$ 2 cm cubes with a wire slicer within 2 h of evaluation. Cheeses were placed into 4-oz (113 g) soufflé cups with lids and tempered to 12°C prior to evaluation. Cheeses were evaluated under white light in a balanced block design by using 3-digit codes. Order of presentation was randomized among panelists. Panelists had access to water and unsalted crackers throughout the evaluation. Panelists evalu-

Figure 1. Flow diagram for Cheddar cheese manufacture by the stirred-curd and milled-curd methods.

ated 4 cheeses per session in 4 sessions, and each cheese was evaluated in duplicate.

Cheese Grading

After 30, 90, and 180 d of ripening, cheeses (0.5 kg) were coded with random numbers and mailed by overnight carrier on blue ice gel packs to 12 industry graders from 6 companies (4 culture suppliers and 2 cheese manufacturers). Cheeses were graded for overall quality, flavor, and texture according to standard

grading practices in which cheeses receive overall quality scores from 1 or 2 experienced individuals, based on the presence or absence of specific predefined defects (Bodyfelt et al., 1988; Delahunty and Drake, 2004).

Statistical Analysis

Analysis of variance with means separation was conducted to determine the effects of cheese manufacture, starter culture, and ripening temperature on composi-

Table 1. Composition of 7-d-old Cheddar cheese manufactured by the milled-curd or stirred-curd method using commercial starters (a, b, c, d) supplied by 4 different companies

Component	Method of manufacture	Culture ¹				SEM
		a	b	c	d	
Fat, ² %	Milled	32.50	32.00	32.75	32.58	0.113
	Stirred	31.25	31.83	30.16	30.16	0.293
Moisture, ³ %	Milled	35.30	36.74	36.15	35.76	0.216
	Stirred	35.45	36.52	36.29	34.52	0.321
Salt, ⁴ %	Milled	1.57 (4.4)	1.63 (4.4)	1.44 (4.0)	1.62 (4.5)	0.030
	Stirred	1.87 (5.2)	1.82 (5.0)	2.02 (5.5)	2.05 (5.9)	0.039
Protein, ⁵ %	Milled	24.68	24.22	24.33	24.16	0.082
	Stirred	26.34	25.78	25.85	26.05	0.089
pH ⁶	Milled	5.15	5.14	5.18	5.12	0.008
	Stirred	5.17	5.13	5.23	5.28	0.023

¹All values are means of data points of 3 trials analyzed in duplicate. Figures in parenthesis are salt-in-moisture of the cheese.

²*P*-values for method (0.001), culture (0.442).

³*P*-values for method (0.147), culture (0.001).

⁴*P*-values for method (0.001), culture (0.492).

⁵*P*-values for method (0.001), culture (0.484).

⁶*P*-values for method (0.001), culture (0.001).

tion and microbial counts (Minitab Inc., State College, PA) by using a GLM. Two-way ANOVA was used for composition, with method and culture as the factors. The bacterial data and WSN were analyzed by using a GLM at different ripening times, with method, ripening temperature, and culture as factors.

All 3 variables (method, culture, and ripening temperature) as well as ripening time were evaluated for their effects on descriptive sensory attributes. Main effects were evaluated (SAS version 8.2, SAS Institute Inc., Cary, NC). Interaction effects were noted whenever desired. The data on grading scores was checked by the usual diagnostics (normal data, constant standard deviations across groups), followed by a blocked 2-way multivariate ANOVA (MANOVA), and any violations in the assumptions of MANOVA were assessed by the Wilk, Lewly-Hotelling, Pillai, and Roy tests.

RESULTS AND DISCUSSION

Composition

The composition of cheese is given in Table 1. The method of manufacture (MC or SC) significantly influenced ($P < 0.001$) fat, protein, salt, and pH of the cheese, whereas moisture and pH were affected by culture type (Table 1). The fat content in SC cheese was lower than in MC cheese because additional pumping and stirring caused damage to the protective milk fat globule membrane, and hence leakage of fat into the whey. The fat loss contributed to a concomitant increase in protein content in the SC cheeses, which was higher ($P < 0.001$) than in the MC cheeses. The high salting pH used in SC cheeses could have

retarded rennet and starter activity, leading to comparatively lower protein levels in the MC cheeses (Farkye, 1995).

