Characterization of Flavor and Texture Development Within Large (291 kg) Blocks of Cheddar Cheese¹

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ABSTRACT

Cheddar cheese is a natural product that has a variable flavor and texture profile. Many companies produce 291-kg blocks of Cheddar cheese, which are subsequently cut and shipped, or stored and subsequently cut. Previous research has shown that compositional differences exist within 291-kg blocks and that these differences may influence flavor and texture development. The objectives of this study were to systematically characterize flavor and texture differences within 291-kg blocks. On 2 different occasions, a 291-kg block was manufactured at each of 4 manufacturing facilities. After 7 d, the 291-kg blocks were sliced into sixteen 18-kg sample portions using a predetermined diagram, and each portion was labeled appropriately (outer corner, inner corner, etc.) and stored at 7°C. Cheese from different locations within the 291-kg blocks was evaluated at 1, 4, 8, and 12 mo. At each time point, two 18-kg portions representing an inside and outside location with the 291-kg block cross-section (from inside to outside) were sampled. The moisture content was lower in the inner than outer locations within the 291-kg blocks. Protein hydrolysis was higher in the inner location and inner locations developed aged Cheddar flavors sulfur, nutty, and brothy more rapidly than the outer locations. However, plant-to-plant differences in aging were often larger than differences caused by block location. These differences were due to differences in cheese manufacturing practices among plants. Dynamic headspace results for flavor volatiles were consistent with descriptive sensory flavor results, documenting differences between inner and outer locations within 291-kg blocks. The inner locations were more fracturable and the outer locations were more cohesive

and had more residual in the mouth. Inner locations had greater fracture strain than outer locations. Documenting the differences in aging of 291-kg blocks of Cheddar cheese is important in understanding how to make a consistent high-quality Cheddar cheese.

Key words: 291-kilogram block, flavor development, texture development

INTRODUCTION

Cheddar cheese is a natural product that has a variable flavor and texture profile. Many companies produce 291-kg blocks of Cheddar cheese. Making these large blocks of Cheddar rather than the more traditional 18-kg blocks reduces labor and handling costs (Mesa-Dishington et al., 1987). However, previous studies have demonstrated that compositional differences exist within 291-kg blocks (Reinbold and Ernstrom, 1988; Reinbold et al., 1992; Barbano, 2001). The process used during pressing and draining the 291-kg blocks may influence the characteristics of the moisture gradient, but generally the gradient from the exterior to the interior is larger in the bottom half of a 291-kg block (Barbano, 2001). These differences in composition may influence flavor and texture development.

In a 291-kg block, the temperature at the outer surfaces of the block will decrease more rapidly than the temperature inside the block. This temperature gradient has an impact on moisture migration (Olabi and Barbano, 2002). Previous studies (Reinbold and Ernstrom, 1988; Reinbold et al., 1992; Barbano, 2001) reported a difference in the moisture content between the inner and outer portions of the 291-kg cheese block. As the temperature of the cheese near the surface of the 291-kg block decreases, the moisture content increases because moisture migrates from the warmer cheese in the center of the block to the colder cheese at the surfaces. Mobility of the moisture appears to be directly related to the ability of CN to hold or release water, which is primarily a function of pH and temperature (Lawrence et al., 2004). Caseins are relatively hydrophobic (Swaigood, 1992),

Received November 13, 2006.

Accepted March 13, 2007.

¹Use of names, names of ingredients, and identification of specific models of equipment is for scientific clarity and does not constitute any endorsement of product by authors, North Carolina State University, or the Southeast Dairy Foods Research Center.

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and lower temperatures cause hydrophobic proteins to favor protein-water interactions (outer location), whereas higher temperatures favor protein-protein interactions (inner location; Lehninger, 1970). Olabi and Barbano (2002) reported that a temperature gradient within cheese caused moisture to move from an area of warm temperature (interior) to an area of cooler temperature (exterior) both with and against the force of gravity in cheeses cooled with a temperature gradient from 27 to 3°C. The result was that the outer portion of the block was higher in moisture than the inner portion (Barbano, 2001; Olabi and Barbano, 2002).

Previous research has also documented a pH gradient initially before cooling of 5.2 at the exterior and 5.38 in the interior to a pH of 5.05 exterior to 4.95 interior in 291-kg cheese blocks (Reinbold et al. 1992). Reinbold et al. (1992) found that during aging, the pH at the interior of the block decreased, whereas there was less change in pH at the exterior of the 291-kg block. Decreasing pH can increase syneresis, which will decrease the moisture content (Pastorino et al., 2003). Proteolysis within the cheese can have dramatic effects on the flavor and texture development and may be different among locations with 291-kg blocks.

There has been no systematic study characterizing flavor and texture development within 291-kg blocks. The objectives of this study were to systematically characterize flavor and texture differences across 291-kg blocks of Cheddar cheese. Instrumental and rheological methods were used as well as descriptive sensory analyses of flavor and texture.

MATERIALS AND METHODS

Cheeses

Blocks of pasteurized milk Cheddar cheeses (291 kg) were produced at 4 commercial manufacturing facilities (2 milled-curd, 2 stirred-curd facilities). One block was sampled at each plant, and this sampling was replicated on a block manufactured at a later date in each plant. Stirred-curd facilities were located on the West coast and in the Midwestern United States. Milled-curd facilities were located in the Midwest and Northeastern United States. Blocks at each facility were cooled using forced-air coolers and kept separate from other blocks to ensure even cooling rates through all 6 faces of each block. Blocks from plant 1 were cooled at 5°C for 14 d, whereas blocks from plants 2 to 4 were cooled at 1°C for 3 to 5 d. All plants used fermentation-produced chymosin. The rennet use rates were as follows: Plant 1 used 23.1 mL, plants 2 and 3 used 37.5 mL, and plant 4 used 21 mL per 454 kg of standardized milk. Only plant 1 did not add enzymes to increase the rate of flavor development during aging. The temperature of the curd when the

Figure 1. Diagram of 18-kg sample portions cut from a 291-kg block. The upper- and outermost top corner of 18-kg portion C represents an outer location (i.e., the point of the arrow facing into the upper, outside corner of the block) and the lower- and bottom-most corner of 18-kg portion D represents an inner location (i.e., the point of the arrow facing into the center of the block). Blocks E to H are mirrored in the top part of the block. Blocks A, C, E, and G are outer blocks; blocks B, D, F, and H are inner blocks.

291-kg blocks were filled was lower (28 to 29°C) for plants 1 and 4, whereas it was higher (32°C) for plants 2 and 3. Plants 1 and 3 did not fortify their milk, plant 2 used only a low level of fortification, and plant 4 used a very high level of fortification with nonfat milk solids and cream. When cooling the 291-kg blocks after pressing, plant 1 used forced air at 5°C, whereas the other 3 plants used 1°C.

After cooling (7 d), each 291-kg block was sliced into sixteen 18-kg portions using a predetermined diagram (Figure 1) and each portion was labeled appropriately (outer corner, inner corner, etc.). Eight 18-kg portions from the upper half of the 291-kg block were vacuum sealed and shipped by refrigerated carrier to North Carolina State University. Upon receipt, the 18-kg portions from the 291-kg blocks were examined for damage and then stored in the dark at 7°C.

The 18-kg portions were trimmed and cut at 1, 4, 8, and 12 mo using steel wires. At each time point, 2 of the 18-kg portions were sampled such that the exterior to interior locations of the upper half of the 291-kg block were represented (locations A and B, C and D, E and F, G and H). Approximately 2 kg of cheese representing an inner location (B, D, F, and H in Figure 1) were cut from each 18-kg portion and 2-kg samples of the outer location blocks (A, C, E, and G in Figure 1) were cut from each

18-kg portion for instrumental and sensory analysis. The rest of the 18-kg portions were discarded. The 2-kg samples from inner portions represented adjacent pieces from the interior of the 291-kg block and the outer 2-kg samples represented the exterior top corners of the 291-kg block. The lower half of the 291-kg block was not sampled.

Proximate Analysis

The pH, fat, moisture, protein, and salt were measured for all (inner and outer) locations in duplicate at each time point using standard methods. Cheese pH was measured using a Xerolyt combination electrode (model HA405; Mettler Toledo, Columbus, OH) and an Accumet pH meter (model AR 25, Fisher Scientific, Pittsburgh, PA) after tempering the cheese to 23°C. Fat content was determined using the Babcock method (Marshall, 1992; method 15.8.A). Cheese moisture was determined gravimetrically in 2 g of cheese in a forced-air oven at 100°C for 24 h (AOAC, 2000; methods 33.2.44, 990.20). Salt content was determined using the Volhard method (Marshall, 1992; method 15.5.B). The Kjeldahl method was used to determine the total nitrogen content of cheese (Lynch et al., 2002). Crude protein was calculated by multiplying total nitrogen by 6.38. Analyses were performed in duplicate.

