Identification and Quantification of Potent Odorants in Regular-Fat and Low-Fat Mild Cheddar Cheese

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The potent odorants of regular-fat and low-fat Cheddar cheese were systematically characterized by aroma extract dilution analysis and gas chromatography/olfactometry of static headspace samples. Quantification of nine odorants using stable isotopes as the internal standards and calculation of odor activity values revealed methional, 2(5)-ethyl-5(2)-methyl-4-hydroxy-3(2*H*)-furanone (homofuraneol), diacetyl, acetic acid, and butyric acid as odorants with high aroma impact. Among the highly volatile compounds, the role of methanethiol and dimethyl sulfide for the nasal perception of the cheese flavor was established. The low-fat Cheddar cheese had a meaty-brothy odor defect that is believed to be caused by significantly higher amounts of 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (Furaneol), homofuraneol, and methional in this cheese.

Keywords: *Cheddar cheese; regular fat; low fat; AEDA; static headspace; odor activity value*

INTRODUCTION

The chemical differences in the aromas of regular- and low-fat Cheddar cheese are not well-known. Aroma differences between low-fat and regular-fat Cheddar cheeses are considered to be partially responsible for the lower consumer acceptance of the reduced-fat cheeses. Although a lot of research on the flavor of Cheddar cheese has been done and more than 230 volatiles have been identified (Maarse and Visscher, 1996), little is known about the actual contribution of these volatiles to the aroma profile. As a consequence of our poor understanding of those compounds that are potent in this cheese, the reproduction of its aroma remains a difficult task (Fox et al., 1995).

In the present investigation we applied aroma extract dilution analysis (AEDA) combined with GC−sniffing of static headspace samples (GCO-H) to screen for the potent odorants in mild Cheddar cheese. AEDA was applied for the first time on Cheddar cheese by Christensen and Reineccius (1995). On the basis of their results, a sensory study showed diacetyl, methional, and butyric acid as important aroma compounds (Dacremont and Vickers, 1994). The authors mention a possible contribution of other compounds that are very volatile and/or were not commercially available for their tests. AEDA was also successfully used in the characterization of the aroma components in Swiss Emmentaler cheese by Preininger et al. (1994). After quantification of the aroma compounds by an isotope dilution assay and calculation of odor activity units (ratio of the concentration to the threshold), they were able to determine the character impact compounds of this cheese (Preininger and Grosch, 1994). Very recently, several groups focused on new and more reliable headspace techniques to further elucidate the volatiles of Cheddar cheese (Yang and Min, 1994; Arora et al., 1995). Headspace analysis alone, however, allows only a partial view of the total aroma composition of a food but is considered a valuable complementary tool to GC−sniffing of food extracts (Milo, 1995).

Our objective was to systematically characterize the odorants of mild regular-fat Cheddar cheese and to compare them to those in low-fat cheese on a quantitative basis. Conclusions about the cause of a meaty-broth-like odor defect observed in the low-fat sample are given on the basis of odor activity values (OAV).

EXPERIMENTAL PROCEDURES

Chemicals. Diethyl ether (anhydrous, 99.8%) and pure samples of compounds **2**−**5**, **7**, **9**, **10**, **12**−**14**, **17**, **19**, **20**, **23**, and **25** (Tables 1 and 2) were purchased from Aldrich (Milwaukee, WI). Compound **1** was obtained from Givaudan Roure (Clifton, NJ), **8** from Firmenich (Princeton, NJ), and **15** from Lancaster (Windham, NJ). Compound **6** was synthesized on the basis of work by Heiba et al. (1974). Odorant **11** was prepared according to the method of Schieberle and Grosch (1991), and **18** and **24** were prepared according to the procedure of Milligan et al. (1963). The labeled compounds in Table 3 were synthesized in our laboratory with the exceptions of d_3-homofuraneol, which was a gift from Dr. Blank (Nestec Ltd., Lausanne, Switzerland), and [$^{13}C_2$]acetic acid, which was purchased from Cambridge Isotope Laboratories (Montreal, Canada).

Cheese. Ten kilograms of a mild regular-fat Cheddar cheese (A) and 10 kg of a cheese with a 40% fat reduction (B) were purchased on the retail market. The samples were vacuum packed in portions of 200−250 g and stored in a blast freezer at −30 °C prior to the experiments.