The SC Cheddar cheese contained a significantly higher ($P < 0.001$) salt content than the MC Cheddar because the former cheese had smaller and more granular curd particles, leading to a larger surface area for salt absorption. The differences in cheese pH caused by starter cultures may be due to the different rates of acid production and the salt tolerance of the different starters.

Microbiology of Cheeses

During Cheddar cheese manufacture, starter bacteria grow rapidly in the cheese milk and curd, reaching 10^8 to 10^9 cfu/g at salting. Following salting, the starter population decreases at a strain-dependent rate to approximately 1% of maximum numbers within 1 mo of ripening (McSweeney et al., 1994), probably because of the low curd pH and high salt concentration in the cheese.

In this study, the initial cell density of starter bacteria reached 10^8 to 10^9 cfu/g and was dependent on the starter type (Table 2). The SC cheeses contained lower initial cell densities compared with MC cheeses, possibly because of the higher salt content in the former (Table 1). Starter cell density also varied because of the starter type used for manufacture. Differences in starter strains supplied by the different culture companies may have accounted for the initial starter population in the cheeses. There was a sharp decline in starter population in all the cheeses between d 1 and

Table 2. Mean cell densities of starter bacteria in Cheddar cheese manufactured by the milled- or stirred-curd method using starters (a, b, c, d) supplied by 4 different starter companies and ripened at 2 or 8°C

Starter	Method of manufacture	Ripening temperature, °C	Starter count, ¹ cfu/g	
			1 d ²	90 d ³
a	Milled	2	4.40×10^8	2.40×10^8
		8	4.40×10^8	3.30×10^8
	Stirred	2	2.90×10^8	4.40×10^7
		8	2.90×10^8	5.10×10^7
b	Milled	2	1.10×10^9	9.30×10^8
		8	1.10×10^9	4.10×10^8
	Stirred	2	1.00×10^9	8.70×10^8
		8	1.00×10^9	2.00×10^8
c	Milled	2	1.80×10^9	1.90×10^8
		8	1.80×10^9	6.50×10^8
	Stirred	2	1.40×10^9	8.80×10^8
		8	1.40×10^9	2.50×10^8
d	Milled	2	4.30×10^9	2.50×10^8
		8	4.30×10^9	1.10×10^9
	Stirred	2	3.40×10^9	1.90×10^9
		8	3.40×10^9	9.60×10^8

¹All values are means of data points of 3 trials analyzed in duplicate.

²P-values for method (0.218), culture (0.001), temperature (1.0).

³P-values for method (0.094), culture (0.001), temperature (0.001).

90 postmanufacture. The decline in starter population was independent of the method of manufacture. However, the rate of starter death was greater in SC cheese than in MC cheese for 3 of the 4 starters, possibly because of the higher salt-in-moisture in the former cheeses; the salt-in-moisture in SC cheeses varied from 5.0 to 5.9% compared with 4.0 to 4.5% in MC cheeses.

All the cheeses had high numbers of NSLAB at 1 d postmanufacture (Table 3). The NSLAB cell densities caused by culture type were significantly different ($P < 0.001$), suggesting the presence of adjunct lactobacilli in the starter cultures. The culture companies confirmed having strains of *Lactobacillus casei* or *Lactobacillus helveticus* in the culture mixtures used in the study. Both *Lb. casei* and *Lb. helveticus* grow in Rogosa agar, which is used to enumerate NSLAB in Cheddar cheese. The starter type and method of manufacture contributed to differences in the cell densities of NSLAB up to the first 180 d of ripening (Table 3). The MC Cheddar cheese manufactured with starter a contained the highest numbers of NSLAB after 180 d of ripening. Other than differences in NSLAB count caused by starter type, differences in the rate of cell lysis could influence NSLAB counts. Thomas (1987) suggested the possibility that NSLAB utilize products of starter cell lysis for growth, and Lane and Fox (1997) attributed differences in cell densities of NSLAB during ripening of Cheddar cheese to differences in their rate of lysis. The influence of ripening temperature on

the NSLAB count was noted in cheese ripened for 270 d, although Shakeel-ur-Rehman et al. (2000) reported no difference in NSLAB counts in Cheddar cheese ripened at 2 or 8°C. The inclusion of adjunct starter and the high initial NSLAB count in this study could explain the differences between this study and the results of Shakeel-ur-Rehman et al. (2000).

