

Characteristic Aroma Components of British Farmhouse Cheddar Cheese

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Aroma components responsible for aroma typical of British Farmhouse Cheddar cheese were studied by aroma extract dilution analysis. Cheese extracts were prepared by direct solvent extraction, high-vacuum transfer, and class fractionation. Most aroma-active components of acidic and neutral/basic fractions have been previously associated with Cheddar cheese flavor. *p*-Cresol was mainly responsible for a “cowy-barny” note, whereas an intense “soil-like” note was due to 2-isopropyl-3-methoxypyrazine. At much lower odor intensity, 2-isobutyl-3-methoxypyrazine contributed a “bell pepper-like” note. Additionally, within the same wedge of cheese, the concentrations of *p*-cresol and 2-isopropyl-3-methoxypyrazine were lower at the narrow end (center) than at the rind side. Direct addition of *p*-cresol (≥ 100 ppb) or 2-isopropyl-3-methoxypyrazine (≥ 3 ppb) in a mild domestic Cheddar cheese resulted in increases in intensities of cowy/phenolic and earthy/bell pepper aroma notes.

Keywords: *British Farmhouse Cheddar cheese; cheese flavor; p-cresol; alkylmethoxypyrazines; aroma extract dilution analysis*

INTRODUCTION

British Farmhouse Cheddar cheeses are regarded as “specialty” products (1). Farmhouse cheeses possess distinctly different flavor attributes from domestic Cheddar cheeses. The typical characteristic flavor of Farmhouse cheeses has been described as unclean “barnyard” and “earthy” (2). In general, unclean flavors in regular Cheddar cheese are considered as defective attributes (3). Many studies have been conducted to determine probable causes of off-flavors in natural cheeses (4–6). However, “mushroom–musty–earthy” odors are preferable in surfaced-ripened cheeses such as Brie and Camembert (7). Karahadien and co-workers (8, 9) reported that the development of secondary metabolites during cheese ripening by *Penicillium* sp. contributed pronounced mushroom–earthy–raw potato notes in Brie and Camembert cheeses. The character-impact odorants included 1-octen-3-ol, 1,5-octandien-3-ol, 1,5-octadien-3-one, 3-octanol, 3-octanone, 2-methylisoborneol, and 2-isopropyl-3-methoxypyrazine. Despite numerous studies on the characterization of the flavor profiles of different cheese varieties (5, 10, 11), to our knowledge, the characteristic aroma components of British Farmhouse Cheddar cheeses have not been studied.

Cheddar cheeses produced in different regions generally possess unique and distinguishing flavor attributes (12). Consumer attitudes and preferences differ from region to region, so flavor attributes that may be

considered undesirable in one region may be favored in a different region. For example, bitterness in Australian and Irish Cheddar cheeses is considered to be desirable (13), whereas in American Cheddar cheeses this attribute is undesirable. As a result, the definition of flavor of Cheddar cheese with respect to subjective analysis is still somewhat ambiguous.

The flavor of natural cheese has been acknowledged as a balanced blend of its constituents such as fatty acids, sulfur compounds, esters, and alcohols (12). However, it is widely accepted that only a few volatile compounds are primarily responsible for the overall aroma of foods (14, 15). Dilution techniques, especially aroma extract dilution analysis (AEDA), have been successfully used to elucidate characteristic aroma compounds in foods (16–19).

Our first objective was to systematically identify the character impact odorants of British Farmhouse Cheddar cheeses by means of sensory evaluation and instrumental analyses. The results will aid in the linking of sensory flavor descriptors with defined references for Cheddar cheese and will lead to a better understanding of Cheddar cheese flavor. The second objective was to quantify the chemical compounds responsible for unclean flavors at different parts of a wedge of British Farmhouse Cheddar cheese. The distribution pattern of selected aroma-active compounds will aid in indicating the source of potential aroma contributors.

MATERIALS AND METHODS

Farmhouse Cheddar Cheeses. Two different brands of Farmhouse Cheddar cheeses (designated samples A and B) were purchased as 2 kg wedges from Whole Foods (Madison, WI). Both cheeses were manufactured from raw milk by large farmstead cheese operations located in Somerset, U.K., and

