Production of Ingredient-Type Cheddar Cheese with Accelerated Flavor Development by Addition of Enzyme-Modified Cheese Powder

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ABSTRACT

Fast-ripened Cheddar cheeses for ingredient purposes were produced by addition of a dried enzyme-modified cheese (EMC; 0.25 and 1 g/100 g of milled curd) at the salting stage during a standard Cheddar cheese-making procedure. Populations of starter and nonstarter lactic acid bacteria (NSLAB), levels of proteolysis and lipolysis, volatile analysis, and flavor development (by quantitative descriptive sensory analysis) were monitored over a 6-mo ripening period. Levels of free AA and free fatty acids were elevated in the experimental cheeses on d 1 because of inclusion of the EMC. Counts of NSLAB were also elevated in the experimental cheeses compared with the control cheese from the start of ripening. Levels of free AA were slightly elevated in the experimental cheeses at 1, 2, and 4 mo, but significantly greater accumulations were detected by 6 mo of ripening, with His, Leu, and glutamate reflecting the greatest increases. Levels of long-chain free fatty acids increased up to 2 mo, indicating an initial stimulation of lipolysis, but had decreased by 6 mo, indicating greater catabolism, probably caused by NSLAB and increased starter lysis. Principal component analysis of the volatile compounds showed few differences in the aroma profiles among the cheeses up to 4 mo of ripening, but a large separation of the cheeses supplemented with EMC relative to the control was observed by 6 mo. Sensory analysis of the cheeses with added EMC showed an acceleration of 2 mo in flavor development compared with the control cheese with the addition of 1 g/100 g of EMC developing a flavor profile at 4 mo similar to the control cheese at 6 mo of ripening. However, atypical Cheddar flavors developed on prolonged storage. This study shows the potential of adding EMC during Cheddar production to produce a fast-ripened ingredient-type Cheddar cheese.

Key words: enzyme-modified cheese, proteolysis, lipolysis, flavor acceleration

INTRODUCTION

Growth in the consumption of convenience foods has been reflected in increased demand for cheese with distinctive characteristics, as well as in the use of cheese as a food ingredient. A lengthy ripening period is required for flavor formation in Cheddar cheese (e.g., up to 2 yr for extra-mature Cheddar), accounting for a considerable part of the overall cost to the cheese manufacturer. Therefore, any reduction of ripening time without adversely affecting quality is of major economic interest. Methods to reduce the ripening time of cheese using various strategies, all offering varying degrees of success, have been reviewed by Wilkinson (1993) and Law (2001). Much of the time required to form a matured, flavored cheese relates to the interaction among key enzymes and their substrates. This study deals with the enhancement of flavor-generating reactions by provision of a concentrated source of substrates [in the form of an enzyme-modified cheese (EMC) powder] during manufacture to provide a novel means of accelerating cheese ripening and flavor development.

In an attempt to accelerate Cheddar cheese ripening, Dulley (1976) added enzymatically active Cheddar cheese slurries to cheese curd at salting. These cheese slurries (consisting of 2 parts fresh salted curd with 1 part solution of 5% NaCl or 0.3% potassium sorbate, mixed and stored at 30°C for 1 wk) were used as a source of enzymes, microorganisms, and flavor precursors to aid in flavor development. The addition of cheese slurry either to the cheese milk or to the salted Cheddar curd (6 g of slurry to 100 g of salted curd) before hooping resulted in higher counts of lactobacilli, a 2% increase in moisture, and a significant advancement in flavor and body development at 3 mo of ripening. Addition of cheese slurry to Swiss cheese (Singh and Kristoffersen, 1971), Ras cheese (Abdel Baky et al., 1982), and Blue cheese (Rabie, 1989) has also been shown to accelerate ripening.

Enzyme-modified cheeses are technologically advanced forms of cheese slurry produced enzymatically from cheese, immature cheese curd, or other dairy substrates as a cost-effective cheese flavor ingredient (Kil-

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cawley et al., 1998). Enzyme-modified cheese technology typically involves simultaneous hydrolysis of protein and fat. The resulting pastes or powders contain high concentrations of free AA (FAA), peptides, FFA, and other catabolic products typically associated with cheese flavor. However, they do not contain residual enzyme activity because they are heat-treated prior to use.

The objective of this study was to accelerate Cheddar cheese ripening by addition of a potentially rate-limiting biochemical substrate (in the form of EMC) during the manufacture of Cheddar cheese. The study involved production of an EMC powder with concentrated levels of key flavor compounds and flavor precursors, and the development of a process to enable its uniform incorporation into the cheese curd and to ensure a balanced but rapid increase in flavor development during the maturation process. The resulting cheeses would be appropriate as table cheeses but are more specifically for use as ingredient-type cheeses suitable for secondary processing.

MATERIALS AND METHODS

Preparation of Spray-Dried EMC

Rennet cheese curd was produced from full-fat milk without the addition of lactic acid starter culture. Rennet cheese curd was chosen as the main substrate because any subsequent flavor development during EMC production could be directly attributed to the enzymes used during the process rather than to any indigenous enzymes from the starter culture(s) present in the cheese curd. This had an additional benefit of greater reproducibility because the curd substrate has less biochemical variability. Rennet (Chymax-Plus; Chr. Hansen Ireland Ltd., Little Island, Co. Cork, Ireland) was added to the milk at a level typical for Cheddar cheese (81 mL in 450 mL of water per 450 kg of full-fat milk) at a temperature of 31.7°C. The milk was agitated for 3 min and left to coagulate. Once the coagulum was formed, it was cut and left to heal for 5 min. It was then heated to a final cooking temperature of 38°C, which was achieved over a 40-min period, with agitation. After 20 min, the whey was drained and the curd went through the normal cheddaring process, followed by milling, salting (2.7 g/100 g), and pressing overnight. Cheeses were then vacuum-packed and stored frozen (-20°C) until required. The curd slurry base used to generate EMC was produced as follows: 39.43 kg of milled rennet curd was added to 25.23 kg of water, 8.865 kg of anhydrous butterfat, 630 g of disodium phosphate, 315 g of trisodium phosphate, 381 g of trisodium citrate, and 147 g of salt (NaCl). These ingredients were mixed in a Stephan universal blender (Stephan und Söhne

