J. Dairy Sci. 87:1999–2010

Characterization of Nutty Flavor in Cheddar Cheese

Y. K. Avsar,[1] Y. Karagul-Yuceer,[2] M. A. Drake,[3]
T. K. Singh,[4] Y. Yoon,[3] and K. R. Cadwallader[4]
[1]Department of Dairy Technology, Mustafa Kemal University, Antakya, Turkey
[2]Department of Food Engineering, Onsekiz Mart University, Canakkale, Turkey
[3]Department of Food Science, Southeast Dairy Foods Research Center, North Carolina State University, Raleigh
[4]Department of Food Science and Human Nutrition, University of Illinois, Urbana

ABSTRACT

The objectives of this study were to determine the volatile components responsible for the sensory perception of nutty flavor in Cheddar cheese. Cheddar cheeses with and without nutty flavors were selected by descriptive sensory analysis. Volatile aroma components from Cheddar cheeses with and without nutty flavors were isolated and characterized using solvent extraction with high vacuum distillation, dynamic headspace analysis, gas chromatography-olfactometry, and gas chromatography-mass spectrometry. More than 50 aroma-active compounds were detected in Cheddar cheeses. Consistent differences were observed between nutty and not nutty Cheddar cheeses. Strecker aldehydes were detected in higher amounts in Cheddar cheeses with nutty flavors compared with Cheddar cheeses without nutty flavors. Strecker aldehydes, dimethyl sulfide, and propionic acid were evaluated in young and aged Cheddar cheese models for nutty flavor by descriptive sensory analysis. Dimethyl sulfide and propionic acid did not contribute to nutty flavor in Cheddar cheese. The addition of Strecker aldehydes to young (<4 mo old) Cheddar cheese models resulted in nutty/malty flavor perceived by sensory analysis. When Strecker aldehydes were incorporated into aged (>9 mo old) Cheddar cheese models, nutty flavor perception increased. Strecker aldehydes contribute to nutty flavor in aged Cheddar cheese.
(**Key words:** Cheddar cheese, cheese flavor, nutty flavor, Strecker aldehyde)

Abbreviation key: DHS-GC-MS = dynamic headspace analysis-gas chromatography-mass spectrometry, **DSE** = direct solvent extraction, **FID** = flame ionization detector, **GC-MS** = gas chromatography-mass spectrometry, **GCO** = gas chromatography-olfactometry, **GCO-DHS** = gas chromatography-olfactometry dynamic headspace analysis, **HVD** = high vacuum distillation, **N** = nutty, **NN** = not nutty.

INTRODUCTION

Cheese flavor is one of the most important criteria determining consumer choice and acceptance. Cheddar cheese flavor varies widely with source, age, and fat content. However, aged Cheddar cheese flavor is characterized by sulfur, brothy, and nutty flavors (Urbach, 1997; Drake et al., 2001). The role of sulfur compounds in Cheddar cheese flavor (Milo and Reineccius, 1997) and their formation from sulfur containing amino acids by bacterial activity (Urbach, 1995; Weimer et al., 1999) or Strecker degradation (Griffith and Hammond, 1989) have been investigated extensively and reviewed (Weimer et al., 1999). Unlike sulfur flavor, knowledge on the nutty flavor of Cheddar cheese is scarce. First of all, defining the sensory term "nutty" appeared to be a difficult task, as the aroma quality in all nuts are not exactly the same (Clark and Nursten, 1977). Drake et al. (2001) developed a defined sensory language for Cheddar cheese flavor. Nutty flavor was defined as the "(nonspecific) nut-like aromatic associated with different nuts." Lightly toasted unsalted nuts, unsalted wheat thins, or roasted peanut oil extract were used as references for nutty flavor. It is not clear whether nutty flavor is a product of a single compound or a combined effect of several compounds. Also, nutty character and the volatile source of nutty flavor may vary with different types of cheese (Clark and Nursten, 1977).

The majority of studies on nutty flavor in cheese have been carried out on Swiss type cheese due to its distinct sweet and nutty notes. A range of compounds, such as ketones, lactones, esters, alcohols, aldehydes, pyrazines, sulfurous compounds, carbonyl compounds, free fatty acids, free amino acids, and salts have been reported to contribute to nutty flavor (Biede and Hammond, 1979a, 1979b; Liardon et al., 1982; Vangtal and Hammon, 1986; Warmke et al., 1996; Preininger et al., 1996; Rychlik and Bosset, 2001a). Specifically, acetic and propionic acids, the major products of propionic

Received October 29, 2003.
Accepted December 12, 2003.
Corresponding author: M. A. Drake; e-mail: mdrake@unity.ncsu.edu.

acid bacteria, were claimed to play an important role in nutty flavors of this particular cheese type. It is important to note that descriptive sensory analysis using a defined sensory language was not conducted in these studies nor were model studies conducted to confirm or pinpoint the exact cause of nutty flavors.

Numerous studies have likewise been conducted to reveal the overall chemical profile of Cheddar cheese flavor and identify the most potent compounds (Milo and Reineccius, 1997; Suriyaphan et al., 2001; O'Riordan and Delahunty, 2001; Zehentbaur and Reineccius, 2002). The presence of pyrazines in Cheddar cheese has been reported in a few studies (Suriyaphan et al., 2001). In addition, 2-acetylthiazoline (Milo and Reineccius, 1997), 2-acetyl-1-pyroline, and 2-acetyl-2-thiazoline (Zehentbauer and Reineccius, 2002) were identified as compounds in Cheddar cheese that exhibited nutty aromas. However, no sensory analysis revealing the relationship between those compounds and the nutty flavor of Cheddar cheese were reported. In their research, Fernandez-Espla and Fox (1998) produced Cheddar cheese with nutty flavor using propionic acid bacteria as an adjunct culture. The researchers stated that this flavor was associated with increased levels of free amino acids and that the cheese flavor resembled that of Swiss cheese. Descriptive sensory analyses were not conducted.

Identifying specific chemical compounds associated with particular flavors requires extensive and specific instrumental and sensory analysis. First, the sensory perceived flavor of a food should be identified by descriptive sensory analysis. Descriptive sensory analysis qualitatively and quantitatively identifies all of the sensory-perceived flavor and tastes present in the food and provides definitions and references for the perceived flavors and tastes (Drake and Civille, 2003). Instrumental analysis can then be conducted to identify volatile compounds that contribute to flavor. The presence of a particular compound, identified by gas chromatography-mass spectrometry, does not necessarily mean the compound plays a role in flavor because many volatile compounds can be present in a food, but concentrations may be below actual sensory thresholds (McGorrin, 2002). Gas chromatography-olfactometry (**GCO**) can assist in identification of compounds that are actually present in the sensory threshold range, and it is often used as a way of further screening volatile compounds that play key roles in flavor (Friedrich and Acree, 1998). However, the aroma of an individual compound identified by instrumental analysis is not necessarily indicative of its role on flavor in a food due to interactions with the matrix and other compounds (Drake and Civille, 2003). Model systems, similar to the actual food, should then be constructed to evaluate the role of specific compound(s) on sensory-perceived flavor. To our knowledge, compounds responsible for nutty flavor in Cheddar cheese have not been reported and specifically linked to sensory perception of nutty flavor in Cheddar cheese. The purpose of this study was, therefore, to identify and quantify volatile compound(s) responsible for nutty flavor in Cheddar cheese.

MATERIALS AND METHODS

Experimental Overview

Cheddar cheeses were screened and selected for initial sensory and instrumental experiments. Due to sample size limitations, additional cheeses were selected for further experiments to confirm observations.

Cheese Selection

Forty 5-kg blocks of Cheddar cheese were purchased on the retail market and screened for nutty flavor by 3 sensory experts, each with more than 150 h of experience in the sensory evaluation of cheese flavor. Cheeses selected (15 total) were 1 to 3 yr old. Cheeses with intense nutty (**N**) flavors (designated as N1, N2, N3, …, N8) and without nutty (**NN**) flavor (designated as NN1, NN2, NN3, …, N7) were selected for volatile aroma analyses and descriptive sensory analysis.