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Table 1. Terms and Definitions Used in the Sensory Evaluation of British Farmhouse Cheddar Cheeses

term	definition	references
cooked whey	aromatics associated with cooked milk	skim milk heated to 85 °C for 30 min
diacetyl	aromatics associated with Cheddar cheese whey	fresh Cheddar whey
lactone	aromatics associated with diacetyl	diacetyl
sulfur	aromatics associated with milkfat	fresh coconut meat, heavy cream, δ -dodecalactone
	aromatics associated with sulfurous compounds	boiled mashed egg, struck match, hydrogen sulfide bubbled through water
brothy	aromatics associated with boiled meat or vegetable soup stock	Knorr beef broth cubes, Knorr vegetable 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
cowy/phenolic	aromatics associated with barns and stock trailers	<i>p</i> -cresol, Band-aids, phenol
earthy/bell pepper	aromatics associated with freshly cut green vegetables	alkyl methoxypyrazines, freshly cut bell pepper
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)
prickle	chemical feeling factor of which the sensation of carbonation on the tongue	soda water

were available commercially almost year round. These cheeses were produced in a traditional fashion as 30 or 60 lb wheels and aged for a minimum of 1 year, with natural bandage rinds (Neal's Yard Dairy, personal communication, 1999). Each cheese was purchased twice within 6 months to assess reproducibility of flavors. All cheese samples were vacuum packed and kept at 5 °C until analysis.

Chemicals. Standard reference compounds identified in Table 2 were obtained from commercial sources. Compounds **1**, **3**, **18**, **20**, **34**, **35**, and **39** were purchased from Aldrich F&F (Milwaukee, WI). Compounds **2**, **4**–**6**, **19**, **23**–**25**, **27**, **28**, **33**, and **36** were supplied by Aldrich (Milwaukee, WI). Compound **7** was received from TCI America Organic Chemicals (Portland, OR). Compounds **8**, **17**, and **21** were from Bedoukian Research, Inc. (Danbury, CT). Compound **11** was purchased from Fisher Scientific (Fair Lawn, NJ). Compounds **22** and **37** were supplied by Sigma (St. Louis, MO). Compound **32** was purchased from Lancaster Synthesis Inc. (Windham, NH). 2-Methyl-3-heptanone, 2-methylvaleric acid, methanol, and anhydrous diethyl ether were supplied by Aldrich.

Sensory Evaluation. Panelists were selected on the basis of interest, time available, and a liking for cheese. Panelists were university students or staff; six were female and five were male, and ages ranged from 20 to 42 years. Panelists evaluated the cheeses using the language and training methods developed for Cheddar cheeses (2). Panelists received 75 h of training on flavor, aroma, or feeling factor terms. Panelists marked responses on 15-point numerical intensity scales anchored on the left with "not" and on the right with "very". Panelists were trained to use the scales using the universal sweetness, acidity, bitter, and salty references described by Meilgaard et al. (20) for the Spectrum method. For each of the other terms, a "definition" reference was identified (Table 1). During training, panelists discussed terms and attributes and learned to consistently use the scale.

Sensory Evaluation of Cheeses. Cheeses were presented in 2 × 2 × 2 cm cubes with three-digit codes. Panelists had free access to water and unsalted crackers throughout evaluations and were instructed to expectorate samples. Order of presentation was balanced among panelists. Panelists evaluated each cheese in duplicate.

Sensory Evaluation of Cheese Models. Each cheese model consisted of 100 g of local mild Cheddar cheese (Birmingham, AL) and a predetermined amount of *p*-cresol or 2-isopropyl-3-methoxypyrazine. All chemical solutions were prepared in ethanol at the suitable concentration with the maximum dose added to cheese being <50 μ L. Chemical solutions were introduced to cheeses by a clean, disposable glass micropipet. Cheese models were kneaded for 5 min and

then molded to a rectangular shape and equilibrated overnight at 5 °C. Cheese models resembled natural cheese and were introduced to the sensory panel as natural cheese. Cheese models were evaluated using the same procedure applied for British Farmhouse Cheddar cheeses.

