JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Characterization of the Key Aroma Compounds in Two Bavarian Wheat Beers by Means of the Sensomics Approach

Daniel Langos,[†] Michael Granvogl,[§] and Peter Schieberle*[,†,§]

[†]Deutsche Forschungsanstalt für Lebensmittelchemie, Lise-Meitner-Strasse 34, D-85354 Freising, Germany

[§]Department für Chemie, Lehrstuhl für Lebensmittelchemie, Technische Universität München, Lise-Meitner-Strasse 34, D-85354 Freising, Germany

ABSTRACT: Application of aroma extract dilution analysis (AEDA) on the volatiles isolated from a commercial Bavarian wheat beer (WB A) eliciting its typical aroma profile, best described by a clove-like, phenolic odor quality, revealed 36 odorants in the flavor dilution (FD) factor range from 16 to 4096. Among them, 2-methoxy-4-vinylphenol (clove-like) and 2-phenylethanol (flowery) showed the highest FD factors. AEDA of a second wheat beer (WB B), somewhat lacking the typical wheat beer odor note, revealed 32 odor-active components in the FD factor range from 32 to 8192. Among them, 2-phenylethanol, (*E*)-*β*-damascenone (cooked apple-like) and 3-methylbutanol (malty) were detected with the highest FD factors. Next, all odorants evaluated with an FD factor ≥32 were quantitated by stable isotope dilution assays in both beers, and the odor activity values (OAVs; ratio of concentration to odor threshold) were calculated. Thereby, ethanol, (*E*)-*β*-damascenone, 3-methylbutyl acetate, ethyl methylpropanoate, and ethyl butanoate showed the highest OAVs in WB A, followed by acetaldehyde, 3-methylbutanol, and dimethyl sulfide. In WB B, ethanol, (*E*)-*β*-damascenone, ethyl methylpropanoate, ethyl butanoate, and 3-methylbutyl acetate showed the highest OAVs. Whereas most aroma compounds were present in the same order of magnitude in both beer samples, in particular, 2-methoxy-4-vinylphenol and 4-vinylphenol (smoky, leather-like) were by factors of 13 and 15, respectively, higher in WB A. For the first time, the overall aroma of wheat beer (WB A) was successfully simulated on the basis of 27 reference compounds in their natural concentrations using water/ethanol (95:5; v/v) as the matrix.

KEYWORDS: *wheat beer, aroma extract dilution analysis, stable isotope dilution assay, odor activity value, aroma recombination, 2-methoxy-4-vinylphenol*

■ INTRODUCTION

Wheat beer is a Bavarian and Austrian specialty beer produced by substituting barley malt by at least 50% of wheat malt. In Germany, it is one of the most popular beers but differs in the overall aroma profile, for example, from lager beer, by a clove-like, slightly phenolic odor note. Due to this unique odor quality, in particular in Bavaria, wheat beer is very much liked by consumers, although today wheat beer is also increasingly exported to many countries around the world. Due to its clove-like odor quality, 2-methoxy-4-vinylphenol was among the first components suggested to be responsible for the typical wheat beer aroma, and Tressl et al.[1] reported on the occurrence of 2-methoxy-4-vinylphenol, 4-vinylphenol, and other phenolic compounds in wheat beer. They proposed that the formation of these phenolics takes place mainly during wort boiling by a decarboxylation of the respective phenolic acids such as ferulic acid.[2] In contrast, Goodey and Tubb[3] investigated the ability of different strains of the top fermenting brewing yeast *Saccharomyces cerevisiae* to decarboxylate hydroxycinnamic acid derivatives. It was found that a so-called POF1 gene is necessary to produce cellular decarboxylase. Wackerbauer et al.[4−6] also proposed that besides a thermal degradation of the acids, such phenols can be generated in beer due to contamination with bacteria or by specific yeast strains used in wort fermentation able to decarboxylate phenolic acids. The concentrations of the phenolic volatiles, however, varied widely: whereas the concentrations of 2-methoxy-4-vinylphenol ranged from 189 to 4373 μg/kg, the amounts of 4-vinylphenol differed even more in 22 beers from only 20 to 2696 μg/kg.[7]

However, although the concentrations of phenolic compounds in beer have been extensively studied, up to now, no information on further compounds contributing to the aroma of wheat beer is available, despite more than 600 volatile compounds already having been reported in different kinds of beer.[8] Meilgaard[9] was the first to evaluate the aroma contribution of single volatiles to the overall aroma of lager beers. A calculation of the odor activity values (OAVs; ratio of concentration to odor threshold) of 239 volatile compounds revealed that besides ethanol, several esters (e.g., 3-methylbutyl acetate and ethyl hexanoate), alcohols (e.g., 3-methylbutanol), dialkyl sulfides (e.g., dimethyl sulfide), and short-chain fatty acids (e.g., butanoic acid) are essential for the aroma of U.S. lager beers. However, compared to lager beer, most previous studies performed on wheat beer solely aimed at optimizing or varying technological procedures rather than at the identification of key aroma compounds. Furthermore, only a few volatile compounds have been quantitated, mainly yeast metabolites, such as higher alcohols, esters, and diacetyl,[10,11] but no evaluation of their contribution to the overall aroma of wheat beer has been done so far.

Received: September 3, 2013
Revised: October 25, 2013
Accepted: October 26, 2013
Published: October 27, 2013

Table 1. Amount of Beer Used for Workup, Selected Ions (m/z) of Analytes and Stable Isotopically Labeled Standards, and Response Factors (R_f) Used for Stable Isotope Dilution Assays

odorant	amount of beer (mL)	analyte (m/z)	standard isotope label	standard m/z	R_f
linalool	500	137	$^{2}H_2$	139	1.02
3-hydroxy-4,5-dimethyl-2(5*H*)-furanone	500	129	$^{13}C_2$	131	1.00
(*E*)-*β*-damascenone	250	191	$^{2}H_{5-8}$	196−199	0.66
ethyl methylpropanoate	250	117	$^{2}H_5$	122	1.00
2-acetyl-1-pyrroline	200	112	$^{2}H_{2-5}$	114−117	0.79
3-(methylthio)propanal	200	105	$^{13}C_3$	108	1.01
2-aminoacetophenone	150	136	$^{2}H_3$	139	0.86
4-hydroxy-2,5-dimethyl-3(2*H*)-furanone	100	129	$^{13}C_2$	131	1.01
3-methylbutanal	80	87	$^{2}H_2$	89	0.72
vanillin	50	153	$^{2}H_3$	156	0.95
2-methoxyphenol	50	125	$^{2}H_3$	128	0.97
γ-nonalactone	20	157	$^{2}H_2$	159	0.97
ethyl butanoate	20	117	$^{2}H_3$	120	1.01
1,1-diethoxyethane	20	73	$^{13}C_2$	75	0.95
ethyl hexanoate	15	145	$^{2}H_3$	148	1.02
ethyl octanoate	14	173	$^{2}H_3$	176	1.01
phenylacetic acid	10	137	$^{13}C_2$	139	0.84
4-vinylphenol	6	121	$^{2}H_4$	125	0.85
3-methylbutanoic acid	6	103	$^{2}H_9$	112	0.68
2-methoxy-4-vinylphenol	5	151	$^{2}H_3$	154	0.94
2-phenylethyl acetate	5	105	$^{13}C_2$	107	0.65
butanoic acid	3	89	$^{2}H_2$	91	0.62
3-methylbutyl acetate	2	131	$^{2}H_2$	133	1.02
3-(methylthio)propanol	2	107	$^{2}H_3$	110	1.00
dimethyl sulfide	1	63	$^{2}H_6$	69	0.93
2-phenylethanol	0.1	105	$^{13}C_2$	107	1.02
3-methylbutanol	0.1	71	$^{2}H_2$	73	0.93
methylpropanol	0.1	57	$^{2}H_2$	59	0.64

In an earlier study by our group, a few aroma compounds of wheat beer were quantitated and their OAVs were calculated.[12] Ethyl butanoate was found to show the highest OAV, followed by 3-methylbutanol, ethyl hexanoate, 2-phenylethanol, 2-methoxy-4-vinylphenol, and 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone.