Chemicals

Diethyl ether (anhydrous, 99.8%), sodium chloride (99%), sodium sulfate (99%), and 2-methyl-3-heptanone (internal standard for neutral/basic fractions) were purchased from Aldrich Chemical Company (St. Louis, MO) and 2-methylpentanoic acid (internal standard for acidic fractions) was obtained from Lancaster (Windham, NH). Aroma compounds listed in Tables 2, 3, 4, and 7 below were provided from the following commercial sources: numbers 1 to 4, 6, 8, 9, 10 to 12, 14 to 20, 22 to 26, 28, 29, 30, 33, 35, 37 to 39, 42 to 46, 50, 52 to 54, 56, 59, 61 to 66 (Aldrich Chemical Co.); numbers 47 and 48 (Sigma, St. Louis, MO), number 13 (Lancaster), and number 59 (Firmenich Inc., Plainsboro, NJ). Sodium bicarbonate (99.7%), hydrochloric acid (36.5%), and acetic acid (number 42) were obtained from Fisher Scientific (Pittsburgh, PA).

Sensory Evaluation of Cheeses

A trained sensory panel (n = 8) evaluated the selected cheeses using a lexicon developed for Cheddar cheese flavor (Drake et al., 2001). Definitions and references for the terms used are given in Table 1. Panelists each received 75 h training on descriptive analysis of cheese

Table 1. Basic sensory terms used for descriptive analysis of cheese and cheese models.[1]

Term	Definition	References
Cooked	Aromatics associated with cooked milk	Skim milk heated to 85°C for 30 min
Whey	Aromatics associated with Cheddar cheese whey	Fresh Cheddar whey
Diacetyl	Aromatics associated with diacetyl	Diacetyl
Lactone	Aromatics associated with milkfat	Fresh coconut meat, heavy cream, δ-dodecalactone
Sulfur	Aromatics associated with sulfurous compounds	Boiled mashed egg, struck match, hydrogen sulfide bubbled through water
Brothy	Aromatics associated with boiled meat or vegetable stock	Knorr beef broth cubes, Knorr vegetables broth cubes, Wyler's low-sodium beef broth cubes, canned potatoes, methional
Free fatty acid	Aromatics associated with short-chain fatty acids	Butanoic acid
Fruity	Aromatics associated with different fruits	Fresh pineapple, canned pineapple juice
Nutty	The nut-like aromatic associated with different nuts	Lightly toasted unsalted nuts, wheat germ, unsalted wheat thins
Sweet	Fundamental taste sensation elicited by sugars	Sucrose (5% in water)
Salty	Fundamental taste sensation elicited by salts	Sodium chloride (0.5% in water)
Sour	Fundamental taste sensation elicited by acids	Citric acid (0.08% in water)
Bitter	Fundamental taste sensation elicited by caffeine, quinine	Caffeine (0.08% in water)
Umami	Fundamental meaty taste elicited by monosodium glutamate (msg)	Monosodium glutamate (msg, 1% in water)

[1]Adapted from Drake et al., 2001.

flavor using the Spectrum descriptive analysis method (Meilgaard et al., 1999). Panelists evaluated cheeses individually in booths with free access to bottled water and unsalted crackers for palate cleansing. Panelists expectorated cheeses following analysis. Cheeses were presented in 2- × 2-cm cubes with 3-digit codes at 10°C. Cheeses were evaluated in triplicate by each panelist.

Sample Preparation

Direct solvent extraction (DSE). Cheese extracts were prepared according to the methods of Milo and Reineccius (1997), with some modifications as described as follows. Cheeses were frozen at −20°C for 24 h and then grated using a hand grater. Freshly grated cheese (100 g) was weighed and divided into 2 Teflon bottles (capacity of 250 mL) equipped with Tefzel closures. Diethyl ether (100 mL) and 20 μL of internal standard solution (0.202 μg/μL of 2-methyl-3-heptanone and 0.228 μg/μL of 2-methylpentanoic acid in methanol for neutral/basic and acidic fractions, respectively) were added to each bottle. The mixture was shaken for 30 min on a Roto mix (Thermolyne, type 50800; Dubuque, IA) at high speed. The bottles were then centrifuged at 3000 × g for 10 min in order to separate the solvent phase from the mixture, which was subsequently collected into a jar. The procedure was repeated 2× with 50 mL of diethyl ether. The solvent phases were combined and kept at −20°C overnight. The extract was dried over anhydrous sodium sulfate and concentrated to 100 mL under a stream of nitrogen gas.

High vacuum distillation (HVD). Separation of volatile compounds from cheese extract was achieved by means of high vacuum distillation as described by Karagul-Yuceer and co-workers (2001). The assembly used was similar to that described by Sen and co-workers (1991). For HVD, the cheese solvent extract was transferred to a 1-L, round-bottom flask and immersed in a Dewar flask containing liquid nitrogen until it was frozen. The flask was then connected to a unit equipped with a rough pump/diffusion pump, as a vacuum source; a receiving tube; and a waste tube. The receiving tube and waste tube were held in separate Dewar flasks containing liquid nitrogen at all times. Distillation was carried out for 4 h under vacuum (approx. 10^{-5} Torr). For the first 2 h, the sample flask was kept at room temperature; for the second 2 h, the sample was kept in a water bath at 50°C. After distillation, the contents of the receiving flask were concentrated to 20 mL under a gentle stream of nitrogen gas. Concentrated distillate was then washed 2× with 15 mL of sodium bicarbonate (0.5 M) and 3× with saturated sodium chloride solution. The upper layer (ether) containing the neutral/basic fraction was collected, dried over anhydrous sodium sulfate, and concentrated to 0.5 mL under a gentle stream of nitrogen gas. To recover acidic volatiles, the bottom layer (aqueous phase) was acidified with hydrochloric acid (18%) to 2 to 2.5 pH, extracted 3× with diethyl ether, and dried over anhydrous sodium sulfate before concentrating to 0.5 mL under a nitrogen gas stream.

Dynamic headspace analysis-gas chromatography-mass spectrometry (DHS-GC-MS). Dynamic headspace analysis was conducted using a CDS 6000 headspace analyzer (CDS Analytical, Inc., Oxford, PA). A cheese slurry mixture (5 g) was prepared by mixing grated cheese with deodorized water (1:2 ratio, wt/wt), to which 2 g of NaCl and 50 ppm of 2-methyl-2-butenal (internal standard) were added. The sample was placed in a dark area for equilibration at room temperature for 60 min. The sample was then placed into the analyzer where helium gas (30 mL/min) was purged into the mixture at 30°C for 30 min. Once the purge process was complete, the trap was dry-purged for 10 min to eliminate moisture prior to desorption and transfer. The transfer line was set at 180°C. The injector temperature was 250°C. Volatiles were thermally desorbed and injected in split mode (1:1 ratio, vol/vol). The chromatographic separation system consisted of an HP GC 5890 and MSD 5972 (Hewlett-Packard, Palo Alto, CA) equipped with a DB5 column (30-m × 0.25-mm i.d. × 0.25-μm film thickness, J&W Scientific, Folsom, CA). The oven temperature was programmed from –20 to 220°C at 8°C/min from –20 to 60°C for 0 min, and 6°C/min from 60 to 220°C for 5 min. Initial and final hold times were 6.5 and 5 min, respectively.

Gas chromatography-olfactometry (GCO) of solvent extracts. Two methods of sniffing were used for solvent extract fractions: postpeak intensity and aroma extract dilution analysis (Friedrich and Acree, 1998; Van Ruth, 2001). For postpeak intensity, an HP5890 series II gas chromatograph (Hewlett-Packard, Palo Alto, CA) equipped with a flame-ionization detector (**FID**), a sniffing port, and a splitless injector was used. From each fraction of every extract, 2 μL was injected into a polar capillary column (DB-WAX 30-m length × 0.25-mm i.d. × 0.25-μm film thickness (d_f); J&W Scientific) and a nonpolar column (DB-5ms 30-m length × 0.25-mm i.d. × 0.25-μm d_f; J&W Scientific). Column effluent was split 1:1 between FID and sniffing port using deactivated fused silica capillaries (1 m length × 0.25 mm i.d.). Gas chromatographic oven temperature was programmed from 40 to 200°C at a rate of 10°C/min, with initial and final hold times of 5 and 15 min, respectively. The FID and sniffing port were maintained at a temperature of 250°C. The sniffing port was supplied with humidified air at 30 mL/min. Three experienced sniffers evaluated the neutral/basic and acid fractions of cheese extract 2× on the 2 different columns, described the odor, and scored the intensity using a 10-point numerical intensity scale. Sniffers each had more than 60 h of experience with GCO and GCO techniques, including scaling and aroma description.