Aroma Analysis. Preparation of Cheese Extracts. Cheese extracts were prepared following the procedure described by Milo and Reineccius (5). Cheese extracts for AEDA were prepared from the first lot of samples A and B. Cheese wedges were trimmed off 1 cm from the rind end. Center and outer portions were obtained by cutting at 7.5 cm from the narrow end. Cheeses were then grated. The mixture of freshly grated cheese (50 g), anhydrous diethyl ether (75 mL), 10 μ L of 2-methylvaleric acid solution (acidic fraction internal standard, 2.920 μ g/ μ L in methanol), and 10 μ L of 2-methyl-3-heptanone solution (neutral/basic fraction internal standard, 0.171 μ g/ μ L in methanol) was constantly shaken for 30 min. The solvent phase was separated from the mixture by centrifugation (3000g, 30 min). The remaining solids were re-extracted twice with ether (2 × 50 mL). The solvent phases were combined, kept at -20 °C overnight, dried over anhydrous sodium sulfate, and subsequently concentrated to 100 mL at 40 °C using a Vigreux column. Volatile and semivolatile components were isolated from the concentrated extract by means of high-vacuum transfer. The apparatus was the same as that described in Suriyaphan et al. (6) except the operating vacuum level was $\sim 5 \times 10^{-5}$ Torr. The extract was distilled at ambient temperature for 2 h followed by an additional 2 h at 50 °C. Volatile compounds were accumulated in a collecting tube immersed in liquid nitrogen. After thawing at ambient temperature, the distillate was concentrated to 25 mL under a gentle stream of nitrogen gas. The distillate was then washed twice with sodium bicarbonate (0.5 mol/L, 2 × 15 mL) and a saturated aqueous solution of NaCl (2 × 5 mL). The solvent phase retaining the neutral–basic (NB) compounds was dried over anhydrous sodium sulfate and concentrated to 0.5 mL. The aqueous phase was acidified to pH 2 using 10% aqueous HCl followed by solvent extraction (3 × 5 mL ether). The solvent phase containing acidic compounds was dried over anhydrous sodium sulfate and concentrated to 1 mL. All NB fractions and acidic (Ac) fractions were kept at -20 °C until analysis.

Gas Chromatography—Olfactometry (GCO) and AEDA. Serial dilutions (1:2) of each fraction were prepared using anhydrous diethyl ether as a diluent. Each dilution was transferred to a 2 mL amber vial equipped with a Teflon-lined screw cap. Dilutions were kept at -20 °C until analyzed. The GCO system consisted of an HP5890 series II GC (Hewlett-Packard Co., Palo Alto, CA) equipped with a DB-FFAP or DB-

5MS capillary column (30 m × 0.32 mm i.d. × 0.25 μm film thickness; J&W Scientific, Folsom, CA), a flame ionization detector (FID), and a sniffing port. For each dilution, 1 μL was injected in the cold on-column mode. The injector temperature was 40 °C. Helium was used as a carrier gas at a velocity of 25 cm/s. Oven temperature was programmed from 40 to 195 °C at 5 °C/min with an initial hold time of 5 min and a final hold time of 40 min. Temperatures of the FID and transfer line were held at 200 °C. GCO was performed by two panelists familiar with dairy products and olfactometry techniques. Each sample was analyzed twice.

GC—Mass Spectrometry (GC-MS). The GC-MS system consisted of an HP 5890 series II GC/HP 5972 mass selective detector (MSD, Hewlett-Packard Co.). All NB fractions were further concentrated under a gentle stream of nitrogen gas to 100 μL prior to GC-MS analysis. Two microliters of each extract was injected (on-column mode, injector temperature = 40 °C) into a DB-Wax column (30 m × 0.25 mm i.d. × 0.25 μm film thickness; J&W Scientific) or an HP-5MS (50 m × 0.20 mm i.d. × 0.33 μm film thickness, Hewlett-Packard Co.). The oven temperature was programmed from 40 to 200 °C at 5 °C/min with initial and final hold times of 5 and 45 min, respectively. MSD condition were as follows: capillary direct interface temperature, 280 °C; ionization energy, 70 eV; mass range, 33–350 amu; scan rate, 5 scans/s. Duplicate analyses were performed on each sample.

Compound Identification. Identifications were based on comparison of GC retention indices, mass spectra, and aroma properties of unknowns with those of authentic standard compounds analyzed under identical experimental conditions.

Compound Quantification. Recovery factors of selected compounds in NB fractions were determined by direct addition of known amounts of authentic standards in 50 g of mild Cheddar cheese prior to solvent extraction—high-vacuum transfer and GC-MS as described previously.

For acidic compounds, the response factors were determined by analysis of a series of known concentrations of authentic standards. Two microliters of each dilution was analyzed by GC-MS using the same procedure as described for an Ac fraction of British Farmhouse Cheddar cheese.