The Sensomics approach involving a calculation of odor activity values followed by the preparation of an aroma recombinate has proven to be a successful concept in characterizing the aroma compounds responsible for the aroma signature of foods.[13] By means of this approach, the specific mixture of odorants able to activate the human odorant receptors when the food is smelled can be characterized. However, a comprehensive investigation on wheat beer aroma has not yet been done. Therefore, the aims of this study were (i) to identify the key odorants in two commercial wheat beers clearly differing in their overall aroma profile; (ii) to quantitate these compounds by stable isotope dilution assays, and (iii) to simulate the aroma of wheat beer by recombining the key odorants in their natural concentrations measured in the beer itself.

■ MATERIALS AND METHODS

Bavarian Wheat Beers. Nine different brands of Bavarian wheat beers were purchased at local supermarkets. Sensory evaluation was done in three sessions on three different batches of all nine brands. As a result of the hedonic evaluation by a consumer panel, two wheat beers were chosen for analysis, because one showed the most pronounced, typical wheat beer aroma (WB A), whereas a second one was ranked by the sensory panel with the lowest intensity of the typical wheat beer aroma (WB B).

Chemicals. Acetaldehyde, 2-aminoacetophenone, 1,1-diethoxyethane, dimethyl sulfide, ethyl hexanoate, ethyl methylpropanoate, ethyl octanoate, 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone, 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone, linalool, 3-methylbutanal, 3-methylbutanoic acid, 3-methylbutanol, 3-methylbutyl acetate, methylpropanol, 3-(methylthio)propanal, 3-(methylthio)propanol, *γ*-nonalactone, octanoic acid, phenylacetic acid, phenylacetaldehyde, phenylethyl acetate, and 4-vinylphenol were purchased from Aldrich (Sigma-Aldrich-Chemie, Taufkirchen, Germany). Acetic acid, butanoic acid, ethanol, 2-methoxyphenol, and vanillin were obtained from VWR (Darmstadt, Germany); ethyl butanoate, hexanoic acid, methylpropanoic acid, and 2-phenylethanol were from Fluka (Sigma-Aldrich-Chemie); and 4-ethyl-2-methoxyphenol, 2-methoxy-4-vinylphenol, and 1-octen-3-one were from Lancaster (Mühlheim/Main, Germany). (*E*)-*β*-Damascenone was generously supplied by Symrise (Holzminden, Germany).

Stable Isotopically Labeled Compounds. The following compounds were prepared as described previously: [$^{2}H_3$]-2-aminoacetophenone and [$^{2}H_4$]-4-vinylphenol;[14] [$^{2}H_2$]-butanoic acid;[15] [$^{2}H_{5-8}$]-(*E*)-*β*-damascenone;[16] [$^{13}C_2$]-1,1-diethoxyethane, [$^{2}H_3$]-ethyl butanoate, [$^{2}H_3$]-ethyl hexanoate, [$^{2}H_2$]-3-methylbutyl acetate, [$^{13}C_2$]-2-phenylethanol, and [$^{13}C_2$]-2-phenylethyl acetate;[17] [$^{2}H_5$]-ethyl methylpropanoate;[18] [$^{2}H_3$]-ethyl octanoate and [$^{2}H_2$]-3-methylbutanol;[19] [$^{13}C_2$]-4-hydroxy-2,5-dimethyl-3(2*H*)-furanone,[20] [$^{13}C_2$]-3-hydroxy-4,5-dimethyl-2(5*H*)-furanone,[21] and [$^{2}H_2$]-linalool;[22] [$^{2}H_3$]-3-(methylthio)propanal and [$^{2}H_3$]-3-(methylthio)propanol;[23] [$^{2}H_3$]-2-methoxyphenol,[24] [$^{2}H_3$]-2-methoxy-4-vinylphenol, and [$^{2}H_9$]-3-methylbutanoic acid;[25] [$^{2}H_2$]-3-methylbutanal;[26] [$^{2}H_2$]-methylpropanol;[27] [$^{2}H_2$]-*γ*-nonalactone;[28] and [$^{2}H_3$]-vanillin.[29]

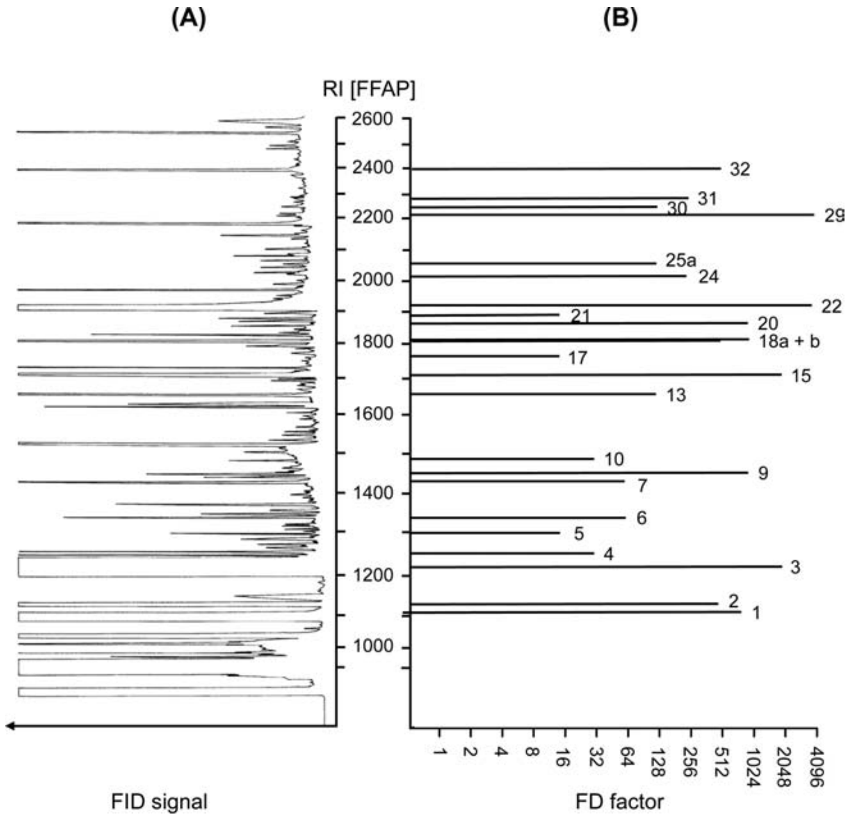

Figure 1. GC-FID chromatogram (A) and flavor dilution chromatogram (B) obtained by application of AEDA on a distillate from wheat beer A containing the neutral/basic volatiles. AEDA was performed on an FFAP capillary column. Numbering is used to assign odorants as identified in Table 2.