Preparation of Cheese Extracts for AEDA. The frozen samples were cut into pieces of about 1 cm^3 and then ground with an Osterizer to a fine powder while frozen in liquid nitrogen. Diethyl ether (400 mL) was added to the cheese powder (250 g of A, 200 g of B) and then stirred under an atmosphere of argon for 2 h (all-glass/Teflon setup). The cheese/solvent mixture was filtered, and the residue was pressed to recover most of the solvent and rinsed with ether (2 × 50 mL). The cheese residue was then re-extracted with diethyl ether (300 mL) by stirring for 1 h. The ether extracts were combined, dried over anhydrous sodium sulfate, and then concentrated to 150−200 mL at 40 °C using a Vigreux column (50 × 1 cm). The volatiles and the solvent were distilled off the low-volatile materials under a high vacuum using the system described by Sen et al. (1991), except with just two

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Table 1. Potent Odorants (FD > 8) in Mild Regular-Fat (A) and Low-Fat (B) Cheddar Cheeses

no.	compound	odor[a]	RI[b] DB-5 ms	RI[b] DB-1701	FD[c] A	FD[c] B
1	homofuraneol[d]	caramel	1128	1310	512	2048
2	butyric acid[d]	sweet, sweaty	1627[f]		512	1024
3	Furaneol[d]	caramel	1051	1240	128	1024
4	δ-decalactone[d]	coconut-like	1500	1744	128	64
5	skatole[d]	fecal	1400	1636	64	64
6	6-(*Z*)-dodecen-γ-lactone[d]	soapy	1675	1922	64	256
7	methional[e]	boiled potato	908	1049	32	32
8	(*E*)-β-damascenone[e]	fruity, peach-like	1391	1510	32	64
9	diacetyl[d]	butter-like	595		16	8
10	nonanal[d]	green, fatty	1102	1208	16	8
11	*trans*-4,5-epoxy-2-(*E*)-decenal[e]	metallic	1383	1562	16	16
12	acetic acid[d]	sour	1455[f]		16	16
13	(*E*)-2-nonenal[e]	cardboard-like	1164	1281	8	32
14	δ-dodecalactone[d]	sweet, soapy	1718	1970	8	16
15	1-octen-3-one[e]	mushroom	977	1070	4	8
16	(*Z*)-2-nonenal[e]	fatty, tallowy	1150	1261	4	8
17	2-acetylthiazoline[e]	roasty, corny	1114	1254	2	8

[a] Odor description at the GC−sniffing port. [b] Retention indices according to Van den Dole and Kratz (1969). [c] Flavour dilution on DB-5 ms capillary column, except for **2** and **12**. [d] Compound identified by comparison with the reference compound on the basis of the following criteria: odor quality at the GC−sniffing port, RI on DB-5 ms and DB-1701, and mass spectra in the electron impact mode. [e] Mass spectra were too weak for an unequivocal interpretation. Compounds were identified on the basis of the remaining criteria in footnote *d*. [f] Retention index and FD determined on DB-Wax capillary.

Table 2. GCO-H of Mild Regular-Fat (A) and Low-Fat (B) Cheddar Cheeses

no.[a]	compound[b]	odor description[c]	RI on RTX 5	vol[d] (mL) A	vol[d] (mL) B
9	diacetyl	butter-like	595	2.5	10.0
18	dimethyl trisulfide	putrid	972	5.0	2.5
19	methanethiol	sulfury	<500	10.0	2.5
20	dimethyl sulfide	cabbage-like	<500	10.0	10.0
21	unknown	mushroom-like	1078	10.0	20.0
22	unknown	perfume, green	1102	10.0	10.0
12	acetic acid	sour	1445	10.0	10.0
23	acetaldehyde	sweet, pungent	<400	20.0	10.0
7	methional	boiled potato-like	910	20.0	20.0
15	1-octen-3-one	mushroom-like	977	20.0	20.0
24	dimethyl tetrasulfide	putrid	1235	20.0	40.0
25	hexanal	green	800	40.0	40.0

[a] The numbers **7**, **9**, **12**, and **15** refer to Table 1. [b] Compounds were identified on the basis of matching odour quality and retention indices with the corresponding reference compounds. [c] Odor description assigned during GCO-H. [d] The smallest headspace volume sufficient to perceive an odorant at the GC−sniffing port.