Proteolysis

The *o*-phthaldialdehyde (OPA) assay, as outlined by Church et al. (1983), was used to determine the degree of protein hydrolysis over time during cheese aging. A 1:20 dilution of grated cheese and water was prepared by taking 1 g of cheese and adding it to 19 g of deionized water and vortexing. Five grams was removed and 10 mL of 0.75 *N* TCA was added. The samples stood for 10 min and were filtered through a Whatman #2 filter paper (Whatman Ltd., Maidstone, UK) to remove particles and obtain the material soluble in the TCA solution. Premade OPA reagent (Pierce, Rockford, IL; 2 mL) was added to a 200- μ L sample and allowed to sit for exactly 2 min to allow the OPA reagent to react with primary amino groups. The absorbance was then measured at 340 nm using a Shimadzu UV-260 spectrophotometer (Shimadzu, Columbia, MD). The following formulas were used to determine the degree of protein hydrolysis:

$$n = \Delta A_{340\text{nm}} / \varepsilon MF,$$

where *n* is the number of bonds cleaved, ε is 6,000 / cm, *M* is the molar concentration of protein in the cheese slurry (0.5 mM), *F* is the dilution factor in the assay procedure (0.0017), and $\Delta A_{340\text{nm}}$ is the absorbance at

340 nm of a sample compared with a reagent blank prepared in water,

$$\% \text{ hydrolysis} = n/194.2 \times 100,$$

where 194.2 is the weighted average of the total number of peptide bonds for CN and whey proteins in cheese. Analyses were conducted in duplicate.

At 12 mo of age, a second method (Kjeldahl soluble nitrogen) that has been more commonly used to determine proteolysis in cheese was used to cross-check the results of the OPA method. Total nitrogen and nitrogen soluble in pH 4.6 acetate buffer and 12% TCA were determined in duplicate as described by using the Kjeldahl total nitrogen method for milk (AOAC, 2000) with the sample preparation and size as described by Bynum and Barbano (1985). The pH 4.6 acetate buffer and 12% TCA-soluble nitrogen were expressed as a percentage of total nitrogen.

Dynamic Headspace Analysis GC-MS

Cheese was frozen and grated using a hand grater. A 50- μ L quantity of internal standard (50 μ L of 2-methyl-3-heptanone and 50 μ L of 2-methyl pentanoic acid in 5 mL of methanol) was added to 50 g of grated cheese. The cheese was then kneaded and thoroughly mixed to distribute the internal standard evenly. This reformed cheese sample was refrigerated at 4°C for overnight equilibration and then frozen at -80°C for at least 24 h. Cheese was then grated again, and 10 g of cheese was added to 20 g of water and thoroughly mixed using a hand homogenizer. A 5-g quantity of cheese slurry was loaded into a needle sparger 25-mL purge-and-trap vial (Tekmar, Vernon, British Columbia, Canada) along with 2 g of salt. The vial was equilibrated at room temperature for 30 min and then placed on a CDS 6000 purge-and-trap apparatus (CDS, Oxford, PA) and purged with nitrogen at 40 mL/min. The initial purge volume was 800 mL (32 min). Concentrated volatile compounds were desorbed from the trap and then transferred by a heated transfer line to the gas chromatograph and desorbed onto a nonpolar DB-5MS column (30 m length $\times$ 0.25 mm i.d. $\times$ 0.25 μ m d_f; J & W Scientific, Folsom, CA) on an HP5890 Series II GC/HP 5972 mass selective detector (Hewlett-Packard, Co., Palo Alto, CA). The oven temperature was programmed at -20 to 60°C at a rate of 4°C/min with a 6-min hold at -20°C, and then 60°C to 220°C at a rate of 6°C/min with a final hold of 5 min. Mass selective detector conditions were as follows: capillary direct interface temperature, 280°C; ionization energy, 70 eV; mass range, 33 to 330 atomic mass units; electron multiplier voltage (Atune+200 V); scan rate, 5 scans/s. Triplicate analyses were performed on each sample.

Based on MS results, concentrations of 2,3-butanedione (diacetyl), 2/3-methyl butanal, 3-hydroxy-2-butanone (acetoin), methyl butanoate, ethyl butanoate, hexanal, methyl hexanoate, ethyl hexanoate, acetic acid, and butanoic acid were calculated using an external standard curve.

For positive identifications, retention indices and mass spectra were compared with those of authentic standard compounds analyzed under identical conditions. Tentative identifications were based on comparing mass spectra of unknown compounds with those in the 1992 National Institute of Standards and Technology (Gaithersburg, MD) mass spectral database or on matching the retention index values against those of authentic standards. For the calculation of retention indices, an *n*-alkane series was used (Van den Dool and Kratz, 1963).

Quantification of Volatile Compounds

Response factors of selected compounds were calculated by direct addition of known amounts of standards to odor-free water prior to dynamic headspace analysis GC-MS. Response factors were determined using a 5-point standard curve ($r^2 > 0.96$) on a DB-5 column using GC-MS. With these response factors, the selected compounds were quantified using the response factor and the area ratio of compound to internal standards. 2,3-Butandione (diacetyl), 2/3-methyl butanal, 3-hydroxy-2-butanone (acetoin), methyl butanoate, ethyl butanoate, hexanal, methyl hexanoate, ethyl hexanoate, acetic acid, and butanoic acid were selected for quantification because these compounds were consistently observed in the samples and have previously been shown to affect the flavor in Cheddar cheese (Christensen and Reineccius, 1995; Milo and Reineccius, 1997; Zehentbauer and Reineccius, 2002; Singh et al., 2003; Carunchia Whetstine et al., 2005). All standards were obtained from Aldrich Chemical Company (St. Louis, MO).

Sensory Evaluation of Cheeses

At each time point (1, 4, 8, and 12 mo), cheeses were sampled for sensory analysis. For sampling, the outer 1 cm of each 18-kg portion was trimmed to eliminate flavors caused by packaging or exposure. Flavor and texture were evaluated on different days in different sessions.

Flavor Analysis

A trained (>100 h each) sensory panel ($n = 14$) evaluated the cheeses using the flavor lexicon developed for Cheddar cheese (Drake et al., 2001). Cheese was presented in 2×2 cm cubes and placed into 4-oz. (120 mL)

soufflé cups with 3-digit codes. Panelists were trained for 100 h on flavor, aroma, and feeling factors using the Spectrum method (Meilgaard et al., 1999). The 15-point numerical Spectrum intensity scale was used to mark panelist responses. On this universal intensity scale, which can be applied to all products and flavor intensities, most Cheddar cheese flavors fall between 0 and 5 (Drake et al., 2001, 2005). During evaluation, panelists had free access to water and unsalted crackers. Four cheeses were evaluated per session. Cheeses were evaluated in duplicate by each panelist.

Texture Analysis

A sensory panel ($n = 14$) with 50 h of training evaluated the cheeses using a previously published texture lexicon (Brown et al., 2003). The scaling technique used for texture analysis was product specific, with a 15-point anchored and referenced scale. The scale was anchored on the right with “very” and on the left with “not.” On this scale, which is specific only to cheeses, texture values fall between 0 and 15. Panelists were provided with cheese references (Brown et al., 2003) during evaluations to minimize variability. At each session, no more than 6 samples were evaluated. Each cheese was cut into 1.27-cm^3 cubes and presented at room temperature in lidded soufflé cups (to minimize moisture loss) with 3-digit random codes. At each session, panelists had free access to spring water and unsalted crackers as well as appropriate references. Each cheese was evaluated in duplicate by each panelist.

Instrumental Texture

Large Strain Analysis. A torsional method was used to determine fracture properties of cored cheese samples. Cheese was cored from each 18-kg portion using a cylindrical metal corer (minimum diameter, 10 mm). The cheese was then cut to a length of 28.7 mm. Plastic disks (Gel Consultants, Raleigh, NC) were glued to the ends of the cylinder using cyanoacrylate glue (Loctite 100, Loctite Corporation, Rocky Hill, CT) to enable the samples to be mounted to the grinding and twisting apparatuses. The cylinders were shaped into a capstan shape having a minimum diameter of 10 mm using a precision grinding machine (Gel Consultants). Samples were twisted using a Haake 550 viscotester (Gebruder Haake GmbH, Karlsruhe, Germany) fitted with a fabricated apparatus that enabled torsional measurement (Truong and Daubert, 2001). Samples were twisted at a rate of 4.5 rpm at 25°C. Samples were twisted at a strain rate of 0.45 s^{-1} until fracture. Six to 8 capstans were produced and measured for each sample. After grinding, sample geometry was measured for calculations. True shear

stress (σ_t) and true shear strain ($\gamma_{t-\text{true}}$) were calculated at fracture based on the method of Diehl et al. (1979) as described by Brown et al. (2003).

Creep and Recovery. Creep and recovery tests were performed using a Stress Tech controlled stress rheometer (ATS Rheosystems, Bordentown, NJ) fitted with 20-mm-diameter smooth parallel plates. Temperature was maintained at 25°C using an induction heating device. Samples were sliced into approximately 2-mm-thick disks. Cyanoacrylate glue (Loctite 100, Loctite Corporation) was placed on the bottom and top of the cheese disk to attach it firmly to the plates of the instrument. After attaching the cheese to the stationary bottom plate, the top plate was lowered onto the sample until a normal force of 1 N was reached. The sample was then trimmed to fit the plate size and a thin film of synthetic lubricant (Superlube, Loctite Corporation) was applied to prevent moisture loss. Tests were conducted at stresses of 400 Pa. This stress was chosen based on a previously conducted stress sweep at 25°C. The creep portion of each test consisted of a stress application for 600 s; the stress was removed and strain recovery was measured for an additional 1,200 s. Three creep and recovery tests were done for each sample.

Relaxation time was determined by the time required for the delayed strain to reach 63.2% of its final value (Steffe, 1996). Compliance (J), defined as the ratio of strain to stress, was monitored as a function of time. Maximum compliance (J_{max}) was the peak compliance reached by the material before the constant stress was removed. Percentage of creep recovery gives an indication of the degree of elasticity in the material and was calculated using the following relationship:

$$\% \text{creep recovery} = \frac{(J_{\text{max}}) - (J_t)}{(J_{\text{max}})} \times 100$$

where J_{max} is the maximum creep compliance and J_t is compliance after recovery.