[$^{13}C_2$]-Acetaldehyde was supplied by Promochem (Wesel, Germany), and [$^{13}C_2$]-phenylacetic acid as well as [$^{2}H_6$]-dimethyl sulfide were from Aldrich (Sigma-Aldrich Chemie).

Isolation of the Volatiles. Wheat beer (250 mL) was filtered through a paper filter to avoid foaming during workup and was extracted with diethyl ether (total volume = 1000 mL). After drying of the extract over anhydrous Na_2SO_4 and filtration, the solution was concentrated to ~100 mL by distilling off the solvent at 38 °C using a Vigreux column (60 cm × 1 cm i.d.). To remove the nonvolatile material, the extract was then subjected to high-vacuum distillation using a solvent-assisted flavor evaporation (SAFE) technique.[30] The distillate obtained was fractionated into the acidic (AV) and the neutral/basic volatiles (NBV) by treatment with aqueous Na_2CO_3 (3 × 50 mL; pH 10.0; 0.5 mol/L).[31] After drying over anhydrous Na_2SO_4, both fractions were concentrated to ~250 μL by micro-distillation and were used for aroma extract dilution analysis (AEDA).

High Resolution Gas Chromatography−Olfactometry (HRGC-O) and High-Resolution Gas Chromatography−Mass Spectrometry (HRGC-MS). HRGC-O was performed by means of a type 8000 gas chromatograph (Fisons Instruments, Mainz, Germany) using two fused silica capillaries: DB-FFAP and DB-5 (both 30 m × 0.25 mm i.d., 0.25 μm film thickness) (J&W Scientific; Agilent, Waldbronn, Germany). Samples were injected by the cold on-column technique at 40 °C. After 2 min, the oven temperature was raised at 6 °C/min to 230 °C and held for 3 min. The flow rate of the carrier gas, helium, was 2.0 mL/min. At the end of the column, the effluent was split into two equal parts by means of a Y-type quick seal glass splitter (Chrompack, Frankfurt, Germany) and two deactivated fused-silica capillaries of the same length (30 cm × 0.32 mm i.d.). One part was directed to a flame ionization detector (FID) held at 230 °C, and the other part was directed to a sniffing port held at 200 °C. Linear retention indices (RI) of the compounds were calculated using a series of *n*-alkanes (C6−C26 (DB-FFAP) and C6−C18 (DB-5)) as previously described.[31]

HRGC-MS was performed by means of a gas chromatograph 5890 series II (Hewlett-Packard, Waldbronn, Germany) connected to a sector field mass spectrometer type MAT 95 S (Finnigan, Bremen, Germany). Using the capillaries described above, mass spectra were generated in the electron impact mode (MS-EI) at 70 eV and in the chemical ionization mode (MS-CI) at 115 eV using isobutane as reactant gas.

Aroma Extract Dilution Analysis. AEDA was performed as described previously.[32]

Quantitation by Stable Isotope Dilution Assays (SIDA). Wheat beer (0.1−500 mL, depending on the concentration of the respective odorant determined in preliminary experiments) was spiked with the internal standards either dissolved in ethanol or diethyl ether (Table 1). The amount of the respective standard was chosen in a similar concentration as the analyte, and the samples were equilibrated for 30 min with stirring. After extraction with diethyl ether (25−500 mL, depending on the initial volume of beer), the volatiles and the labeled internal standards were isolated by SAFE distillation.[30]

GC-MS was performed using a 431 gas chromatograph (Varian, Darmstadt, Germany) equipped with an FFAP column and coupled to a Varian mass spectrometer 220. Mass spectra were generated in the chemical ionization mode at 70 eV using methanol as the reactant gas.

If overlapping peaks were observed, two-dimensional gas chromatography−mass spectrometry (GC/GC-MS) was performed using a GC Trace 2000 (ThermoQuest, Egelsbach, Germany) equipped with an FFAP column in the first dimension coupled to a Varian GC CP 3800 equipped with an OV-1701 column in the second dimension (both 30 m × 0.25 mm i.d., 0.25 μm film thickness) (J&W Scientific). Heart cuts containing the respective odorants and the internal standards were transferred to the second column by means of the ThermoQuest moving capillary stream switching and a cold-trap cooled to −100 °C. The second GC was coupled to a Varian ion trap MS Saturn 2000. Mass spectra were generated in the chemical ionization mode at 70 eV using methanol as reactant gas. Samples were injected by means of a Combi PAL autosampler (CTC Analytics, Zwingen, Switzerland).

Response factors (R_f; Table 1) were determined by analyzing mixtures of known amounts of the unlabeled target compound and the respective isotopically labeled internal standard in five different ratios (5:1, 3:1, 1:1, 1:3, and 1:5) by either GC-MS or GC/GC-MS.

Quantitation of Dimethyl Sulfide. Quantitation of dimethyl sulfide was done by a SIDA using static headspace GC-MS. Wheat beer (1.2 mL) was pipetted into a headspace vial (20 mL), spiked with [$^2H_6$]-dimethyl sulfide, and equilibrated for 30 min at room temperature. Then, an aliquot of the headspace volume (2 mL) was withdrawn using a gastight syringe (Innovative Labor Systeme, Stützerbach, Germany). The volatiles were cryofocused in a Thermo cold trap 915 and were then transferred onto a DB-5 fused silica capillary (30 m × 0.25 mm i.d., 1.00 μm film thickness) (J&W Scientific) installed in a Thermo Trace GC Ultra coupled to a Varian MS 2100 T. Mass spectra were generated in the chemical ionization mode at 70 eV using methanol as reactant gas.

Quantitation of Ethanol and Acetaldehyde. Ethanol and acetaldehyde were quantitated using enzyme kits (R-Biopharm AG, Darmstadt, Germany). First, wheat beer (10 mL) was degassed using a paper filter. Then, for ethanol quantitation, the beer was diluted with water 999:1 (v/v), and for acetaldehyde quantitation the undiluted beer was used.