GmbH & Co., Hameln, Germany), heated to 80°C, and held for 40 min at a continuous shear rate of 1,500 rpm. One-third of this mixture was removed and homogenized in a 2-stage homogenizer (APV Gaulin Lab 60, Unna, Germany) at 10/2 MPa, then spray-dried using an Anhydro dryer (model Lab 3; Anhydro, Copenhagen, Denmark; drying capacity of 10 kg/h), with an air inlet temperature of 184°C and an air outlet temperature of 92°C to yield a powder of >>2 to 3% moisture. Under these conditions, the final dried product had a slightly tacky nature, which aided its incorporation into the milled cheese curd during salting. This powder was designated "rennet powder" and was used in the control cheeses. The remaining two-thirds of the slurry was transferred to a slow gate agitator tank at >>50°C and used to make the EMC powder. The enzymes and dose rates detailed below were chosen on the basis of previous studies investigating their activities (Kilcawley et al., 2002a,b) and preliminary work on EMC production (Kilcawley, 2002). Once the temperature of the slurry had reached 45°C, enzymes were added in the following concentrations: Debitrase DBP20 (Danisco, Copenhagen, Denmark) 25 g, Glutaminase F (Amano Enzyme Europe, Chipping Norton, UK) 200 g, Lipomod 338 (Biocatalysis, Ltd., Cardiff, UK) 50 g. This mixture was incubated at 45°C with constant agitation. After 6 h, 10 g of Lipomod 229 (Biocatalysis, Ltd.) was added and incubation continued for a further 26 h. An extra 4 kg of water was added to the tank, and the enzymes were inactivated by heating the mixture to 80°C for 40 min. This mixture was homogenized and spray-dried as described above to yield powders of 2 to 3% moisture. This powder was designated "EMC powder" and was added to the experimental cheeses. The powders were placed in sealed sterile bags and stored at ambient temperature until required.

Cheese Manufacture and Cheese-Making Strains

The strains used were *Lactococcus lactis* ssp. *lactis* 303 and *Lactococcus lactis* ssp. *cremoris* 227 (Chr. Hansen Ireland Ltd.) grown overnight at 21°C in heat-treated (95°C for 30 min), reconstituted low-heat skim milk powder (10 g/100 mL). Three separate cheese trials were performed from different milks. Cheddar cheese was made using a standard cheese-making procedure (Wilkinson et al., 1994) until salting. The milled curd was divided into 3 equal parts. Rennet powder and EMC powder were blended with the salt (2.7 g/100 g) and added to the milled curd. The control cheese (C) contained 1 g/100 g of rennet powder. Experimental cheese A contained 0.75 g/100 g of rennet powder and 0.25 g/100 g of EMC powder, and experimental cheese B contained 1 g/100 g of EMC powder. The rennet pow-

der was added to account for compositional variations caused by the addition of EMC. The cheeses were pressed, vacuum-packed, and ripened at 8°C for 6 mo. All cheeses were sampled at 1 d, at 2 wk, and at 1, 2, 3, 4, 5, and 6 mo of ripening for the various analyses.

Microbiological Analysis

The microbiological analysis of cheese during ripening was carried out in duplicate at each sampling time. Starter lactococci were enumerated on LM17 agar (Terzaghi and Sandine, 1975) after incubation for 3 d at 30°C. Nonstarter lactic acid bacteria (NSLAB) were enumerated on *Lactobacillus* selective agar (Rogosa et al., 1951) incubated aerobically with an overlay for 5 d at 30°C.

Compositional Analysis

Samples of EMC powder and Cheddar cheese were taken at 1 and 14 d, respectively, for duplicate determinations of protein (IDF, 1964), fat (IIRS, 1955), moisture (IDF, 1982), and salt (Fox, 1963). The pH of grated cheese (10 g) macerated in 10 mL of distilled water was measured.

Assessment of Proteolysis

Proteolysis was monitored throughout ripening by duplicate determinations of the levels of nitrogen soluble in water at pH 4.6 (**pH 4.6-SN**) using the method of Fenelon et al. (2000), and the results are expressed as the percentage of total N. Nitrogen soluble in phosphotungstic acid (5%; **PTA-N**) was measured as described by Jarrett et al. (1982), with results expressed as the percentage of total N. Individual FAA were determined on the pH 4.6-SN extracts as described by Fenelon et al. (2000), with results expressed as milligrams per gram of cheese. The FAA content of the EMC powder and molecular mass distribution of the soluble peptides by gel permeation of the EMC powder were determined on the pH 4.6-SN fractions as described by Fenelon et al. (2000). Results are expressed as the percentage of peptide material in various molecular mass ranges (Wilkinson et al., 1992).

Individual FFA Analysis

Individual FFA were extracted from the cheese and quantified according to the method of De Jong and Badings (1990) with modifications as described in Hannon et al. (2006). All samples were analyzed in triplicate at 1, 2, 4, and 6 mo of ripening.

Dynamic Headspace Analysis

The headspace volatile compounds of the cheeses were isolated by a dynamic headspace analyzer (Tekmar 3000 concentrator; JVA Analytical Ltd., Dublin, Ireland) as described in Hannon et al. (2006). Analyses were repeated in triplicate on freshly prepared homogenate for all samples for all trials at 1, 2, 4, and 6 mo of ripening.

Descriptive Sensory Analysis

Descriptive sensory analysis was carried out as described in Hannon et al. (2003, 2005) at 1, 2, 4, and 6 mo. A final vocabulary of 9 odor and 19 flavor terms was used to describe the cheeses. Descriptive sensory assessment of the cheeses (in duplicate) was performed in individual booths under controlled red lighting at the Sensory Laboratory, University College, Cork, which conforms to international standards. Panelists had free access to deionized water and unsalted crackers to aid in palate cleansing between samples. Twelve sessions took place, with 6 cheeses tasted each time during a 6-d period. Cheeses were randomly assigned to days in a balanced block design, whereas order of tasting within each day was balanced to account for first-order and carryover effects (MacFie et al., 1989). Sensory attributes were scored on unstructured 100-mm line scales labeled at both ends (at 5 and 95%) with the extremes of each descriptive term. Data were recorded and stored using Compusense Five-Release 3.0, v 3.8 (Compusense, Guelph, Ontario, Canada).

Statistical Analysis

A randomized complete block design that incorporated the 3 treatments and 3 blocks (replicate trials) was used for analysis of the response variables relating to the compositions of the cheeses. Analysis of variance of the randomized complete block design was carried out using the GLM procedure of SAS (SAS Institute, 1995), in which the effect of treatment and replicates were estimated for all response variables. Duncan's multiple comparisons test was used as a guide for paired comparisons of the treatment means. Differences among treatments that are described subsequently as being significant were determined at $P < 0.05$.

The experimental design for the response variables pH 4.6-SN, PTA-N, concentration of total FAA, and concentration of total FFA was a split plot with 3 replicates. The main plot factor was treatment (i.e., level of EMC addition) and the subplot factor was ripening time. Analysis of variance for the split-plot design was carried out using the GLM procedure of SAS (SAS Insti-

tute, 1995). Statistically significant differences ($P < 0.05$) between different treatment levels were determined by Fisher's least significant differences test.

