JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Characterization of Aroma Compounds Responsible for the Rosy/Floral Flavor in Cheddar Cheese

MARY E. CARUNCHIA WHETSTINE,† KEITH R. CADWALLADER,§ AND MARYANNE DRAKE*,†

Department of Food Science, Southeast Dairy Foods Research Center, North Carolina State University, Raleigh, North Carolina 27695, and Department of Food Science and Human Nutrition, University of Illinois, Urbana, Illinois 61801

The aroma-active compounds that contribute to the rosy/floral flavor in Cheddar cheese were characterized using both instrumental and sensory techniques. Two cheeses (>12 months old) with rosy/floral flavor and two Cheddar cheeses of similar ages without rosy/floral flavors were selected. After direct solvent extraction/solvent-assisted flavor evaporation and separation into neutral/basic and acidic fractions, samples were analyzed by gas chromatography−olfactometry with aroma extract dilution analysis. Selected compounds were quantified using internal standard methodology. Some of the intense aroma-active compounds in the neutral basic fraction of the rosy/floral cheeses included 2-phenethanol (rosy), phenylethyl acetate (rosy), and phenylacetaldehyde (rosy/floral). Quantification, threshold analysis, and sensory analysis of model cheeses confirmed that increased concentrations of phenylacetaldehyde and phenylacetic acid caused rosy/floral flavor when spiked into Cheddar cheese.

KEYWORDS: Cheddar cheese flavor; GC-O; sensory analysis; threshold determination; model systems

INTRODUCTION

The flavor of Cheddar cheese is a key parameter for consumer acceptance and marketing. Using instrumental and sensory analysis, many aspects of Cheddar flavor have been characterized. Early research focused solely on instrumental evaluation of amino acids and fatty acids (*1*). This information is an important clue into Cheddar flavor because many flavor compounds are derived from amino acids and formed during amino acid catabolism (*2*, *3*). The breakdown of aromatic amino acids via the Strecker degradation pathway is one way that unclean off-flavors may be formed (*2*, *4*).

The term unclean is undefined and encompasses many flavors including cowy/barny and earthy (*2*, *4*), and is typically perceived as a negative attribute by consumers (*5*). Cheddar cheeses may exhibit a rosy/floral type of unclean flavor that is typically detected after swallowing, leaving a lingering, and generally undesirable aftertaste. Rosy/floral flavor has been previously documented in Camembert, Gruyere, and Cheddar cheeses (*2*, *6*−*8*). Drake et al. (*9*) developed a referenced sensory language to document Cheddar cheese flavor. Rosy/floral flavor was identified as a distinct flavor in Cheddar cheese.

Lactic acid bacteria can synthesize phenylacetaldehyde and 2-phenethanol, which have a rosy aroma, from the catabolism of aromatic amino acids, especially phenylalanine (*2*, *8*, *10*). The enzyme-mediated transamination, decarboxylation, and reduction reactions of aromatic amino acids are the source of these two compounds (*11*). However, it is likely that additional compounds may also contribute to the rosy/floral flavor. Although previous studies have indicated the presence of rosy/floral flavor (*2*, *6*−*8*), no study has systematically studied the chemical sources of this flavor. The application of analytical sensory techniques such as descriptive sensory analysis and threshold determination in conjunction with instrumental methods such as gas chromatography−olfactometry (GC-O) and gas chromatography−mass spectrometry (GC-MS) provides a powerful and comprehensive way to identify and characterize flavor and flavor sources. These methods have been successfully utilized previously to identify flavor compounds in Cheddar cheese (*5*, *12*, *13*). The objectives of this study were to systematically characterize and quantify the compound(s) responsible for causing rosy/floral flavor in Cheddar cheese using both instrumental and sensory analysis and to confirm the impact of the identified compounds using model system analysis.

MATERIALS AND METHODS

Cheeses. Twenty blocks (18 kg) of commercial Cheddar cheese (9−18 months old, moisture content = 36.7−38.0%, pH 5.5−5.9) were collected and screened for rosy/floral flavor by a descriptive sensory analysis panel. This age range of cheeses was selected because previous research indicated that high intensities of rosy/floral flavor were more likely to be present in aged Cheddar cheese (*2*, *9*). Two cheeses with rosy/floral flavor (12 and 17 months old) and two cheeses without rosy/floral flavor (18 months old) were selected and analyzed for volatile aroma compounds.

* Author to whom correspondence should be addressed [telephone (919) 513-4598; fax (919) 515-7924; e-mail mdrake@unity.ncsu.edu].
† North Carolina State University.
§ University of Illinois.

10.1021/jf048278o CCC: $30.25
Published on Web 03/16/2005

Chemicals. Ethyl ether (anhydrous, 99.8%), sodium chloride (99%), sodium sulfate (99%), 2-methyl-3-heptanone, and 2-methylpentanoic acid (internal standards for neutral/basic fraction and acidic fractions, respectively) were obtained from Aldrich Chemical Co. (St. Louis, MO). Aroma compounds listed in **Table 3** that were positively identified were provided by Aldrich Chemical Co. with the following exceptions: no. **19** (Lancaster, Windham, NH) and no. **25** (TCI America, Portland, OR). Sodium bicarbonate (99.7%) (w/w) and hydrochloric acid (36.5%) (w/w) were obtained from Fisher Scientific (Pittsburgh, PA).

Sample Preparation. *Direct Solvent Extraction.* Cheese extracts were prepared according to the methods of Milo and Reineccius (*12*), with some modifications described as follows. Cheese samples were stored frozen (−18 °C). Samples were then thawed overnight (4 °C) and grated using a hand grater. Freshly grated cheese (150 g) was weighed and divided into two Teflon bottles (capacity of 250 mL) with Tefzel closures in duplicate (300 g total for each cheese) (Nalgene, Rochester, NY). Ethyl ether (50 mL) and 20 μL of internal standard (50 μL of 2-methyl-3-heptanone and 50 μL of 2-methylpentanoic acid in 5 mL of methanol) were added to each bottle. The pH was lowered to ∼3.0 using 2 M HCl. 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 735*g* for 10 min to separate the solvent phase from the mixture, which was subsequently collected into a glass jar. The procedure was repeated twice with 50 mL of ethyl ether. After the third extraction, the pH was raised to ∼10.0 using 2 M NaOH, and the extraction procedure was repeated. The solvent phases were combined and kept at −20 °C overnight. The extract was concentrated to 120 mL using a Vigreux column.