Statistical Analysis

Chemical and sensory data were analyzed using the GLM procedure of SAS (version 8.2, 2001; SAS Institute, Cary, NC). A split-plot model was used with cheese plant ($n = 4$), replicate ($n = 2$), and location within 291-kg block ($n = 2$) as the whole-plot category variables and the linear and quadratic form of time of aging and the interactions of time of cheese aging with plant, replicate, and location as subplot variables, with time as a continuous variable. Replicate was not nested within plant because other analytical factors were included in the replicate variation that were independent of plant. Statistical significance of the individual terms in the model was deter-

mined when the F -test for the model was ≤ 0.05 . The interaction of plant $\times$ location $\times$ replicate was used as the error term to determine whether the effect of plant, replicate, location, or their interaction was significant. The full model error was used to test the significance of the subplot terms. Because time of cheese aging was treated as a continuous variable in the ANOVA model, the linear and quadratic terms for time would be correlated. Distortion of the ANOVA by multicollinearity of these terms in the model was minimized by centering the time of cheese aging data using a mathematical transformation (Glantz and Slinker, 2001). The time variable was transformed as follows: time = month of aging - [(last month - first month)/2]. This transformation made the data set orthogonal with respect to time of aging. The full model with all interaction terms in the whole and the subplot was run for each parameter (e.g., bitterness, moisture, etc.). After running the full model, terms that were not significant were removed in a step-wise fashion, starting with nonsignificant terms in the subplot with the lowest type III sum of squares. Least squares means were calculated and reported for plant and location within 291-kg block. Analysis of the data with this model allowed us to determine the impact of plant and location (inner vs. outer) within 291-kg block.

Of the 4 cheese plants in the study, 2 used only the stirred-curd method and 2 used only the milled-curd method of Cheddar cheese manufacture. Because no manufacturing plants used both methods, it was not possible to clearly separate the impact of curd type from plant of manufacture because plant and curd type were not independent. Therefore, all statistical analyses were done with manufacturing plant in the model without curd type.

RESULTS

Cheese Composition

Cheese manufacturing plant explained the largest portion of variation in the moisture content of the cheese (Table 1), and the cheese from plant 1 had higher moisture than cheeses from the other plants (Table 2). A moisture difference was observed between portions from the exterior and interior (i.e., location within block; Table 1) of the 291-kg block (Table 2). The magnitude of the differences in moisture between inner and outer locations within the 291-kg blocks in our study (Table 2) were much smaller than those reported in previous studies (Reinbold and Ernstom, 1988; Reinbold et al., 1992; Barbano, 2001).

Temperature-induced moisture migration has been studied extensively. As the temperature of the cheese block cools, the moisture content increases, causing a moisture gradient between the cooler outer locations and

Table 1. Sums of squares (type III) and probability values (in parentheses) for the ANOVA analysis of the impact of plant, location within 291-kg block (inner vs. outer), and aging time (1, 4, 8, and 12 mo) on moisture, pH, fat, fat dry basis, protein, and salt content of portions cut from 291-kg blocks of Cheddar cheeses during 12 mo at 7°C

Factor	df	Moisture, %	pH	Fat, % (wet basis)	Fat, % (dry basis)	Protein, %	Salt, %	Protein hydrolysis, %
Plant	3	17* (<0.01)	0.19* (<0.01)	13.6* (<0.01)	13.5* (<0.01)	19.3* (<0.01)	0.78* (<0.01)	366* (<0.01)
Replicate	1	0.01 (0.96)	0.88* (<0.01)	2.0* (0.04)	3.6* (0.02)	0.81* (0.01)	0.25* (<0.01)	2* (<0.01)
Location	1	9* (<0.01)	<0.01 (0.88)	6.8* (<0.01)	0.6 (0.39)	0.36 (0.10)	<0.01 (0.95)	76* (<0.01)
Plant × replicate	3	13* (<0.01)	0.24* (<0.01)	6.6* (<0.01)	14.3* (<0.01)	11.7* (<0.01)	0.45* (<0.01)	11* (<0.01)
Plant × location	3	NS ¹	NS	NS	NS	NS	NS	18* (0.01)
Plant × replicate × location	4	7.2 (0.08)	0.07 (0.18)	1.5 (0.52)	1.5 (0.73)	2.8* (0.01)	0.03 (0.74)	4.7 (0.44)
Time	1	0.55 (0.43)	0.11* (<0.01)	3.0* (0.01)	2.9* (0.04)	2.3* (<0.01)	0.24* (<0.01)	905* (<0.01)
Time × plant	3	NS	NS	NS	NS	NS	NS	18* (<0.01)
Time × location	1	NS	NS	NS	NS	NS	NS	13* (<0.01)
Time × time	1	NS	NS	NS	NS	NS	NS	65* (<0.01)
Time × time × replicate	1	NS	NS	NS	NS	NS	NS	NS
Error ²	111	102	1.5	54	83	16	1.5	132
R ²		0.28	0.50	0.37	0.29	0.69	0.53	0.92

¹NS = nonsignificant terms removed from the model.²Degrees of freedom of the error term for percentage of protein hydrolysis = 104.**P* < 0.05.

warmer inner locations. Barbano (2001) found that the outer portions of 291-kg cheese blocks of reduced-fat Cheddar cheese always displayed higher moisture content (about 5%) than the inner portions and that moisture migration occurred, moving up, down, and laterally within the block. However, the size of the moisture gradi-

ent was shown to be much smaller in the top half of a 291-kg block than in the bottom half (Barbano, 2001). Olabi and Barbano (2002) confirmed these findings and determined that moisture migrated from a warmer to cooler temperature both with and against gravity. In our study, the inner locations with the 291-kg blocks did not

Table 2. Least squares mean values of moisture, pH, fat, fat dry basis, protein, salt content, and percentage of protein hydrolysis of cheese cut from 291-kg blocks of Cheddar cheeses averaged across 12 mo of aging at 7°C

Variable	Moisture, %	pH	Fat, % (wet basis)	Fat, % (dry basis)	Protein, %	Salt, %	Protein hydrolysis, ¹ %
Plant							
Plant 1	36.5 ^a	5.23 ^a	35.5 ^c	55.9 ^{bc}	23.4 ^b	1.73 ^c	6.40 ^b
Plant 2	35.6 ^b	5.20 ^a	35.7 ^{bc}	55.6 ^c	23.6 ^a	1.93 ^a	9.31 ^a
Plant 3	35.8 ^b	5.24 ^a	36.4 ^a	56.5 ^a	22.7 ^c	1.76 ^{bc}	6.57 ^b
Plant 4	35.6 ^b	5.14 ^b	36.0 ^b	56.0 ^b	22.8 ^c	1.79 ^b	4.48 ^c
Location within 291-kg block ²							
Inner	35.6 ^b	5.20 ^a	36.1 ^a	56.1 ^a	23.2 ^a	1.80 ^a	7.47 ^a
Outer	36.1 ^a	5.20 ^a	35.7 ^b	55.9 ^a	23.1 ^a	1.80 ^a	5.91 ^b

^{a-c}Means in a column (within plant or inner vs. outer location) followed by different letters are different (*P* < 0.05).¹Percentage of protein hydrolysis measured by the *o*-phthaldialdehyde test.²Inner = interior of 291-kg block; outer = exterior of 291-kg block.

Table 3. Least squares mean values of moisture, protein, pH 4.6-soluble nitrogen, and 12% TCA-soluble nitrogen, expressed as a percentage of total nitrogen content, of cheese cut from 291-kg blocks of Cheddar cheeses after aging for 12 mo at 7°C

Variable	Moisture, %	Protein, %	pH 4.6-soluble nitrogen, %	12% TCA-soluble nitrogen, %
Plant				
Plant 1	36.51 ^a	23.22 ^{ab}	25.77 ^b	17.57 ^c
Plant 2	35.04 ^b	23.72 ^a	28.90 ^a	21.51 ^a
Plant 3	35.32 ^b	22.60 ^c	28.68 ^a	19.08 ^b
Plant 4	35.73 ^{ab}	22.74 ^{bc}	24.23 ^b	14.78 ^d
Location within 291-kg block ¹				
Inner	35.32 ^b	23.16 ^a	27.63 ^a	18.65 ^a
Outer	35.99 ^a	22.98 ^a	26.16 ^b	17.82 ^b

^{a-d}Means in a column (within plant or inner vs. outer location within 291-kg block) followed by different letters are different ($P < 0.05$).

¹Inner = interior of 291-kg block; outer = exterior of 291-kg block.

cool as quickly as the outer locations; consequently, the inner locations had a lower moisture content than the outer locations, and this is consistent with previous reports (Reinbold and Ernstrom, 1988; Reinbold et al., 1992; Barbano, 2001). As expected, there was no change (i.e., the linear and quadratic term for time was not significant; Table 1) in the total moisture content over the aging period. No plant $\times$ location within block interaction effect ($P > 0.05$) was detected (Table 1), confirming that the temperature-driven moisture migration within the 291-kg blocks was happening in the same way in all 4 plants, regardless of differences from plant to plant in moisture level or cheese manufacturing practice.