Table 3. Selected Ions, Calibration Factors, and Thin Film Fused Silica Capillaries Used for Quantification of Odorants by GC/MS-EI

odorant[a]	*m/z*	std	*m/z*	calibrn factor	capillary
homofuraneol (**1**)	142	**d$_3$-1**	145	0.69	DB-1701
butyric acid (**2**)	60	**d$_4$-2**	62	0.55	DB-Wax
Furaneol (**3**)	128	**^{13}C$_2$-3**	130	1.00	DB-1701
δ-decalactone (**4**)	99	**d$_5$-4**	102	0.87	DB-Wax
skatole (**5**)	130	**d$_2$-5**	133	0.52	DB-5 ms
(*Z*)-6-dodecen-γ-lactone (**6**)[b]	197	**d$_2$-6**	199	1.00	DB-Wax
methional (**7**)	104	**d$_3$-7**	107	2.33	DB-1701
diacetyl (**9**)	43	**^{13}C$_4$-9**	45	1.00	DB-Wax
acetic acid (**12**)	60	**^{13}C$_2$-12**	62	0.73	DB-Wax

[a] The numbers of the odorants and the standards refer to Table 1. [b] This odorant was quantified using GC/MS-CI.

cold traps immersed in liquid nitrogen. Once the pressure was below 6×10^{-5} mbar, the crude extract was dropwise released through a feeding funnel into the distillation flask kept at 40 °C. After the addition was completed, the temperature was raised to 60 °C for 1 h. The trapped distillate was subsequently washed with sodium bicarbonate (0.5 mol/L; 2 × 50 mL) and a saturated solution of sodium chloride in water (3 × 15 mL). The organic phase containing the neutral and basic volatiles was dried over anhydrous Na_2SO_4 and then concentrated to 2 mL by distillation and finally to 0.5 mL by evaporation at room temperature. The aqueous phase was acidified with hydrochloric acid (18 %) to pH 1.5 and reextracted with diethyl ether (3 × 40 mL) to recover acidic volatiles. After drying over anhydrous Na_2SO_4, it was concentrated to 0.5 mL.

Cheese Extracts for the Quantification of Odorants. *Odorants **2**, **4**, **6**, **9**, and **12**.* Fifty grams of the powdered cheese was suspended in 150 mL of diethyl ether spiked with the following amounts of the corresponding labeled standards: 604 μg of *d*$_4$-**2**, 92 μg of *d*$_5$-**4**, 73 μg of *d*$_2$-**6**, 26 μg of [^{13}C$_2$]-**9**, and 4112 μg of [^{13}C$_2$]-**12**. The suspension was stirred for 2.5 h before the solvent was filtered off and the extract submitted to the sublimation procedure described above. The recovered distillate was dried over Na_2SO_4, and after concentration to 0.2 mL, an aliquot of 1 μL was injected.

*Odorants **1**, **3**, **5**, and **7**.* Two hundred grams of the powdered cheese was suspended in 500 mL of diethyl ether containing 20.0 μg of *d*$_3$-**1**, 29.4 μg of [^{13}C$_2$]-**3**, 3.7 μg of *d*$_2$-**5**, and 0.85 μg of *d*$_3$-**7**. After stirring under argon for 3 h, the slurry was filtered off and the extract sublimed; the distillate was washed with sodium bicarbonate (0.5 mol/L; 3 × 20 mL) and a saturated solution of sodium chloride (2 × 15 mL). After drying over Na_2SO_4, the extract was concentrated to 0.1 mL.

High-Resolution Gas Chromatography/Olfactometry (HRGC/O). For HRGC, a Hewlett-Packard (Little Falls, PA) Model 5890A Series II gas chromatograph was used, equipped with the following fused silica capillary columns: DB-5 ms (30 m × 0.25 mm, film thickness 0.5 μm), DB-1701 (30 m × 0.25 mm, film thickness 0.5 μm), and DB-Wax (30 m × 0.25 mm, film thickness 0.5 μm). The three columns were purchased from J&W Scientific (Rancho Cordoba, CA). The capillary columns were connected to a HP splitter allowing the effluent to be split 1:1 by using deactivated uncoated restriction capillaries (25 cm × 0.1 mm) leading to the FID (set at 250 °C) and the sniffing port (set at 200 °C, not humidified). Usually, 1 μL of the concentrated extracts was injected in the splitless mode. The injector was held at 200 °C; the initial oven temperature of 40 °C was held for 2 min, then raised at 6 °C/min to 180 °C and from there at 10 °C/min to 250 °C (230 °C for DB-Wax). AEDA was performed by two experienced sniffers. The series of stepwise-diluted extracts (addition of diethyl ether to the extract at a ratio of 1:1, v/v) was sniffed on a single day by each subject. In a preliminary experiment it was found that when a cold on-column injection technique was used, the same odors were perceived at the sniffing port as with the splitless injection, which was used routinely.