Aroma extract dilution analysis of solvent extract fractions was conducted using an HP6890 series GC (Agilent Technologies Inc., Palo Alto, CA) equipped with a DB-FFAP (15-m × 0.32-mm i.d. × 0.25-μm film thickness; J&W Scientific), a cool on-column injector, an FID, and a sniff port (DATU Technology Transfer, Geneva, NY). Oven temperature was programmed from 35 to 225°C at 10°C/min, with an initial and final hold times of 5 and 15 min, respectively. Helium was used as a carrier gas at 10 mL/min (velocity of 70 cm/s). Solvent extracts were diluted stepwise with diethyl ether at a ratio of 1/3. The dilution procedure was followed until no odorants were detected by sniffers. The highest dilution was reported as the flavor dilution factor (Grosch, 1993).

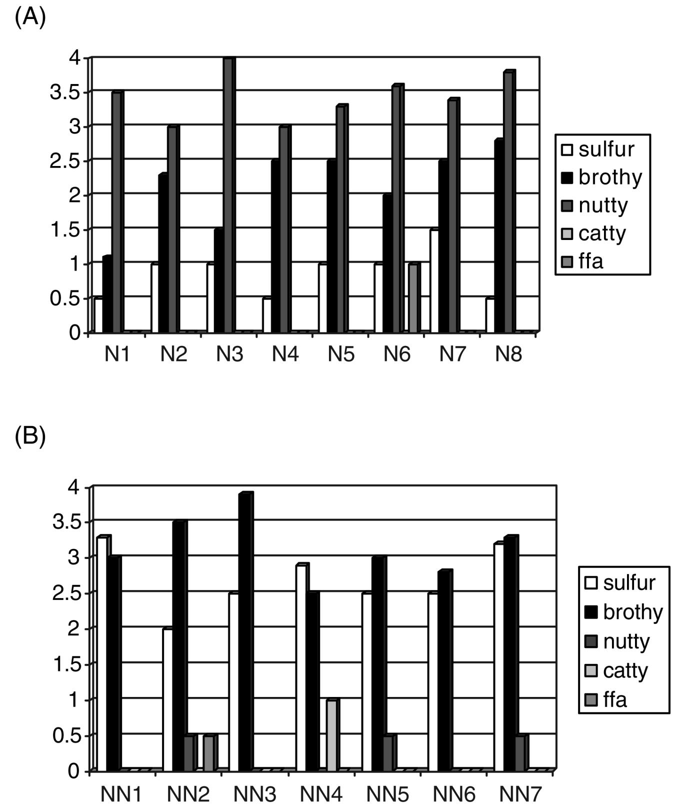

Figure 1. Mean scores of aged/developed flavors for selected nutty (A) and not nutty (B) cheeses. Flavors include: sulfur, brothy, nutty, catty, and free fatty acid (ffa). All Cheddar cheeses were greater than 1 yr old. Flavor intensities are scored on a 10-point scale (Drake et al., 2001).

Gas chromatography-olfactometry of dynamic headspace analysis (GCO-DHS). For the dynamic headspace sampling, a grated cheese sample (10 g), in duplicate, was first equilibrated in a 3-neck glass purge vessel (280-mL volume, jacketed; Custom Glass Shop) to 45°C for 20 min, followed by purging of headspace volatiles on to adsorbent Tenax TA 60/80 (200 mg/trap) using nitrogen (flow rate 50 mL/min, ultra high purity). Flavor dilution analysis was performed by varying headspace purge times (25, 5, and 1 min), as described by Cadwallader and Baek (1998). Volatiles adsorbed on the Tenax were thermally desorbed (splitless-mode; Thermal Desorption system TDS2, Gerstel GmbH & Co. KG, Germany) and cryo-focused before injection (–150°C, solvent venting mode; cooled injection system

Table 2. Odor-active volatiles in neutral/basic fractions of not nutty (NN) and nutty (N) Cheddar cheeses by postpeak intensity (10-point scale).

		RI[2]			Odor intensity[4]			
No.	Compound[1]	DB-5	DB-Wax	Odor[3]	NN1	NN2	N1	N2
1	2,3-Butanedione[A]	623	979	Buttery	1.3	0.5	0.25	3.5
2	1-Hexen-3-one[B]	747	1099	Rubbery	1.0	. . .	1.25	. . .
3	Hexanal[A]	805	1073	Green/cut grass	1.8	. . .	. . .	0.5
4	Ethylbutanoate[A]	808	1022	Fruity, bubblegum	2.8	2.3	1.75	2.0
5	Unknown	829	. . .	Plastic, coffee like	. . .	1	2.0	1.5
6	2-Methyl-3-furanthiol[B]	873	. . .	Vitamin	1.5	4	. . .	3.8
7	Unknown	890	. . .	Soil like, mushroom	. . .	. . .	1.5	. . .
8	(Z)-4-heptenal[B]	904	1216	Fatty, rancid	. . .	1	. . .	. . .
9	Methional[A]	911	1450	Potato	5.0	3.8	3.25	8.0
10	2-Acetyl-1-pyrroline[B]	925	1323	Popcorn	0.5	. . .	0.75	5.0
11	Ethylpentanoate[B]	929	1137	Fruity, bubblegum	. . .	2.3	. . .	. . .
12	Dimethyltrisulfide[B]	970	1365	Sulfur like	. . .	1	. . .	. . .
13	1-Octen-3-one[B]	982	1295	Mushroom	3.5	2.8	2.0	4.5
14	Ethyl hexanoate[A]	1000	1224	Fruity, sweet	0.5	1	2.25	1.5
15	Unknown	1005	. . .	Nutty, dirty	0.8	. . .	2.25	4.5
16	(E)-2-octenal[B]	1039	. . .	Dirty, stale	. . .	. . .	0.5	1.5
17	Phenylacetaldehyde[A]	1051	1638	Rosey, floral	2.0	. . .	. . .	4.5
18	Tetramethylpyrazine[A]	1075	. . .	Nutty	. . .	3.3	2.5	4.5
19	*p*-Cresol[A]	1084	2078	Fecal, band aid	. . .	2	. . .	4.5
20	2-Isopropyl-3-methoxy pyrazine[B]	1091	1425	Dirty, nutty	3.5	0.5	5.5	4.5
21	Unknown	1100	. . .	Fatty	3.0	2.5	. . .	. . .
22	2-Acetyl-2-thiazoline[B]	1115	1725	Popcorn, roasted nutty	3.3	3	3.25	5.0
23	2,3-Diethyl-5-methylpyrazine[B]	1151	. . .	Sweet, nutty	. . .	0.8	0.75	3.5
24	(E, Z) 2,6-Nonadienal[B]	1153	1583	Cucumber	1.5	1.8	0.5	2.0
25	(E) 2-Nonenal[B]	1168	1535	Hay, tobacco	1.0	2.8	3.0	2.5
26	2-Isobutyl-3-methoxypyrazine[B]	1192	. . .	Nutty	1.0	2.0	4.5	4.5
27	Unknown	1245	. . .	Dirty, fatty	0.8	1.5	2.0	3.3
28	δ-Octalactone[A]	1306	1977	Dirty, lactone	1.3	2.3	2.0	3.3
29	(E, E) 2,4-Decadienal[B]	1332	1313	Frier oil	2.5	. . .	. . .	. . .
30	γ-Nonalactone[A]	1344	2020	Coconut	1.0	2.0	2.0	1.0
31	Unknown	1369	. . .	Lactone, sweet, nutty	0.5	2.5	1.5	1.8
32	Unknown	1440	. . .	Lactone, dirty	. . .	. . .	0.5	2.5
33	3-Methylindole[A]	1473	2489	Fecal	. . .	1.3	. . .	5.0
34	Unknown	1501	. . .	Brothy, dirty	1.0	. . .	1.0	5.0
35	δ-Decalactone[A]	1547	2179	Coconut	0.5	. . .	2.75	2.5
36	Unknown	1658	. . .	Coconut	0.5	2.8	0.5	1.5
37	γ-Dodecalactone[A]	1668	2366	Coconut	. . .	. . .	0.5	4.0
38	6-Dodecenyl-γ-lactone[A]	1701	2390	Coconut	2.0	2.0	3.5	4.0
39	δ-Dodecalactone[A]	1730	. . .	Coconut	. . .	. . .	2.5	2.5
40	Unknown	1780	. . .	Coconut	2.0	0.3	1.0	1.3
41	Unknown	1821	. . .	Coconut	. . .	. . .	0.5	2.0

[1]Compounds designated with a superscript A were positively identified (retention indices, odor, mass-spectrometry); those designated with a superscript B were tentatively identified (retention indices, odor).