Analysis of variance was used to test the ability of 1) the descriptive vocabulary attributes, 2) the levels of individual FAA, and 3) the levels of individual FFA to discriminate among cheeses, using SPSS version 11.0 for Windows XP (SPSS Inc., Chicago, IL). A statistical model was constructed and fitted with terms to account for variations attributable to treatment (EMC addition), age (ripening time), and the interaction of treatment and age. Duncan's multiple comparisons test was used for paired comparisons of the treatment means. Differences among treatments that are described subsequently as being significant were determined at $P < 0.05$.

All attribute scores and volatile compounds were subsequently averaged across replicates, standardized (1/SD of the mean score for each attribute), and analyzed using principal component analysis (PCA; Piggott and Sharman, 1986). Analysis of variance was used to investigate how each principal component (PC) discriminated among the characters of the cheeses. Principal component analysis was carried out using Unscrambler v 6.1 (CAMO AS, Trondheim, Norway) and ANOVA and hierarchical cluster analysis using SPSS v 8.0 (SPSS Inc.).

RESULTS AND DISCUSSION

Composition and Proteolysis of EMC

The EMC powder had a low moisture content (2.23%), a high fat content (56.1%), and a high salt-in-moisture content (S/M; 13.45%). The composition of the rennet powder was similar (data not shown). The molecular weight distributions of peptides in the pH 4.6-SN extracts of the EMC powder are as follows: >20 kDa: 0.21%; 10–20 kDa: 0.48%; 3–10 kDa: 6.31%; 2–3 kDa: 7.31%; 1–2 kDa: 16.12%; 0.5–1 kDa: 15.64%; <0.5 kDa: 53.73%. Extensive proteolysis was evident from the amount of peptide material with a molecular weight of <0.5 kDa (53.73%) and can be primarily attributed to the enzymes Glutaminase F and Lipomod 229, which contain substantial amounts of proteinase side activities (Kilcawley, 2002; Kilcawley et al., 2002a,b). The predominant FAA in the EMC powder were Leu (4.9 mg/g), Val (2.5 mg/g), Phe (2.0 mg/g), Lys (1.8 mg/g), and Glu (1.7 mg/g) and are a result of the high levels of general aminopeptidase and glutaminase activities present in the Debritrax DBP20 and Glutaminase F, respectively (Kilcawley et al., 2002a,b).

Cheese Composition

The compositions of the cheeses (Table 1) were within the ranges acceptable for Cheddar cheese. Rennet powder was added to the control cheese and cheese A in an effort to eliminate compositional differences among the cheeses. No significant differences ($P > 0.05$) were found among the cheeses for salt, moisture, salt-in-moisture, pH, and protein values because of the inclusion of the EMC or rennet powder. However, addition of EMC powder resulted in higher fat levels ($P < 0.05$) in the experimental cheeses (28.31 and 28.41%) compared with the control (27.80%). Total FAA and FFA were elevated in the experimental cheeses compared with the control cheese at d 1, with levels increasing with higher addition of EMC powder in comparison with rennet powder (Table 2). The increase in these compounds at d 1 also represents additional microbial growth substrates as well as substrates for flavor-forming reactions. Higher fat levels in cheese may increase the fat–water–protein interface for flavor-forming reactions to occur, as well as acting as a possible reservoir for fat-soluble compounds (Manning, 1974; Olson and Johnson, 1990; Lawrence et al., 1993).

Cheese Microbiology

The mean populations of starter (from 3 separate trials) decreased from $>2 \times 10^9$ cfu/g of cheese at 1 d to $>2 \times 10^7$ cfu/g of cheese after 6 mo of ripening in the control and experimental cheeses. Similar trends for starter populations have been reported by others (Jordan and Cogan, 1993; Lane and Fox, 1996; Hannon et al., 2003, 2005). Previous studies have shown that the starter culture *Lactobacillus lactis* ssp. *lactis* 303 does not lyse during cheese ripening, with viability remaining constant (O'Donovan et al., 1996). However, other studies have shown that *Lactobacillus lactis* ssp. *cremoris* 227 (previously known as G11) releases intracellular enzymes during the early stages of ripening, with a concomitant decrease in viability (Wilkinson et al., 1994).

Nonstarter lactic acid bacteria increased from $>10^2$ cfu/g of cheese at 1 d to $>10^7$ cfu/g of cheese in all cheeses after 6 mo of ripening. However, the populations detected were 2.5 and 3.3 times greater in cheeses A and B, respectively, compared with the control cheese on d 1 and remained higher throughout ripening, especially from 4 to 6 mo. The higher levels of NSLAB represent a large increase in the enzyme content within the cheese matrix, especially after 2 mo of ripening, which may have contributed to flavor development in the experimental cheeses. This increase in NSLAB is attributed to the addition of EMC powder, which may provide growth substrates, because the control cheese con-

Table 1. Composition (%) of 14-d-old Cheddar cheeses made with added rennet and enzyme-modified cheese (EMC) powder¹

Cheese	Salt	Moisture	S/M	Fat	Protein	pH
Control	1.71 ^a	39.04 ^a	4.40 ^a	27.80 ^a	26.22 ^a	5.03 ^a
A	1.67 ^a	38.78 ^a	4.33 ^a	28.31 ^b	26.05 ^a	5.04 ^a
B	1.69 ^a	38.77 ^a	4.30 ^a	28.41 ^b	26.01 ^a	5.03 ^a
SED	0.02	0.32	0.27	0.15	0.23	0.04

^{a,b}Values within a column with different superscript letters differ ($P < 0.05$).

¹A = 0.25% EMC; B = 1.0% EMC. S/M = salt in moisture; SED = standard error of the difference of cheese data; degrees of freedom = 4.

tained rennet powder but this was not hydrolyzed by the peptidase or lipase enzymes. The increase could also be due to the higher fat content in the experimental cheeses; Fenelon et al. (2000) reported greater NSLAB populations in cheeses with a higher fat content.

Assessment of Proteolysis

The concentration of N soluble at pH 4.6 increased significantly in all cheeses during maturation (Tables 2, 3). Significant differences were observed for addition of EMC powder ($P < 0.0005$), ripening time ($P < 0.0001$), and their interaction ($P < 0.008$). At all ripening times, the differences in pH 4.6-SN among the cheeses were numerically very small; however, statistical analysis found these differences to be significant. At 1 d, the

levels of pH 4.6-SN expressed as a percentage of total nitrogen were higher in the experimental cheeses (3.4 and 3.9%) compared with the control cheese (2.9%). This increase was most likely a direct result of the addition of hydrolyzed casein in the EMC powder. As maturation progressed up to 4 mo, levels of pH 4.6-SN remained higher in the experimental cheeses, although the rate of accumulation was similar in all cheeses. From 4 to 6 mo of ripening, the rates of accumulation were higher in the experimental cheeses. The concentration of N soluble at pH 4.6 is primarily due to the coagulant, and because the retained or residual activity of chymosin should be similar in all cheeses, large differences in proteolysis at this level were not expected.