Proteolysis

Starter type affected the WSN content of cheeses during the entire ripening period. The method of manufacture did not affect the WSN contents in the cheese during the first 90 d of ripening (Table 4). The influence of ripening temperature was not seen in cheese ripened for 7 d. After 270 d of ripening, cheese manufactured by the MC method using starter b contained the highest concentration of WSN (i.e., 46%). The high level of WSN may be due to proteolytic enzymes produced by the starter at the later stages of ripening. Production of such enzymes may have been suppressed by the higher salt content in cheeses made by the SC method. Cheeses made with culture a by either the MC or SC method contained the lowest level of WSN after 270 d at both ripening temperatures (2 or 8°C). Levels of WSN were higher in cheese ripened at 8°C at similar ages for all sampling intervals compared with those ripened at 2°C because of the effects of temperature on the activities of proteolytic enzymes in cheese. Residual chymosin and indigenous plasmin are responsible for the production of 90% of WSN during the ripening of Cheddar cheese (Shakeel-ur-Rehman et al., 1998), and the activities of these enzymes are different at 2 or 8°C.

Reversed-phase HPLC profiles of the water-soluble fractions of cheese showed differences in the peptide profiles attributable to the type of culture and method of manufacture (Figure 2A and 2B). Reversed-phase HPLC of water-soluble fractions of cheeses made by the SC method (Figure 2A) showed similar peptide profiles for cultures c and d and were different from profiles for cultures a and d, especially in the region for peptides eluting at approximately 14 to 18 min. The RP-HPLC profiles of WSN of cheeses made by the MC method (Figure 2B) were identical to cheeses made with the same cultures but using the SC method. These results suggest that culture did influence the RP-HPLC peptide profiles of cheeses. High-performance liquid chromatography is a useful technique for studying the peptidase activities of starters (Tan et al., 1993). In addition, different starters have been shown to influence the RP-HPLC profiles of the WSN fraction of model Cheddar cheeses (Farkye et al., 1995; Shakeel-ur-Rehman et al., 1998). The individual RP-

Table 3. Numbers of nonstarter lactic acid bacteria (NSLAB) in 1-, 90-, 180-, and 270-d-old Cheddar cheese manufactured by the milled- or stirred-curd method using starters (a, b, c, d) supplied by 4 different companies and ripened at 2 or 8°C

Starter	Method of manufacture	Ripening temperature, °C	NSLAB count, ¹ cfu/g			
			1 d ²	90 d ³	180 d ⁴	270 d ⁵
a	Milled	2	2.80×10^7	1.40×10^8	1.50×10^8	8.30×10^6
		8	2.79×10^7	1.90×10^8	1.46×10^8	5.40×10^6
	Stirred	2	2.70×10^7	5.20×10^7	2.90×10^7	5.44×10^6
		8	2.68×10^7	2.58×10^7	3.74×10^7	5.30×10^6
b	Milled	2	8.20×10^6	5.00×10^6	3.60×10^6	1.3×10^6
		8	8.20×10^6	5.60×10^6	1.36×10^7	1.40×10^7
	Stirred	2	7.30×10^6	2.40×10^6	2.50×10^6	1.80×10^6
		8	7.30×10^6	8.30×10^6	5.11×10^6	5.10×10^6
c	Milled	2	1.80×10^7	3.50×10^7	1.50×10^7	7.90×10^6
		8	1.84×10^7	3.70×10^7	2.76×10^7	1.00×10^7
	Stirred	2	2.79×10^7	1.80×10^7	1.30×10^7	5.40×10^6
		8	2.26×10^7	2.49×10^7	1.52×10^7	4.00×10^6
d	Milled	2	2.30×10^7	1.80×10^7	3.80×10^7	2.10×10^7
		8	5.50×10^7	6.70×10^7	4.71×10^7	3.21×10^7
	Stirred	2	3.00×10^7	5.10×10^7	1.90×10^7	1.10×10^7
		8	3.05×10^7	5.11×10^7	4.09×10^7	1.10×10^7

¹All values are means of data points of 3 trials analyzed in duplicate.²*P*-values for method (0.089), culture (0.001), temperature (1.0).³*P*-values for method (0.001), culture (0.001), temperature (0.610).⁴*P*-values for method (0.002), culture (0.001), temperature (0.253).⁵*P*-values for method (0.001), culture (0.001), temperature (0.001).