[2]Retention indices (RI) calculated from gas chromatography-olfactometry results.

[3]Odor description at the sniffing port during gas chromatography-olfactometry.

[4]Odor intensities represent the mean from extracts evaluated in duplicate by 3 sniffers.

CIS4, Gerstel GmbH & Co.) for GCO analysis. The GCO system consisted of an HP6890 series GC (Agilent Technologies Inc.) equipped with a DB-FFAP (15-m × 0.53-mm i.d. × 1-μ film thickness; J&W Scientific), an FID, and a sniffing port. Oven temperature was programmed from 30 to 225°C at 10°C/min, with initial and final hold times of 2 and 10 min, respectively. Helium was used as a carrier gas at 10 mL/min (velocity of 70 cm/s). Two experienced sniffers evaluated each sample/purge time combination. A flavor dilution factor was calculated for each odorant by dividing the highest purge time tested (25 min) by the purge time at which it was last detected by GCO-DHS (e.g., either 25, 5, or 1 min).

For the quantification of selected headspace volatiles, 5 μL of internal standard solution (1.03 μL/μL of 2-methyl-3-heptanone in methanol) was added to grated cheese (25 g), in duplicate. The mixture was quickly kneaded (10 to 15 s) and reformed into a block, wrapped in aluminum foil, and kept at 4°C for 180 min for equilibration. At the end of the equilibration period, the cheese block was grated, and 10-g cheese aliquot, in duplicate, was first equilibrated in a 3-neck glass purge vessel (280-mL volume) to 45°C for 20 min followed by

Table 3. Odor-active volatiles in the acidic fractions of not nutty (NN) and nutty (N) Cheddar cheeses by postpeak intensity (10-point scale).

No.	Compound[1]	R^2 DB-5	R^2 DB-Wax	Odor[3]	Odor intensity[4] NN1	NN2	N1	N2
42	Acetic acid[A]	625	1448	Vinegar	3.9	. . .	3.0	4.2
43	Propionic acid[A]	916	1498	Sour	. . .	. . .	3.5	4.0
44	Isobutyric acid	966	1520	Sour	6.4	2.9	2.0	4.2
45	Butanoic acid[A]	844	1624	Cheesy	. . .	2.3	3.3	3.5
46	Pentanoic acid[A]	933	1720	Sweaty	5.8	. . .	3.0	4.0
47	Hexanoic acid[A]	1036	1862	Sweaty	2.5	2.0	6.0	5.9
48	Maltol[A]	1060	1991	Burnt sugar	. . .	7	. . .	. . .
49	Unknown	1077		Medicinal	. . .	. . .	4.7	6.3
50	Sotolon[B]	1145	2196	Curry	3.4	3.2	4.5	6.2
51	Unknown	1190		Chemical	4	9	. . .	10

[1]Compounds designated with a superscript A were positively identified (Retention indices, odor, mass-spectrometry); those designated with a superscript B were tentatively identified.

[2]Retention indices (RI) calculated from gas chromatography-olfactometry results.

[3]Odor description at the sniffing port during gas chromatography-olfactometry.

[4]Odor intensities represent the mean from extracts evaluated in duplicate by 3 sniffers.

purging of headspace volatiles on to adsorbent Tenax TA 60/80 (200 mg/trap) using nitrogen (flow rate 50 mL/min, ultra high purity). Volatiles adsorbed on the Tenax were thermally desorbed (splitless-mode; Thermal Desorption system TDS2) and cryo-focused before injection (−150°C, solvent venting mode; cooled injection system CIS4) for GC-MS analysis. The GC-MS system consisted of an HP6890 series GC/5973 mass selective detector (Agilent Technologies Inc.), and separation of desorbed volatiles was performed on a DB-FFAP (30-m × 0.25-mm i.d. × 0.25-μm film thickness; J&W Scientific). Oven temperature was programmed from 20 to 225°C at 4°C/min, with initial and final hold times of 5 and 15 min, respectively. Helium was used as a carrier gas at 1.2 mL/min. The MSD conditions were as follows: capillary direct interface temperature, 280°C; ionization energy, 70 eV; mass range, 35 to 300 amu; scan rate, 5.27 scans/s.

Gas chromatography-mass spectrometry. For GC-MS analysis of solvent extracts, a HP5890 Series II GC/HP 5972 mass selective detector (MSD, Hewlett-Packard) was used. Separations were performed on fused silica capillary column (DB-WAX 30-m length × 0.25-mm i.d. × 0.25-μm d_f, J&W Scientific). Helium gas was used as a carrier at a constant flow of 1 mL/min. Oven temperature was programmed from 40 to 200°C at a rate of 5°C/min, with initial and final hold times of 5 and 45 min, respectively. The MSD conditions were as follows: capillary direct interface temperature, 280°C; ionization energy, 70 eV; mass range, 33 to 330 amu; EM voltage (Atune+200 V); scan rate, 5 scans/s. Each extract (2 μL) was injected in the splitless mode. Duplicate analyses were performed on each sample. Based on MS results, relative concentrations of the compounds identified positively were calculated.

Identification of Odorants

For positive identifications, retention indices, mass spectra, and odor properties of unknowns 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 National Institute of Standards and Technology (NIST, 1992) mass spectral database or on matching the retention indices values and odor properties of unknowns against those of authentic standards. For the calculation of retention indices, an n-alkane series was used (Van den Dool and Kratz, 1963).

Sensory Evaluation of Cheese Models

Cheese models were prepared from mild Cheddar cheese (≤4 mo age) or aged Cheddar cheese (≥1 yr), previously confirmed by descriptive sensory analysis to be free of nutty flavor. The cheeses were grated and portioned (50 g). All chemical solutions to be tested were prepared in ethanol at the suitable concentration so that maximum volume to be added did not exceed 100 μL. Chemical solutions were introduced by a clean, disposable micropipette. After addition of the chemicals, cheese models were kneaded for 3 min, then molded to a rectangular shape, and equilibrated overnight at refrigerator temperature (5°C). Cheese models were evaluated by descriptive sensory analysis using the same procedure applied to nutty and not nutty Cheddar cheeses.

Freshly grated young or aged Cheddar cheeses were separately spiked with 2-methylbutanal (0 to 1000 ppb), 3-methylbutanal (0 to 1000 ppb), 2-methylpropanal (0

Table 4. Aroma-active compounds in the neutral/basic fractions of not nutty (NN) and nutty (N) Cheddar cheeses by aroma extract dilution analysis.