The salt content (Table 2) of the cheese differed from plant to plant, and the plant of manufacture explained most of the variation (i.e., highest type III sum of squares in Table 1) in salt content. However, no difference ($P > 0.05$) in salt content between the inner and outer locations (Tables 1 and 2) was detected.

The cheese pH was lower in plant 4 than in the other 3 plants (Table 2). However, the differences in pH between plant 4 and the other 3 plants were not large; the model explained only 50% of the variation (Table 1). Most of that variation was explained by replicate, and very little of the variation was explained by location within block, despite significant differences in moisture caused by location within the 291-kg block. Cheese pH decreased ($P < 0.05$) with time from an average of 5.23 to 5.15. The downward trend in cheese pH with time for all 4 plants was similar (e.g., no interaction between plant and time). A previous study (Reinbold et al., 1992) found that a pH gradient was present initially in 291-kg blocks, but after cooling for 240 h, the pH had equilibrated between the inner and outer portions. Because an initial (prior to cutting the 291-kg blocks) pH measurement was not taken in our study, it is not known whether there were

large differences in the pH of the inner and outer locations initially.

The fat content of cheese on a wet basis differed from plant to plant (Tables 1 and 2); this would be driven by plant-to-plant differences in the fat-to-CN ratio in the milk used for cheese making and differences in fat loss in the whey during cheese making. Differences in fat content (on a wet basis only) between the inner and outer locations (inner $>$ outer) were consistently observed throughout aging (Tables 3 and 4). Because fat within the structure of cheese does not move and moisture did move with time, it was not surprising that fat on a wet basis was lower in the outer portions of the cheese (Table 2), where the moisture content was high. On average, the fat content on a wet basis increased ($P < 0.05$) with time but the changes in grand mean values (mean of all cheeses) with time were small and variable (35.6, 36.2, 35.6, and 36.2% at 1, 4, 8, and 12 mo, respectively). Although there was a slight but significant trend for an increase in fat content with time, the variation from month to month was large, and this probably represents inconsistency in composition within the 4 quarters of the upper half of the 291-kg block that could not be distinguished from a time effect in the design of our study. These differences in fat content attributable to location were not detected when fat was expressed on a dry basis (Tables 1 and 2), confirming that differences in wet fat content were due to a dilution effect caused by moisture migration during cooling. The protein content of the cheese differed from plant to plant, but this was driven by differences in moisture content of the cheese and protein and fat content differences from plant to plant in the milk used to make the cheese (Tables 1 and 2).

Table 4. Sums of squares (type III) and probability values (in parentheses) for the ANOVA analysis of the impact of plant, location within 291-kg block (inner vs. outer), and aging time (1, 4, 8, and 12 mo) on sensory flavor¹ of cheese cut from 291-kg blocks of Cheddar cheeses after aging for 12 mo at 7°C

Factor	df	Sensory flavor attribute									
		Whey	Diacetyl	Milk fat/ lactone	Sulfur	Brothy	Nutty	Sour	Salty	Sweet	Umami
Plant	3	186* (<0.01)	26* (<0.01)	10* (<0.02)	37* (<0.01)	216* (<0.01)	111* (<0.01)	3* (<0.01)	18* (<0.01)	22* (<0.01)	28* (<0.01)
Replicate	1	38* (<0.01)	1 (0.30)	0.01 (0.87)	44* (<0.01)	32* (<0.01)	2* (0.01)	0.01 (0.87)	0.2 (0.48)	11* (<0.01)	22* (<0.01)
Location	1	12* (<0.01)	4* (<0.01)	2* (<0.01)	4* (0.02)	9* (0.05)	11* (<0.01)	0.1 (0.41)	1* (0.05)	4* (<0.01)	5* (<0.01)
Panelist	18	292* (<0.01)	107* (<0.01)	140* (<0.01)	105* (<0.01)	206* (<0.01)	132* (<0.01)	74* (<0.01)	38* (<0.01)	93* (<0.01)	141* (<0.01)
Plant × replicate	3	NS ²	NS	NS	5* (<0.01)	NS	4* (0.03)	2* (0.02)	2* (0.02)	NS	3* (0.02)
Location × replicate	1	3* (0.05)	2* (<0.01)	2* (<0.01)	NS	2 (0.17)	NS	NS	NS	NS	NS
Plant × location	3	NS	2* (0.02)	NS	NS	NS	NS	NS	NS	NS	NS
Plant × location × replicate	9	8 (0.38)	3* (0.04)	6* (0.05)	3 (0.40)	14 (0.23)	1 (0.89)	1 (0.80)	2 (0.17)	3 (0.50)	1 (0.58)
Time	1	725* (<0.01)	71* (<0.01)	9* (<0.01)	496* (<0.01)	875* (<0.01)	148* (<0.01)	2* (<0.01)	13* (<0.01)	131* (<0.01)	292* (<0.01)
Time × plant	3	31* (<0.01)	5* (<0.01)	5* (<0.01)	4* (0.02)	10* (0.04)	14* (<0.01)	3* (<0.01)	3* (<0.01)	2* (0.05)	NS
Time × location	1	NS	NS	NS	NS	NS	2* (0.02)	NS	NS	2* (<0.01)	NS
Time × replicate	1	18* (<0.01)	3* (<0.01)	NS	NS	NS	NS	6* (<0.01)	5* (<0.01)	6* (<0.01)	1* (0.04)
Time × plant × location	3	NS	3* (0.02)	NS	NS	NS	NS	NS	NS	NS	NS
Time × plant × replicate	3	NS	NS	NS	NS	NS	9* (<0.01)	5* (<0.01)	NS	NS	NS
Time × time	1	4* (0.02)	7* (<0.01)	3* (<0.01)	3* (<0.01)	43* (<0.01)	10* (<0.01)	3* (<0.01)	NS	NS	NS
Time × time × plant	3	22* (<0.01)	NS	NS	3 (0.09)	NS	8* (<0.01)	2 (0.06)	NS	NS	NS
Time × time × replicate	1	33* (<0.01)	2* (<0.01)	NS	37* (<0.01)	52* (<0.01)	7* (<0.01)	NS	NS	NS	NS
Time × time × location	1	NS	NS	NS	NS	NS	NS	1* (0.05)	NS	NS	NS
Error ³ R ²		1,215 0.57	362 0.42	501 0.27	640 0.57	1,842 0.49	636 0.48	337 0.25	340 0.18	429 0.38	395 0.56

¹Fruity, FFA, catty, and bitter tastes were not detected in cheeses. Cooked flavor is not shown because there were no significant differences among the cheeses.

²NS = nonsignificant terms removed from the model.

³Degrees of freedom in the error term varied from 1,484 to 1,497, depending on the nonsignificant terms removed from the model for each sensory flavor attribute.

* $P < 0.05$.

Degree of Protein Hydrolysis

There were large differences in proteolysis during aging among the different plants (Tables 1 and 2), and the statistical model explained a large amount ($r^2 = 0.92$) of the variation in the data. Cheese from plant 2 had more than twice as much proteolysis, averaged across

12 mo, as plant 4 (Table 2) when measured by the OPA test. More proteolysis occurred in the inner locations than in the outer locations within the 291-kg blocks. There was also a significant increase in the percentage of protein hydrolysis for all plants with aging time (Figure 2). There was a significant linear and quadratic component (Table 1) to this age-dependent increase, and

Figure 2. Change in the percentage of protein hydrolysis of inner and outer locations within 291-kg blocks of Cheddar cheese during 12 mo of aging at 7°C (◆ = inner location; ■ = outer location).

there was a time $\times$ plant and time $\times$ location within block interaction. The cheeses with lower moisture (inner location) had a higher degree of hydrolysis. This was related to the temperature gradient during cooling, with lower moisture inner locations remaining at a higher temperature for a longer time than the outer locations.

The pH 4.6 acetate-soluble nitrogen and 12% TCA-soluble nitrogen content of the cheeses expressed as a percentage of total nitrogen are shown in Table 3. These analyses were conducted in a different laboratory from the laboratory where the OPA testing was done. The differences among plants and treatments for moisture and protein tests on the 12-mo samples analyzed at Cornell (Table 3) were consistent with those in the North Carolina State laboratory (Table 2). Both the pH 4.6 and 12% TCA-soluble nitrogen were different among plants and locations within block. There was more proteolysis in the inner than the outer location across all plants, but the influence of the plant on overall proteolysis was much larger than location within block (Table 3). During the first 30 to 60 d of aging, most of the proteolysis is done by the residual chymosin in the cheese (Grappin et al., 1985). Cheeses from plants 2 and 3 had a higher rate of rennet addition, a higher cheese temperature when the cheese was filled into the forms, and also the highest soluble nitrogen content at 12 mo. The cheese in the internal portion of the 291-kg block stays warmer

longer and this may allow more degradation of intact CN by chymosin. The TCA-soluble nitrogen is more selective for lower molecular weight proteolysis products and reflects the completeness or depth of proteolysis caused by the action of proteases and peptides of lysed starter and nonstarter bacteria and their extracellular enzymes, which is sometimes called secondary proteolysis (Rank et al., 1985). The pH 4.6 and 12% TCA-soluble nitrogen were lowest in cheese from plant 4, and this may be due to the combination of a low level of rennet use, a high level of milk fortification, and a lower cheese temperature when the 290-kg blocks were filled.