Solvent-Assisted Flavor Evaporation (SAFE). Volatile compounds from cheese extracts were distilled using SAFE (Ace Glassware, Vineland, NJ). The assembly used was similar to that described by Engel et al. (*14*). The SAFE apparatus was connected to a receiving tube and a waste tube. The glassware was then connected to a rough pump/diffusion pump as the vacuum source. The receiving tube and waste tube were held in separate Dewar flasks containing liquid nitrogen at all times. Distillation was carried out for 2 h under vacuum (.∼10^{-5} Torr). Liquid solvent extract was loaded into the top of the SAFE apparatus and released into the vacuum dropwise until all of the extract had been placed under vacuum conditions. The SAFE apparatus was kept thermostated at 50 °C with a circulating water bath. After distillation, the distillate was concentrated to 20 mL under a stream of nitrogen gas. Concentrated distillate was then washed twice with 3 mL of sodium bicarbonate (0.5M) and vigorously shaken. It was then washed three times with 2 mL of saturated sodium chloride solution. After each wash step, the solution was shaken and the upper layer (ether) containing the neutral/basic fraction was collected using a pipet. The upper (neutral/basic) layers were then pooled, and the extract was dried over anhydrous sodium sulfate and concentrated to 0.5 mL under a stream of nitrogen gas. Acidic volatiles were recovered by acidifying the bottom layer (aqueous phase) with hydrochloric acid (18%) to pH 2−2.5 and extracting the sample three times with 15 mL of ethyl ether. The acidified extract was dried over anhydrous sodium sulfate before concentration to 0.5 mL under a nitrogen gas stream.

Gas Chromatography−Olfactometry. A semiquantitative GC-O technique, aroma extract dilution analysis (AEDA), was used to determine which aroma active compounds contribute to flavor (*15*, *16*). AEDA was performed using an HP5890 series II gas chromatograph (Hewlett-Packard Co., Palo Alto, CA) equipped with a flame ionization detector (FID), a sniffing port, and splitless injector. Both the neutral/basic and acidic fractions were analyzed from every extraction. Two microliters 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, Folsom, CA) 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 the FID and the sniffing port using deactivated fused silica capillaries (1 m length × 0.25 mm i.d.). The GC oven temperature was programmed from 40 to 200 °C at a rate of 10 °C/min with an initial hold for 3 min and a final hold of 20 min. 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. The extracts were diluted stepwise with diethyl ether at a ratio of 1:3 (v/v). Two experienced sniffers, each with >50 h training on GC-O, were used for AEDA. The dilution procedure was followed until sniffers detected no odorants. The highest dilution was reported as the flavor dilution (FD) factor (*15*).

Gas Chromatography−Mass Spectrometry. For GC-MS analysis of the solvent extracts, an HP5890 series II GC/HP 5972 mass selective detector (MSD, Hewlett-Packard, Co.) was used. Separations were performed on a fused silica capillary column (DB5MS 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. The 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. MSD conditions were as follows: capillary direct interface temperature, 280 °C; ionization energy, 70 eV; mass range, 33−330 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. On the basis of MS results, relative concentrations of the compounds were calculated.

For positive identifications, retention indices (RI), 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 (1992) mass spectral database or on matching the RI values and odor properties of unknowns against those of authentic standards. For the calculation of retention indices, an *n*-alkane series was used (*17*).

Quantification of Odorants. Response factors of selected compounds were calculated by direct addition of known amounts of standards to odor-free water prior to solvent extraction (SAFE). Response factors for neutral/basic compounds were determined using a five-point standard curve ($R^2 > 0.985$) on a DB-5 column using GC-MS and for acidic compounds on a DB-Wax column using GC-FID. Using these response factors, the selected compounds were quantified using the response factor and the area ratio of compound to internal standards. Phenylacetaldehyde, 2-phenethanol, and phenylacetic acid were all above the detection limits of the GC-MS, but phenyl ethyl acetate was not, and therefore was not quantified. 2-Methylphenethanol was used as the internal standard for 2-phenethanol. 4-Methylacetophenone was used as the internal standard for phenylacetaldehyde, and *m*-tolyacetic acid was used as the internal standard for phenylacetic acid. All standards were obtained from Aldrich Chemical Co. (St. Louis, MO).

Sensory Evaluation of Cheeses. A sensory panel ($n = 8$) evaluated the cheeses using the lexicon developed for Cheddar cheese (*9*). The definitions and references for the terms used are given in **Table 1**. Panelists were trained for 75 h on flavor, aroma, and feeling factors using the Spectrum method (*18*). Cheeses were presented in 2 × 2 cm cubes with three-digit codes. The 15-point numerical Spectrum intensity scale was used to mark the panelist responses. During evaluation, panelists had free access to water and unsalted crackers. Cheeses were evaluated in duplicate by each panelist. Data were analyzed by analysis of variance with means separation (SAS Statistical Analysis Software, version 8.2, SAS Institute, Cary, NC).