Nitrogen soluble in phosphotungstic acid is a measure of di-, tri-, and tetra-peptides and FAA. Similar to

Table 2. Analysis of indices of ripening in Cheddar cheese made with the addition of rennet and enzyme-modified cheese (EMC) powder¹

Component	Ripening time	Cheese					
		C	(SD)	A	(SD)	B	(SD)
pH 4.6-SN (% TN)	1 d	2.9 ^a	(0.14)	3.4 ^{ab}	(0.11)	3.9 ^b	(0.20)
	1 mo	8.9 ^a	(0.51)	9.3 ^{ab}	(0.55)	9.8 ^b	(0.26)
	2 mo	12.0 ^a	(0.72)	12.3 ^{ab}	(0.44)	12.8 ^b	(0.26)
	4 mo	17.3 ^a	(0.47)	17.9 ^b	(0.36)	18.3 ^b	(0.40)
	6 mo	22.2 ^a	(0.50)	23.0 ^b	(0.25)	24.6 ^c	(0.32)
PTA-N (% TN)	1 d	0.55 ^a	(0.01)	0.66 ^{ab}	(0.02)	0.76 ^b	(0.02)
	1 mo	1.06 ^a	(0.02)	1.20 ^{ab}	(0.05)	1.29 ^b	(0.02)
	2 mo	1.55 ^a	(0.03)	1.64 ^{ab}	(0.05)	1.71 ^b	(0.04)
	4 mo	2.02 ^a	(0.06)	2.12 ^b	(0.04)	2.49 ^c	(0.19)
	6 mo	3.91 ^a	(0.15)	4.12 ^b	(0.08)	4.41 ^c	(0.05)
Total AA (mg/g of cheese)	1 d	0.69 ^a	(0.03)	0.84 ^{ab}	(0.06)	1.01 ^b	(0.03)
	1 mo	1.87 ^a	(0.03)	2.00 ^{ab}	(0.04)	2.15 ^b	(0.04)
	2 mo	2.49 ^a	(0.07)	2.74 ^b	(0.16)	2.91 ^b	(0.10)
	4 mo	3.92 ^a	(0.32)	4.53 ^b	(0.35)	5.00 ^c	(0.42)
	6 mo	8.07 ^a	(0.76)	9.90 ^b	(0.95)	10.51 ^c	(0.55)
Total fatty acids (mg/g of cheese)	1 d	0.8 ^a	(0.14)	0.9 ^a	(0.08)	1.3 ^b	(0.11)
	1 mo	1.2 ^a	(0.15)	1.6 ^b	(0.06)	1.9 ^c	(0.05)
	2 mo	1.7 ^a	(0.07)	1.9 ^a	(0.08)	1.9 ^a	(0.06)
	4 mo	0.9 ^a	(0.11)	1.1 ^a	(0.13)	1.5 ^b	(0.12)
	6 mo	0.9 ^a	(0.13)	0.8 ^a	(0.12)	1.2 ^b	(0.12)

^{a-c}Values within a row not followed by the same superscript letter differ ($P < 0.05$).

¹The results shown are the average of 3 replicate trials. C = control cheese: 1% rennet powder; A = 0.25% EMC and 0.75% rennet powder; B = 1% EMC; SN = soluble nitrogen; TN = total nitrogen; PTA-N = phosphotungstic acid-soluble nitrogen.

Table 3. Mean squares and probabilities (*P*) for age-related changes of ripening in Cheddar cheese made with the addition of rennet and enzyme-modified cheese (EMC) powder¹

Factor	pH 4.6-SN			PTA-SN			Total FAA			Total FFA		
	df	MS	<i>P</i>	df	MS	<i>P</i>	df	MS	<i>P</i>	df	MS	<i>P</i>
Main plot												
Treatment	3	11.5	0.0005	3	1.55	<0.0001	3	9.19	0.0001	2	0.74	0.0062
Error	6	0.4		6	0.03		6	0.12		4	0.03	
Subplot												
Time	3	251.6	<0.0001	3	25.55	<0.0001	3	157.1	<0.0001	4	1.30	0.0001
Interaction (treatment × time)	9	1.2	0.0078	9	0.23	0.01	9	0.67	0.0068	8	0.06	0.8636
Error	24	0.36		24	0.07		24	0.19		24	0.12	

¹SN = Soluble nitrogen; PTA-SN = phosphotungstic acid-soluble nitrogen; FAA = free AA.

trends for pH 4.6-SN, the levels of PTA-N were higher in the experimental cheeses compared with the control cheese throughout ripening (Tables 2, 3). Significant differences were observed because of the addition of EMC powder ($P < 0.0001$), time ($P < 0.0001$), and their interaction ($P < 0.01$). At 1 d, levels of PTA-N were higher in the experimental cheeses (0.66 and 0.76%) compared with the control cheese (0.55%). These differences were attributed to the addition of EMC powder, which contained high levels of short peptides (53.73% <0.5 kDa) and FAA (Kilcawley et al., 1998). Up to 2 mo of ripening, the levels of PTA-N increased at a similar rate in all the cheeses, with the highest levels detected in the experimental cheeses. After 2 mo, levels of PTA-N increased in experimental cheeses at a higher rate than in the control. In cheese, large water-insoluble casein-derived peptides are converted to smaller water-soluble peptides through the action of chymosin, plasmin, and cell-wall-associated proteinases over the first 3 mo of ripening. After this time, the smaller water-soluble peptides are rapidly degraded by lactococcal peptidases released on lysis, as well as by NSLAB, to smaller peptides and FAA soluble in water and PTA (Fox and Wallace, 1997). Hence, the higher levels of PTA-N in the experimental cheeses may be due to peptidases released from *L. lactis* ssp. *cremoris* 227 after 2 mo of ripening, because studies have shown that 227 slowly releases intracellular enzymes (Wilkinson et al., 1994b). It is also possible that the higher numbers of NSLAB present in the experimental cheeses also contributed to the increased PTA-N.

Individual FAA Analysis

The results of the individual FAA are shown in Figure 1. Statistical analysis of the individual FAA revealed significant differences ($P < 0.05$) caused by treatment, ripening time, and the interaction of treatment and ripening time (data not shown). In agreement with previous studies (Fenelon et al., 1999; Hannon et al., 2003,

2005, 2006), the principal FAA were glutamate, Leu, Phe, Val, Lys, and Ser. At 1 d of ripening, all individual FAA were elevated in the experimental cheeses compared with the control cheese, reflecting the enhancement of FAA caused by addition of the EMC powder to the cheeses. Higher levels of all individual FAA were detected at all stages of ripening in cheeses containing the EMC powder. However, a large accumulation of FAA was not observed until the end of ripening (from 4 to 6 mo) in all cheeses. The higher levels of FAA observed toward the end of ripening are attributed to starter lysis as well as the high levels of NSLAB. According to Fox (1989), the greatest source of FAA is via the proteolytic activities of the starters. Starter numbers were observed to drop 2 logs between 3 and 6 mo of ripening, suggesting that possible lysis of the starter lactococci could also be enhancing conversion of peptides to FAA. Levels of NSLAB were 1 log greater at 4 mo than at 2 mo in all cheeses, and continued to increase for the remainder of ripening. The higher levels of FAA observed in the experimental cheeses may be due in part to the higher NSLAB in these cheeses.