Determination of Odor Thresholds. For the calculation of OAVs, odor thresholds were determined in tap water.[33]

Aroma Recombinate. Carbonized tap water was adjusted to a pH of 4.2 (the same pH as in wheat beer) using hydrochloric acid (32%). For WB A, the following mixture of 27 purified odorants, dissolved in ethanol, was added to 900 mL of acidified, carbonized tap water in a graduated flask (1 L): acetic acid (275000 μg), 3-methylbutanol (58300 μg), methylpropanol (23000 μg), 2-phenylethanol (21800 μg), 3-(methylthio)propanol (4480 μg), 3-methylbutyl acetate (4330 μg), 2-methoxy-4-vinylphenol (2010 μg), acetaldehyde (1630 μg), butanoic acid (1180 μg), 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (1130 μg), 4-vinylphenol (854 μg), 3-methylbutanoic acid (744 μg), 2-phenylethyl acetate (532 μg), ethyl octanoate (223 μg), ethyl hexanoate (127 μg), ethyl butanoate (113 μg), dimethyl sulfide (50.2 μg), γ-nonalactone (43.8 μg), 1,1-diethoxyethane (22.9 μg), vanillin (21.3 μg), 3-methylbutanal (18.4 μg), ethyl methylpropanoate (4.6 μg), 3-(methylthio)propanal (3.0 μg), (*R*)-linalool (2.8 μg), 2-aminoacetophenone (2.5 μg), 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (1.7 μg), and (*E*)-β-damascenone (1.3 μg). The ethanol content was finally adjusted to 40 g/L, and the flask was filled to 1 L with acidified, carbonized tap water.

Aroma Profile Analysis. The sensory evaluation of the wheat beer samples and of the wheat beer aroma recombinate was performed by 10 trained panelists recruited from the German Research Center for Food Chemistry. The assessors were regularly trained in orthonasal odor perception.[33] The panelists were asked to evaluate the intensity of the odor attributes flowery, clove-like/phenolic, malty, cabbage-like, pungent, caramel-like, and fruity/banana-like on a seven point linear scale from 0 (not perceivable) over 0.5, 1.0, 1.5, 2.0, and 2.5 to 3 (strongly perceivable). The attributes were defined in a presession by the panelists as most relevant to describe the overall aroma of wheat beer. Samples (15 mL) were presented in covered glass vessels (i.d. = 40 mm, total volume = 45 mL) at room temperature, and the results obtained in two sessions were averaged.

RESULTS AND DISCUSSION

Identification of Aroma-Active Compounds in Wheat Beer A (WB A). Isolation of the volatile compounds from WB A gave an extract eliciting a strong wheat beer-like smell, with odor attributes such as clove-like, fruity, malty, and flowery predominating, when an aliquot was evaluated by the sensory panel on a strip of filter paper. To avoid interferences in GC runs among volatiles with identical retention indices but differences in their amounts, the distillate was separated into neutral/basic and acidic volatiles, and the odor-active compounds were detected in both fractions by AEDA.

The gas chromatogram of the volatiles present in the neutral/basic fraction (Figure 1A) was compared to the flavor dilution chromatogram obtained by GC-O (Figure 1B). Due to the extremely different odor thresholds in air, many major volatiles did not result in an odor impression at the sniffing port, whereas others smelled even though they did not show an FID signal. By sniffing of serial dilutions in the AEDA, 23 aroma compounds were finally assigned an FD factor in the range between 16 and 4096 (Figure 1B). The highest FD factors were determined for compounds **22** (flowery) and **29** (clove-like). Somewhat lower FD factors were determined for **3** (malty) and **15** (cooked potato-like). By applying AEDA on the extract of the acidic volatiles, 13 additional aroma-active compounds were found in the FD factor range between 16 and 2048 (Figure 2). The highest FD factors were assigned to compounds **8** (sour, pungent) and **14** (sweaty).

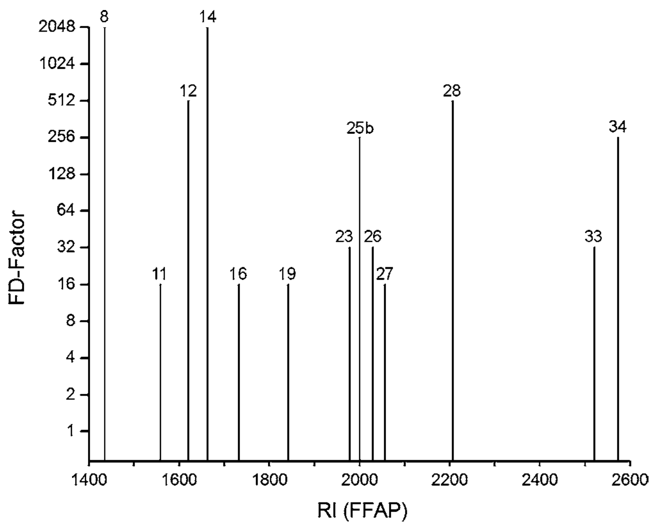

Figure 2. Flavor dilution chromatogram of a distillate from wheat beer A containing the acidic volatiles. AEDA was performed on an FFAP capillary column. Numbering is used to assign odorants as identified in Table 2.

To identify the compounds responsible for the odors perceived during GC-O, first, the neutral/basic volatiles of WB A were further separated by column chromatography on silica.[31] Then, the respective odorants were located again by GC-O, and their retention indices were determined on two columns of different polarity. Additionally, mass spectra (MS-EI, MS-CI) were recorded, which were compared to mass spectra of food odorants available in an in-house database, all verified by reference compounds. In addition, the odor intensity and odor quality of each analyte were compared to the odor attributes of the reference compound, because only by this approach can it be verified that no coeluting compound is identified on the basis of the MS and RI data, whereas the odor is elicited by a coeluting trace compound not detectable by MS.

In this way, 2-phenylethanol (**22**; flowery) and 2-methoxy-4-vinylphenol (**29**; clove-like) were identified with the highest FD factor among the neutral/basic volatiles (Table 2). The second highest FD factor of 2048 was determined for 3-methylbutanol (**3**; malty) and 3-(methylthio)propanol (**15**; cooked potato-like), whereas methylpropanol (**1**; malty), 3-(methylthio)-propanal (**9**; cooked potato-like), 2-phenylethyl acetate (**18a**; flowery), and 2-methoxyphenol (**20**; smoky, woody) showed a somewhat lower FD factor of 1024 (Table 2). Among the acidic

Table 2. Most Odor-Active Volatiles in Bavarian Wheat Beer A (FD Factor ≥ 16)

			RI[a] on		
no.[b]	odorant[c]	odor quality[d]	FFAP	SE-54	FD factor[e]
1	methylpropanol	malty	1100	<700	1024
2	3-methylbutyl acetate	fruity, banana-like	1130	881	512
3	3-methylbutanol	malty	1225	758	2048
4	ethyl hexanoate	fruity	1246	1007	32
5	1-octen-3-one[f]	mushroom-like	1300	969	16
6	2-acetyl-1-pyrroline[f]	roasty, popcorn-like	1317	923	64
7	ethyl octanoate	fruity	1430	1168	64
8	acetic acid[g]	sour, pungent	1435	<700	2048
9	3-(methylthio)propanal	cooked potato-like	1452	915	1024
10	linalool	flowery, citrus-like	1533	1107	32
11	methylpropanoic acid[g]	sweaty	1559	823	16
12	butanoic acid[g]	sweaty	1621	862	512
13	phenylacetaldehyde	honey-like	1662	1048	64
14	2- and 3-methylbutanoic acid[g]	sweaty	1663	881	2048
15	3-(methylthio)propanol	cooked potato-like	1710	92	2048
16	unknown[g]	earthy	1732	nd[h]	16
17	unknown	roasty	1747	1168	16
18a	2-phenylethyl acetate	flowery	1816	1260	1024
18b	(*E*)-*β*-damascenone	cooked apple-like	1816	1379	512
19	hexanoic acid[g]	sweaty, goat-like	1842	1029	16
20	2-methoxyphenol	smoky, woody	1863	1093	1024
21	unknown	fruity	1884	1354	16
22	2-phenylethanol	flowery	1922	1125	4096
23	unknown[g]	earthy, fatty	2000	nd	256
24	4-ethyl-2-methoxyphenol	smoky	2012	1154	256
25a	*γ*-nonalactone	coconut-like	2029	1367	128
25b	4-hydroxy-2,5-dimethyl-3(2*H*)-furanone[g]	caramel-like	2029	1075	32
26	octanoic acid[g]	sweaty, goat-like	2056	1283	16
27	unknown[g]	caramel-like	2127	1032	16
28	3-hydroxy-4,5-dimethyl-2(5*H*)-furanone[g]	seasoning-like, spicy	2206	1119	512
29	2-methoxy-4-vinylphenol	clove-like	2212	1321	4096
30	2-aminoacetophenone	foxy	2235	1354	128
31	unknown	metallic, geranium-like	2276	1491	256
32	4-vinylphenol	smoky, leather-like	2393	1228	512
33	phenylacetic acid[g]	honey-like	2520	1262	32
34	vanillin[g]	vanilla-like	2573	1392	256