Threshold Determination. Best estimate thresholds of phenylacetaldehyde, 2-phenethanol, phenylethyl acetate, and phenylacetic acid were determined using the ASTM ascending forced choice method of limits procedure E679-79 (*19*). Thresholds of phenylacetaldehyde, phenylacetic acid, 2-phenethanol, and phenylethyl acetate were determined orthonasally in both deodorized water and pH 5.5 buffer. Deodorized water was prepared by taking deionized water and boiling to two-thirds of the original volume. The buffer was prepared using 250 mL of 0.2 M Tris acid maleate (24.2 g of Tris and 23.2 g of maleic acid/L) and 66 mL of 0.2 N NaOH made up to 1 L in deodorized water. Stock solutions of phenylacetaldehyde, phenylethyl acetate, 2-phenethanol, and phenylacetic acid were prepared in methanol. Aliquots of these stock solutions were placed into either water or pH 5.5 buffer. These solutions were serially diluted (factor of 3), and 15 mL of each was poured into clean, labeled 56 mL plastic cups. Panelists ($n = 80$) were given these concentrations in a series with either two deodorized water blanks or two pH 5.5 buffer blanks. Seven ascending series were tested

Table 1. Cheddar Cheese Lexicon and References[a]

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 (2,3-butanedione)
lactone	aromatics associates with milk fat	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
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
catty	aromatics associated with tomcat urine	2-mercapto-2-methylpentan-4-one, 20 ppm
cowy/phenolic	aromatics associated with barns and stock trailers	*p*-cresol, Band-aids, phenol
fecal/mothball	aromatics associated with feces and mothballs	skatole, indole, naphthalene
rosy/floral	aromatics associated with flowers, generally rose-like, detected in aftertaste	
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 (1% in water)

[a] Data from Drake et al. (*9*).

each day. Series were presented in ascending concentration, and each series was presented in a randomized order and evaluated by panelists using the ASTM method with the modification detailed by Lawless et al. (*19*).

Subjects were briefly instructed prior to testing. Subjects were told to open the cups and briefly sniff the headspace of each cup in the series. Subjects rested for 1 min between each set of three and were also instructed to sniff their sleeve to assist cleaning their nasal passageways between cups. The individual best estimate threshold was calculated as the geometric mean of the last concentration with an incorrect response and the first concentration with a correct response. Group thresholds were calculated as the geometric mean of the individual best estimate thresholds.

Sensory Evaluation of Cheese Models. Sensory analysis of cheese models was conducted to pinpoint the compound(s) responsible for the rosy/floral aroma and flavor in Cheddar cheese. Cheese models were prepared from a commercial Cheddar cheese (5 months, 37.0 ± 0.3% moisture, pH 5.6 ± 0.2) that did not have a rosy/floral flavor, purchased from a local grocery store. Chemicals tested (phenylacetaldehyde, phenylacetic acid, 2-phenethanol, and phenylethyl acetate) were prepared in methanol (aroma evaluation) or 95% ethanol (flavor evaluation) across the concentration range found in the cheeses. The cheeses were grated and portioned (25 g), and chemicals were introduced by a clean, disposable micropipet. After the addition of the chemicals, cheese models were kneaded for 3 min and then molded to a rectangular shape and equilibrated for 24 h 5 °C. Cheese models were evaluated for aroma or flavor by sensory analysis using the same procedure applied for descriptive analysis of Cheddar cheeses.

RESULTS AND DISCUSSION

Sensory Analysis. Selected cheeses displayed the desired sensory properties with two cheeses having distinct rosy flavors and two cheeses of similar age without rosy flavor (**Table 2**). Rosy cheeses had significantly higher intensities of rosy/floral flavor as well as higher intensities of brothy notes (**Table 2**). The cheeses were characterized by typical aged Cheddar cheese flavors (*9*). There were no other consistent differences in the flavor of rosy and nonrosy cheeses ($p < 0.05$).

Gas Chromatography−Olfactometry. Fifty odor-active compounds were identified in the rosy and nonrosy cheeses. Nineteen compounds were positively identified by comparing mass spectra of authentic standards, 26 were tentatively identified by comparing RI and odor properties with standards, and 5 remained unknown (**Table 3**). With the exception of compounds **11**, **44**, and **49**, all of these compounds have been previously identified in Cheddar cheese (*5*, *12*, *20*, *21*). AEDA is a semiquantitative GC-O technique. Extracts were serially diluted by a factor of 3, and this is referred to as the flavor dilution factor ($\log_3$ FD). Using this method, we determined which compounds had higher aroma activities in the rosy cheeses to tentatively identify if these compounds were potentially responsible for causing rosy/floral flavor based on their aroma character. There were several compounds that had relatively high $\log_3$ FD values in all four of the cheeses, including butanoic acid (cheesy/rancid), methional (potato), dimethyl trisulfide (cabbage), phenylacetaldehyde (rosy), 2-phenethanol (rosy), 2-methoxyphenol (smoky), 2-acetyl-2-thiazoline (popcorn), and phenylethyl acetate (rosy). Additionally, phenylacetic acid was examined because of its rosy aroma character, although no differences for this compound were observed between the rosy and nonrosy cheeses based on AEDA results. All of these compounds have been previously identified in Cheddar cheese (*1*, *5*, *22*).

Table 2. Sensory Analysis of Cheeses[a]

attribute	rosy 1	rosy 2	nonrosy 1	nonrosy 2
cooked	2.04ab	2.12a	2.20a	2.24a
whey	1.44a	0.88b	1.25ab	1.16ab
diacetyl	ND[b]	ND	ND	ND
milk fat/lactone	2.12ab	2.34a	1.94b	1.99ab
fruity	ND	ND	ND	ND
sulfur	1.78b	2.46a	1.91b	1.75b
free fatty acid	ND	ND	ND	ND
brothy	3.09a	3.08a	2.66b	2.39b
nutty	0.50b	1.04a	0.77ab	0.56b
catty	ND	0.52a	ND	ND
rosy/floral	3.00a	3.00a	0.50b	0.50b
sour	3.49c	3.53c	3.70b	3.99a
sweet	1.49b	1.90a	1.79ab	1.53ab
salty	3.72a	3.81a	3.88a	3.94a
bitter	0.99a	ND	ND	0.98a
umami	1.38bc	1.93a	1.54b	1.14c

[a] Intensities are scored on a 15-point scale where 0 = none and 15 = very high. Means in a row followed by different letters are different ($p < 0.05$). [b] Not detected.