RESULTS AND DISCUSSION

Sensory Evaluation. Sensory data (Figure 1) revealed that both samples exhibited several uncommon Cheddar cheese flavors, described as “earthy/bell pepper” and “cowy/phenolic”. An earthy flavor has been regarded as a desirable attribute for some aged cheeses (21). Bell pepper flavor is undoubtedly an uncommon occurrence in regular Cheddar cheese. Cowy/phenolic flavor has been classified as a defect attribute in cheeses (22). Sensory evaluation on multiple samples of cheeses indicated that these flavors are consistent with and comparable to typical characteristics of British Farmhouse Cheddar cheeses previously described by Drake and co-workers (2). In our study, sensory panelists agreed that there were some differences in several basic flavor characteristics between the two cheeses. Sample A exhibited less bitter, sweet, prickle, nutty, free fatty acid, and fruity flavors than sample B (Figure 1). Bitterness, sweetness, and fruitiness normally are regarded as defects in regular Cheddar cheeses (13). It is well established that bitterness in Cheddar cheese is highly related to specific peptides that contained high amounts of certain hydrophilic amino acids such as glutamic acid and serine (23). Proteolytic enzymes produced by psychrotrophic bacteria in raw milk or nonstarter bacteria from raw milk are responsible for bitterness in Cheddar cheeses (24, 25). British Farmhouse Cheddar cheeses are made from raw milk (Neil’s Yard Dairy, personal communication, 1999). Sweet flavors in aged cheese are also due to proteolytic activity.

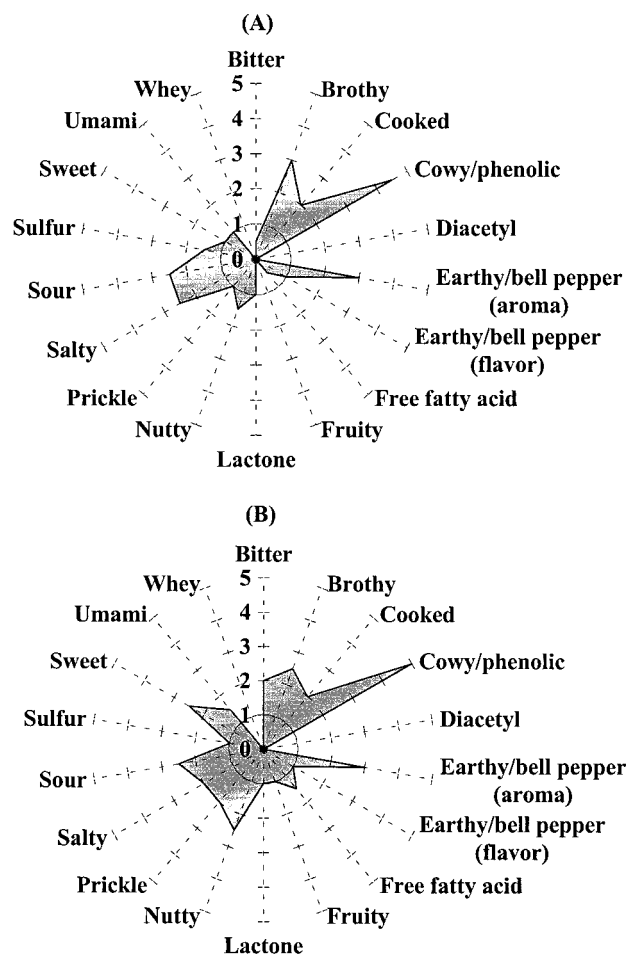


Figure 1. Sensory profiles of British Farmhouse Cheddar cheeses.

In addition to peptides, several other compounds were reported as major contributors to sweetness in certain varieties of cheeses. For instance, 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (Furaneol) and 5-ethyl-4-hydroxy-3(2*H*)-furanone were important contributors to sweetness in Emmental cheeses (26). Fruity notes in Cheddar cheese were associated with high levels of esters, especially ethyl butanoate and ethyl hexanoate. These esters were produced by non-lactic acid bacteria contamination during the manufacture of cheese (27).

AEDA. AEDA of the volatile fraction of British Farmhouse Cheddar cheeses revealed 27 odorants in the FD factor range of 9–729 (Table 2). These included 3 nitrogen-containing compounds, 3 esters, 6 acids, 1 phenolic compound, 4 alcohols, 1 sulfur-containing compound, 2 aldehydes, 5 ketones, and 2 miscellaneous compounds. Because the aim of the present study was to identify compounds responsible for the typical flavor of British Farmhouse Cheddar cheese, the following discussion will focus on the most potent aroma compounds and compounds possessing targeted flavor properties (earthy/bell pepper and cowy/phenolic) that were previously identified by sensory analysis.