				Flavor dilution factor[4]			
No.	Compound[1]	RI[2]	Odor[3]	NN1	NN2	N1	N2
52	2/3-Methylbutanal	928	*Dark chocolate, malty*	9	3	9	27
1	2,3-Butanedione	980	*Buttery*	9	3	3	9
4	Ethyl butanoate	1029	*Fruity, bubble gum*	27	27	81	27
2	1-Hexen-3-one	1093	*Plastic water bottle*	27	9	9	9
11	Ethyl pentanoate	1134	*Fruity*	. . .	27	9	3
14	Ethyl hexanoate	1232	*Fruity, ripe berry*	9	3	81	3
8	(Z)-4-Heptenal	1233	*Rancid*	9	. . .	. . .	3
53	3-Hydroxy-2-butanone	1273	*Buttery, sour*	9	3	9	27
13	1-Octen-3-one	1295	*Mushroom*	81	9	9	3
10	2-Acetyl-1-pyrolline	1336	*Popcorn, roasty*	. . .	3	. . .	3
12	Dimethyltrisulfide	1371	*Sulfurous, cabbage*	3	3	9	9
20	3-Isopropyl-2-methoxypyrazine	1427	*Earthy, soil*	27	9	9	9
16	(E)-2-Octenal	1436	*Fresh, milky*	3	9		9
9	Methional	1451	*Potato*	243	243	729	243
54	(Z)-2-Nonenal	1492	*Stale, hay*	3	. . .	. . .	3
25	(E)-2-Nonenal	1535	*Stale, hay*	3	3	. . .	3
55	Unknown	1565	*Melon, stale*	9	. . .	. . .	3
56	3-Hydroxy-2-pentanone	1576	*Buttery*	. . .	9	. . .	. . .
24	(E,Z)-2,6-Nonadienal	1589	*Cucumber*	3	. . .	. . .	3
17	Phenylacetaldehyde	1646	*Rosy, styrene*	3	. . .	. . .	3
57	Unknown	1721	*Stale, metallic, green*	9	27	9	9
58	Unknown	1732	*Hay, saffron*	3	3	3	3
22	2-Acetyl-2-thiazoline	1760	*Popcorn, roasty*	81	9	3	3
59	β-Damascenone	1822	*Applesauce*	81	81	27	27
60	Unknown	1856	*Tobacco, hay*	27	. . .	. . .	3
61	2-Phenylethanol	1901	*Rosy, floral*	3	3	3	9
28	δ-Octalactone	1976	*Floral, peachy*	27	27	9	27
30	γ-Nonalactone	1998	*Floral, peachy*	3	3	9	9
62	δ-Nonalactone	2023	*Coconut oil*	9	9	3	3
19	p-Cresol	2047	*Dung, stable*	9	27	27	27
63	γ-Decalactone	2137	*Coconut*	9	27	9	81
35	δ-Decalactone	2190	*Coconut, peachy*	81	243	27	81
64	o-Aminoacetophenone	2202	*Corn tortilla, musky*	27	81	9	27
37	γ-Dodecalactone	2380	*Coconut, peachy*	81	81	3	9
38	(Z)-6-Dodecenyl-γ-lactone	2393	*Coconut, peachy*	729	729	243	243
33	3-Methylindole	2481	*Fecal, mothballs*	27	27	9	27

[1]Compounds sniffed during gas chromatography-olfactometry.

[2]Retention indices (RI) calculated from gas chromatography-olfactometry results on DB-FFAP column.

[3]Odor description at the sniffing port during gas chromatography-olfactometry.

[4]Flavor dilution factor on DB-FFAP column.

to 1000 ppb), propionic acid (0 to 250 ppm), and dimethyl sulfide (0 to 1000 ppb) across a concentration range reasonably expected in Cheddar cheese based on results from this study and on previous literature with Cheddar or Swiss cheeses (Dunn and Lindsay, 1985; Rychlik and Bosset, 2001). These compounds were selected based on the GCO-DHS results and, in the case of propionic acid, hypotheses from many previous studies (Preinenger et al., 1996; Rychlik and Bosset, 2001). Both young and aged Cheddar cheeses were used in the model studies. We hypothesized that nutty flavor perception might require the appropriate balance of other minor compounds that are present in aged Cheddar but not present in young Cheddar cheese, because Cheddar cheeses with intense nutty flavors are generally >9 mo of age.

Statistical Analysis

Sensory data and relevant instrumental quantitative results were analyzed using ANOVA (PROC GLM) with means separation to determine differences among treatments (SAS version 8.2, Cary, NC). Significance was established at $P < 0.05$.

RESULTS AND DISCUSSION

Sensory Analysis of Cheeses

Selected cheeses exhibited several common aged Cheddar cheese flavors (Drake et al., 2001). Not nutty cheeses had sulfur and brothy notes predominantly. Nutty cheeses also exhibited sulfur and brothy flavors but had high intensities of nutty flavor (Figure 1). No

Table 5. Relative abundance of selected potent odorants in neutral/basic fractions of not nutty (NN) and nutty (N) Cheddar cheeses.

	R1[1]		Mean ± SE (μg/kg)[2]			
			Non-nutty		Nutty	
Compounds	DB-5	DB-Wax	NN1	NN2	N1	N2
2,3-Butanedione	623	979	ND[3]	ND	ND	78.7 ± 5.1
3-Hydroxy-2-butanone	718	1268	172.3 ± 3.1	133.7 ± 8.6	99.1 ± 0.5	227.5 ± 24.8
Hexanal	805	1073	35.3 ± 5.7	ND	ND	9.8 ± 2.7
Ethylbutanoate	808	1022	ND	ND	ND	6.0 ± 0.2
Methional	911	1450	0.4 ± 0.02	ND	ND	18.0 ± 2.4
Ethyl hexanoate	1000	1224	29.8 ± 0.2	31.8 ± 0.6	75.9 ± 10.2	60.3 ± 5.5
Trimethylpyrazine	1005	—	0.09 ± 0.0002	0.3 ± 0.08	0.11 ± 0.0004	74.3 ± 6.2
Phenylacetaldehyde	1051	1638	1.8 ± 0.6	ND	8.6 ± 8.6	40.7 ± 3.1
p-Cresol	1084	2078	4.6 ± 4.2	11.9 ± 1.4	33.5 ± 0.7	0.6 ± 0.001
δ-Octalactone	1306	1977	63.2 ± 15.4	58.0 ± 19.7	58.0 ± 4.8	48.3 ± 2.9
γ-Nonalactone	1344	2020	2.0 ± 0.06	2.4 ± 0.9	2.3 ± 0.37	2.4 ± 0.14
3-Methylindole	1473	2489	ND	ND	ND	0.5 ± 0.06
δ-Decalactone	1547	2179	409.3 ± 158.6	439.0 ± 184.5	357.7 ± 22.1	382.8 ± 54.5
γ-Dodecalactone	1668	2366	29.7 ± 13.3	22.9 ± 5.9	27.9 ± 0.95	46.1 ± 11.4
6-Dodecenyl-γ-lactone	1701	2390	21.3 ± 7.9	22.1 ± 7.0	23.6 ± 2.4	28.3 ± 16.2
δ-Dodecalactone	1730		144.2 ± 65.6	183.7 ± 42.5	159.5 ± 6.8	141.9 ± 41.7

[1]Retention indices (RI) calculated from gas chromatography-olfactometry results.

[2]Mean relative abundance = concentration of internal standard × peak area of compound/peak area of the internal standard. Data are mean values of duplication.

[3]ND = Not detected.

differences were detected in basic tastes of cheeses ($P > 0.05$) (data not shown). All cheeses selected were >1 yr of age.

Volatile Compounds Detected by DSE-HVD

Odor properties of 41 compounds were described in the neutral/basic fractions of cheeses (Table 2). Of the compounds, 16 were positively identified, 14 were tentatively identified, and 11 compounds remained unknown. Identified compounds included 7 aldehydes, 6 lactones, 3 ketones, 3 esters, 3 sulfurous compounds, and 7 nitrogen containing compounds. Twelve odor-active compounds were detected in acidic fractions (Table 3), including 6 fatty acids, 1 pyrane, 1 furanone, and 2 unknowns. These compounds have been reported in Cheddar cheese previously (Christensen and Reineccius, 1995; Milo and Reineccius, 1997; Suriyaphan et al., 2001), and their formation pathways were reviewed elsewhere (Urbach, 1997; McSweeney and Sousa, 2000).

Postpeak intensity sniffing scores indicated that there were no consistent differences between the nutty and not nutty cheeses (Tables 2 and 3). Aroma extract dilution analyses of the neutral/basic fractions of cheeses are summarized in Table 4. As mentioned earlier, this analysis was conducted with a different column (FFAP) and cool on-column injection. As might be

Table 6. Relative abundance of selected volatiles in acidic fractions of not nutty (NN) and nutty (N) Cheddar cheeses.