Butanoic acid is an important compound in Cheddar cheese, formed via lipolysis (*4*), and increases during cheese aging (*23*). The sulfur compound dimethyl trisulfide is derived from the amino acid methionine (*8*, *10*) and also increases as cheese ages (*1*). 2-Methoxyphenol (guaiacol) has a smoky aroma and has been identified in strongly flavored cheeses (*5*, *11*) and can be

Table 3. Potent Odorants in Rosy/Floral Cheddar Cheeses

no.	compound	fraction	odor[c]	RI[a]		log 3 FD factor[b]				method of identification[d]
				DB-5MS	DB-Wax	rosy 1	rosy 2	nonrosy 1	nonrosy 2	
1	acetic acid	Ac	vinegar	600	1393	2	5	5	3	RI, odor, MS
2	2,3-butanedione (diacetyl)	NB	buttery	670	956	5	3	4	4	RI, odor, MS
3	3-methylbutanal	NB	malty	686	925	1	5	3	ND	RI, odor, MS
4	2-methylbutanal	NB	dark chocolate	688		<1	<1	3	<1	RI, odor, MS
5	3-hydroxy-2-butanone	NB	buttery	715	990	1	1	3	2	RI, odor, MS
6	ethyl butyrate	NB	bubblegum	730	1000	ND	<1	4	4	RI, odor
7	hexanal	NB	green/sweet	810	1048	3	4	<1	<1	RI, odor, MS
8	unk	NB	skunk	827	1067	<1	6	3	5	odor
9	butanoic acid	Ac	rancid cheese		1610	5	7	7	6	RI, odor, MS
10	unk	NB	fruity/solvent	857	1037	2	4	2	2	odor
11	2-methyl-3-furanthiol[e]	NB	brothy/vitamin	875		2	4	3	2	RI, odor
12	(*Z*)-4-heptenal	NB	fatty/fishy	903	1220	<1	2	2	<1	RI, odor
13	pentanoic acid	Ac	sour/cheesy/beefy	925	1713	3	ND	<1	ND	RI, odor, MS
14	methional	NB	potato	911	1429	4	8	7	7	RI, odor, MS
15	2-acetyl-1-pyrroline[f]	NB	popcorn	925	1317	<1	4	<1	<1	RI, odor
16	methyl hexanoate	NB	citrus	938	1118	<1	<1	1	<1	RI, odor
17	unknown	NB	burnt	962		<1	2	ND	<1	odor
18	dimethyl trisulfide	NB	cabbage/sulfur	973	1362	2	6	1	<1	RI, odor
19	1-octen-3-one	NB	metallic/mushroom	983	1270	2	5	3	3	RI, odor
20	ethyl hexanoate	NB	fruity/citrus	996	1221	3	6	3	2	RI, odor, MS
21	octanal	NB	green/citrus	1008	1273	1	<1	1	ND	RI, odor, MS
22	hexanoic acid	Ac	sweaty	1019	1861	<1	<1	2	<1	RI, odor, MS
23	phenylacetaldehyde	NB	floral/honey	1044	1619	3	6	3	3	RI, odor, MS
24	2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (Furaneol)	Ac	burnt sugar	1071	2054	3	3	3	<1	RI, odor
25	2-methoxyphenol (guaiacol)	NB	burnt/smoky	1092	1480	1	7	3	7	RI, odor, MS
26	nonanal	NB	fatty/floral	1100		4	3	3	3	RI, odor, MS
27	2-acetyl-2-thiazoline	NB	popcorn	1111	1793	5	7	3	3	RI, odor
28	3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (sotolon)	Ac	curry/maple/spicy	1127	2210	5	5	4	<1	RI, odor
29	(*Z*)-2-nonenal	NB	green/floral	1130	1528	2	5	ND	ND	RI, odor
30	2-ethyl-4-hydroxy-5-methyl-(2*H*)-furanone (homofuraneol)	Ac	burnt sugar	1142		5	4	3	2	RI, odor
31	2-phenethanol	NB	floral/rosy	1150		9	8	4	6	RI, odor, MS
32	(*E*, *Z*)-2,6-nonadienal[f]	NB	cucumber	1154	1573	5	2	1	<1	RI, odor
33	(*E*)-2-nonenal	NB	old books/paper	1164	1568	5	6	3	4	RI, odor, MS
34	(*E*,*E*)-2,4-nonadienal	NB	fatty	1224		1	4	2	<1	RI, odor
35	phenylethyl acetate	NB	fatty/rosy	1260		8	7	3	6	RI, odor
36	phenylacetic acid	Ac	honey	1265	2569	<1	<1	<1	<1	RI, odor, MS
37	δ-octalactone	NB	coconut	1287		1	4	1	4	RI, odor, MS
38	(*E*,*Z*)-2,4-decadienal[f]	NB	fatty	1316		ND	ND	3	ND	RI, odor
39	*o*-aminoacetophenone	NB	grape/stale	1315		3	2	2	4	RI, odor
40	(*E*,*E*)-2,4-decadienal	NB	fatty	1325		2	4	3	3	RI, odor
41	γ-nonalactone	NB	coconut/cilantro	1360		<1	3	3	ND	RI, odor
42	unknown	NB	grain/floral	1397	1745	3	4	2	3	odor
43	3-methylindole	NB	fecal/mothball	1399		2	5	5	ND	RI, odor
44	3-methoxy-4-hydroxybenz-aldehyde (vanillin)[e]	Ac	vanilla	1430		ND	<1	<1	6	RI, odor
45	δ-decalactone	NB	peach	1481		5	<1	3	2	RI, odor, MS
46	γ-decalactone	NB	coconut	1508		<1	5	5	4	RI, odor
47	6-(*Z*)-dodecen-γ-lactone	NB	soapy/waxy	1603		6	5	5	4	RI, odor
48	δ-dodecalactone	NB	peach	1705		5	4	5	6	RI, odor, MS
49	nonanoic acid[e]	Ac	sweaty		2053	1	3	ND	<1	RI, odor, MS
50	unknown	Ac	caramelized/maple		>2400	4	3	<1	1	odor