Four odorants in both cheeses were detected at the highest FD factor of 729. These were 2-isopropyl-3-methoxy-pyrazine (earthy, soil), 3-(methylthio)propanal (potato), *p*-cresol (cowy, barny, medicine), and δ -dodecalactone (cheesy, coconut). Within this group, 3-(methylthio)propanal and δ -dodecalactone have been commonly found in a regular Cheddar cheese (5, 6). 2-Isopropyl-3-methoxy-pyrazine and 2-acetylpyrazine

Table 2. Potent Odorants in British Farmhouse Cheddar Cheese

no.	compound	fraction ^a	odor ^b	RI ^c		FD factor ^d	method of identification ^e
				DB-FFAP	DB-5MS		
1	2-isopropyl-3-methoxypyrazine	N/B	earthy, soil	1412	1094	729	RI, odor, MS
2	3-(methylthiol)propanal	N/B	baked potato	1441	911	729	RI, odor, MS
3	<i>p</i> -cresol	N/B	cowy, barny, medicine	2082	1074	729	RI, odor, MS
4	δ -dodecalactone	N/B	cheesy, coconut	2400	1728	729	RI, odor, MS
5	butanoic acid	Ac	fecal, cheesy	1628	825	243	RI, odor, MS
6	isovaleric acid	Ac	Swiss cheese, fecal	1657	873	243	RI, odor, MS
7	2-phenylethanol	N/B	rosy	1912	1112	243	RI, odor, MS
8	ethyl octanoate	N/B	fruity	1404	1196	81	RI, odor, MS
9	unknown	N/B	plastic, styrene-like	1456		81	
10	unknown	N/B	hay, melon, stale	1488		81	
11	acetic acid	N/B	vinegar	1452	637	81	RI, odor, MS
12	unknown	N/B	mushroom	1555		81	
13	unknown	N/B	hay, sweet, stale	1703		81	
14	unknown	N/B	musty	1797		81	
15	β -damascenone	N/B	applesauce	1809	1391	81	RI, odor
16	unknown	N/B	saffron	1966		81	
17	octanoic acid	Ac	body odor, sweat	2044	1281	81	RI, odor, MS
18	3-hydroxy-4,5-dimethyl-2(5 <i>H</i>)-furanone (sotolon)	Ac	curry, seasoning	2192	1107	81	RI, odor
19	phenylacetic acid	Ac	rosy	2569	1262	81	RI, odor, MS
20	ethyl butanoate	N/B	bubble gum, fruity	1031	803	27	RI, odor, MS
21	ethyl hexanoate	N/B	fruity	1221	1000	27	RI, odor, MS
22	dimethyl trisulfide	N/B	sulfurous, cabbage	1358	967	27	RI, odor, MS
23	phenylacetaldehyde	N/B	rosy, styrene	1631	1045	27	RI, odor, MS
24	pentanoic acid	Ac	Swiss cheese	1723	1041	27	RI, odor, MS
25	guaiacol	N/B	spice, smoky	1854	1089	27	RI, odor, MS
26	unknown	N/B	spicy, stale, woody, hay	1872		27	
27	γ -decalactone	N/B	coconut	2152	1467	27	RI, odor, MS
28	δ -decalactone	N/B	peachy, coconut	2209	1490	27	RI, odor, MS
29	unknown	N/B	fruity	1046		9	
30	unknown	N/B	plastic	1090		9	
31	unknown	N/B	sweet	1128		9	
32	1-octen-3-one	N/B	mushroom	1285	981	9	RI, odor, MS
33	2-acetylpyrazine	N/B	popcorn	1319	965	9	RI, odor, MS
34	2-isobutyl-3-methoxypyrazine	N/B	bell pepper-like	1506	1174	9	RI, odor, MS
35	linalool	N/B	sweet, floral	1522	1099	9	RI, odor, MS
36	(<i>E,Z</i>)-2,6-nonadienal	N/B	melon-like, cucumber	1571	1151	9	RI, odor, MS
37	geosmin	N/B	earthy, moistened soil	1576	1417	9	RI, odor, MS
38	unknown	N/B	waxy, cheesy	1824		9	
39	2,5-dimethyl-4-hydroxy-3(2 <i>H</i>)-furanone (Furaneol)	Ac	sweet, burnt sugar	2025	1065	9	RI, odor, MS

^a N/B, neutral/basic fraction; Ac, acidic fraction. ^b Odor description at the GC-sniffing port during GCO. ^c Retention indices were calculated from GCO data. ^d FD factor, flavor dilution factors on DB-FFAP column. ^e Compounds were identified by comparison with the authentic standards on the following criteria: retention index (RI) on DB-FFAP and DB-5MS columns, odor property at the GC-sniffing port, and mass spectra in the electron impact mode.

(popcorn) have been reported as minor components of old Cheddar cheese (12). Zehentbauer and Reineccius (28) recently reported the occurrence of alkylmethoxypyrazines as minor constituents in a full-fat Cheddar cheese. Methoxyalkylpyrazines generally have exceptionally potent and penetrating odors (29). Dunn and Lindsay (3) suggested that the development of 2-isopropyl-3-methoxypyrazine resulted from microbial Strecker degradation reactions in aged Cheddar cheese. Precursors for alkylmethoxypyrazines are reportedly branched-chain amino acids such as leucine (30). In addition, geosmin, methylisoborneol, and 2-isopropyl-3-methoxypyrazine were reported as major contributors to earthy/musty flavors in water, vegetables, and dairy products (21, 31).