Flavor Analysis

Descriptive Sensory Analysis of Flavor. Cheeses were characterized by high intensities of diacetyl, cooked, whey, and milk fat/lactone flavors at 1 mo. These flavors are commonly found in young Cheddars (Drake et al., 2001). There were significant manufacturing plant effects for all sensory attributes listed in Table 4, and manufacturing site clearly played a key role in the flavor profiles of cheese. No influence ($P > 0.05$) of the factors in our study on cooked flavor was detected (Table 5). Whey and diacetyl flavors decreased over time for all treatments (Figures 3 and 4), as did milk fat flavor (data not shown). There was an interaction ($P < 0.05$) of plant

Table 5. Least squares mean values of sensory flavor of cheese cut from 291-kg blocks of Cheddar cheeses during 12 mo of aging at 7°C by plant and inner vs. outer location¹

Variable	Sensory flavor attribute										
	Cooked	Whey	Diacetyl	Milk fat/ lactone	Sulfur	Brothy	Nutty	Sour	Salty	Sweet	Umami
Plant											
Plant 1	2.38 ^a	1.92 ^b	0.46 ^b	2.52 ^b	1.33 ^b	1.84 ^c	0.50 ^c	3.37 ^{ab}	3.66 ^c	1.79 ^c	1.38 ^c
Plant 2	2.37 ^a	1.46 ^d	0.31 ^c	2.68 ^a	1.43 ^a	2.37 ^a	1.23 ^a	3.42 ^a	3.93 ^a	2.06 ^a	1.62 ^a
Plant 3	2.43 ^a	1.73 ^c	0.35 ^c	2.55 ^b	1.44 ^a	2.00 ^b	0.76 ^b	3.33 ^b	3.74 ^b	1.90 ^b	1.49 ^b
Plant 4	2.46 ^a	2.59 ^a	0.66 ^a	2.70 ^a	0.96 ^c	1.31 ^d	0.35 ^d	3.42 ^a	3.70 ^{bc}	1.75 ^c	1.25 ^a
Location within 291-kg block ²											
Inner	2.37 ^a	1.84 ^b	0.40 ^b	2.57 ^a	1.34 ^a	1.95 ^a	0.79 ^a	3.40 ^a	3.78 ^a	1.92 ^a	1.49 ^a
Outer	2.40 ^a	2.02 ^a	0.50 ^a	2.65 ^a	1.24 ^b	1.80 ^b	0.63 ^b	3.36 ^a	3.73 ^a	1.83 ^b	1.38 ^b

^{a-d}Means in a column (within plant, milled vs. stirred curd, or inner vs. outer location) followed by different letters are different ($P < 0.05$).

¹Fruity, FFA, catty, and bitter taste were not detected in cheeses.

²Inner = interior of 291-kg block; outer = exterior of 291-kg block.

by both the linear and quadratic terms for time (Table 4) for whey flavor; this can be seen in Figure 3, with whey flavor not decreasing as rapidly in cheese from plant 4. Plant-to-plant differences in whey flavor (Figure 3) may be related to differences in the milk standardization and fortification strategies used in the different cheese plants prior to cheese making. Those plants using a higher level of milk solids fortification (e.g., plant 4) may maintain a higher whey flavor intensity longer. Whey flavor intensity was slightly higher in the outer

locations of 291-kg blocks (Table 5), which is consistent with this being a zone of higher moisture content.

Diacetyl flavor intensity was influenced by location within block (Table 4), with higher diacetyl flavor in the outer locations within the 291-kg blocks (Table 5). There was a significant plant $\times$ time interaction (Table 4); this can be seen in Figure 4, with the difference in diacetyl flavor among plants being larger initially and then becoming more similar among plants by 12 mo of age. These are young, undeveloped flavors and are commonly

Figure 3. Change in whey flavor from a 291-kg block of Cheddar cheese aged 12 mo at 7°C. Data were averaged across block location because this parameter had no significant effect for this attribute (♦ = plant 1; ■ = plant 2; ▲ = plant 3; × = plant 4).

Figure 4. Change in diacetyl flavor from a 291-kg block of Cheddar cheese aged 12 mo at 7°C. Data were averaged across block location (◆ = plant 1; ■ = plant 2; ▲ = plant 3; × = plant 4).

found in Cheddar cheese <4 mo old. With aging time, the intensities of these flavors (i.e., whey, diacetyl, and milk fat/lactone) decreased, consistent with previous studies (Drake et al., 2001; Rehman et al., 2003).

Fruity, FFA, or catty flavors were not detected in any of the cheeses. There was an effect ($P < 0.05$) of the interaction of the linear and quadratic terms for time $\times$ plant on nutty flavor (Table 4). Nutty flavor was more intense in the inner than the outer location within 291-kg blocks (Table 5). High nutty flavor intensities are usually observed only in Cheddar cheeses > 8 mo old (Avsar et al., 2004). The inner locations (Table 5) had higher intensities of aged flavors (nutty, brothy, and sulfur) and the outer locations had higher intensities of young, undeveloped flavors (whey and diacetyl).

There was a significant effect ($P < 0.05$) of plant and time of aging (Table 4) on all the basic tastes, with the rate of increase with aging time differing among plants (data not shown). Bitter taste was not detected in these cheeses. The inner locations were sweeter than the outer locations (Table 5). There were also significant differences in umami intensity between the inner and outer locations within 291-kg blocks. The inner locations had higher umami intensities than the outer locations. Umami is typically higher in aged Cheddar cheeses (Drake et al., 2001), and umami was positively correlated with the aged flavors sulfur, brothy, and nutty ($r^2 > 0.90$) in this study (data not shown). There was no difference

in salty flavor due to location within the 291-kg block, which is consistent with the fact that no differences in salt content were detected among block locations (Tables 2 and 5). In general, even though there were differences in basic tastes among the cheeses, the differences may not be of practical significance, because differences among the means were very small and variation caused by panelists effects (Table 4) accounted for most of the explained variation in the basic tastes.

Instrumental Volatile Analysis. Ten volatile compounds were quantified. Two different groups of flavor compounds were clearly identified: compounds that contribute mainly to young, undeveloped flavors (cooked, whey, diacetyl, and milk fat/lactone flavors) and compounds that contribute to aged, developed flavors (sulfur, brothy, and nutty flavors; Tables 6 and 7). 2,3-Butanedione, 3-hydroxy-2-butanone, and acetic acid all contribute to young flavors (Singh et al., 2003), whereas esters and aldehydes contribute more to aged flavors (Singh et al., 2003). In contrast to sensory perception of flavor, production facility did not have a strong direct influence on the level of volatile compounds, but manufacturing plant was significant in various interactions terms in combination with location within block and time for many of the volatile compounds (Table 6). 2,3-Butanedione (diacetyl), 3-hydroxy-2-butanone (acetoin), acetic acid, and butanoic acid were detected at high concentrations at 1 mo, after which the concentrations decreased (Table 6)

Table 6. Sums of the squares (type III) and probability values (in parentheses) for the ANOVA analysis of the impact of plant, location within 291-kg block (inner vs. outer), and aging time (1, 4, 8, and 12 mo) on the concentration of aroma-active compounds identified in cheese cut from 291-kg blocks of Cheddar cheeses during 12 mo of aging at 7°C