[a]RI, linear retention index. [b]Numbering refers to Figures 1 and 2. [c]Compound was identified by comparison with reference substance on the basis of the following criteria: retention indeces (RI) on the capillaries detailed in the table, mass spectra obtained by MS-EI and MS-CI, odor quality as well as odor intensity perceived at the sniffing port. [d]Odor quality perceived at the sniffing port. [e]FD, flavor dilution factor. [f]MS signals were too weak for an unequivocal interpretation. Compound was identified on the basis of the remaining criteria given in footnote *b*. [g]Compound was identified in the fraction of the acidic volatiles. [h]nd, not determined.

volatiles, the most odor-active compounds were identified as acetic acid (**8**; sour, pungent) as well as 2- and 3-methylbutanoic acid (**14**; both sweaty). Compounds with somewhat lower FD factors were identified as butanoic acid (**12**; sweaty), 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (sotolon; **28**; seasoning-like, spicy), and vanillin (**34**; vanilla-like) (Table 2).

By application of AEDA, compounds are ranked on the basis of their odor thresholds in air, and it has to be kept in mind that the entire amount of each compound present in the volume injected onto the GC column is vaporized in the sniffing port during GC-O. To get closer to the situation in wheat beer, 25 of the odor-active compounds showing FD factors ≥32 as well as seven additional compounds (acetaldehyde, 1,1-diethoxyethane, dimethyl sulfide, ethanol, ethyl butanoate, ethyl methylpropanoate, and 3-methylbutanal) were quantitated. The latter, very volatile, aroma compounds were previously also identified as important odorants with high OAVs in a Pilsner-type beer.[34] As expected, ethanol appeared with the highest concentration in WB A (40100 mg/L), and also acetic acid (275 mg/L), 3-methylbutanol (58.3 mg/L), methylpropanol (23.1 mg/L), and 2-phenylethanol (21.1 mg/L) showed high concentrations (Table 3). The odorants with the lowest amounts were (*E*)-*β*-damascenone (1.33 μg/L) and 4-ethyl-2-methoxyphenol (0.77 μg/L). Among the phenolic compounds, 2-methoxy-4-vinylphenol showed the highest concentration (2020 μg/L), followed by 4-vinylphenol (882 μg/L), whereas 2-methoxyphenol (1.61 μg/L) and 4-ethyl-2-methoxyphenol (0.77 μg/L) were present only in trace amounts. To get a closer insight into the role of the single odorants in the overall wheat beer aroma, odor thresholds were determined. Because there is no appropriate matrix available, which especially simulates the interactions of odorants with the nonvolatile beer constituents, odor thresholds in water were used for the calculation of the OAVs (Table 4). Thereby, ethanol showed the highest OAV (1610), and next in rank were the cooked apple-like smelling (*E*)-*β*-damascenone (325), the fruity, banana-like smelling 3-methylbutyl acetate (231), and the fruity smelling esters ethyl methylpropanoate (225) and ethyl butanoate (115). Additionally, acetaldehyde, 3-methylbutanol, dimethyl sulfide, 3-methylbutanal, 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone, ethyl hexanoate, and 2-phenylethanol were found to be important contributors to the overall aroma of wheat beer A. In contrast, 2-methoxyphenol, phenylacetic acid, and 4-ethyl-2-methoxyphenol showed OAVs < 1 (Table 4), and, thus, these should not contribute to the wheat beer aroma.

Aroma Simulation of Wheat Beer A. To verify that the odorants with high OAVs contribute to the aroma of Bavarian wheat beer, an aroma recombinate was prepared for wheat beer A by the following procedure: all odorants with an OAV ≥ 1 (altogether 27 aroma compounds plus ethanol) were dissolved in acidified, carbonized tap water in their natural concentrations. A sensory panel of 10 trained panelists performed a descriptive profile test of the recombinate, and the intensities of the odor attributes of the original beer and the recombinate were scaled in different sessions. On average, the intensities of the seven odor attributes were rated with nearly the same values. Furthermore, the panelists judged the similarity between the overall aroma of the original wheat beer A and that of the recombinate on a scale from 0 to 3. The results of the aroma simulation corroborated the successful identification and quantitation experiments, because the similarity of the wheat beer (Figure 3A) and the aroma recombinate (Figure 3B) was rated with 2.7. The results showed that, because the aroma

Table 3. Concentrations of Potent Odorants in Bavarian Wheat Beer A (WB A) and Wheat Beer B (WB B)

odorant	concn[a] (μg/L) WB A	WB B
ethanol	40100000	40800000
acetic acid	275000	915000
3-methylbutanol	58300	54300
methylpropanol	23100	14500
2-phenylethanol	21100	27200
3-(methylthio)propanol	4490	1550
3-methylbutyl acetate	4390	1910
2-methoxy-4-vinylphenol	2020	159
acetaldehyde	1720	520
butanoic acid	1180	820
4-hydroxy-2,5-dimethyl-3(2*H*)-furanone	1110	1130
4-vinylphenol	882	60.2
3-methylbutanoic acid	794	1620
2-phenylethyl acetate	518	560
phenylacetic acid	463	256
ethyl octanoate	220	157
ethyl hexanoate	129	206
ethyl butanoate	115	142
dimethyl sulfide	49.6	28.1
γ-nonalactone	42.4	84.1
1,1-diethoxyethane	23.2	20.2
vanillin	21.6	15.6
3-methylbutanal	18.8	14.7
ethyl methylpropanoate	4.52	13.0
3-(methylthio)propanal	3.12	2.60
linalool	2.79	10.7
2-aminoacetophenone	2.41	1.89
3-hydroxy-4,5-dimethyl-2(5*H*)-furanone	1.80	1.72
2-methoxyphenol	1.61	1.61
(*E*)-β-damascenone	1.29	3.60
4-ethyl-2-methoxyphenol	0.77	na
2-acetyl-1-pyrroline	<1.0	na
γ-decalactone	na	1.30
δ-decalactone	na	2.71
geraniol	na	4.79
δ-octalactone	na	2.01
methylpropanoic acid	na	893

[a]Mean values of triplicates. na, not analyzed, because compound was not detected during AEDA.