Production of FAA is no longer considered as the rate-limiting step in flavor development, but rather the enzymatic or chemical modification of these FAA (Yvon and Rijnen, 2001). However, FAA are considered to contribute to the background flavor of cheese (Urbach, 1993); hence, increasing their levels in cheese is a prerequisite step in accelerating flavor development. Free AA such as His, Met, Val, Arg, Ile, Leu, Phe, and Tyr are associated with a bitter taste; Ala, Gly, Ser, Pro, and Thr have a sweet taste; and His, glutamate, and Asn are sour (McSweeney and Sousa, 2000). Catabolism of branched-chain, aromatic, and sulfur-containing FAA have been implicated in the flavor development of cheese, resulting in the production of potent volatile aroma compounds that enhance the cheese aroma (Banks et al., 2001; Yvon and Rijnen, 2001). Many of these key FAA (Leu, Ile, Val, Met, and His) were significantly higher in the experimental cheeses than in

Figure 1. Levels of individual free AA detected in cheeses made with the addition of rennet and enzyme-modified cheese (EMC) powder. Control cheese C (solid bars); 0.25% EMC, A (cross-hatched bars); or 1.0% EMC, B (open bars) at (A) 1, (B) 2, (C) 4, and (D) 6 mo of ripening. The values presented are the means of 3 replicate trials. Significant differences between individual free AA at each ripening time are indicated by lowercase letters (a–c) on the figure. Free AA not sharing a common letter differ ($P < 0.05$).

the control cheeses after 4 mo of ripening and are likely to be responsible for some of the differences in the flavor and aroma of these cheeses. Levels of glutamate were significantly higher by 6 mo of ripening in cheeses containing EMC and may be acting as a flavor potentiator in these cheeses.

Individual FFA Analysis

Higher levels of total FFA were detected in cheeses containing EMC powder on d 1 (Table 2) and were attributed to the enhanced lipolysis of the EMC powder compared with the rennet powder. Individual FFA detected in the cheeses at 1, 2, 4, and 6 mo of ripening are presented in Figure 2. The concentrations of short- and medium-chain FFA (4:0, 8:0, 10:0, and 12:0) were lower than the long-chain FFA (14:0, 16:0, 18:0, and 18:1) at all time points, a pattern previously reported by Hannon et al. (2006) and Collins et al. (2003). Because the EMC was highly lipolysed, it would contain not only FFA but also mono- and diacylglycerides. Starter culture bacteria can contribute to the production of FFA during aging because their esterases hydrolyze mainly mono- and diacylglycerides rather than triacylglycerides (Stadhouders and Veringa, 1973; Holland et al., 2002). Hence, increases in FFA detected in the cheeses at 1 mo of ripening may not only be due to the addition of EMC powder, but also to higher levels of mono- and diacylglycerides, which act as substrates for the starter bacteria. As with levels of individual FFA, levels of FFA detected at any stage of ripening are the result of hydrolysis and catabolism. Levels of 4:0 increased from d 1 but changed very little over ripening in all the cheeses and probably contributed to the “rancid” flavor of these cheeses. Hexanoic acid levels (6:0) were negligible in all cheeses at all time points (data not shown), suggesting perhaps high catabolism to 2-pentanone. The levels of 8:0 detected were extremely low throughout ripening, also possibly due to rapid catabolism to produce 2-heptanone via β -oxidation and contributing to the “soapy” or “rancid” flavors of the cheeses. Levels of 10:0 and 12:0 were numerically similar throughout ripening and have been described as having a “rancid” and “fatty” flavor note (Sablé and Cotteceau, 1999). Levels of the longer chain FFA were extremely high at 1 and 2 mo, but lower levels were detected at 4 and 6 mo, suggesting that significant catabolism occurred after 2 mo. Because of their larger flavor thresholds, long-chain FFA are not thought to contribute significantly to the flavor profiles. However, perhaps their presence at high levels contributed to the perception of atypical Cheddar notes detected in these cheeses toward the end of ripening. Although FFA contribute to the flavor of the cheeses, it is their catabolic

Figure 2. Levels of individual FFA detected in cheeses made with the addition of rennet and enzyme-modified cheese (EMC) powder. Control cheese C (solid bars); 0.25% EMC, A (cross-hatched bars); or 1.0% EMC, B (open bars) at (A) 1, (B) 2, (C) 4, and (D) 6 mo of ripening. The values presented are the means of 3 replicate trials. Differences between individual FFA at each ripening time are indicated by lowercase letters (a–c) on the figure. Free fatty acids not sharing a common letter differ ($P < 0.05$).

products—the lactones, methyl ketones, alcohols, and esters formed by reactions with methanol and ethanol—that contribute to creating the diversity of flavor in cheese.

Assessment of Volatile Compounds

The volatile compounds detected in the cheeses at 1, 2, 4, and 6 mo of ripening are given in Table 4. Twenty-one compounds, consisting of 2 alcohols, 7 aldehydes, 2 esters, 6 ketones, and 4 sulfur compounds, were identified in all the cheeses at each ripening time analyzed. All of these compounds have previously been identified in Cheddar cheese (Maarse et al., 1994) and were included in the data set for PCA. Analysis of variance showed that the first 2 PC significantly discriminated ($P < 0.05$) between the cheeses, accounting for a cumulative variation of 68%. A biplot of PC1 and PC2 is presented in Figure 3. Because all compounds were detected in the cheeses at all stages of ripening, it was the balance of concentrations of the compounds that characterized the volatile profile at any particular stage of ripening, as suggested by Bosset and Gauch (1993).

Principal component 1 (43%) separated the cheeses on the basis of age, with the young cheeses scoring negatively and the older cheeses scoring positively on PC1. Few differences were detected among the cheeses in terms of their volatile profile at 1 and 2 mo of ripening across PC1. Greater separation of the cheeses was detected between 4 and 6 mo of ripening, when the control cheese was grouped away from cheeses containing the EMC powder. Up to 4 mo of ripening, all the cheeses contained high concentrations of dimethyl disulfide, dimethyl sulfide, carbon disulfide, heptanal, 2-butanol, ethyl butanoate, and 2-hexanone. The cheeses at 1 mo, scoring negatively on PC2, contained lower proportions of these compounds. The biplot reflects the change in the volatile profiles as the cheeses aged, from a relatively weak aroma at 1 mo to a more intense aroma at 2 and 4 mo, mainly characterized by sulfur compounds. However, the profile changed little between 2 and 4 mo of ripening. At 6 mo of ripening, cheeses containing the EMC powder were well separated from the control cheese and were characterized as containing high levels of ketones, methanethiol, pentanal, 3 methyl-butanol, 2-pentanol, and ethyl acetate. These data suggest that the catabolic products of lipolysis largely characterize the volatile profiles of cheeses containing the EMC powder.