HRGC/Mass Spectrometry (HRGC/MS). Identification and quantification of the aroma compounds were performed with a 5890 GC (Hewlett-Packard) coupled to a 5970 mass spectrometer (Hewlett-Packard) run in the electron impact mode at 70 eV. Capillary columns for GC/MS were identical to those used for AEDA.

Gas Chromatography−Olfactometry of Static Headspace Samples (GCO-H). Analysis of the headspace samples was performed with a CP 9001 gas chromatograph connected to the purge and trap system TCT/PTI 4001 (Chrompack, Frankfurt, Germany) as reported by Milo and Grosch (1995). Powdered cheese (25 g), regular or reduced fat, was placed in a headspace vessel (volume, 240 mL), sealed by a septum, and held at 25 °C in a water bath. After an incubation time of at least 1 h but not more than 2.5 h, the headspace volumes listed in Table 2 were injected into the purge and trap system by means of a gastight syringe. After desorbing and cryofocusing for 10 min, the trap was instantly heated and the GC run started. The capillary column RTX 5 (30 m × 0.53 μm, film thickness 1.5 μm; Amchro, Sulzbach/Taunus, Germany) was held at 0 °C for 1 min and then ramped at 8 °C/min to 250 °C.

RESULTS AND DISCUSSION

Regular- and reduced-fat Cheddar cheeses had significantly different aroma characteristics. While the regular-fat cheese exhibited a butter-like, sweet-fruity, acidic aroma, the low-fat product had a distinct meaty-brothy character.

The results of AEDA of the neutral/basic fraction and the acidic fraction of the volatiles in regular-fat Cheddar cheese (A) are summarized in Table 1. GC−sniffing of the extract containing the neutral/basic volatiles revealed 12 odorants within the FD range 8−512. Two caramel-like smelling compounds were among those with the highest FD factors (>128). On the basis of matching retention indices with reference substances on DB-5 ms, DB-1701, and DB-Wax (data not shown) and their unique smell, they were first tentatively identified as 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (Furaneol; registered trademark of Firmenich, Geneva, Switzerland) and 2(5)-ethyl-5(2)-methyl-4-hydroxy-3-(2*H*)-furanone (homofuraneol). Their presence was subsequently verified by HRGC/MS-EI. Due to the high polarity of both compounds, they were also found in the acidic fraction by AEDA. Consequently, their high FD values determined on DB-5 ms are still underestimated. To our knowledge, this is the first time these compounds have been reported in Cheddar cheese. Their high impact on the aroma of Swiss Emmentaler cheese was very recently elucidated by Preininger and Grosch (1994). Other compounds with high FD factors were butyric acid, δ-decalactone, skatole, 6-(*Z*)-dodecen-γ-lactone, and methional. The role of methional and butyric acid was also documented by AEDA on aged Cheddar cheese (Christensen and Reineccius, 1995). With the exception of acetic acid, however, esters and other acids found by them could not be detected by GC−sniffing of extracts prepared from the mild Cheddar cheese samples used in our experiments.

A comparison of regular- and low-fat Cheddar cheese based on AEDA data shows significantly higher FD factors for homofuraneol (**1**) and Furaneol (**3**) in the low-fat Cheddar cheese. This suggests that they had a greater impact on the aroma of the low-fat cheese (Table 1). The differences in FD for the other odorants were rather small and will be discussed later on the basis of quantitative results.

GCO-H was performed to determine the contribution of highly volatile odorants to the aroma of the Cheddar cheeses. Eight of the 12 compounds listed in Table 2 were assessed only by GC−sniffing of headspace samples (**18**−**25**). According to the minimal headspace volume sufficient to perceive a certain odorant by GCO-H, diacetyl, methanethiol, dimethyl sulfide, dimethyl trisulfide, and acetic acid, in addition to those found by AEDA, contribute to the aroma profile of the mild Cheddar cheese. On the basis of the headspace analysis, the reduced-fat Cheddar cheese (B) seems to have higher contents of sulfury compounds (**18**, **19**).