	RI[1]		Mean ± standard error (μg/kg)[2]			
			Not nutty		Nutty	
Compound	DB-5	DB-Wax	NN1	NN2	N1	N2
Acetic acid	625	1448	8939.2 ± 283.4	8541.4 ± 2016.1	9639.5 ± 340	7530.0 ± 863.1
Propionic acid	916	1498	200.0 ± 15.0	168.9 ± 10.1	148.6 ± 2.9	119.8 ± 7.1
Butanoic acid	844	1624	19,638.5 ± 2480.3	30,026.6 ± 7420.9	17,680.0 ± 1678.1	14,520.6 ± 2092.1
Pentanoic acid	933	1720	422.9 ± 32.4	1165.9 ± 5.4	351.3 ± 14.2	357.9 ± 15.3
Hexanoic acid	1036	1862	8223.8 ± 921.2	19,404.4 ± 1177.8	8729.0 ± 316.3	7837.0 ± 1092.3
Maltol	1060	1943	1.7 ± 0.6	2.3 ± 0.3	1.7 ± 0.2	3.3 ± 0.2
Octanoic acid	1270	2030	3871.1 ± 51.0	7920.3 ± 20.4	5097.1 ± 966.5	3537.4 ± 483.5
Decanoic acid		2250	2716.9 ± 141.4	5022.8 ± 466.8	4337.1 ±	2004.5 ± 524.9
Dodecanoic acid		2438	109.8 ± 57.3	309.1 ± 5.6	447.4 ± 43.6	141.0 ± 59.4

[1]Retention indices (RI) calculated from gas chromatography-olfactometry results.

[2]Mean relative abundance = concentration of internal standard × peak area of compound/peak area of the internal standard. Data are mean values of duplication.

Table 7. Aroma-active components of not nutty (NN) and nutty (N) Cheddar cheeses by gas chromatography-olfactometry (GCO) of dynamic headspace samples.

	RI[1]		Flavor dilution factor[3] (odor intensity)		
			Not nutty		Nutty
Compound	FFAP	Odor[2]	NN3	NN4	N3
65 Dimethylsulfide	706	Cut cabbage	5 (*1*)	5 (*1*)	25 (*3*)
66 2-Methylpropanal	770	Dark chocolate	—	5 (*3*)	25 (*3*)
52 2/3 Methylbutanal	915	Dark chocolate	25 (*1*)	25 (*1*)	25 (*4*)
1 2,3 butanedione	980	Buttery	25 (*1*)	25 (*1*)	25 (*3*)
4 Ethyl butyrate	1052	Fruity, bubble gum	25 (3)	25 (1)	25 (3)
14 Ethyl hexanoate	1244	Fruity, berry-like	1 (2)	5 (1)	25 (3)
13 1-octen-3-one	1320	Mushroom	25 (2)	25 (1)	25 (3)
12 Dimethyltrisulfide	1386	Sulfurous, cabbage	25 (2)	25 (1)	25 (4)
9 Methional	1497	Potato	25 (2)	25 (2)	25 (4)

[1]Retention indices (RI) on DB-FFAP calculated from GCO results.

[2]Odor description at the gas chromatography-sniffing port during GC.

[3]Flavor dilution factor = highest purge time tested (25 min) divided by lowest purge time in which odorant was last detected by GCO (either 25, 5, or 1 min). Numbers in parentheses represent postpeak odor intensities.

expected, additional highly volatile components, 2/3-methylbutanal, were detected. However, consistent differences between nutty and not nutty cheeses were not identified. Relative abundance of selected neutral/basic compounds (Tables 5 and 6) support GCO results, with no clear consistent differences.

Volatile Compounds Detected by GCO-DHS

Dynamic headspace analysis was conducted as a subsequent alternative volatile recovery technique, because each technique has potential limitations (Zehentbauer and Reineccius, 2002). Highly volatile compounds are not well recovered by DSE-HVD. We hypothesized that there may be additional key compounds, not recovered by DSE-HVD, that might contribute to nutty flavor. In this analysis, due to the limitation in cheese sample size, 3 additional cheeses (N3, NN3, and NN4) were selected. Cheeses were evaluated by descriptive analysis techniques described previously (Figure 1). In addition to 7 compounds identified previously, 2 additional highly volatile compounds, 2-methylpropanal and dimethylsulfide, were identified by GCO-DHS (Table 7). Use of purge time as a dilution factor with DHA-DHS revealed that there were distinct differences between the headspace compositions of the not nutty and nutty cheeses, particularly dimethylsulfide and the Strecker aldehydes, 2-methylpropanal and 2/3-methylbutanal. To further confirm these findings, 9 additional Cheddar cheeses (5 nutty Cheddar cheeses, 4 not nutty Cheddar cheeses, all aged ≥1 yr) were selected, evaluated by descriptive sensory analysis, and then analyzed by DHA-GC-MS for Strecker aldehydes. Descriptive sensory analysis confirmed the presence or absence of nutty flavors in the nutty and not nutty Cheddar cheeses, respectively (data not shown). In agreement with previous findings, 2-methylpropanal was consistently detected in nutty Cheddar cheeses and was not found in 3 of the 4 not nutty cheeses (Table 8). The other Strecker aldehydes, 2/3-methylbutanal, were also detected in some of the nutty and not nutty Cheddar cheeses, but they were present at higher concentrations in nutty Cheddar cheeses.

Model Cheeses

The addition of propionic acid did not enhance nutty flavors in young or aged Cheddar cheeses. The addition of as little as 50 ppm resulted in a sour/vinegar aroma and a sour/vinegar flavor (data not shown). This result conflicts with those reported for Swiss-type cheese in which propionic acid was claimed to be one of the key

Table 8. Relative abundance of Strecker aldehydes in selected nutty (N) and not nutty (NN) Cheddar cheeses.

Cheddar cheese	Relative abundance[1] of 2-methylpropanal (μg/kg) (ppb)	Relative abundance of 2/3-methylbutanal (μg/kg) (ppb)
N4	220 ± 48[c3]	73 ± 21[b3]
N5	280 ± 85[c]	ND[2]
N6	335 ± 145[c]	ND
N7	1153 ± 311[a3]	210 ± 85[a]
N8	687 ± 65[b]	ND
NN4	90 ± 85[d]	ND
NN5	ND	ND
NN6	ND	ND
NN7	ND	53 ± 34[b]

[1]Mean relative abundance = concentration of internal standard × peak area of compound/peak area of the internal standard. Data are mean values of triplicate replications.

[3]Means in a column followed by different letters are different (P < 0.05).

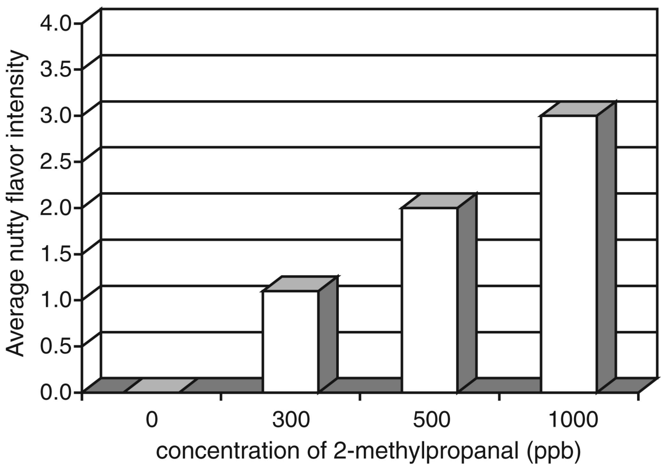

Figure 2. The relationship between 2-methylpropanal concentration and nutty flavor intensity in aged Cheddar cheese models.