Sulfur compounds originate from Met and are major contributors to the Cheddar cheese aroma (Urbach, 1993; McSweeney and Sousa, 2000; Yvon and Rijnen, 2001; Thierry and Maillard, 2002). Methionine is converted to methanethiol, which is the direct precursor of compounds such as dimethyl disulfide (Arfi et al., 2003). Levels of Met were very similar in all the cheeses throughout ripening; hence, few differences were detected in the catabolic products up to 4 mo of ripening. However, higher levels of dimethyl sulfide, dimethyl

Table 4. Results of volatile analysis of Cheddar cheese made with added rennet and enzyme-modified cheese (EMC) powder, showing the averaged peak areas (in arbitrary units $\times 1,000$) of triplicate determinations, and coefficients of variation¹ at 1, 2, 4, and 6 mo of ripening²

Compound	C				A				B			
	1 mo	2 mo	4 mo	6 mo	1 mo	2 mo	4 mo	6 mo	1 mo	2 mo	4 mo	6 mo
Alcohols												
2-Butanol	31 (26)	347 (22)	62 (34)	93 (36)	37 (28)	400 (34)	72 (20)	148 (19)	41 (41)	599 (43)	77 (37)	159 (39)
2-Pentanol	53 (17)	58 (26)	58 (27)	74 (16)	58 (28)	69 (20)	74 (27)	83 (16)	45 (24)	51 (17)	85 (19)	95 (20)
Aldehydes												
Butanal	91 (25)	39 (27)	30 (22)	27 (23)	27 (21)	36 (33)	35 (20)	38 (23)	27 (20)	37 (21)	42 (18)	39 (24)
Pentanal	210 (32)	299 (31)	517 (21)	700 (25)	245 (21)	306 (24)	425 (20)	603 (22)	262 (18)	377 (28)	480 (18)	882 (27)
Hexanal	847 (11)	886 (15)	1,004 (21)	105 (17)	812 (10)	822 (11)	1,136 (26)	931 (17)	819 (09)	754 (08)	1,209 (27)	898 (19)
Heptanal	166 (16)	150 (16)	183 (11)	169 (23)	163 (25)	170 (28)	175 (26)	140 (23)	133 (28)	190 (11)	161 (26)	158 (24)
Octanal	46 (29)	82 (18)	40 (13)	87 (20)	57 (20)	125 (13)	44 (23)	66 (22)	42 (30)	32 (20)	37 (28)	54 (20)
2-ME-butanol	15 (33)	10 (15)	8 (26)	8 (13)	15 (22)	14 (15)	10 (25)	9 (26)	14 (20)	16 (26)	10 (11)	9 (23)
3-ME-butanol	59 (26)	113 (23)	226 (26)	233 (18)	82 (27)	126 (18)	241 (24)	1,040 (18)	88 (22)	212 (23)	299 (14)	1,431 (24)
Esters												
Ethyl acetate	41 (16)	81 (28)	50 (13)	65 (31)	135 (26)	99 (21)	63 (22)	142 (13)	157 (15)	70 (21)	102 (24)	248 (15)
Ethyl butanoate	19 (26)	32 (14)	41 (16)	24 (10)	29 (20)	71 (23)	53 (21)	35 (22)	35 (11)	65 (22)	54 (16)	32 (21)
Ketones												
Acetone	507 (25)	1,125 (25)	770 (20)	529 (21)	1,210 (33)	774 (17)	853 (25)	1,766 (21)	1,788 (27)	1,313 (25)	726 (27)	1,789 (18)
2-Butanone	405 (37)	1,899 (25)	1,756 (23)	2,120 (08)	1,273 (17)	2,296 (32)	2,206 (25)	12,916 (18)	1,405 (30)	2,269 (20)	2,165 (16)	13,719 (32)
2-Pentanone	152 (35)	205 (28)	318 (23)	612 (25)	150 (23)	240 (24)	378 (22)	741 (16)	155 (20)	235 (19)	392 (12)	739 (23)
2-Hexanone	23 (27)	35 (16)	26 (20)	20 (20)	27 (27)	43 (27)	27 (20)	27 (18)	29 (25)	41 (24)	30 (15)	22 (25)
2-Heptanone	107 (08)	120 (18)	274 (18)	243 (20)	96 (22)	152 (17)	177 (42)	255 (20)	101 (26)	144 (12)	185 (28)	280 (27)
2,3-Butanedione	78 (19)	353 (16)	118 (20)	302 (16)	67 (20)	196 (20)	287 (29)	220 (26)	114 (24)	204 (23)	53 (27)	371 (24)
Sulfur compounds												
Methanethiol	4,716 (16)	6,538 (26)	4,576 (25)	5,431 (20)	4,323 (10)	5,936 (20)	5,788 (22)	9,181 (20)	6,446 (11)	5,043 (22)	4,970 (35)	7,267 (21)
Carbon disulfide	335 (25)	423 (28)	226 (24)	159 (22)	410 (20)	510 (19)	467 (23)	186 (19)	420 (25)	536 (23)	342 (18)	198 (10)
Dimethyl sulfide	27 (17)	64 (10)	69 (06)	25 (51)	59 (21)	70 (25)	70 (24)	44 (21)	46 (29)	73 (23)	83 (17)	43 (15)
Dimethyl disulfide	31 (24)	57 (22)	54 (25)	30 (16)	35 (23)	56 (24)	57 (23)	47 (20)	37 (19)	51 (19)	60 (15)	47 (18)

¹Coefficients of variation obtained from a total of 9 measurements.²C = Control cheese; A = cheese with 0.25% EMC; B = cheese with 1.0% EMC.

Figure 3. Principal component analysis showing the first 2 principal components of dynamic headspace volatile data for Cheddar cheeses made with the addition of rennet and enzyme-modified cheese (EMC) powder. C = control cheese; A = 0.25% EMC; B = 1.0% EMC, at 1, 2, 4, and 6 mo of ripening. The values presented are the means of 3 replicate trials.

disulfide, and carbon disulfide were associated with the cheeses at 2 mo of ripening, whereas higher levels of methanethiol were associated with the 6-mo-old cheeses containing EMC powder. This may have been due to higher levels of Met becoming available toward the end of ripening. The concentration of methanethiol has been reported to increase during maturation (Manning et al., 1976; Barlow et al., 1989), and in the absence of methanethiol, the Cheddar flavor is absent (Manning, 1974). However, Urbach (1993) concluded that the presence of methanethiol is a necessary but not sufficient condition for the Cheddar flavor. Carbon disulfide has a sweet, pleasant odor when pure; dimethyl sulfide has a “cauliflower” or “very ripe cheese” odor; dimethyl trisulfide has a “penetrating,” “over-ripened cheese” odor; and methanethiol has a cooked cabbage odor (Sablé and Cottenceau, 1999).