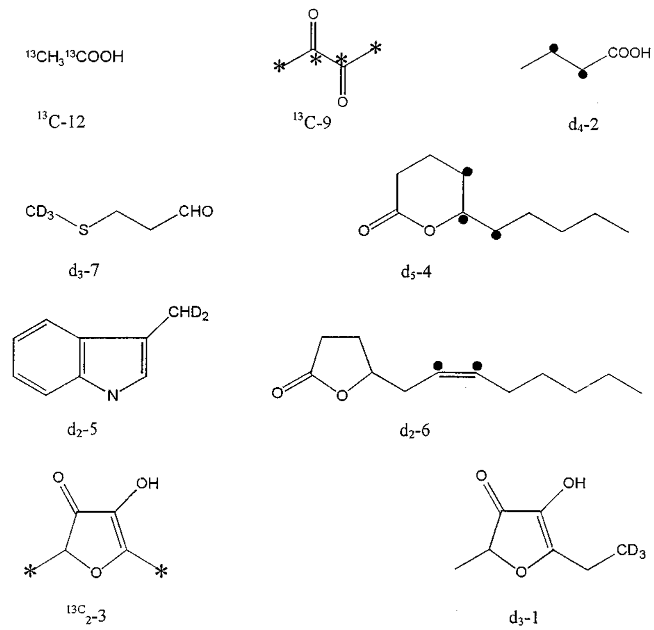

Figure 1. Structures of the nine labeled standards used for the isotope dilution assays. (The numbers refer to Table 3; * indicates ^{13}C labeling, and ● indicates position of deuterium.)

Table 4. Concentration of Selected Odorants Important to Cheddar Cheese Aroma Depending on the Extraction Time

	concn of odorants (μg/kg) after extraction time of				
compound	1 h	2 h	3 h	4 h	6 h
acetic acid	108900	104600	106900	108900	117000
butyric acid	11200	11100	11000	11300	11800
δ-decalactone	1708	2278	2074	1989	2659
Furaneol	29.8	30.1	30.4	27.0	31.5

To overcome the limitations implicit in AEDA (Grosch, 1993), a total of nine compounds were quantified using stable isotope dilution assays (IDA). The structures of these standards are shown in Figure 1. To be a stable standard, **3**, **9**, and **12** needed to be ^{13}C-labeled as opposed to being deuterium-labeled. The synthesis of **2**−**7** will be published later. The ions used for the quantification by means of GC/MS-EI, the capillaries, and the calibration factors are given in Table 3.

In a preliminary experiment, the extraction efficiency was tested to see whether the labeled standard was in equilibrium with the analyte. This condition assures a complete extraction of cheese odorants and indicates a complete penetration of the sample material with the spiked standards. We chose four compounds (acetic acid, butyric acid, Furaneol, and δ-decalactone) covering a wide range of polarity and determined their concentrations depending on the extraction time. Using 50 g of regular-fat Cheddar cheese powder and 150 mL of diethyl ether as described under Cheese Extracts for the Quantification of Odorants, a stirring time of 1 h was sufficient to reach equilibrium for three of the four compounds (Table 4). The δ-decalactone concentration of 1708 μg/kg was a little low after 1 h of extraction. We therefore chose to extract the cheese for 2.5 h. The

Table 5. Concentration of Odorants in Mild Regular-Fat (A) and Low-Fat (B) Cheddar Cheeses

compound	concn (μg/kg)	
	A	B
acetic acid	96200	121000
butyric acid	10120	16600
δ-decalactone	2350	1060
6-(Z)-dodecen-γ-lactone	268	263
diacetyl	210	251
homofuraneol	52	132
furaneol	52	160
skatole	3.8	5.2
methional	3.0	5.9

[a] The data are mean values of duplicates.