Factor	df	Aroma-active compound									
		2,3-Butanedione (diacetyl)	3-Methyl butanal	Acetic acid	3-Hydroxy- 2-butanone (acetoin)	Methyl butanoate	Ethyl butanoate	Butanoic acid	Hexanal	Methyl hexanoate	Ethyl hexanoate
Plant	3	1.1×10^7 (0.82)	50 (0.97)	1.1×10^{11} (0.99)	3.2×10^6 (0.63)	20 (0.50)	1.2×10^8 (<0.10)	1.4×10^7 (0.25)	1.21×10^5 (0.79)	3.4×10^6 (0.99)	6.0×10^4 (0.48)
Replicate	1	4.5×10^6 (0.56)	71 (0.61)	2.6×10^{11} (0.69)	2.7×10^5 (0.71)	6 (0.41)	1.3×10^8 (0.01)	1.6×10^6 (0.46)	$1.45 \times 10^{5*}$ (0.29)	6.4×10^7 (0.41)	$1.7 \times 10^{5*}$ (0.02)
Location	1	1.4×10^7 (0.31)	18 (0.79)	6.4×10^{10} (0.84)	8.0×10^5 (0.52)	55 (0.06)	2.1×10^7 (0.23)	2.26×10^6 (0.36)	1.3×10^3 (0.92)	9.0×10^4 (0.97)	4.0×10^3 (0.90)
Plant × replicate	3	NS ¹	753* (<0.01)	3.5×10^{12} (<0.01)	NS	388* (<0.01)	$8.0 \times 10^{7*}$ (0.03)	$1.5 \times 10^{7*}$ (0.02)	NS	$3.3 \times 10^{8*}$	NS
Plant × location	3	$5.3 \times 10^{7*}$ (0.05)	740* (<0.01)	$6.9 \times 10^{12*}$ (<0.01)	$1.0 \times 10^{7*}$ (<0.01)	203* (<0.01)	NS	$1.3 \times 10^{7*}$ (0.04)	$2.7 \times 10^{5*}$ (0.01)	$1.9 \times 10^{8*}$ (<0.01)	NS
Replicate × location	1	2.2×10^7 (0.07)	179* (0.01)	NS	NS	75* (<0.01)	NS	NS	$1.3 \times 10^{5*}$ (0.02)	$6.4 \times 10^{7*}$ (<0.01)	NS
Plant × replicate × location	3 to 10	7.0×10^7 (0.11)	660* (<0.01)	$5.7 \times 10^{12*}$ (<0.01)	$1.2 \times 10^{7*}$ (<0.01)	20 (0.42)	8.6×10^7 (0.19)	9.4×10^6 (0.20)	$6.5 \times 10^{5*}$ (<0.01)	$2.1 \times 10^{8*}$ (<0.01)	2.3×10^5 (0.59)
Time	1	$3.0 \times 10^{8*}$ (<0.01)	644* (<0.01)	$1.3 \times 10^{13*}$ (<0.01)	$3.7 \times 10^{7*}$ (<0.01)	1,365* (<0.01)	NS	$4.0 \times 10^{7*}$ (<0.01)	NS	$3.0 \times 10^{8*}$ (<0.01)	$1.3 \times 10^{5*}$ (0.03)
Time × plant	3	NS	1,264* (<0.01)	$6.5 \times 10^{12*}$ (<0.01)	$1.1 \times 10^{7*}$ (<0.01)	704* (<0.01)	NS	$1.5 \times 10^{7*}$ (0.02)	NS	$3.5 \times 10^{8*}$ (<0.01)	NS
Time × location	1	NS	426* (<0.01)	$2.9 \times 10^{12*}$ (<0.01)	NS	73* (<0.01)	NS	NS	NS	$8.3 \times 10^{7*}$ (<0.01)	NS
Time × replicate	1	$1 \times 10^{8*}$ (<0.01)	590* (<0.01)	$7.6 \times 10^{12*}$ (<0.01)	NS	363* (<0.01)	NS	$2.0 \times 10^{7*}$ (<0.01)	$1.5 \times 10^{5*}$ (0.03)	$6.7 \times 10^{7*}$ (<0.01)	NS
Time × plant × location	3 or 4	NS	1,188* (<0.01)	$8.1 \times 10^{12*}$ (<0.01)	$1.8 \times 10^{7*}$ (<0.01)	308* (<0.01)	NS	$1.9 \times 10^{7*}$ (0.02)	NS	$4.1 \times 10^{8*}$ (<0.01)	NS
Time × plant × replicate	3	NS	1,054* (<0.01)	$4.3 \times 10^{12*}$ (<0.01)	NS	519* (<0.01)	NS	$1.8 \times 10^{7*}$ (<0.01)	NS	$3.3 \times 10^{8*}$ (<0.01)	NS
Time × time	1	$1.8 \times 10^{8*}$ (<0.01)	82 (0.08)	$4.7 \times 10^{12*}$ (<0.01)	$9.2 \times 10^{6*}$ (<0.01)	387* (<0.01)	$8.3 \times 10^{7*}$ (<0.01)	NS	NS	$7.2 \times 10^{7*}$ (<0.01)	$6.3 \times 10^{5*}$ (<0.01)
Time × time × plant	3	NS	451* (<0.01)	$2.9 \times 10^{12*}$ (<0.01)	$1.1 \times 10^{7*}$ (<0.01)	290* (<0.01)	$7.7 \times 10^{7*}$ (0.03)	NS	$2.3 \times 10^{5*}$ (0.03)	$1.0 \times 10^{8*}$ (<0.01)	NS
Time × time × location	1	NS	222* (<0.01)	$1.8 \times 10^{12*}$ (<0.01)	NS	NS	NS	NS	NS	$4.1 \times 10^{7*}$ (<0.01)	NS
Time × time × replicate	1 or 2	$5.7 \times 10^{7*}$ (<0.01)	83 (0.09)	$5.0 \times 10^{12*}$ (<0.01)	NS	101* (<0.01)	$8.3 \times 10^{7*}$ (<0.01)	$1.6 \times 10^{7*}$ (<0.01)	$2.4 \times 10^{5*}$ (<0.01)	NS	NS
Error ² R ²		131 0.50	126 0.75	124 0.76	131 0.64	130 0.87	127 0.26	133 0.50	130 0.35	124 0.77	144 0.25

¹NS = not significant term removed from the model.²Degrees of freedom in the error term varied from 124 to 144, depending on the number of nonsignificant terms removed from the model for each aroma-active compound.**P* < 0.05.

Table 7. Least squares mean values of volatile concentrations ($\mu\text{g/kg}$) of aroma-active compounds identified in cheese cut from 291-kg blocks of Cheddar cheeses during 12 mo of aging at 7°C

Variable	Aroma-active compound									
	2,3-Butanedione (diacetyl)	3-Methyl butanal	Acetic acid	3-Hydroxy-2 butanone (acetoin)	Methyl butanoate	Ethyl butanoate	Butanoic acid	Hexanal	Methyl hexanoate	Ethyl hexanoate
Retention index on DB-5 MS column	668	686	700	718	723	776	850	853	996	1,000
Threshold, ¹ $\mu\text{g/kg}$	9	1.5	36,000	2,900	31	4.7	860	18	1.7	90
Plant										
Plant 1	860 ^a	1.6 ^a	28,000 ^a	393 ^a	1.8 ^b	1,862 ^a	74 ^a	158 ^a	336 ^a	132 ^a
Plant 2	1,519 ^a	1.3 ^a	620,000 ^a	355 ^a	1.6 ^b	379 ^a	579 ^a	116 ^a	99 ^a	89 ^a
Plant 3	1,151 ^a	6.1 ^a	120,000 ^a	291 ^a	5.1 ^a	716 ^a	437 ^a	140 ^a	3,055 ^a	128 ^a
Plant 4	1,520 ^a	1.3 ^a	160,000 ^a	686 ^a	1.3 ^b	366 ^a	938 ^a	129 ^a	933 ^a	94 ^a
Location within 291-kg block ²										
Inner	946 ^a	3.6 ^a	110,000 ^a	358 ^a	3.0 ^a	1,220 ^a	378 ^a	132 ^a	1,776 ^a	109 ^a
Outer	1,579 ^a	1.5 ^a	350,000 ^a	505 ^a	1.9 ^b	442 ^a	636 ^a	139 ^a	436 ^a	112 ^a

^{a,b}Means in a column (within plant or inner vs. outer location) followed by different letters are different ($P < 0.05$).

¹Thresholds reported orthonasally in water by Rychlik et al. (1998).

²Inner = interior of 291-kg block; outer = exterior of 291-kg block.

sharply with time (Figure 5), as confirmed by the fact that the quadratic term for time was significant for 2,3-butanedione (diacetyl), 3-hydroxy-2-butanone (acetoin), and acetic acid (Table 6). The decrease in butanoic acid with time was more linear (Table 6 and Figure 5). 2,3-Butanedione (diacetyl) and 3-hydroxy-2-butanone (ace-

toin) likely contributed to the buttery/diacetyl flavors (Singh et al., 2003) present in the cheeses after 1 mo of aging. The acetoin (3-hydroxy-2-butanone) content of the cheeses was highly variable and differed greatly among plants at 1 mo (range from 342 to 2,358 $\mu\text{g/kg}$) but by 8 and 12 mo the levels among plants were virtually

Figure 5. Change in volatile concentration from a 291-kg block of Cheddar cheese aged 12 mo at 7°C. There were no significant differences in concentration between inner and outer locations ($P > 0.05$; ◆ = acetic acid; ■ = 2,3-butanedione (diacetyl); ▲ = 3-hydroxy-2-butanone (acetoin); × = butanoic acid).

identical (Table 6, time $\times$ time and time $\times$ plant interactions). Acetic acid concentrations in cheese were highly variable and differences were mostly due to complex interaction effects among time, plant, and location within block (Table 6). Both acetic and butanoic acids have previously been identified in mild Cheddar cheese (Milo and Reineccius, 1997). Variation in butanoic acid content of the cheeses was mostly explained by interactions of time, plant, and block location (Table 6), with butanoic acid decreasing with time of aging (Figure 5). All these compounds (2,3-butanedione, 3-hydroxy-2-butanone, acetic acid, and butanoic acid) were present above the sensory threshold at 1 mo. Milo and Reineccius (1997) reported these compounds to be key aroma-active compounds in mild Cheddar cheese.

Prior to 4 mo of aging, 3-methyl butanal was not detected (data not shown). 3-Methyl butanal is one of the compounds that causes a nutty flavor in Cheddar cheese (Avsar et al., 2004). Nutty flavor was higher in the inner location within 291-kg blocks (Table 5), but 3-methyl butanal concentrations were not detected to be higher ($P > 0.05$) in this location within the 291-kg blocks (Tables 6 and 7).

Prior to 8 mo of aging, the esters methyl hexanoate and methyl butanoate were not detected in the cheeses. The concentration of methyl hexanoate and methyl butanoate increased rapidly between 8 and 12 mo of aging, as seen by the significant effects of linear and quadratic terms for time (Table 6). Methyl butanoate concentration was higher in the inner than outer locations within the 291-kg blocks (Table 7), whereas no differences in methyl hexanoate (Table 7) could be detected because of high variation in the data. The esters were likely formed from short- and medium-chain FFA (Singh et al., 2003) and are also not usually present in high concentrations in Cheddars <8 mo old. No differences were detected in the concentrations of ethyl butanoate or ethyl hexanoate. The compounds that contributed to aged flavor (esters and aldehydes) were present in the highest concentrations after 12 mo of aging (data not shown). Because of the high degree of variation in the instrumental volatile analysis data, it was difficult to detect any significant differences in these compounds attributable to location within block. However, the significant time-dependent changes in young and aged cheese flavor compounds (Table 6) generally supported the time-dependent changes in descriptive sensory flavor analysis results (Table 4).