High levels of aldehydes were detected, and these contributed strongly to the volatile profiles of these cheeses. The straight-chain aldehydes (butanal, pentanal, hexanal, heptanal, and octanal) can be formed during β -oxidation of unsaturated fatty acids and contribute to the green-grass-like and herbaceous aromas (Moio et al., 1993). The branched-chain AA Ile and Leu

are responsible for the evolution of the branched-chain aldehydes, producing 2-methyl-butanal and 3-methyl-butanal, respectively, and can be subsequently converted to branched alcohols (McSweeney and Sousa, 2000; Yvon and Rijnen, 2001). However, the branched-chain alcohols were not detected in any of the cheeses.

By the end of ripening, the character of cheeses containing the EMC powder was predominated by a high level of ketones and correlated well with the reduction of FFA reported earlier. Methyl ketones are derived from FFA (<14:0) by β -oxidation, and can also be converted to their corresponding secondary alcohols (McSweeney and Sousa, 2000). According to Urbach (1993), methyl ketones are products of fat lipolysis in cheeses that are not mold ripened. When the amount of lipolysis is small, the level of methyl ketones is low and these compounds contribute only to the background flavor (Urbach, 1993). In this study the experimental cheeses were associated with high levels of methyl ketones at 6 mo of ripening. Levels of acetone (a normal constituent of milk and cheese) were higher in cheeses containing the EMC powder, and their levels decreased during ripening and then increased toward the end of ripening. 2,3-Butanedione (diacetyl) is formed in dairy

Table 5. Results of the quantitative sensory descriptive analysis of Cheddar cheese made with the addition of rennet and enzyme-modified cheese powder (EMC), showing the averaged attribute scores (1 to 100) and standard deviations¹ at 1, 2, 4, and 6 mo of ripening²

Attribute	C				A				B				ANOVA ³		
	1 mo	2 mo	4 mo	6 mo	1 mo	2 mo	4 mo	6 mo	1 mo	2 mo	4 mo	6 mo	Treat	Age	Treat × age
Odor															
Pungent	6 (2)	8 (1)	10 (3)	21 (6)	10 (1)	8 (2)	13 (4)	20 (7)	9 (1)	16 (1)	10 (2)	21 (6)	***	***	NS
Sulfur	4 (2)	7 (2)	7 (1)	11 (4)	5 (2)	6 (1)	8 (2)	10 (3)	6 (1)	9 (2)	6 (2)	11 (3)	**	***	NS
Sweaty	13 (1)	16 (2)	15 (1)	21 (3)	14 (3)	12 (2)	18 (2)	19 (2)	15 (3)	19 (2)	18 (3)	20 (4)	**	***	NS
Rancid	8 (2)	10 (2)	10 (2)	15 (3)	11 (1)	10 (3)	11 (1)	16 (2)	11 (2)	14 (1)	12 (1)	15 (1)	***	***	NS
Fruity	2 (3)	14 (5)	15 (3)	12 (2)	17 (3)	19 (1)	22 (3)	21 (3)	17 (1)	21 (4)	25 (2)	24 (5)	*	*	NS
Caramel	9 (1)	7 (1)	7 (2)	7 (1)	8 (2)	6 (1)	6 (1)	7 (1)	7 (1)	7 (1)	7 (1)	7 (1)	NS	**	***
Butter	29 (2)	26 (1)	25 (3)	18 (3)	25 (3)	25 (2)	22 (1)	19 (3)	25 (2)	21 (2)	26 (2)	17 (2)	***	***	NS
Moldy	1 (1)	1 (1)	2 (1)	3 (2)	2 (1)	4 (1)	5 (1)	7 (3)	2 (1)	5 (1)	9 (2)	13 (2)	*	NS	NS
Soapy	2 (1)	2 (1)	2 (1)	2 (1)	3 (2)	5 (1)	7 (2)	9 (3)	3 (2)	8 (2)	10 (1)	12 (3)	*	*	NS
Flavor															
Pungent	7 (3)	9 (3)	13 (4)	28 (5)	11 (2)	10 (2)	15 (6)	29 (9)	11 (2)	22 (2)	14 (4)	31 (5)	***	***	NS
Rancid	13 (2)	15 (3)	14 (4)	14 (4)	18 (1)	14 (2)	19 (8)	27 (8)	20 (3)	22 (3)	18 (5)	31 (6)	**	***	NS
Moldy	7 (1)	5 (1)	6 (3)	5 (2)	6 (1)	6 (2)	7 (5)	14 (7)	7 (2)	10 (2)	12 (3)	14 (6)	*	**	NS
Onion	3 (1)	4 (2)	5 (1)	12 (4)	4 (1)	4 (1)	6 (1)	8 (2)	4 (1)	8 (1)	5 (1)	9 (2)	***	***	NS
Caramel	4 (1)	7 (1)	7 (2)	6 (1)	5 (1)	6 (1)	7 (2)	5 (1)	4 (1)	5 (1)	7 (1)	6 (2)	NS	*	NS
Fruity	5 (2)	7 (2)	8 (1)	13 (3)	6 (1)	8 (1)	9 (3)	12 (3)	5 (1)	10 (2)	8 (1)	10 (2)	***	***	NS
Buttery	31 (1)	34 (1)	28 (5)	17 (3)	29 (4)	31 (3)	25 (2)	19 (7)	28 (1)	22 (1)	27 (4)	15 (2)	***	***	NS
Lard	19 (7)	18 (4)	3 (1)	3 (1)	21 (5)	17 (5)	8 (1)	5 (1)	22 (5)	12 (4)	8 (1)	8 (2)	***	***	NS
Soapy	4 (1)	4 (1)	5 (3)	7 (4)	5 (1)	7 (1)	8 (3)	12 (3)	6 (1)	7 (2)	10 (2)	15 (4)	*	**	NS
Processed	35 (9)	23 (6)	14 (3)	4 (1)	27 (6)	24 (4)	12 (6)	6 (1)	29 (9)	15 (1)	14 (2)	7 (1)	***	***	NS
Sweet	7 (2)	8 (2)	7 (2)	7 (2)	6 (1)	6 (1)	5 (2)	6 (2)	6 (1)	6 (1)	6 (1)	5 (1)	NS	NS	NS
Salty	32 (3)	29 (4)	34 (1)	34 (1)	31 (5)	30 (2)	32 (3)	33 (1)	32 (4)	33 (5)	34 (1)	33 (6)	NS	NS	NS
Acidic	20 (4)	25 (2)	29 (6)	35 (2)	25 (1)	27 (4)	29 (4)	33 (3)	23 (4)	32 (2)	31 (2)	36 (3)	***	***	NS
Bitter	13 (3)	17 (5)	22 (5)	28 (3)	18 (3)	17 (4)	23 (6)	31 (9)	18 (4)	28 (6)	28 (4)	32 (9)	*	***	NS
Astringent	16 (3)	16 (2)	19 (2)	23 (1)	17 (2)	17 (3)	19 (2)	24 (4)	16 (3)	22 (5)	20 (2)	22 (2)	*	***	NS
Strength	30 (2)	32 (2)	38 (4)	54 (2)	31 (1)	35 (2)	41 (4)	53 (8)	35 (4)	42 (2)	48 (3)	56 (7)	***	***	NS
Balanced	36 (1)	40 (3)	33 (5)	33 (4)	36 (3)	37 (1)	32 (6)	30 (5)	33 (1)	32 (5)	32 (5)	26 (4)	*	*	NS
AF-metallic	2 (1)	3 (1)	5 (1)	6 (1)	3 (1)	4 (1)	4 (1)	7 (2)	3 (1)	6 (1)	5 (1)	7 (2)	*	***	NS
AF-rancid	6 (1)	7 (1)	8 (2)	13 (2)	9 (1)	9 (1)	11 (2)	16 (3)	12 (3)	13 (3)	11 (3)	22 (4)	**	***	NS