Table 4. Orthonasal Odor Thresholds and Odor Activity Values (OAVs) of Aroma Compounds in Wheat Beer A (WB A) and Wheat Beer B (WB B)

odorant	odor threshold[b] (μg/L water)	OAV[a] WB A	WB B
ethanol	24900	1610	1640
(*E*)-β-damascenone	0.004	325	900
3-methylbutyl acetate	19	231	101
ethyl methylpropanoate	0.02	225	650
ethyl butanoate	1.0	115	142
acetaldehyde	25.0	69	21
3-methylbutanol	1000	58	54
dimethyl sulfide	1.0	50	28
3-methylbutanal	0.4	47	37
4-hydroxy-2,5-dimethyl-3(2*H*)-furanone	25.0	44	45
ethyl hexanoate	5.0	29	41
2-phenylethanol	1000	21	27
2-methoxy-4-vinylphenol	100	20	2
linalool	0.14	20	76
3-(methylthio)propanol	250	18	6
2-aminoacetophenone	0.2	13	10
4-vinylphenol	78	11	<1
3-hydroxy-4,5-dimethyl-2(5*H*)-furanone	0.3	6	6
vanillin	4.9	5	<1
methylpropanol	8300	3	2
ethyl octanoate	70	3	2
acetic acid	180000	2	5
2-phenylethyl acetate	356	2	2
γ-nonalactone	27	2	3
3-(methylthio)propanal	1.8	2	2
butanoic acid	1000	1	<1
3-methylbutanoic acid	740	1	2
1,1-diethoxyethane	25	1	<1
2-methoxyphenol	2.5	<1	<1
phenylacetic acid	1000	<1	<1
4-ethyl-2-methoxyphenol	16	<1	nd
methylpropanoic acid	1000	nd	<1
geraniol	3.2	nd	1
γ-decalactone	2.6	nd	<1
δ-decalactone	51	nd	<1
δ-octalactone	103	nd	<1

[a]OAVs were calculated by dividing the concentration by the respective odor threshold. nd, not determined, because not detectable by GC-olfactometry in WB A. [b]Odor thresholds taken from ref 35.

simulation was performed simply in acidified, carbonized water, the nonvolatile fraction of wheat beer obviously should have only little influence on the odor perception, for example, by influencing the aroma release.

Comparison of the Odorants in Wheat Beers A and B. To get an insight into the compounds responsible for the difference in the overall aroma of both wheat beers, first, the odor-active compounds in a distillate from wheat beer B (WB B) were located by application of AEDA. The data revealed 35 odor-active regions (data not shown), all of which could be identified on the basis of data from reference compounds. The highest FD factors were found for 2-phenylethanol (8192), 3-methylbutanol (4096), and (*E*)-β-damascenone (4096), followed by 2- and 3-methylbutanoic acid (2048). Compared to the results obtained for WB A, nearly all odorants were identical in both beer samples, however, with clear differences in the FD factors for some compounds. Therefore, almost all odorants quantitated in WB A were also determined in WB B (Table 3). A comparison of the concentrations indicated the most pronounced differences for 2-methoxy-4-vinylphenol and 4-vinylphenol, which were clearly higher in WB A. Further compounds higher in WB A were 3-(methylthio)propanol, 3-methylbutyl acetate, and acetaldehyde, whereas 2- and 3-methylbutanoic acid, ethyl hexanoate, γ-nonalactone, linalool, ethyl methylpropanoate, and β-damascenone were higher in WB B (Table 3). A calculation of the OAVs (Table 4) clearly indicated that in particular the higher OAVs of 2-methoxy-4-vinylphenol and 4-vinylphenol in WB A compared to the higher OAVs of (*E*)-β-damascenone, ethyl methylpropanoate, and linalool in WB B can be suggested as the reason for the differences in the overall aroma of both beers. These data corroborate the important role of the two phenolic compounds in the overall aroma of wheat beer eliciting a strongly pronounced aroma.

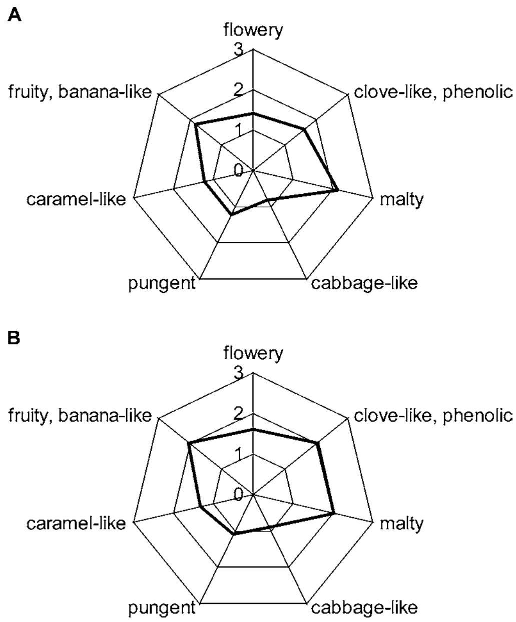

Figure 3. Aroma profile analysis of wheat beer A (A) and of the respective aroma recombinate (B). The intensities of the selected descriptors were evaluated by 10 panelists in each case; results were averaged.

2-Methoxy-4-vinylphenol and 4-vinylphenol were found to show the most significant differences between the two brands of wheat beer. Because three different batches of the two brands were analyzed, this statement confirms the key role of both odorants in the aroma differences, at least for the brands analyzed. It has been shown before that the decomposition of ferulic acid leads to 2-methoxy-4-vinylphenol, and the precursor of 4-vinylphenol is *p*-coumaric acid (Figure 4).[2] Vanbeneden

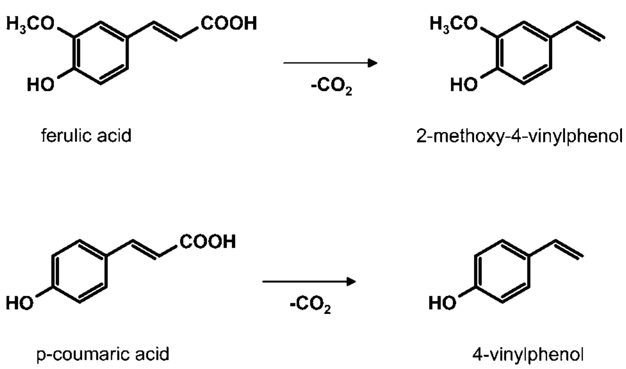

Figure 4. Reaction pathway leading to 2-methoxy-4-vinylphenol and 4-vinylphenol by either a thermally or a yeast-induced decarboxylation of the corresponding phenolic acids.

et al.[36] found that the concentrations of both compounds can vary widely in wheat beer, for example, 2-methoxy-4-vinyphenol between 0.165 and 1.96 mg/kg and 4-vinylphenol between 0.046 and 0.846 mg/kg. McMurrough et al.[37] compared the concentrations of the precursor ferulic acid and 2-methoxy-4-vinylphenol in ale, lager, and wheat beer. They found that only in wheat beer was the concentration of 2-methoxy-4-vinylphenol above its odor threshold, whereas the concentration of ferulic acid was low at the same time, indicating that this precursor was almost completely degraded. Coghe et al.[38] proposed that the amounts of 2-methoxy-4-vinylphenol mainly depend on the yeast strain used for fermentation. Thus, the concentrations of the phenolic compounds obviously depend on the amounts of phenolic acids in the wort and, also, the yeast strain used in beer fermentation.