HPLC peptide profiles of cultures did show differences when cheeses were made by the SC or MC method. The method of manufacture influenced the salt concentration in the cheeses (Table 1) and possibly starter

death (Table 2), which explains the differences in RP-HPLC peptide profiles caused by the method of manufacture.

Sensory Analysis

Cheese age, method of manufacture, and starter and ripening temperatures all influenced specific flavor attributes (Tables 5, 6, 7, and 8) of the cheeses ($P < 0.05$). Whey and milk fat flavors and salty taste decreased with age, whereas sulfur and brothy flavors and sourness, sweetness, and bitterness tastes increased with age (Table 5). Because cheese age influenced the majority of the sensory attributes that were studied, it can be concluded that duration of ripening is the most important factor in the development of cheese flavor made by either the SC or MC method, regardless of the starter type or ripening temperature.

The method of manufacture influenced only diacetyl flavor and sour and salty tastes ($P < 0.05$; Table 6). Diacetyl flavor and salty taste were higher in the SC cheese, whereas sour taste was significantly higher in the MC cheese. The salt level in SC cheeses was higher than in MC cheeses (Table 1), consistent with the higher saltiness perceived by sensory panelists. The lower sourness perceived in SC Cheddar compared with MC Cheddar may be due to lower acidity in the former cheeses as a result of higher salt levels or simply that the sour taste perception was ameliorated in the SC cheeses by the higher salty taste intensities.

Table 4. Concentration of water-soluble N (WSN; as % of total N) in 7-, 90-, and 270-d-old Cheddar cheese manufactured by the milled- or stirred-curd method using cultures (a, b, c, and d) supplied by 4 different companies and ripened at 2 or 8°C

Starter	Method of manufacture	Ripening temperature, °C	WSN, ¹ as % of total N		
			7 d ²	90 d ³	270 d ⁴
a	Milled	2	8.0	21.8	26.5
		8	7.0	21.8	25.9
	Stirred	2	13.8	17.2	20.7
		8	13.6	25.9	26.3
b	Milled	2	8.8	21.1	38.5
		8	11.1	22.8	46.7
	Stirred	2	8.5	21.1	30.8
		8	9.1	21.0	32.4
c	Milled	2	7.3	19.0	29.4
		8	8.1	22.1	30.2
	Stirred	2	6.9	16.4	26.5
		8	6.8	21.1	30.1
d	Milled	2	9.7	17.1	28.7
		8	9.5	20.6	31.6
	Stirred	2	7.5	18.1	25.3
		8	8.3	18.8	26.9

¹All values are means of data points of 3 trials analyzed in duplicate.²*P*-values for method (0.143), culture (0.001), temperature (0.410).³*P*-values for method (0.150), culture (0.001), temperature (0.001).⁴*P*-values for method (0.001), culture (0.001), temperature (0.001).

Figure 2. A) Reversed-phase HPLC patterns of the water-soluble nitrogen fraction of 270-d-old Cheddar cheese manufactured by the stirred-curd method using different starters (a, b, c, d) and ripened at 8°C. B) Reversed-phase HPLC patterns of the water-soluble nitrogen fraction of 270-d-old Cheddar cheese manufactured by the milled-curd method using starters (a, b, c, d) and ripened at 8°C.

The flavor attributes that were significantly influenced by starter type are shown in Table 7. Cheese made with starter b had higher sulfur flavor notes (a good characteristic of sharp Cheddar) and also contained higher levels of WSN (Table 4) after 270 d of ripening, suggesting that starter b may contain en-

zymes and may utilize metabolic pathways to release sulfur compounds in the cheese. Cheddar cheese made with starter c had the highest scores for cooked, whey, and umami flavors and for bitter taste, independent of the method of manufacture. Starter c cheeses had the highest NSLAB cell density (Table 3) during ripen-

Table 5. Effect of ripening time on descriptive flavor attributes of cheese

Descriptor	Ripening time, d		
	90	180	270
Cooked	2.2 ^a	2.0 ^{ab}	1.7 ^b
Whey	3.5 ^a	2.4 ^b	1.8 ^c
Diacetyl	1.4 ^a	1.2 ^a	0.7 ^a
Milk fat	2.0 ^a	2.0 ^a	1.7 ^a
Sulfur	0.3 ^c	1.0 ^b	1.2 ^a
Brothy	1.0 ^b	1.0 ^b	2.0 ^a
Sweet	1.5 ^b	1.5 ^b	2.0 ^a
Sour	3.0 ^a	3.2 ^a	3.2 ^a
Salty	4.0 ^a	4.0 ^a	3.5 ^b
Bitter	0.4 ^b	0.6 ^b	1.0 ^a
Umami	0.5 ^a	0.5 ^a	0.7 ^a