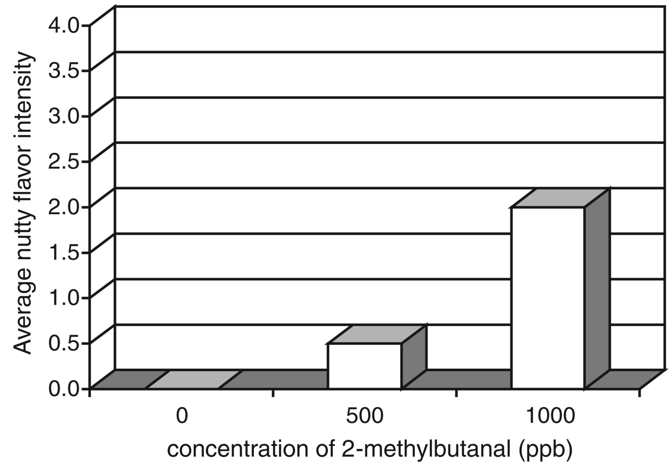

Figure 3. The relationship between 2-methylbutanal concentration and nutty flavor intensity in aged Cheddar cheese models.

compounds responsible for nutty flavor (Vangtal and Hammond, 1986; Lawlor et al., 2002). It was also reported that Cheddar cheese produced with different levels of propionibacterium as an adjunct culture had an increasingly sweet and nutty flavor (Fernandez-Espla and Fox, 1998). Again, descriptive sensory analysis was not used to interpret instrumental results in these studies. In agreement with our results, organoleptic results of Kurtz et al. (1959) indicated that it is improbable that propionic acid itself is the nutty flavor component. Similarly, Dacremont and Vickers (1994) claimed that propionic acid had no effect on Cheddar cheese aroma. The addition of dimethyl sulfide likewise did not result in enhanced nutty flavors. The addition of dimethyl sulfide increased cooked and brothy flavors in young and aged Cheddar cheese models compared with control cheeses without added dimethyl sulfide ($P < 0.05$) (data not shown).

The addition of the Strecker aldehydes 2-methylpropanal, 2-methylbutanal, and 3-methylbutanal did increase nutty flavors in aged Cheddar cheese (Figures 2, 3, and 4), but in young Cheddar cheeses, descriptive panelists described the flavor as nutty/malty. These compounds have been previously reported in Cheddar cheese (Dunn and Lindsay, 1985; Christensen and Reineccius, 1995; Zehentbauer and Reinecccius, 2002). Strecker aldehydes have also been reported as prevalent constituents of Swiss cheese (Preininger et al. 1996; Thierry et al. 1999; Rychlik and Bosset, 2002a; Rychlik and Bosset, 2002b) and Parmesan cheese (Barbieri et al., 1994; Qian and Reineccius, 2002; Qian and Reineccius, 2003). Interestingly, these cheeses are characterized by intense nutty flavors.

To our knowledge, this is the first report that Strecker aldehydes are the key components responsible for nutty flavor of Cheddar cheese. Dunn and Lindsay (1985) reported that these aldehydes gave Cheddar cheese unclean and harsh flavors. A defined descriptive sensory language with a trained descriptive panel was not used to confirm their findings. Strecker aldehydes alone exhibit an aroma described as green, malty, chocolate, and almond (Singh et al., 2003). Their lack of typical nutty aroma when alone may explain why they have not previously been associated with nutty flavor in Cheddar cheese. The aroma of a compound is not necessarily indicative of its role in flavor of a food because food matrix parameters (pH, ionic strength, and other solutes), as well as other volatile compounds at or below threshold, can all impact perceived flavor (Drake and Civille, 2003; Singh et al., 2003). Hence, descriptive sensory analysis with a defined sensory language is necessary both on the food that is studied and with

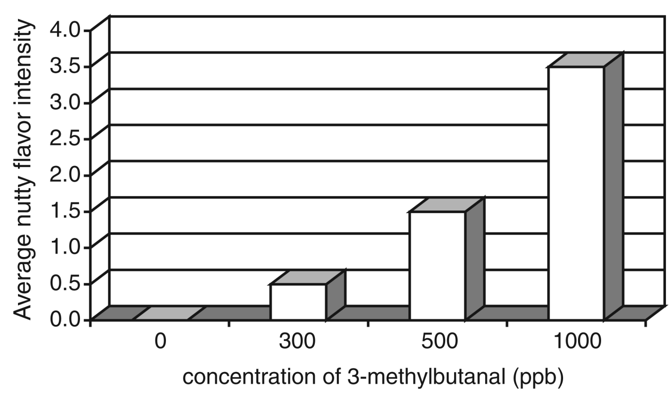

Figure 4. The relationship between 3-methylbutanal concentration and nutty flavor intensity in aged Cheddar cheese models.

subsequent model systems to evaluate volatile components.

Formation of 2-methylpropanal and 2/3-methylbutanal via Strecker degradation in model solutions at high pH (Griffith and Hammand, 1989) or low pH (Pipis-Nicolau et al., 2000) was already reported. In these studies, valine, isoleucine, and leucine amino acids were found to be responsible for the formation of 2-methylpropanal, 2-methylbutanal, and 3-methylbutanal, respectively. These 3 Strecker aldehydes were reported to be the strongest contributors to chocolate flavor (Counet et al., 2002). Strecker aldehydes have also been reported to be the source of malty off-flavors in milk (Miller et al., 1974).

Braun and Olsen (1986) reported that low-fat Cheddar cheeses between 2 and 6 mo of age with increased levels of Strecker aldehydes (500 to 1000 ppb) from added cell-free extracts had a malty flavor. Descriptive sensory analysis with a defined sensory language was not used to evaluate cheeses. However, their results do concur with our findings on the addition of the Strecker aldehydes to young Cheddar cheese models. Sensory panelists reported that these cheeses exhibited a malty/nutty character. When Strecker aldehydes were added to aged Cheddar cheeses (>1 yr), nutty flavors were reported by sensory panelists. Panelists also noted that these flavors were identical to "typical" nutty flavors observed in Cheddar cheeses. Sensory analysis of cheese models revealed that the 3 Strecker aldehydes (2-methylpropanal, 2-methylbutanal, 3-methylbutanal) can contribute to nutty flavors in aged (>9 mo) Cheddar cheeses. However, quantitative data (Table 8) suggested that 2-methylpropanal may be more important because it was more prevalent in nutty cheeses and present at higher concentrations than the other Strecker aldehydes. Cheddar cheese flavor is very complex and is composed of many volatile compounds present in an appropriate balance (Singh et al., 2003). Many complex biochemical reactions occur throughout the aging process to generate a host of volatile aroma-active compounds that contribute to flavor (Singh et al., 2003). Results with cheese models also suggest that an appropriate balance of other compounds at or below sensory threshold are required in addition to Strecker aldehydes for the sensory perception of nutty flavor in Cheddar cheeses. Nutty flavor typically develops slowly in Cheddar cheese. Cheddar cheeses with intense nutty flavors selected for this study were all greater than 1 yr of age.

CONCLUSIONS

It is indisputably accepted that Cheddar cheese flavor is due to numerous volatile components. While some of these compounds contribute to background flavor, some others are predominant and strongly impact the flavor. The present research revealed the importance of some Strecker aldehydes, 2/3-methylbutanal and 2-methylpropanal, in nutty flavor of Cheddar cheese. As the formation of these compounds requires certain amino acids (valine, isoleucine, and leucine), in order to produce Cheddar cheese with enhanced or accelerated nutty flavor, 3 methods should be explored: i) the use of starter bacteria capable of releasing these certain amino acids, ii) additions of these certain amino acids into cheese milk or cheese slurry, and/or iii) accelerating the conversion rate of these amino acids into aroma compounds.

ACKNOWLEDGMENTS

Funding provided by Dairy Management, Inc. The use of tradenames does not imply endorsement nor the lack of endorsement for those not mentioned. Paper number FSR 04-10 of the Department of Food Science, North Carolina State University.

REFERENCES

Barbieri, G., L. Bolzoni, M. Careri, A. Mangia, G. Parolari, S. Spagnoli, and R. Virgilli. 1994. Study of volatile fraction of Parmesan cheese. J. Agric. Food Chem. 42:1170–1176.

Biede, S. L., and E. G. Hammond. 1979a. Swiss cheese flavor: I. Chemical analysis. J. Dairy Sci. 62:227–237.

Biede, S. L., and E. G. Hammond. 1979b. Swiss cheese flavor: II. Organoleptic analysis. J. Dairy Sci. 62:238–248.

Braun, S. D., and N. F. Olson. 1986. Microencapsulation of cell-free extracts to demonstrate the feasibility of heterogeneous enzyme system and co-factor recycling for development of flavor in cheese. J. Dairy Sci. 69:1202–1208.