Due to its low odor threshold, (*E*)-*β*-damascenone is one of the most important odor-active compounds in both wheat beers and has already earlier been identified also as a contributor to the aroma of a Pilsner-type beer.[34] The norisoprenoid has also been identified as an important odor-active compound in barley malt[39] and is, thus, obviously transferred into the wort during mash production. (*E*)-*β*-Damascenone is probably liberated from a glycosidic precursor, which had already been isolated from white wine[40] and from rose blossoms,[41] but not yet from cereals.

Esters, such as 3-methylbutyl acetate, ethyl methylpropanoate, ethyl butanoate, ethyl hexanoate, and ethyl octanoate, all showing fruity odor qualities, are typical metabolites of yeast.[42] Besides these esters, other important odorants, such as 3-methylbutanol and 2-phenylethanol as well as 2- and 3-methylbutanoic acid, are also known to be formed by yeast metabolism via the Ehrlich pathway.[42,43] The alcohols and carboxylic acids also contribute to the aroma of other fermented foods, such as rye sourdough,[44] whiskey,[31] or Williams Christ pear brandy.[45]

Dimethyl sulfide, another important aroma compound in wheat beer, was previously suggested[46] to have a positive influence on the overall beer aroma, whereas higher concentrations may lead to off-flavors. 4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone was shown to be responsible for the caramel-like note of dark beer and malt,[12,39] and it is proposed that the furanone is formed from fructose-1,6-diphosphate,[47] a yeast metabolite. Linalool and also geraniol originate from hops, and both have been characterized as key odorant in hops[48] and Pilsner-type beer.[34,49] The higher OAV of linalool in WB B suggests an addition of aroma hops at a later stage of brewing, that is, in the whirlpool.

A careful inspection of the literature shows that the current study is the first to identify all aroma-active odorants in Bavarian wheat beer by means of the Sensomics concept, resulting in an aroma recombinate clearly mimicking the overall aroma. Although the conclusion drawn on the importance of the odorants identified and quantitated is based on the analysis of only two brands of wheat beer, the results are an important basis for further research activities, for example, to vary recipes and/or processing conditions to maintain a good beer flavor as well as to establish alcohol-reduced, alcohol-free, or gluten-free wheat beers.

■ AUTHOR INFORMATION

Corresponding Author

*(P.S.) Phone: +49 8161 71 2932. Fax: +49 8161 71 2970. E-mail: peter.schieberle@ch.tum.de.

Notes

The authors declare no competing financial interest.

■ REFERENCES

(1) Tressl, R.; Kossa, T.; Renner, R. Gas-chromatographic-mass spectrometric studies of volatile substances of hops, wort, and beer and their genesis. II. Formation of phenols and oxygen-containing heterocyclic compounds in beer. *Eur. Brew. Conv. Proc. Congr.* **1975**, *15*, 737−756.

(2) Fiddler, W.; Parker, W. E.; Wasserman, A. E.; Doerr, R. C. Thermal decomposition of ferulic acid. *J. Agric. Food Chem.* **1967**, *15*, 757−761.

(3) Goodey, A. R.; Tubb, R. S. Genetic and biochemical analysis of the ability of *Saccharomyces cerevisiae* to decarboxylate cinnamic acids. *J. Gen. Microbiol.* **1982**, *128*, 2615−2620.

(4) Wackerbauer, K.; Kraemer, P.; Siepert, J. Phenolic aromatic substances in beer. Phenolic carboxylic acid and phenols in the raw

material and beer preparation (in German). *Brauwelt* **1982**, *122*, 618−620, 622, 624, 626.

(5) Wackerbauer, K.; Kossa, T.; Tressl, R. Phenol formation by yeasts (in German). *Monatsschr. Brau.* **1978**, *31*, 52−55.

(6) Wackerbauer, K.; Kraemer, P. Formation of phenols during fermentation (in German). *Monatsschr. Brau.* **1983**, *36*, 242−247.

(7) Wackerbauer, K.; Kraemer, P. Bavarian wheat beer − an alternative. Production and composition (in German). *Brauwelt* **1982**, *122* (758−760), 762.

(8) VCF Volatile Compounds in Food 14.1. TNO, Innovation for Life; Zeist, The Netherlands, 2013; http://www.vcf-online.nl/VcfHome.cfm.

(9) Meilgaard, M. C. Flavor chemistry of beer. II. Flavor and threshold of 239 aroma volatiles. *Tech. Q. Master Brew. Assoc. Am.* **1975**, *12*, 151−168.

(10) Kieninger, H.; Schweiger, F. Technology of top-fermented wheat beers (in German). *Brauwelt* **1976**, *116*, 370−375.

(11) Kieninger, H.; Narziss, L.; Miedaner, H.; Hecht, S. Changes of quality-determining compounds in the preparation of Bavarian wheat beers (in German). *Monatsschr. Brau.* **1984**, *37* (9−18), 55.

(12) Schieberle, P. Primary odorants of pale lager beer. Differences to other beers and changes during storage. *Z. Lebensm. Unters. Forsch.* **1991**, *193*, 558−565.

(13) Schieberle, P.; Hofmann, T. Mapping the combinatorial code of food flavors by means of molecular sensory science approach. In *Food Flavors. Chemical, Sensory and Technological Properties*; Chemical and Functional Properties of Food Components Series; Jelen, H., Ed.; CRC Press: Boca Raton, FL, 2012; pp 413−438.

(14) Jezussek, M. Aroma formation during boiling of brown rice (*Oryza sativa* L.) as well as of leaves of *Pandanus amaryllifolius* Roxb. (in German). Ph.D. thesis, Technical University of Munich, Munich, Germany, 2002.

(15) Kirchhoff, E. Characterization of key aroma compounds in rye bread − investigations about the influence of the manufacturing process and the dough composition (in German). Ph.D. thesis, Technical University of Munich, Munich, Germany, 2000.

(16) Sen, A.; Laskawy, G.; Schieberle, P.; Grosch, W. Quantitative determination of *β*-damascenone in foods using a stable isotope dilution assay. *J. Agric. Food Chem.* **1991**, *39*, 757−759.

(17) Guth, H. Quantitation and sensory studies of character impact odorants of different white wine varieties. *J. Agric. Food Chem.* **1997**, *45*, 3027−3032.

(18) Guth, H.; Grosch, W. Quantitation of potent odorants of virgin olive oil by stable isotope dilution assays. *J. Am. Oil Chem. Soc.* **1993**, *70*, 513−518.

(19) Gassenmeier, K.; Schieberle, P. Potent aromatic compounds in the crumb of wheat bread (French type). Influence of pre-ferments and studies on the formation of key odorants during dough processing. *Z. Lebensm. Unters. Forsch.* **1995**, *201*, 241−248.