The presence of *p*-cresol has been considered as a defect in a variety of cheeses (3, 21). Phenolic compounds were found to be a component of ointment used to treat infected cows (32). Dunn and Lindsay (3) reported that particular Strecker degradation products contributed unclean flavors in well-aged Cheddar cheeses. For instance, phenethyl alcohol and phenylacetaldehyde caused unclean, rose-like flavors, whereas *p*-cresol was described as barny and horse blanket-like and was

related to unclean flavors in Cheddar cheeses. They proposed that utilization of starter cultures possessing high proteolytic activity promoted the degradation of bitter peptides to amino acids, which are essential substrates in Strecker degradation reactions. In addition, Badings and co workers (33) suggested that the salt-tolerant lactobacilli in the rennet produced *p*-cresol in Gouda cheese.

Another significant odorant found in the cheese extracts was identified as 2-isobutyl-3-methoxypyrazine (bell pepper-like). This compound may impact the overall aroma of British Farmhouse Cheddar cheeses due to its extremely low odor threshold, 2 ppb in water (34). In contrast to its extremely low odor threshold, the FD factor of 2-isobutyl-3-methoxypyrazine in Farmhouse cheese was not high (FD = 9), indicating that it was a trace constituent. In general, quantification of 2-isobutyl-3-methoxypyrazine requires a special analytical technique such as stable isotope dilution analysis and use of a double-focusing mass spectrometer detector (35).

Quantification of Selected Aroma-Impact Compounds. As expected, the GC-MS response of 2-isobutyl-3-methoxypyrazine was too low (<1 ppb) to accurately

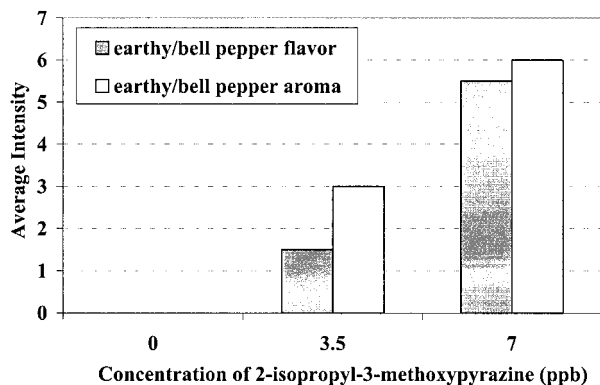


Figure 2. Relationship between 2-isopropyl-3-methoxy-pyrazine concentration and earthy/bell pepper aroma and flavor intensity in a cheese model.

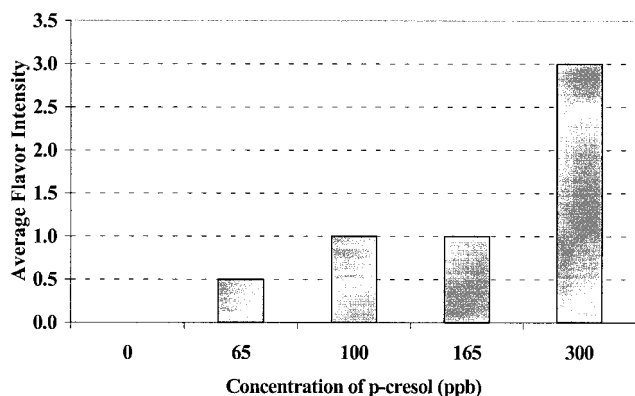


Figure 3. Relationship between *p*-cresol concentration and cowy/phenolic flavor intensity in a cheese model.

determine its content in the cheese extracts. 2-Isopropyl-3-methoxy-pyrazine was found at a high enough level to allow for quantification in sample B. Its average concentration was very low (3.25 ± 1 ppb). *p*-Cresol was found in cheese samples A and B at levels of 99 ± 6 and 132 ± 13 ppb, respectively. These concentrations were lower than the detection threshold of *p*-cresol (300 ppb in cheese) published by Dunn and Lindsay (3). To clarify our results, we directly added 2-isopropyl-3-methoxy-pyrazine (3.5–7 ppb) and *p*-cresol (65–300 ppb) into a mild cheese model. The earthy/bell pepper flavor and aroma were detectable in cheese models containing 3.5 ppb of 2-isopropyl-3-methoxy-pyrazine (Figure 2). Similarly, panelists were able to detect a cowy/phenolic flavor when the amount of *p*-cresol in the cheese model was ≥ 100 ppb (Figure 3).