[a] Retention indices were calculated from GC-O data. [b] Flavor dilution factors were determined on a DB-5MS column for NB compounds and on a DB-WAX column for Ac compounds. [c] Odor description at the GC–sniffing port. [d] Compounds were identified by comparison with the authentic standards on the following criteria: retention index (RI) on DB-WAX and DB-5MS columns, odor property at the GC–sniffing port, and mass spectra in the electron impact mode. Positive identifications indicate that mass spectral data were compared with authentic standards. [e] Compound not previously identified in Cheddar cheese (*11*). [f] Compounds identified by comparing RI with previously published standards (*21*).

formed from the degradation of aromatic amino acids (*24*). 2-Acetyl-2-thiazoline is a thermally generated compound (*25*) with a popcorn aroma and has been identified in milk powders and cheese (*26*). Although these compounds all had high $\log_3$ FD values, there were no consistent differences in the $\log_3$ FD values of these compounds in the rosy and nonrosy cheeses, nor do they exhibit rosy/floral aromas; therefore, we can conclude that these compounds, although important in the overall flavor profile of aged Cheddar cheese, are not responsible for rosy/floral flavor.

During volatile compound extraction, the pH of the cheeses was raised to facilitate extraction of basic compounds such as phenylethylamine and phenylethanol amine. Phenylethylamine was not detected by GC-MS or by GC-O analysis, and we concluded that this compound was not present at significant concentration in these cheeses. Phenylethanol amine was

Table 4. Quantification and Sensory Orthonasal Threshold Values of Selected Compounds ($n = 2$)

compound	RI on DB-5MS column	concn (ppb)				exptl threshold (ppb)		reported threshold (ppb)
		rosy 1	rosy 2	nonrosy 1	nonrosy 2	water	pH 5.5 buffer	
phenylacetaldehyde	1044[a]	3700 ± 1200	10000 ± 3200	1300 ± 200	1900 ± 900	2 ± 0.8	0.8 ± 0.7	40[b]
2-phenethanol	1150[a]	600 ± 300	200 ± 10	100 ± 100	400 ± 300	122 ± 0.9	176 ± 0.9	240[b]
phenylethyl acetate	1260[a]	ND	ND	ND	ND	19 ± 0.6	61 ± 0.9	20[c]
phenylacetic acid	1265[d]	400 ± 300	40 ± 30	1 ± 2	30 ± 40	464 ± 0.9	17 ± 0.8	1000[d]

[a] Retention indices calculated from mass spectrometry results on a DB-5MS column. [b] Threshold reported orthonasally in water by Dunn and Lindsay (*2*). [c] Thresholds reported orthonasally in water by Rychlik et al. (*35*). [d] Retention index calculated from FID results on a DB-WAX column.

detected in the cheeses by GC-MS only. Due to the lack of aroma of this compound as determined by both GC-O and preliminary threshold tests, it was not further pursued as a source of rosy/floral flavor (data not shown).

The compounds phenylacetaldehyde, 2-phenethanol, phenylethyl acetate, and phenylacetic acid all have rosy aromas and have been previously associated with unclean flavors in cheeses (*2*, *6*, *7*, *27*). These compounds are formed by the Strecker degradation of aromatic amino acids, especially phenylalanine (*10*, *26*). The Strecker reaction is a key reaction that produces many flavor compounds found in aged cheeses (*28*). Roger et al. (*6*) found that the concentration of free phenylalanine increased with time during the ripening of Camembert cheese. This is true in all types of cheeses, including Cheddar (*29*, *30*).

The most abundant free amino acids in Cheddar cheese are glutamic acid, leucine, valine, isoleucine, and phenylalanine (*31*). To a lesser extent, tyrosine and tryptophan are also present. The degradation of aromatic amino acids (phenylalanine, tyrosine, and tryptophan) may form compounds that contribute to unclean flavors in Cheddar cheese, including phenylacetaldehyde, 2-phenethanol, phenol, *p*-cresol, indole, and 3-methylindole (*3*). On the other hand, the degradation of branched chain amino acids can form compounds that contribute to flavors that are considered to be more typical in Cheddar flavor such as sulfur and brothy notes caused by the formation of methanethiol (sulfur) and isovaleric acid (cheesy), respectively (*3*).

During cheese ripening, amino acids are liberated via proteolysis (*31*). This process is usually quite slow and may account for why rosy/floral flavor does not occur in young cheeses. Once the amino acids are in their free form, the degradation of aromatic amino acids can occur readily. This can occur by Strecker degradation via transamination reactions that require an α-ketoacid as an amino group acceptor (*3*, *10*, *29*). Aminotransferases have been identified from many lactic acid bacteria, including *Lactococcus lactis* subsp. *cremoris* and *Lactobacillus paracasei* that catalyze the transamination of aromatic amino acids, including phenylalanine (*3*, *8*, *32*). Additionally, decarboxylases and aromatic hydroxyl acid dehydrogenases can also cause the catabolism of aromatic amino acids (*33*). It has been hypothesized that by inactivating the aromatic aminotransferase, off-flavor formation could be prevented (*3*). Broadbent et al. (*34*) found that by genetically modifying a starter culture to produce more hydroxyl acid dehydrogenase, the concentration of 2-phenethanol in resulting cheeses was less than that in a control cheese. The overexpression of this enzyme prevented the spontaneous degradation of aromatic amino acids. Although the alteration of this enzyme may reduce rosy/floral flavor in Cheddar cheese, without further study, it is impossible to fully characterize these effects on the overall flavor of Cheddar cheese.

Quantification of Selected Odorants. Although phenylacetaldehyde, 2-phenethanol, phenylacetic acid, and phenylethyl acetate have been linked to floral notes in dairy products, there has been no systematic study of this flavor in Cheddar cheese. Because these compounds are the most likely sources of rosy/floral flavor in Cheddar, on the basis of both the previous literature and AEDA data, they were quantified using internal standard curves (**Table 4**). Phenylacetaldehyde, 2-phenethanol, and phenylacetic acid were all above the detection limits of the GC-MS, but phenylethyl acetate was not and, therefore, was not quantified. There were consistent differences in the concentration range of phenylacetaldehyde and phenylacetic acid in the rosy versus nonrosy cheeses (**Table 4**).