Cadwallader, K. R., and H. H. Baek. 1998. Aroma-impact components in cooked tail meat of freshwater crayfish (*Procambarus clarkii*). Pages 271–279 in Proc. of Ninth International Flavor Conference, George Charalambous Memorial Symposium. C. Mussinan, E. Contis, C. T. Ho, T. Parliment, A. Spanier, and F. Shahidi, eds. Elsevier Science BV, Amsterdam, The Netherlands.

Christensen, K. R., and G. A. Reineccius. 1995. Aroma extract dilution analysis of aged Cheddar cheese. J. Food Sci. 60:218–220.

Clark, R. G., and H. E. Nursten. 1977. Analysis of the sensory term 'nutty' and list of compounds claimed to be nutty. Int. Flavours Food Additives 8:197–201.

Counet, C., C. Delphine, C. Ouwerx, and S. Collin. 2002. Use of gas chromatography-olfactometry to identify key odorant compounds in dark chocolate. Comparison of samples before and after conching. J. Agric. Food Chem. 50:2385–2391.

Dacremont, C., and Z. Vickers. 1994. Concept matching technique for assessing importance of volatile compounds for Cheddar cheese aroma. J. Food Sci. 59:981–985.

Drake, M. A., S. C. McIngvale, P. D. Gerard, K. R. Cadwallader, and G. V. Civille. 2001. Development of a descriptive language for Cheddar cheese. J. Food Sci. 66:1422–1427.

Drake, M. A., and G. V. Civille. 2003. Flavor Lexicons. Compr. Rev. Food Sci. 2:33–40.

Dunn, H. C., and R. C. Lindsay. 1985. Evaluation of the role of microbial strecker-derived aroma compounds in unclean-type flavors of Cheddar cheese. J. Dairy Sci. 68:2859–2874.

Fernandez-Espla, M. D., and P. F. Fox. 1998. Effect of adding *Propionibacterium shermanii NCDO 853* or *Lactobacillus casei* spp. *casei*

IFPL 731 on proteolysis and flavor development of Cheddar cheese. J. Agric. Food Chem. 46:1228–1234.
Friedrich, J. E., and T. E. Acree. 1998. Gas chromatography olfactometry of dairy products. Int. Dairy J. 8:235–241.
Griffith, R., and E. G. Hammond. 1989. Generation of Swiss cheese flavor components by the reaction of amino acids with carbonyl compounds. J. Dairy Sci. 72:604–613.
Grosch, W. 1993. Detection of potent odorants in foods by aroma extract dilution analysis. Trans. Food Sci. Technol. 4:68–73.
Karagul-Yuceer, Y., M. A. Drake, and K. R. Cadwallader. 2001. Aroma-active components of nonfat dry milk. J. Agric. Food Chem. 49:2948–2953.
Kurtz, F. E., J. A. Hupfer, E. A. Corbin, R. E. Hargrove, and H. E. Walter. 1959. Interrelationship between pH, populations of *Propionibacterium shermanii*, levels of free fatty acids, and the flavor ratings of Swiss cheese. J. Dairy Sci. 42:1008–1019.
Lawlor, J. B., C. M. Delahunty, M. G. Wilkinson, and J. Sheehan. 2002. Relationships between the gross, non-volatile, and volatile compositions and the sensory attributes of eight hard-type cheeses. Int. Dairy J. 12:493–509.
Liardon, R., J. O. Bosset, and B. Blanc. 1982. The aroma composition of Swiss Gruyere cheese. I. The alkaline volatile components. Lebensm.-Wiss. Technol. 15:143–147.
McGorrin, R. J. 2002. Character impact compounds: flavors and off-flavors in foods. Pages 375–413 in Flavor, Fragrance, and Odor Analysis. R. Marsili, ed. Marcel Dekker, Inc. New York, NY.
McSweeney, P. L. H., and M. J. Sousa. 2000. Biochemical pathways for the production of flavour compounds in cheeses during ripening: A review. Lait 80:293–324.
Meilgaard, M. M., G. V. Civille, and T. Carr. 1999. Sensory Evaluation Techniques. 3rd ed. CRC Press, New York, NY.
Miller, A., M. E. Morgan, and L. M. Libbey. 1974. *Lactobacillus maltaromicus*, a new species producing a malty aroma. Int. J. Syst. Bacteriol. 24:346–354.
Milo, C., and G. A. Reineccius. 1997. Identification and quantification of potent odorants in regular-fat and low-fat mild Cheddar cheese. J. Agric. Food Chem. 45:3590–3594.
O'Riordan, P. J., and C. M. Delahunty. 2001. Comparison of volatile compounds released during the consumption of Cheddar cheese with compounds extracted by vacuum distillation using gas chromatography-olfactometry. Flavour Fragr. J. 16:425–434.
Pipis-Nicolau, L., G. de Revel, A. Bertrand, and A. Maujean. 2000. Formation of flavor components by the reaction of amino acid and carbonyl compounds in mild conditions. J. Agric. Food Chem. 48:3761–3766.
Preininger, M., R. Warmke, and W. Grosch. 1996. Identification of character impacts flavor compounds of Swiss cheese by sensory studies of models. Z. Lebensm.Unters.Forrsh. 202:30–34.
Qian, M., and G. A. Reineccius. 2002. Identification of aroma compounds in Parmigianno Reggiano by gas chromatography-olfactometry. J. Dairy Sci. 85:1362–1369.
Qian, M., and G. A. Reineccius. 2003. Quantification of aroma compounds in Parmigiano Reggiano Cheese by a dynamic headspace gas chromatography-mass spectrometry technique and calculation of odor activity value. J. Dairy Sci. 86:770–776.
Rychlik, M., and J. O. Bosset. 2001a. Flavour and off-flavour compounds of Swiss Gruyere cheese. Evaluation of potent odorants. Int. Dairy J. 11:895–901.
Rychlik, M., and J. O. Bosset. 2001b. Flavour and off-flavour compounds of Swiss Gruyere cheese. Identification of key odorants by quantitative instrumental and sensory studies. Int. Dairy J. 11:903–910.
Sen, A., G. Laskawy, P. Schierberle, and W. Grosch. 1991. Quantitative determination of (E)-β-damascenone in foods using stable isotope dilution assay. J. Agric. Food Chem. 39:757–759.
Singh, T., M. A. Drake, and K. R. Cadwallader. 2004. Flavor of Cheddar Cheese: A chemical and sensory perspective. Compr. Rev. Food Sci. 2:139–162.
Suriyaphan, O., M. A. Drake, and K. R. Cadwallader. 1999. Identification of volatile off-flavors in reduced-fat Cheddar cheeses containing lecithin. Lebensm.-Wiss. Technol. 32:250–254.
Suriyaphan, O., M. A. Drake, X. Q. Chen, and K. R. Cadwallader. 2001. Characteristic aroma components of British Farmhouse Cheddar cheese. J. Agric. Food Chem. 49:1382–1387.
Urbach, G. 1995. Contribution of lactic acid bacteria to flavour compound formation in dairy products. Int. Dairy J. 5:877–903.
Urbach, G., 1997. The chemical and biochemical basis of cheese and milk aroma. Pages 253–298 in Microbiology and Biochemistry of Cheese and Fermented Milk. B. A. Law, ed. Chapman and Hall, London, UK.
Vangtal, A., and E. G. Hammond. 1986. Correlation of the flavor characteristics of Swiss-type cheeses with chemical parameters. J. Dairy Sci. 69:2982–2993.
Van den Dool, H., and P. D. Kratz. 1963. A generalization of the retention index system including linear programmed gas liquid partition chromatography. J. Chromatogr. 11:463–471.
Van Ruth, S. M. 2001. Methods for gas chromatography olfactometry: A review. Biomol. Eng. 17:121–128.
Warmke, R., H. D. Belitz, and W. Grosch. 1996. Evaluation of taste compounds of Swiss cheese (Emmentaller). Z. Lebensm.Unters. Forrsh. 203:230–235.
Weimer, B., K. Seefeldt, and B. Dias, 1999. Sulfur metabolism in bacteria associated with cheese. Antonie van Leeuwenhoek 76:247–261.
Zehentbauer, G., and G. A. Reineccius. 2002. Determination of key aroma components of Cheddar cheese using dynamic headspace dilution assay. Flavour Fragr. J. 17:300–305.