To investigate the probable source of these two compounds in British Farmhouse Cheddar cheeses, we prepared cheese extracts from two different locations (center and outer) obtained from the same wedge of sample B. Concentrations of primarily acidic compounds such as butanoic acid, hexanoic acid, and octanoic acid were uniform throughout the wedge of cheese (Table 3). In contrast, differences in levels of 2-isopropyl-3-methoxy-pyrazine and *p*-cresol were observed. More specifically, the extract from the outer portion of the cheese had higher concentrations of both compounds. This result indicated the possible development of these particular pyrazine and phenolic compounds at the rind followed by the migration of these compounds toward the center of the cheese during the ripening period. There was a fine mold growth on the rinds of the studied cheeses, so the development of these pyrazine and

Table 3. Concentration of Selected Compounds in a British Farmhouse Cheddar Cheese (Sample B)

compound	av concn (ppb)	
	outer	center
<i>p</i> -cresol	300 ± 100	25 ± 5
2-isopropyl-3-methoxy-pyrazine	12 ± 3	<1
butanoic acid	60200 ± 8090	66200 ± 3200
hexanoic acid	21000 ± 420	21200 ± 110
octanoic acid	7810 ± 2020	8120 ± 145

phenolic compounds might be associated with the growth of natural molds on the rind of the cheeses.

In summary, the results of this study revealed that earthy/bell pepper and cowy/phenolic flavors were characteristic sensory attributes of British Farmhouse Cheddar cheeses. The cowy/phenolic flavor was caused by the presence of *p*-cresol, whereas 2-isopropyl-3-methoxy-pyrazine and 2-isobutyl-3-methoxy-pyrazine were responsible for earthy/bell pepper flavors. Higher concentrations of these compounds at the rind of the cheese suggest that they were formed on or near the surface of the cheese.

LITERATURE CITED

- O'Connell, J. J.; Henchion, M. M. The marketing of Irish farmhouse cheese. *J. Soc. Dairy Technol.* **1994**, *47*, 51–53.
- Drake, M. A.; McIngvale, S. C.; Cadwallader, K. R.; Civille, G. V. Development of a descriptive language for Cheddar cheese. *Book of Abstracts*, IFT Annual Meeting, Dallas, TX; IFT: Chicago, IL, 2000; p 12.
- Dunn, H. C.; Lindsay, R. C. Evaluation of the role of microbial Strecker-derived aroma compounds in uncleaned-type flavors of Cheddar cheese. *J. Dairy Sci.* **1985**, *68*, 2859–2874.
- Stark, W.; Forss, D. A. A compound responsible for mushroom flavor in dairy products. *J. Dairy Res.* **1964**, *31*, 253–259.
- Milo, C.; Reineccius, G. A. Identification and quantification of potent odorants in regular-fat and low-fat mild Cheddar cheese. *J. Agric. Food Chem.* **1997**, *45*, 3590–3594.
- 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.
- Kosikowski, F. V. In *Cheese and Fermented Milk Foods*, 2nd ed.; F. V. Kosikowski and Associates: Brooktondale, NY, 1982; pp 329–333.
- Karahadian, C.; Josephson, D. B.; Lindsay, R. C. Contribution of *Penicillium* sp. to the flavor of Brie and Camembert cheese. *J. Dairy Sci.* **1985**, *68*, 1865–1877.
- Karahadian, C.; Josephson, D. B.; Lindsay, R. C. Volatile compounds from *Penicillium* sp. contributing musty-earthly notes to Brie and Camembert cheese flavors. *J. Agric. Food Chem.* **1985**, *33*, 339–343.
- Kubickova, J.; Grosch, W. Quantification of potent odorants in Camembert cheese and calculation of their odour activity values. *Int. Dairy J.* **1998**, *8*, 17–23.
- Sable S.; Cottenceau, G. Current knowledge of soft cheeses flavor and related compounds. *J. Agric. Food Chem.* **1999**, *47*, 4825–4836.
- McGugan, W. A. Cheddar cheese flavors: A review of current progress. *J. Agric. Food Chem.* **1975**, *23*, 1047–1050.
- McSweeney, P. L. H. The flavour of milk and dairy products: III. Cheese: taste. *Int. J. Dairy Technol.* **1997**, *50*, 123–128.
- Acree, T. E. Bioassays for flavors. In *Flavor Science—Sensible Principles and Techniques*; Acree, T. E., Teranishi, R., Eds.; ACS Professional Books; American Chemical Society: Washington, DC, 1993; pp 1–20.