Threshold Determination. Thresholds of phenylacetaldehyde, 2-phenethanol, phenylethyl acetate, and phenylacetic acid were determined experimentally in both deodorized water and a pH 5.5 buffer system (**Table 4**). The experimental threshold values were much lower than those previously reported in the literature (*2*, *6*, *35*). Threshold values can vary tremendously depending upon the format used as well as the number of panelists (*18*). The previously published values were not performed using ASTM methodology, and the number of panelists was either very small or else not specified, which may account for the substantial differences.

In the rosy cheeses, phenylacetaldehyde, 2-phenethanol, and phenylacetic acid were all above the threshold concentration range. Phenylacetaldehyde was also above threshold concentration range in the nonrosy cheeses, whereas 2-phenethanol and phenylacetic acid were near or below threshold concentration levels in the nonrosy cheeses.

Model System Confirmation. The impact of selected compounds on rosy/floral flavor was confirmed by model system analysis (**Table 5**). These compounds were spiked both singly and in combination and were evaluated using a descriptive sensory analysis panel. Although all of these compounds exhibited a rosy aroma when smelled individually, all of them do not appear to contribute to rosy/floral flavor in Cheddar cheese. Phenylethyl acetate did not produce a rosy/floral flavor when spiked back into Cheddar cheese at the levels at which it was naturally present in rosy/floral-flavored Cheddar cheese. 2-Phenethanol also did not produce rosy/floral flavors, but did contribute a yeasty aroma and flavor to the cheese models when added at the levels found naturally in cheese. Lee and Richard (*36*) found that among different species of microorganisms, only yeasts were able to form 2-phenethanol from phenylalanine in smear-ripened cheeses. Subsequently, it has been found that low levels (<100 ppb) are formed from lactic acid bacteria (*8*). Because yeast ripening is not utilized in Cheddar cheese production and aging, as in smear-ripened cheeses, 2-phenethanol does not contribute to the rosy flavor in Cheddar cheese to the extent that it does in cheeses such as Camembert (*6*).

Phenylacetaldehyde and phenylacetic acid contributed to rosy/floral flavors in Cheddar cheese. Although phenylacetaldehyde alone exhibited a close similarity to the rosy/floral flavor found naturally in cheese, when phenylacetic acid and phenylacetaldehyde were combined, they contributed more intensity of this flavor to the cheese. Even at the lowest levels found in rosy/

Table 5. Rosy/Floral Intensity and Similarity to Natural Rosy/Floral Cheeses in Cheddar Cheese Model Systems[a]

compound	concn added	intensity[b]	overall similarity[c]
rosey cheese (+ control)	0	2.0c	N/A
base cheese (– control)	0	ND	0c
base cheese + ethanol	300 μL	ND	0c
phenylacetaldehyde	6.85 ppm	2.5c	9.5a
2-phenethanol	400 ppb	ND	0c
phenylethyl acetate	100 ppb	ND	0c
phenylacetic acid	220 ppb	1.0d	7.8b
phenylacetaldehyde + 2-phenethanol + phenylethyl acetate + phenylacetic acid	6.85 ppm/400 ppb/100 ppb/220 ppb	5.0a	9.5a
2-phenethanol + phenylethyl acetate + phenylacetic acid	400 ppb/100 ppb/220 ppb	1.0d	7.5b
phenylacetaldehyde + phenylethyl acetate + phenylacetic acid	6.85 ppm/100 ppb/220 ppb	3.5b	9.5a
phenylacetaldehyde + 2-phenethanol + phenylacetic acid	6.85 ppm/400 ppb/220 ppb	5.0a	9.5a
phenylacetaldehyde + 2-phenethanol + phenylethyl acetate	6.85 ppm/400 ppb/100 ppb	3.0b	9.5a
phenylacetaldehyde + phenylacetic acid	6.85 ppm/220 ppb	4.5a	9.5a
phenylacetaldehyde + phenylacetic acid	3.71 ppm/40 ppb	2.0c	9.5a
phenylacetaldehyde + phenylacetic acid	6.85 ppm/40 ppb	2.8bc	9.5a
phenylacetaldehyde + phenylacetic acid	10.0 ppm/40 ppb	3.5b	9.5a
phenylacetaldehyde + phenylacetic acid	6.85 ppm/220 ppb	2.5c	9.5a
phenylacetaldehyde + phenylacetic acid	3.71 ppm/220 ppb	2.0c	9.5a
phenylacetaldehyde + phenylacetic acid	10.0 ppm/220 ppb	4.0a	9.5a
phenylacetaldehyde + phenylacetic acid	10.0 ppm/400 ppb	5.0a	9.5a
phenylacetaldehyde + phenylacetic acid	3.71 ppm/400 ppb	1.3d	9.5a
phenylacetaldehyde + phenylacetic acid	6.85 ppm/400 ppb	3.0b	9.5a

[a] Compounds were added to mild Cheddar cheese as described in the text. Means in a column followed by different letters are different ($p < 0.05$). [b] Rosy/floral flavor intensity based on the 10-point Spectrum scale (*18*). [c] Based on a 10-point similarity scale: 1 = different, 10 = same, where the reference was Cheddar cheese that naturally exhibited rosy/floral flavor.