¹Standard deviations obtained from a total of 6 measurements. AF = aftertaste flavor.²C = Control cheese; A = cheese with 0.25% EMC; B = cheese with 1.0% EMC.³Analysis of variance performed using the GLM procedure of SAS (SAS Institute, 1995). Treat = addition level; age = ripening time.NS = $P > 0.05$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

products from citrate by the starter and NSLAB and may contribute to the buttery, nutlike note present in all the cheeses. Reduction of 2,3-butanedione leads to acetoin, which is further reduced to butanone and subsequently to 2-butanol by NSLAB (Hugenholtz et al., 2000). Because the cheeses containing EMC powder had higher levels of NSLAB, this may account for the higher levels of 2-butanone and 2-butanol detected in them. These compounds have been widely reported in Cheddar cheese (Urbach, 1993). Levels of 2-butanone continued to increase in all cheeses, whereas levels of 2-butanol increased up to 2 mo and then decreased. This may be indicative of the reducing conditions changing in the cheeses as they mature.

The principal compounds responsible for fruitiness are esters, especially compounds such as ethyl butanoate, formed by the esterification of fatty acids with ethanol. Ethyl butanoate was associated with the cheeses at 2 mo of ripening. The levels of ethyl acetate detected were higher than those of ethyl butanoate,

and were associated with the cheeses containing EMC powder at the end of ripening. Ethyl acetate is derived from the deamination or decarboxylation, or both, of the AA Ser, Ala, and Gly (Wallace and Fox, 1997). Ethyl acetate has been described as having a solvent or fruity flavor note in cheese (Sablé and Cottenceau, 1999).

Descriptive Sensory Analysis of Cheese

Table 5 shows that the individual scores of the cheeses averaged across assessors and replicates for the 9 odor and 19 flavor terms rated by descriptive sensory analysis were pertinent to these cheeses. A biplot of the scores of the cheeses and loadings of the attributes is presented in Figure 4. Analysis of variance found that the first 2 PC significantly discriminated ($P < 0.05$) among the cheeses and accounted for a cumulative variation of 83%. Principal component 1 (75%) separated the cheeses on the basis of age, with the younger cheeses scoring negatively and the older cheeses scoring

Figure 4. Principal component analysis showing the first 2 principal components of the descriptive sensory analysis of Cheddar cheeses made with the addition of rennet and enzyme-modified cheese (EMC) powder. C = control cheese; A = 0.25% EMC; B = 1.0% EMC at 1, 2, 4, and 6 mo of ripening. The values presented are the means of 3 replicate trials. O = odor; F = flavor; AF = aftertaste flavor.

positively. Principal component 2 (8%) separated the cheeses according to treatment, with the control cheese scoring positively and cheeses containing the EMC powder scoring negatively. Because PC1 contributed mostly to the variation among the cheeses, the relative positions across this axis were most important. The control cheese at 6 mo had a position similar to the cheeses containing EMC powder at 4 mo of ripening across PC1, suggesting an acceleration of 2 mo in flavor development. The younger cheeses, at 1 and 2 mo of ripening, were all similar in terms of flavor and were characterized by a “buttery,” “caramel” odor and a “buttery,” “processed,” “balanced,” “lard” flavor. The “lard” flavor probably results from the higher levels of FFA detected in these cheeses up to 2 mo of ripening, but decreases with hydrolysis of the fat as the cheeses mature. Between 2 and 4 mo of ripening, there was a large development of flavor, because the samples were well separated across PC1, probably because of the high levels of NSLAB and starter lysis at this stage of ripening. By 6 mo of ripening, the experimental cheeses were associated with the highest levels of most attributes, reflecting an intense flavor development attributable to the addition of EMC powder. The separation of cheeses achieved by the volatile analysis did not follow the same pattern as the separation achieved by the sensory analysis. This highlights the point that volatile compounds detected following purge-and-trap extraction contrib-

ute only in part to the perception of flavor in the final cheese.

The development of atypical flavors in the experimental cheeses at 6 mo of ripening indicated that these cheeses had exceeded their optimal ripening time. Therefore, it would have been more prudent to stop ripening at 4 mo to achieve a more balanced flavor profile. This study clearly demonstrates the possibility of producing a mature ingredient-type Cheddar cheese through the addition of EMC powder during production. This mechanism has the potential to create a range of ingredient cheese flavors by careful selection of EMC types and dosage rates, with minimal alteration to typical Cheddar manufacturing protocols.

CONCLUSIONS

Addition of 0.25 or 1 g/100 g of EMC powder to Cheddar cheese curd during manufacture had a beneficial effect on the development of cheese flavor up to 4 mo of ripening, as determined by PCA of the sensory data. Levels of NSLAB were greater in the experimental cheeses compared with the control. Although primary proteolysis patterns were unaffected (as indicated by the levels of pH 4.6-SN accumulation), addition of the EMC powder enhanced secondary proteolysis, especially after 2 mo of ripening, primarily because of the increase in available substrate for peptidases released

from starter culture lysis or whole cells of NSLAB or both. The addition of EMC powder to the experimental cheeses provided increased levels of individual FFA and FAA, which could contribute directly to the flavor of the cheeses while also acting as extra substrates for the production of volatile compounds. By the end of ripening, the low flavor threshold catabolic products of lipolysis clearly predominated in the experimental cheeses. Inclusion of the EMC powder enhanced flavor development (by 2 mo). This study highlights a relatively inexpensive method of accelerating cheese ripening.

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