^{a-c}Means in a row followed by different superscript letters are different ($P < 0.05$).

ing, suggesting a possible role of NSLAB in these flavors and tastes. Ripening temperature (2 or 8°C) influenced only brothiness (Table 8), which was higher in cheeses ripened at 2°C, probably because of the lower activities of cheese enzymes at 2°C than at 8°C.

Cheese Grading

Statistical analysis of the flavor scores assigned to cheeses by the 12 industry cheese graders did not provide any evidence for a difference caused by the method of manufacture ($P = 0.631$), the starter type ($P = 0.073$), or a method $\times$ starter interaction ($P = 0.242$; results not shown). The data were further analyzed by the Wilk, Lawley-Hotelling, Pillai, and Roy statistical tests to verify any violations of MANOVA assumptions. The data were also checked by the usual diagnostics (normal data, constant standard deviations across groups) and passed the diagnostic screening. Grading is an extremely useful tool to rapidly assess overall cheese quality. However, the technique is not well standardized and can vary significantly from individual to individual. Further, cheeses can receive the same grades and yet vary widely in specific flavor attributes and intensities (Singh et al., 2003; Delahunty and Drake,

Table 6. Effect of method of manufacture (stirred vs. milled) on descriptive flavor attributes of cheeses¹

Descriptor	Stirred	Milled
Diacetyl	1.4 ^a	1.0 ^b
Sour	3.0 ^a	3.3 ^b
Salty	4.0 ^a	3.6 ^b

^{a,b}Means in a row followed by different superscript letters are different ($P < 0.05$).

¹Only attributes for which there was a significant method effect ($P < 0.05$) are given.

Table 7. Effect of starter type (a, b, c, d) on descriptive flavor attributes of cheeses

Descriptor	a	b	c	d
Cooked	2.2 ^a	1.9 ^b	1.9 ^b	1.9 ^b
Whey	2.0 ^b	2.2 ^b	2.4 ^a	2.5 ^a
Sulfur	0.85 ^b	1.2 ^a	0.85 ^b	0.7 ^b
Brothy	1.4 ^a	1.4 ^a	0.8 ^b	1.0 ^b
Milk fat	1.0 ^a	1.0 ^a	0.5 ^b	0.5 ^b
Umami	1.5 ^b	1.5 ^b	2.0 ^a	0.5 ^b
Bitter	0.0 ^a	0.5 ^b	0.75 ^a	0.75 ^a

^{a,b}Means in a row followed by different letters are different ($P < 0.05$).

2004; Drake, 2004), resulting in a technique that, although applicable to certain industry situations, is not optimal for research and specific communication.

CONCLUSIONS

The starter type used for Cheddar cheese manufacture affected cell densities of both starter and NSLAB in cheese during ripening. Salt, protein, and fat contents of the cheeses were dependent on the method of manufacture. Starter type, method of manufacture, and ripening temperature influenced cheese proteolysis. Diacetyl flavor and salty and sour tastes in cheese were influenced by the method of manufacture. Starter type influenced the cooked, whey, sulfur, brothy, milk fat, and umami flavors and the bitterness taste in cheese, suggesting the importance of starter selection to achieve the desirable flavor attributes in MC or SC Cheddar cheeses. The study shows that the starter type used for manufacture is more important than the method of manufacture in the flavor development of Cheddar cheese.

ACKNOWLEDGMENTS

Thanks to Booris Yim and Therese Considine for their assistance in cheese making, Matt Arnold for assistance with HPLC analysis, and Andrew Schaffner for statistical analyses. The financial support of the California Dairy Research Foundation (Davis, CA) is greatly appreciated.

Table 8. Effect of storage temperature on sensory attributes of cheeses¹

Descriptor	2°C	8°C
Brothy	1.5 ^a	1.0 ^b

^{a,b}Means in a row followed by different letters are different ($P < 0.05$).