(20) Sen, A.; Schieberle, P.; Grosch, W. Quantitative determination of 2,5-dimethyl-4-hydroxy-3-(2*H*)-furanone and its methyl ether using a stable isotope dilution assay. *Lebensm. Wiss. Technol.* **1991**, *24*, 364−369.

(21) Blank, I.; Schieberle, P.; Grosch, W. Quantification of the flavour compounds 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone and 5-ethyl-3-hydroxy-4-methyl-2(5*H*)-furanone by a stable isotope dilution assay. In *Progress in Flavour Precursor Studies*; Schreier, P., Winterhalter, P., Eds.; Allured Publishing: Carol Stream, IL, 1993; pp 103−109.

(22) Steinhaus, M.; Fritsch, H. T.; Schieberle, P. Quantitation of (*R*)- and (*S*)-linalool in beer using solid phase microextraction (SPME) in combination with a stable isotope dilution assay (SIDA). *J. Agric. Food Chem.* **2003**, *51*, 7100−7105.

(23) Sen, A.; Grosch, W. Synthesis of six deuterated sulfur-containing odorants for use as internal standards in quantification assays. *Z. Lebensm. Unters. Forsch.* **1991**, *192*, 541−547.

(24) Cerny, C.; Grosch, W. Quantification of character-impact odor compounds of roasted beef. *Z. Lebensm. Unters. Forsch.* **1993**, *196*, 417−422.

(25) Guth, H.; Grosch, W. Identification of the character impact odorants of stewed beef juice by instrumental analysis and sensory studies. *J. Agric. Food Chem.* **1994**, *42*, 2862−2866.

(26) Schieberle, P.; Grosch, W. Changes in the concentrations of potent crust odorants during storage of white bread. *Flavour Fragrance J.* **1992**, *7*, 213−218.

(27) Guth, H.; Grosch, W. 12-Methyltridecanal, a species-specific odorant of stewed beef. *Lebensm. Wiss. Technol.* **1993**, *26*, 171−177.

(28) Poisson, L.; Schieberle, P. Characterization of the key aroma compounds in an American bourbon whisky by quantitative measurements, aroma recombination, and omission studies. *J. Agric. Food Chem.* **2008**, *56*, 5820−5826.

(29) Semmelroch, P.; Laskawy, G.; Blank, I.; Grosch, W. Determination of potent odorants in roasted coffee by stable isotope dilution assays. *Flavour Fragrance J.* **1995**, *10*, 1−7.

(30) 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 matrixes. *Eur. Food Res. Technol.* **1999**, *209*, 237−241.

(31) Schieberle, P. Primary odorants in popcorn. *J. Agric. Food Chem.* **1991**, *39*, 1141−1144.

(32) Schieberle, P. Recent developments in methods for analysis of flavor compounds and their precurors. In *Characterization of Food: Emerging Methods*; Goankar, A. G., Ed.; Elsevier Science: Amsterdam, The Netherlands, 1995; pp 403−431.

(33) Czerny, M.; Christlbauer, M.; Christlbauer, M.; Fischer, A.; Granvogl, M.; Hammer, M.; Hartl, C.; Hernandez, N. M.; Schieberle, P. Re-investigation on odour thresholds of key food aroma compounds and development of an aroma language based on odour qualities of defined aqueous odorant solutions. *Eur. Food Res. Technol.* **2008**, *228*, 265−273.

(34) Fritsch, H. T.; Schieberle, P. Identification based on quantitative measurements and aroma recombination of the character impact odorants in a Bavarian Pilsner-type beer. *J. Agric. Food Chem.* **2005**, *53*, 7544−7551.

(35) Rychlik, M.; Schieberle, P.; Grosch, W. *Compilation of Odor Thresholds, Odor Qualities and Retention Indices of Key Food Odorants*; Deutsche Forschungsanstalt für Lebensmittelchemie: Garching, Germany, 1998.

(36) Vanbeneden, N.; Gils, F.; Delvaux, F.; Delvaux, F. R. Formation of 4-vinyl and 4-ethyl derivatives from hydroxycinnamic acids: occurrence of volatile phenolic flavour compounds in beer and distribution of Pad1-activity among brewing yeasts. *Food Chem.* **2007**, *107*, 221−230.

(37) McMurrough, I.; Madigan, D.; Donnelly, D.; Hurley, J.; Doyle, A.-M.; Hennigan, G.; McNulty, N.; Smyth, M. R. Control of ferulic acid and 4-vinyl guaiacol in brewing. *J. Inst. Brew.* **1996**, *102*, 327−332.

(38) Coghe, S.; Benoot, K.; Delvaux, F.; Vanderhaegen, B.; Delvaux, F. R. Ferulic acid release and 4-vinylguaiacol formation during brewing and fermentation: indications for feruloyl esterase activity in *Saccharomyces cerevisiae*. *J. Agric. Food Chem.* **2004**, *52*, 602−608.

(39) Fickert, B.; Schieberle, P. Identification of the key odorants in barley malt (caramalt) using GC-MS techniques and odor dilution analyses. *Nahrung* **1998**, *42*, 371−375.

(40) Baderschneider, B.; Skouroumounis, G.; Winterhalter, P. Isolation of two glucosidic precursors of *β*-damascenone from Riesling wine. *Nat. Prod. Lett.* **1997**, *10*, 111−114.

(41) Straubinger, M.; Knapp, H.; Oka, N.; Watanabe, N.; Winterhalter, P. Isolation of a glucosidic *β*-damascenone precursor from rose petals. *J. Agric. Food Chem.* **1997**, *45*, 4053−4056.

(42) Neubauer, O.; Fromherz, K. The decomposition of amino acids in yeast fermentation (in German). *Z. Physiol. Chem.* **1911**, *70*, 326−350.

(43) Äyräpää, T. Formation of phenethyl alcohol from ^{14}C-labelled phenylalanine. *J. Inst. Brew.* **1965**, *71*, 341−347.

(44) Kirchhoff, E.; Schieberle, P. Influence of drying of rye sourdough on the concentration of key aroma compounds in sourdough and bread crumb (in German). *Getreide, Mehl Brot* **1998**, *52*, 273−275.

(45) Willner, B.; Granvogl, M.; Schieberle, P. Characterization of the key aroma compounds in Bartlett pear brandies by means of the Sensomics concept. *J. Agric. Food Chem.* **2013**, *61*, 9583−9593.

(46) White, F. H. The origin and control of dimethyl sulfide in beer. *Brew. Dig.* **1977**, *52*, 38−44, 46−48, 50.

(47) Schieberle, P. Formation of Furaneol in heat-processed foods. In *Flavour Precursors. Thermal and Enzymatic Conversions*; Teranishi, R., Takeoka, G. R., Güntert, M., Eds.; ACS Symposium Series 490; American Chemical Society: Washington, DC, 1992; pp 164−174.

(48) 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.

(49) Steinhaus, M.; Wilhelm, W.; Schieberle, P. Comparison of the most odour-active volatiles in different hop varieties by application of a comparative aroma extract dilution analysis. *Eur. Food Res. Technol.* **2007**, *226*, 45−55.