Texture Analysis

Descriptive Sensory Analysis. When sensory texture attributes of the cheeses were plotted as a function of time of aging, most attributes increased slightly in

value from 1 to 4 mo of aging and then remained the same after 4 mo (data not shown). Two exceptions to this were hand springiness and hand rate of recovery. Both these attributes demonstrated large decreases ($P < 0.05$) in value with age that were predominately linear in character for all treatments (Table 8). The inner locations within 291-kg blocks had higher hand firmness (Tables 8 and 9) than outer locations, which is consistent with the lower moisture content of the inner location. There was a significant time $\times$ block location interaction (Table 8), with the magnitude of the difference in hand firmness between the inner and outer locations decreasing with time of aging. The outer locations were more cohesive, had a smoother mass, and had more residual mouth coating than inner locations with 291-kg blocks (Table 9). Initially, there was no difference in cohesiveness between the inner and outer locations, but cohesiveness for the inner and outer locations changed with aging (time $\times$ location within block interaction), with the outer locations developing more cohesiveness (Figure 6). Fracturability changed very little with time of aging. No differences in mouth firmness attributable to plant, location within block, or time were detected. After 8 mo, all cheeses were more cohesive, were more adhesive, and had a smoother mouth coating (data not shown) because of the significant impact of time (Table 8).

Rheological Analysis

Large Strain Analysis. There was no effect of production facility on true shear stress, strain, or modulus (data not shown). Fracture stress did not change with time of aging, whereas strain decreased from 0.55 to 0.23 and modulus increased from 69 to 216 kPa with time. In this study, as modulus increased, hand springiness and hand rate of recovery decreased. Previous research has shown that the fracture modulus is negatively correlated with hand springiness and hand rate of recovery for young cheeses (Brown et al., 2003). Location within the 291-kg block had an impact on fracture strain, with the least squares means for inner and outer locations being 0.43 and 0.32, respectively. Fracture strain decreased with time of aging and was higher for the outer location (0.43) than the inner location (0.32) within the 291-kg block. Our data are consistent with those of Brown et al. (2003), who found that fracture strain was highly correlated with firmness and fracturability and similar trends were observed ($r^2 > 0.8$).

Creep and Recovery Analysis. No significant effects ($P > 0.05$) were observed among plants (data not shown) on measures of creep compliance or recovery. Significant time-dependent decreases from 1 to 12 mo of aging in percentage of creep recovery (from 49.2 to 34.8%), relaxation time from 128 to 79 s, $J_{\max}$ (from 0.0035 to 0.0023

Table 8. Sums of squares (type III) and probability values (in parentheses) for the ANOVA analysis of the impact of plant, location within 291-kg block (inner vs. outer), and aging time (1, 4, 8, and 12 mo) on sensory texture of cheese cut from 291-kg blocks of Cheddar cheeses during 12 mo of aging at 7°C

Factor	df	Sensory texture attribute									
		Hand firmness	Hand springiness	Hand rate of recovery	Mouth firmness	Fracturability	Breakdown	Cohesiveness	Adhesiveness	Mouth coating	Smoothness of mass
Plant	3	16 (0.12)	420* (0.01)	333* (<0.01)	NS ¹	15 (0.77)	622 (0.73)	35 (0.14)	154* (<0.01)	78* (<0.01)	134* (0.02)
Replicate	1	8 (0.07)	899* (<0.01)	495* (<0.01)	NS	50 (0.10)	641 (0.27)	34* (0.03)	0.1 (0.89)	0.1 (0.88)	5 (0.27)
Location	1	109* (<0.01)	107* (0.03)	78* (0.03)	NS	29 (0.18)	638 (0.27)	26* (0.05)	2 (0.46)	29* (<0.01)	34* (0.04)
Panelist	9	302* (<0.01)	713* (<0.01)	303* (0.03)	NS	153* (<0.01)	2,730 (0.61)	156* (<0.01)	202* (<0.01)	152* (<0.01)	146* (<0.01)
Plant × replicate	3	121* (<0.01)	454* (<0.01)	364* (<0.01)	NS	NS	NS	159* (<0.01)	23* (0.02)	70* (<0.01)	74* (<0.01)
Plant × location	3	46* (<0.01)	48 (0.09)	NS	NS	10 (0.07)	NS	NS	NS	NS	15 (0.06)
Plant × replicate × location	4 to 10	6 (0.56)	41 (0.22)	58 (0.08)	NS	94* (<0.01)	4,700 (0.25)	32 (0.13)	23 (0.18)	10 (0.39)	8 (0.24)
Time	1	NS	2,830* (<0.01)	1,481* (<0.01)	NS	46* (<0.01)	NS	506* (<0.01)	647* (<0.01)	136* (<0.01)	263* (<0.01)
Time × plant	3	25* (<0.01)	NS	NS	NS	37* (<0.01)	NS	36* (<0.01)	17* (0.05)	NS	22 (0.01)
Time × location	1	16* (<0.01)	NS	NS	NS	NS	NS	15* (0.02)	21* (<0.01)	NS	NS
Time × replicate	1	NS	663* (<0.01)	529* (<0.01)	NS	NS	NS	151* (<0.01)	253* (<0.01)	110* (<0.01)	192* (<0.01)
Time × plant × location	3	NS	NS	NS	NS	NS	NS	27* (0.02)	19* (0.04)	20* (<0.01)	20* (0.04)
Time × plant × replicate	3 to 6	38* (<0.01)	NS	100.0* (<0.01)	NS	25* (<0.01)	NS	35* (<0.01)	33* (<0.01)	14* (0.02)	35* (<0.01)
Time × time	1	23* (<0.01)	237* (<0.01)	122* (<0.01)	NS	NS	NS	255* (<0.01)	373* (<0.01)	70* (<0.01)	186* (<0.01)
Time × time × plant	3	51* (<0.01)	NS	NS	NS	10 (0.06)	NS	28* (0.02)	NS	NS	NS
Time × time × replicate	1	NS	235* (<0.01)	91* (<0.01)	NS	6* (0.04)	NS	NS	51* (<0.01)	14* (<0.01)	45 (<0.01)
Error ²		1,875	7,140	4,961	668,830	1,364	373,694	2,772	2,212	1,361	1,947
R ²		0.30	0.49	0.46	0.02	0.28	0.02	0.39	0.39	0.39	0.41

¹NS = nonsignificant terms removed from the model.

²Degrees of freedom in the error term varied from 975 to 993, depending on the number of nonsignificant terms removed from the model for each sensory texture attribute.

**P* < 0.05.

Table 9. Least squares mean values of sensory texture of cheese cut from 291-kg blocks of Cheddar cheeses during 12 mo of aging at 7°C

Variable	Sensory texture attribute									
	Hand firmness	Hand springiness	Hand rate of recovery	Mouth firmness	Fracturability	Breakdown	Cohesiveness	Adhesiveness	Smoothness of mass	Mouth coating
Plant										
Plant 1	10.2 ^a	7.0 ^a	5.8 ^{ab}	8.8 ^a	5.7 ^a	9.6 ^a	9.4 ^a	8.5 ^b	8.6 ^{bc}	9.7 ^b
Plant 2	10.1 ^a	5.6 ^b	4.5 ^c	11.85 ^a	6.0 ^a	9.9 ^a	9.7 ^a	9.1 ^a	8.8 ^b	9.7 ^b
Plant 3	9.7 ^a	7.0 ^a	5.5 ^b	8.1 ^a	5.2 ^a	10.2 ^a	10.3 ^a	9.2 ^a	9.4 ^a	10.3 ^a
Plant 4	10.3 ^a	7.3 ^a	6.0 ^a	8.8 ^a	5.7 ^a	11.7 ^a	9.0 ^a	8.3 ^b	8.4 ^c	9.5 ^b
Location within 291-kg block ¹										
Inner	10.4 ^a	6.4 ^b	5.2 ^b	10.5 ^a	5.8 ^a	9.6 ^a	9.5 ^b	8.7 ^a	8.6 ^b	9.6 ^b
Outer	9.8 ^b	7.1 ^a	5.7 ^a	8.3 ^a	5.5 ^a	11.1 ^a	9.8 ^a	8.8 ^a	9.0 ^a	10.0 ^a

^{a-c}Means in a column (within plant or inner vs. outer location) followed by different letters are different ($P < 0.05$).

¹Inner = interior of 291-kg block; outer = exterior of 291-kg block.

Pa^{-1}), and γ_{rec} (from 0.0025 to 0.0013 Pa^{-1}). This is expected because during aging, there is an increase in proteolysis, a decrease in hand springiness and hand rate of recovery, and an increase in fracturability. This indicates that the cheese network is breaking down to some extent (as expected during aging). As the cheese network breaks down, the elastic elements of the cheese decrease, and the recovery declines as well. The percentage of creep recovery and J_r are indicators of the viscoelastic properties of cheese and the ability of the cheese to recover from a deformation (Brown et al., 2003). Therefore, during aging as the cheese networks break down, the elastic elements decrease and the cheese becomes more viscous, which is reflected in the decrease

of J_r and creep recovery. There was an effect ($P < 0.05$) of location within block, with the values being higher for the inner location for percentage of creep recovery (44 vs. 40%), J_r (5.26×10^{-6} vs. 3.67×10^{-6} Pa^{-1}), and J_{max} (0.0033 vs. 0.0024 Pa^{-1}).