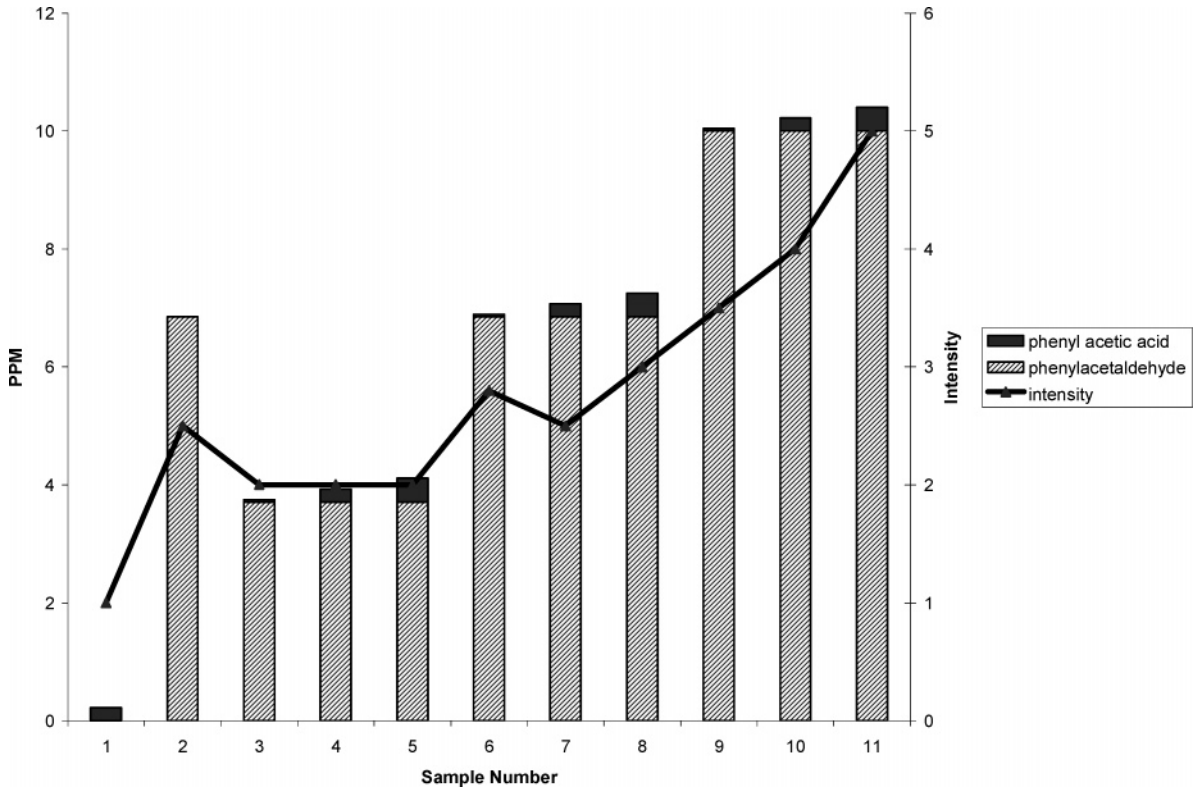

Figure 1. Impact of the addition of phenylacetaldehyde and phenylacetic acid on rosy/floral flavor of cheese models.

floral cheese, the combination of these two compounds plays a role in rosy/floral flavor.

Phenylacetaldehyde clearly plays a more important role in the rosy/floral flavor than phenylacetic acid (**Figure 1**). When phenylacetaldehyde was added to the model cheese at the lowest concentration, adding more phenylacetic acid did not affect rosy/floral intensity. When phenylacetaldehyde was added to the model at the mean concentration found naturally in cheese, the intensity of rosy/floral flavor increased. Again, the amount of phenylacetic acid did not linearly increase the intensity. However, when phenylacetaldehyde was added at the highest concentration, there was a clear linear dose/response effect on the intensity of rosy/flavor aroma when phenylacetic acid was added.

Compounds identified in this study and others are important to aged Cheddar cheese flavor, and some compounds have been linked to specific flavors (*2*, *5*). This study systematically confirms that a combination of phenylacetaldehyde and phen-

ylacetic acid contributes to rosy/floral flavor in Cheddar cheese. This study also provides us with a chemical anchor for this sensory attribute. Because this flavor is not desirable and it is formed from the catabolism of aromatic amino acids, methods should be explored to minimize the degradation or formation of these amino acids.

LITERATURE CITED

(1) Aston, J.; Dulley, J. Cheddar cheese flavour. *Aust. J. Dairy Technol.* **1982**, June, 59−64.

(2) Dunn, H.; Linsday, R. Evaluation of the role of microbial Strecker-derived aroma compounds in unclean-type flavors of Cheddar cheese. *J. Dairy Sci.* **1985**, *68*, 2859−2874.

(3) Rijnen, L.; Delacroix-Buchet, A.; Demaizieres, D.; Le Quere, J.; Gripon, J.; Yvon, M. Inactivation of lactococcal aromatic aminotransferase prevents the formation of floral aroma compounds from aromatic amino acids in semi-hard cheese. *Int. Dairy J.* **1999**, *9*, 877−885.

(4) Yvon, M.; Rijnen, L. Cheese flavor formation by amino acid catabolism. *Int. Dairy J.* **2001**, *11*, 185−201.

(5) Suriyaphan, O.; Drake, M. A.; Chen, X. Q.; Cadwallader, K. R. Characteristic aroma components of British Farmhouse Cheddar cheese. *J. Agric. Food Chem.* **2001**, *49*, 1382−1387.

(6) Roger, S.; Degas, C.; Gripon, J. Production of phenyl ethyl alcohol and its esters during ripening of traditional Camembert. *Food Chem.* **1998**, *28*, 129−140.

(7) Rychlik, M.; Bosset, J. Flavour and off-flavour compounds of Swiss Gruyere cheese. Identification of key odorants by quantitative instrumental and sensory studies. *Int. Dairy J.* **2001**, *11*, 903−910.

(8) Williams, A.; Noble, J.; Banks, J. Catabolism of amino acids by lactic acid bacteria isolated from Cheddar cheese. *Int. Dairy J.* **2001**, *11*, 203−215.

(9) Drake, M. A.; McIngvale, S.; Gerard, P.; Cadwallader, K. R.; Civille, G. V. Development of a descriptive language for Cheddar cheese. *J. Food Sci*. **2001**, *66*, 1422−1427.

(10) Urbach, G. Contribution of lactic acid bacteria to flavour compound formation in dairy products. *Int. Dairy J.* **1995**, *5*, 877−903.