¹Only attributes for which there was a storage temperature effect ($P < 0.05$) are given.

REFERENCES

- Bodyfelt, F. W., J. Tobias, and G. M. Trout. 1988. The Sensory Evaluation of Dairy Products. Van Nostrand Reinhold, London, UK.
- Cogan, T. M., and T. P. Beresford. 2002. Microbiology of hard cheese. Pages 515–560 in *Dairy Microbiology Handbook: The Microbiology of Milk and Milk Products*. R. K. Robinson, ed. John Wiley and Sons Inc., New York, NY.
- Delahunty, C. M., and M. A. Drake. 2004. Sensory character of cheese and its evaluation. Pages 455–487 in *Cheese: Chemistry, Physics, and Microbiology*. 3rd ed. Vol. 1: General Aspects. P. F. Fox, P. L. H. McSweeney, T. M. Cogan, and T. P. Guinee, ed. Elsevier, London, UK.
- Drake, M. A. 2004. Defining dairy flavors. *J. Dairy Sci.* 87:777–784.
- Drake, M. A., and G. V. Cville. 2003. Flavor lexicons. *Compr. Rev. Food Sci. Food Saf.* 1:33–38.
- Drake, M. A., S. C. McIngvale, K. R. Cadwallader, and G. V. Cville. 2001. Development of a descriptive sensory language for Cheddar cheese. *J. Food Sci.* 66:1422–1427.
- Farkye, N. Y. 1995. Contribution of milk clotting enzymes and plasmin to cheese ripening. Pages 195–207 in *Chemistry of Structure-Function Relationships in Cheese*. E. L. Malin and M. H. Tunick, ed. Plenum Press, New York, NY.
- Farkye, N. Y., S. A. Madkor, and G. H. Atkins. 1995. Proteolytic ability of some lactic acid bacteria in a model cheese system. *Int. Dairy J.* 5:715–725.
- Kuchroo, C. N., and P. F. Fox. 1982. Soluble nitrogen in Cheddar cheese: Comparison of extraction procedures. *Milchwissenschaft* 37:331–335.
- Lane, C. N., and P. F. Fox. 1997. Role of starter enzymes during ripening of Cheddar cheese made from pasteurized milk under controlled microbiological conditions. *Int. Dairy J.* 7:53–63.
- Law, J., and A. Haandrikman. 1997. Proteolytic enzymes of lactic acid bacteria. *Int. Dairy J.* 7:1–11.
- Marshall, T. R., ed. 1992. *Standard Methods for the Examination of Dairy Products*. 15th ed. Am. Publ. Health Assoc. Inc., Washington, DC.
- McSweeney, P. L. H., E. M. Walsh, P. F. Fox, T. M. Cogan, F. D. Drinan, and M. Castelo-Gonzalez. 1994. A procedure for the manufacture of Cheddar cheese under controlled bacteriological conditions and the effect of adjunct lactobacilli on cheese quality. *Irish J. Agric. Food Res.* 33:183–192.
- Meilgaard, M., G. V. Cville, and B. T. Carr. 1999. Descriptive analysis techniques. Pages 161–170 in *Sensory Evaluation Techniques*. 3rd ed. CRC Press, New York, NY.
- Shakeel-Ur-Rehman, E. P. Feeney, P. L. H. McSweeney, and P. F. Fox. 1998. Inhibition of coagulant in cheese using pepstatin. *Int. Dairy J.* 8:987–992.
- Shakeel-ur-Rehman, P. L. H. McSweeney, J. M. Banks, and P. F. Fox. 2000. Effect of ripening temperature on the growth and significance of non-starter lactic acid bacteria in Cheddar cheese made from raw or pasteurized milk. *Int. Dairy J.* 10:33–44.
- Singh, T., M. A. Drake, and K. R. Cadwallader. 2003. Flavor of Cheddar cheese: A chemical and sensory perspective. *Compr. Rev. Food Sci. Food Saf.* 2:139–162.
- Tan, P. S. T., B. Pooman, and W. N. Konings. 1993. Proteolytic enzymes of *Lactococcus lactis*. *J. Dairy Res.* 60:269–286.
- Thomas, T. D. 1987. Acetate production from lactate and citrate by non-starter bacteria in Cheddar cheese. *N. Z. J. Dairy Sci. Technol.* 22:25–38.