DISCUSSION

Cheese Manufacturing Conditions

As would be expected, some important cheese manufacturing procedures and conditions differed from one commercial cheese plant to another (listed in the materials and methods section), and that may be why the term

Figure 6. Change in cohesiveness measured by sensory texture in inner and outer locations of Cheddar cheese removed from a 291-kg block aged 12 mo at 7°C (◆ = inner location; ■ = outer location).

for plant in the ANOVA model explained a large amount of the variation in flavor scores (Table 4). Those conditions that might have influenced flavor and texture development are rennet type and use rate, addition of enzymes to increase flavor development during aging, temperature of the curd going into the 291-kg mold, fortification of milk for cheese making with nonfat milk solids, and cooling conditions after pressing. Those differences in conditions specific to the production facility would be expected to influence the overall flavor and texture development during aging.

Flavor Development

The main flavor reactions in Cheddar cheese are lipolysis and proteolysis. Flavor development in Cheddar can be greatly affected by the conditions during cheese making, the aging conditions, and the initial composition of the cheese as well as how these composition parameters change throughout aging (Banks et al., 1995; Chen et al., 1998). When looking solely at moisture content, it was unexpected that the inner locations within 291-kg blocks generally had more intense aged Cheddar cheese flavors given that the moisture content of these locations within 291-kg blocks was lower than at the outer locations (Table 5). The salt-to-moisture ratio can also influence proteolysis, but there was very little difference in the salt-to-moisture ratio in inner vs. outer locations within block in our study. During the first few days after manufacture, the salt-to-moisture ratio is the main influence controlling the water activity of the cheese (Lawrence et al., 2004). The water activity influences bacterial growth and enzyme activity, which are the main drivers for proteolysis (Lawrence et al., 2004), with all other factors being equal. Differences in fat content were noted (inner > outer), but the differences were very small (Table 2). However, even though there were differences in the fat content between inner and outer locations, proteolysis was likely the main reaction controlling flavor development in these Cheddar cheeses during 12 mo of aging. Proteolysis is crucial in the formation of flavor compounds. The catabolism of AA results in many different aroma-active compounds that have previously been shown to be important in Cheddar cheese flavor (Christensen and Reineccius, 1995; Milo and Reineccius, 1997; Singh et al., 2003). About half of all flavor compounds in cheese result from the breakdown of AA, especially Met and Leu (Yvon and Rijnen, 2001).

As mentioned, the inner location within the 291-kg blocks displayed higher percentages of protein hydrolysis, pH 4.6 acetate buffer, and TCA-soluble nitrogen, even though these cheeses had lower moisture. This clearly affected aged Cheddar flavor formation in the inner and outer locations (Tables 4 and 5) within the

291-kg blocks in this study. The outer locations not only had higher intensities of the young, undeveloped flavors cooked and diacetyl, but also had lower intensities of the aged, developed flavors sulfur and brothy. These results suggest that aged Cheddar flavor developed more rapidly in the inner locations (more protein hydrolysis and less moisture) than in the outer locations (less protein hydrolysis and more moisture). A direct example of this was nutty flavor formation. This flavor is formed by the aldehydes 2/3-methyl butanal and 3-methyl propanal, which are formed from the Strecker degradation of Leu (Singh et al., 2003; Avsar et al., 2004). Prior to 4 mo of aging, 3-methyl butanal was not detected. It takes several months for this compound to be formed at concentrations above sensory thresholds, which is why nutty flavor is not commonly observed in Cheddar cheeses <8 mo old (Avsar et al., 2004). The presence of this compound was correlated with nutty flavor in this study as well ($r^2 > 0.65$, $P < 0.05$).

What caused more proteolysis and flavor development in the inner locations of the 291-kg blocks? The combination of a higher rennet use rate and higher temperature of the cheese going into the 291-kg blocks (i.e., longer time to cool) for plants 2 and 3 caused more proteolysis during aging (Table 1, plant $\times$ time interaction; Figure 2) and more aged cheese flavor development (Tables 4 and 5). The lowest proteolysis in plant 4 was caused by the combination of the lowest rennet use rate per unit of milk, the high level of milk fortification (i.e., even less rennet per unit of CN), the lower temperature of curd going into the hoop, and the low cooling temperature. Cheese produced in plant 2 had the highest level of proteolysis (Tables 2 and 3) and also had the highest nutty and brothy flavors (Table 5). Plant 4 had the highest intensity of young cheese flavors (Table 5), and this was probably related to the high level of fortification of the milk and low rennet use rate. The time it takes for the inner portion to cool is much longer compared with the outer portion of the block (Reinbold et al., 1992). Depending on the cooling conditions, it can take as long as 12 to 14 d for the interior portion of 291-kg blocks to cool completely, depending on the material used for the cheese hoop (Reinbold et al., 1992). Temperature has a large influence on the rate of proteolysis, and it is likely that chymosin produced more primary proteolysis in the inner portion of the block because of the higher temperature for a longer time than in the outer portion of the 291-kg blocks. This is supported by the higher pH 4.6 acetate buffer soluble nitrogen (which is increased mostly by chymosin activity) observed in the inner location within the 291-kg blocks (Table 3). Also, the higher temperature in the interior portion of the block may favor the growth of a different mixture of nonstarter lactic acid bacteria compared with exterior locations, and

this could influence peptidase activity (higher 12% TCA-soluble nitrogen) and the development of other volatile flavor compounds.

Texture Development

Although proteolysis was likely the driver of differences in flavor formation among different locations within the 291-kg block of cheese, other factors appeared to influence texture. The pH of the cheese influences CN solubility (Lawrence et al., 2004). At pH 5.35, CN hydration is maximized (Lawrence et al., 2004), which would result in a softer texture. However, in our study, the pH was very similar for inner and outer locations within 291-kg blocks (Table 2), and differences in pH did not appear to influence texture. In our study, moisture was likely the main influence in texture differences between locations within 291-kg blocks. The relative ratios of fat, protein, and moisture affect the texture (Hort and Le Grys, 2001). Increased moisture contributed to a softer texture (outer locations), whereas lower moisture contributed to more brittle cheeses (inner locations). These properties were evident by both sensory and rheological measurements. Higher moisture can weaken the protein network because the volume fraction of the protein is lower when more water is present (Lawrence et al., 2004). Increased brittleness is consistent with the fracture strain being higher ($P < 0.05$) in the outer location than in the inner location (0.43 vs. 0.32).

From the proximate analysis results, one can deduce that the moisture gradient had an impact on texture. The inner locations within 291-kg blocks were consistently more firm and fracturable. This result was not expected because the inner locations within 291-kg blocks had a higher degree of protein hydrolysis, which should have made the texture less firm than the outer locations, which had less proteolysis (Lucey et al., 2003). Extensive proteolysis may severely break down the CN structure, resulting in a less firm texture (Lucey et al., 2003); however, in all the cheeses in the current study, hand firmness was almost constant during 12 mo of aging, whereas there were large increases in proteolysis. Therefore, proteolysis may be less important with respect to changes in firmness than for some other texture attributes, such as hand springiness and hand rate of recovery, which decrease greatly with increasing proteolysis.

The higher moisture content of the outer locations within 291-kg blocks also influenced the sensory texture attributes. A higher moisture content is associated with a less rigid cheese matrix (Lucey et al., 2003). The outer locations within 291-kg blocks were more springy and cohesive, and had a more smooth mass in the mouth and mouth coating than the inner locations (Table 9).

The inner locations were firmer (hand only; Table 9). Firmness, mouth fracturability, and hand fracturability were highly correlated with each other and were negatively correlated with the breakdown terms (breakdown, cohesiveness, adhesiveness). These findings were in agreement with those observed by Brown et al. (2003) for Mozzarella cheese using the same texture lexicon.

CONCLUSIONS

Large differences were observed in both the cheese flavor and volatile profiles among cheese plants. These differences among plants were caused in part by differences in rennet use rate, time-temperature relationships during cooling, and the level of milk fortification. Differences also were observed between the inner and outer locations within the 291-kg blocks. The inner locations developed aged flavors more rapidly and intensely than the outer locations. A moisture gradient was present (outer > inner), and this undoubtedly influenced flavor and texture. However, the degree of protein hydrolysis was greater in the inner locations than the outer locations. The inner locations within the 291-kg blocks developed more aged flavor. It is likely that the higher temperature of the cheese in the inner location within the 291-kg block for a longer time during cooling caused more aged cheese flavor to develop in the inner location of the blocks. There were also differences in the inner and outer locations for sensory texture. The inner locations were firmer and the outer locations were more cohesive in the mouth. The moisture gradient appeared to have more impact on texture than on flavor in the inner vs. outer locations within the 291-kg blocks. The differences in flavor and texture development observed between inner and outer locations from different 291-kg blocks could be very important when determining end product use. Industrially, cheese can have many applications, such as food ingredients, cheese shreds or slices, or further aging prior to shredding, slicing, or direct consumption. The inner locations, with a firmer texture and more flavor, could be used in some applications, whereas the softer, less flavorful outer blocks may be better suited to other applications. However, the logistics of sorting cheese for different uses based on location within a 291-kg block is probably not practical in a high-speed industrial cheese-cutting operation; therefore, it would be better to develop a technological solution that could achieve more uniform composition and flavor within the 291-kg blocks.

ACKNOWLEDGMENTS

Funding provided in part by the California Dairy Research Foundation (Davis, CA) and Dairy Management,

Inc. (Rosemont, IL). Our sincere thanks to the companies that participated in this study. Manuscript FSR0708-2007 of the Department of Food Science, North Carolina State University. The use of trade names in the publication does not imply endorsement by these organizations or criticisms of ones not mentioned.

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