- (15) Grosch, W. Detection of potent odorants in food by aroma extraction dilution analysis. *Trends Food Sci. Technol.* **1993**, *17*, 142–144.
- (16) Guth, H.; Grosch, W. Identification of the character impact odorants of stewed beef juice by instrumental analyses and sensory studies. *J. Agric. Food Chem.* **1994**, *42*, 2862–2866.
- (17) Christensen, K. R.; Reineccius, G. A. Aroma extract dilution analysis of aged Cheddar cheese. *J. Food Sci.* **1995**, *60*, 218–220.
- (18) Baek, H. H.; Cadwallader, K. R. Aroma volatiles in cooked alligator meat. *J. Food Sci.* **1997**, *62*, 321–325.
- (19) Steinhaus, M.; Schieberle, P. Comparison of the most odor-active compounds in fresh and dried hop cones (*Humulus lupulus* L. variety Spalter select) based on GC-olfactometry and odor dilution techniques. *J. Agric. Food Chem.* **2000**, *48*, 1776–1783.
- (20) Meilgaard, M.; Civille, G. V.; Carr, B. T. Selection and training of panel members. In *Sensory Evaluation Techniques*, 2nd ed.; CRC Press: Boca Raton, FL, 1991; pp 174–176.
- (21) Nijssen, B. Off-Flavors. In *Volatile Compounds in Foods and Beverages*; Maarse, H., Ed.; Dekker: New York, 1991; pp 689–735.
- (22) Ramshaw, E. H. Aspects of the flavour of phenol, methylphenol and ethylphenol. *CSIRO Food Res. Q.* **1985**, *45*, 20–22.
- (23) Lee, K.-P. D.; Warthesen, J. J. Preparative methods of isolating bitter peptides from Cheddar cheeses. *J. Agric. Food Chem.* **1996**, *44*, 1058–1063.
- (24) Hick, C. L. Effect of milk quality and low-temperature storage on cheese yield—a summation. *J. Dairy Sci.* **1986**, *69*, 649–657.
- (25) Wendorff, B.; Johnson, M.; Olson, N. The great Cheddar debate. *Dairy Pipeline* **1998**, *10*, 1–3.
- (26) Urbach, G. The flavour of milk and dairy products: II. Cheese: contribution of volatile compounds. *Int. J. Dairy Technol.* **1997**, *50*, 79–89.
- (27) McGugan, W. A.; Blais, J. A.; Boulet, M.; Giroux, R. N.; Elliot, J. A.; Emmons, D. B. Ethanol, ethyl esters, and volatile fatty acids in fruity Cheddar cheese. *Can. Inst. Food Sci. Technol. J.* **1975**, *8*, 196–198.
- (28) Zehentbauer, G. N.; Reineccius, G. A. Identification of key odorants in regular-fat Cheddar cheese by dynamic-headspace-dilution-assay (DHDA). *Book of Abstracts*; IFT Annual Meeting, Dallas, TX; IFT: Chicago, IL, 2000; p 14.
- (29) Lindsay, R. C. Flavors. In *Food Chemistry*, 3rd ed.; Fennema, O. R., Ed.; Dekker: New York, 1996; pp 724–762.
- (30) Murray, K. E.; Shipton, J.; Whitfield, F. B. 2-Methoxypyrazines and the flavor of green peas (*Pisum sativum*). *Chem. Ind. (London)* **1970**, 897–898.
- (31) Murray, K. E.; Whitfield, F. B. The occurrence of 3-alkyl-2-methoxypyrazine in raw vegetables. *J. Sci. Food Agric.* **1975**, *26*, 973–986.
- (32) Schlegel, J. A.; Babel, F. J. Flavor imparted to dairy products by phenol derivatives. *J. Dairy Sci.* **1963**, *46*, 190–194.
- (33) Badings, H. T.; Stadhouders, J.; Van Duin, H. Phenolic flavor in cheese. *J. Dairy Sci.* **1968**, *51*, 31–35.
- (34) Buttery, R. G.; Seifert, R. M.; Lundin, R. E.; Gaudagni, D. G.; Ling, L. C. Characterization of some volatile constituents of bell peppers. *J. Agric. Food Chem.* **1969**, *17*, 1322–1327.
- (35) Allen, M. S.; Lacey, M. J.; Boyd, S. Determination of methoxypyrazines in red wines by stable isotope dilution gas chromatography–mass spectrometry. *J. Agric. Food Chem.* **1994**, *42*, 1734–1738.

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