(11) Singh, T.; Drake, M. A.; Cadwallader, K. R. Flavor of Cheddar cheese: A chemical and sensory perspective. *Compr. Rev. Food Sci. Food Saf.* **2003**, *2*, 139−162.

(12) Milo, C.; Reineccius, G. Identification and quantification of potent odorants in regular-fat and low-fat mild Cheddar cheese. *J. Agric. Food Chem.* **1997**, *45*, 3590−3594.

(13) Zehentbauer, G.; Reineccius, G. Determination of key aroma components of Cheddar cheese using dynamic headspace dilution assay. *Flavour Fragrance J.* **2002**, *17*, 300−305.

(14) Engel, W.; Bahr, W.; Schieberle, P. Solvent assisted flavour evaporation—a new and versatile technique for the careful and direct isolation of aroma compounds from complex food matrices. *Eur. Food Res. Technol.* **1999**, *209*, 237−241.

(15) Grosch, W. Detection of potent odorants in foods by aroma extract dilution analysis. *Trends Food Sci. Technol.* **1993**, *4*, 68−73.

(16) Van Ruth, S. Methods for gas chromatography−olfactometry: a review. *Biomol. Eng.* **2001**, *17*, 121−128.

(17) Van den Dool, H.; Kratz, P. A generalization of the retention index system including linear programmed gas liquid partition chromatography. *J. Chromatogr.* **1963**, *11*, 463−471.

(18) Meilgaard, M. M.; Civille, G. V.; Carr, B. T. Selection and training of panel members. In *Sensory Evaluation Techniques*, 3rd ed.; CRC Press: Boca Raton, FL, 1999; pp 174−176.

(19) Lawless, H.; Harono, C.; Hernandez, S. Thresholds and suprathreshold intensity functions for capsaicin in oil and aqueous based carriers. *J. Sensory Stud.* **2000**, *15*, 437−447.

(20) Christensen, K.; Reinecccius, G.; Aroma extract dilution analysis of aged Cheddar cheese. *J. Food Sci.* **1995**, *60*, 218−220.

(21) Avsar, Y.; Karagul-Yuceer, Y.; Drake, M. A.; Singh, T.; Yoon, Y. Cadwallader, K. R. Characterization of nutty flavor in Cheddar cheese. *J. Dairy Sci.* **2004**, *87*, 1999−2010.

(22) Suriyaphan, O.; Drake, M. A.; Cadwallader, K. R. Identification of volatile off-flavors in reduced-fat Cheddar cheeses containing lecithin. *Lebensm.-Wiss. -Technol.* **1999**, *32*, 250−254.

(23) Chin, H.; Bernhard, R.; Rosenberg, M. Solid-phase microextraction for cheese volatile compound analysis. *J. Food Sci.* **1996**, *61*, 1118−1121.

(24) Randhir, R.; Shetty, K. Developmental stimulation of total phenolics and related antioxidant activity in light- and dark-germinated corn by natural elicitors. *Process Biochem.* **2004**, in press.

(25) Forss, D. Review of the progress of dairy sciences: Mechanisms of formation of aroma compounds in milk and milk products. *J. Dairy Res*. **1979**, *46*, 691−706.

(26) Karagul-Yuceer, Y.; Cadwallader, K. R.; Drake, M. A. Volatile flavor components of stored nonfat dry milk. *J. Agric. Food Chem.* **2002**, *50*, 305−312.

(27) Kubickova, J.; Grosch, W. Evaluation of potent odorants of Camembert cheese by dilution and concentration techniques. *Int. Dairy J.* **1997**, *7*, 65−70.

(28) Weenen, H.; van der Ven, J. The formation of Strecker aldehydes. In *Aroma Active Compounds in Foods—Chemistry and Sensory Properties*; Takeoka, G., Guntert, M., Engle, K., Eds.; American Chemical Society: Washington, DC, 2001; pp 183−195.

(29) Yvon, M.; Berthelot, S.; Gripon, J. Adding α-ketoglutarate to semi-hard cheese curd highly enhances the conversion of amino acids to aroma compounds. *Int. Dairy J.* **1998**, *8*, 889−898.

(30) Tavaria, F.; Dahl, S.; Carballo, F.; Malcata, F. Amino acid catabolism and generation of volatiles by lactic acid bacteria. *J. Dairy Sci.* **2002**, *85*, 2462−2470.

(31) Wallace, J.; Fox, P. Effect of adding free amino acids to Cheddar cheese curd on proteolysis, flavour and texture development. *Int. Dairy J.* **1997**, *7*, 157−167.

(32) Williams, A.; Noble, J.; Tammam, J.; Lloyd, D.; Banks, J. Factors affecting the activity of enzymes involved in peptide and amino acid catabolism in non-started lactic acid bacteria isolated from Cheddar cheese. *Int. Dairy J.* **2002**, *10*, 841−852.

(33) Gummalla, S.; Broadbent, J. Tyrosine and phenylalanine catabolism by *Lactobacillus* cheese flavor adjuncts. *J. Dairy Sci.* **2001**, *84*, 1011−1019.

(34) Broadbent, J.; Gummalla, S.; Hughes, J.; Johnson, M.; Rankin, S.; Drake, M. A. *Appl. Environ. Microbiol.* **2004**, *70*, 4814−4820.

(35) Rychlik, M.; Schieberle, P.; Grosch, W. *Compilation of Thresholds, Odor Qualitities, and Retention Indices of Key Food Odorants*; Deutsche Forschungsanstalt fur Lebensmittelchemie and Instiut fur Lebensmittelchemie der Technischen Universitat Munchen: Garching, Germany, 1998.

(36) Lee, C.; Richard, J. Catabolism of L-phenylalanine by some microorganisms of cheese origin. *J. Dairy Res.* **1984**, *51*, 461−469.

Received for review October 17, 2004. Revised manuscript received February 11, 2005. Accepted February 13, 2005. Funding provided by Dairy Management, Inc., and the California Dairy Research Foundation. Manuscript 05-04 of the Department of Food Science, North Carolina State University. The use of trade names does not imply endorsement by these organizations or criticisms of ones not mentioned.

JF048278O