Table 6. Odor Activity Values of Potent Odorants in Mild Regular-Fat (A) and Low-Fat (B) Cheddar Cheeses

odorant[a]	n-OAV[b] (oil) A	n-OAV[b] (water) A	n-OAV[b] (oil) B	n-OAV[b] (water) B	rn-OAV[b] (oil) A	rn-OAV[b] (oil) B
homofuraneol (**1**)	9	45	22	115	36	88
butyric acid (**2**)	75	3.5	118	6	15	25
Furaneol (**3**)	2	5	6	16	12	36
δ-decalactone (**4**)	6	23	2.5	11	1.5	<1
skatole (**5**)	<1	25	<1	35	<1	<1
6-(Z)-dodecen-γ-lactone (**6**)	1	nd	1	nd	nd	nd
methional (**7**)	15	15	30	30	60	120
diacetyl (**9**)	21	14	24	17	21	24
acetic acid (**12**)	nd	3	nd	4	14	17

[a] The numbering refers to Table 1. [b] The odor activity values were calculated by dividing the concentration by, respectively, the nasal (n-OAV) and the retronasal (rn-OAV) threshold either in oil or in water. The nasal and retronasal thresholds in oil for odorants **1**, **3**, **5**, **7**, and **9** were obtained from Preininger and Grosch (1994), the retronasal thresholds for **2** and **12** from Siek et al. (1969), and the nasal thresholds for **2** and **6** from Schieberle et al. (1993). The nasal thresholds in water for odorants **2**, **7**, **9**, and **12** were determined by Guth and Grosch (1994), for **1** and **3** by Semmelroch and Grosch (1995), for **4** by Engel et al. (1988), and for **5** in our group (0.15 mg/L) (unpublished data).

results further illustrate the high precision of the IDA. Even with a slight influence due to the different extraction times, the standard deviation for Furaneol was only 5% from a mean value of 29.76 μg/kg.

Among all of the compounds determined, acetic acid and butyric acid were by far the most predominant odorants in both cheeses, followed by δ-decalactone (Table 5). In good agreement with the results from the AEDA experiments, the concentrations of homofuraneol and Furaneol in the low-fat cheese (B) were 2.5 and 3 times higher, respectively, than in the regular-far cheese (A). Methional and skatole were also found at higher concentrations in low-fat cheese (B) (2- and 1.4-fold). On the contrary, δ-decalactone in low-fat cheese (B) was about 45% of the amount in regular-fat cheese (A).

To gain a better insight into the importance of the odorants, OAVs were calculated using the quantitative data from Table 5 and published threshold values in oil and water. On the basis of their nasal thresholds in oil, butyric acid, diacetyl, and methional are the most potent odorants, while skatole seems to have the least impact on the aroma (Table 6). The OAVs of these odorants based on their thresholds in water showed a different ranking. Homofuraneol, followed by skatole and δ-decalactone, had the highest OAVs, whereas butyric acid had a relatively low nasal OAV in water. This change in importance can be explained by the differences in solubility in oil versus water of skatole and butyric acid. The calculated OAV for acetic acid suggested that it is as important as butyric acid to the aroma. The contribution of homofuraneol is also reflected by the OAV based on the retronasal threshold in oil. Therefore, we regard this compound and possibly skatole at the concentrations determined above, in addition to odorants **2**, **7**, and **9**, as important for the pleasant aroma of mild regular-fat Cheddar cheese. Our results confirm the work by Vickers and Dacremont (1994), who suggested that compounds **2**, **7**, and **9** were primarily responsible for the Cheddar cheese aroma.

In the low-fat Cheddar cheese (B), the retronasal OAVs for homofuraneol, methional, and Furaneol showed by far the highest values. The meaty-brothy odor observed in our low-fat samples is therefore likely due to a higher level of these odorants. Methional and Furaneol were indeed recently identified as potent odorants in beef broth by Guth and Grosch (1994).

CONCLUSIONS

On the basis of quantitative data, OAVs were calculated for the potent odorants of regular- and low-fat Cheddar cheeses. These results show that acetic acid, butyric acid, methional, diacetyl, and homofuraneol are primary contributors to the pleasant mild aroma of Cheddar cheese. The contribution of highly volatile sulfur compounds such as methanethiol and dimethyl sulfide to the nasal perception of the Cheddar cheese flavor is obvious on the basis of the static headspace sniffing. The meaty-brothy odor characteristic of low-fat Cheddar cheese is by and large caused by higher levels of methional, Furaneol, and especially homofuraneol. The Furaneol-type odorants are known to be formed by certain strains of lactobacilli (Preininger, 1995). We assume that the higher water content in the low-fat cheese and a possibly higher microbial activity are the reasons for the elevated amounts of these compounds.

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