Determination of Key Aroma Compounds in the Crumb of a Three-Stage Sourdough Rye Bread by Stable Isotope Dilution Assays and Sensory Studies

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An investigation of the volatile fraction of a freshly prepared sourdough rye bread crumb by means of the aroma extract dilution analysis (AEDA), followed by identification experiments, revealed 22 flavor compounds in the flavor dilution (FD) factor range of 128 to 2048. Quantitations performed by stable isotope dilution assays (SIDA) and a calculation of odor activity values (OAV; ratio of concentration to odor threshold) revealed the following as contributors to the overall crumb flavor: 3-methylbutanal (malty), (*E*)-2-nonenal (green, fatty), (*E*,*E*)-2,4-decadienal (fatty, waxy), hexanal (green), acetic acid (sour, pungent), phenylacetaldehyde (honey-like), methional (boiled potato-like), vanillin (vanilla-like), 2,3-butandione (buttery), 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (spicy), and 2- and 3-methylbutanoic acid (sweaty). Using either citrate buffer, starch, or deodorized crumb as model matrixes, the typical malty and sour rye bread crumb flavor was reproduced by adding a mixture of 20 reference odorants in the "natural" concentrations as quantitatively determined in the fresh crumb.

Keywords: *Sourdough rye bread; crumb flavor; aroma extract dilution analysis; stable isotope dilution assay; aroma recombination*

INTRODUCTION

The typical flavor of bread is formed as a result of enzymatic reactions occurring during dough fermentation by yeasts and/or lactic acid bacteria followed by thermal reactions induced during baking. Because of its lack in gluten proteins, rye flour has to be pre-fermented as "sourdough" using special starter cultures before the bread can be manufactured from the dough. This sourdough procedure is one of the main reasons for the differences in the overall aromas of wheat and rye breads. Whereas the microbial activities are believed to generate mainly precursors for the formation of, in particular, the rye crust odorants, volatiles formed by the microorganisms, like 2,3-butandione, are suggested to directly influence the flavor of the bread crumb.

At the present time, more than 99 volatiles are known to be present in different rye breads (*1*). Methods based on GC/olfactometry and using dilution to odor threshold approaches (*2*), are good tools for identification experiments on odorants which are involved in the flavor impression elicited by a certain food. By applying aroma extract dilution analysis (AEDA) on a freshly prepared extract of rye bread crumb, 30 odor-active volatiles have previously been suggested as key odorants in rye crumb flavor, in particular, phenylacetaldehyde, (*E*)-2-nonenal, (*E*,*E*)-2,4-decadienal, and acetic acid (*3*).

As previously shown for several foods, such as butter (*4*), grapefruit juice (*5*), or baguette wheat bread (*6*), the contribution of single odorants to the overall aroma cannot be estimated on the basis of dilution to odor threshold techniques alone, such as AEDA. To establish the results of GC/O, quantitative measurements on the flavor compounds suggested by AEDA have to be performed followed by flavor reconstitution studies.

Also, many parameters are known to modify the overall aroma generated during production of rye sourdough breads, such as the type of starter cultures, fermentation time, type of flour, addition of baker's yeast etc. However, to establish the influence of the recipe and the processing parameters on rye crumb flavor, first the key aroma compounds must be clarified. The purpose of the following study was, therefore, to elucidate the flavor compounds in a fresh rye bread crumb by application of AEDA, followed by quantitation and flavor reconstitution experiments.

MATERIALS AND METHODS

Rye Bread. Freshly baked rye bread, made by a three-stage sourdough procedure (*3*), was purchased from a local bakery and freed from the crust about 2 h after baking. Ingredients were rye flour type 997, three-stage rye sourdough, water, and sea salt.

Chemicals. The reference compounds listed in the tables were obtained from the sources given in parentheses: nos. 1−7, 9, 12, 15, 16, 18−22, 24, 26, 38, 39, 43, and 44 (Aldrich, Steinheim, Germany); nos. 10, 14, and 28 (Lancaster, Mühlheim, Germany); nos. 36, 37, 40, 41, and 45 (Merck, Darmstadt, Germany); and nos. 8 and 25 were gifts from Harmann & Reimer, Holzminden, Germany. The following compounds were synthesized according to the literature cited: no. 11 (*7*), no. 13 (*8*), no. 17 (*9*), and no. 27 (*10*). No. 23 was isolated according to Gassenmeier and Schieberle (*11*).

The isotopically labeled internal standards used were either labeled with deuterium or with carbon-13 and were synthesized as described in the previous publications given in parentheses. The structures are displayed in Figure 2: d-2 (*12*); c-4 (*13*); c-5 (*14*); d-6 (*15*); d-7 and d-26 (*16*); d-8 (*17*); d-16 (*18*); d-17, d-18, d-24, and d-27 (*19*); c-20 (*20*); d-25 (*21*); d-37 (*3*); d-38 (*22*); c-43 (*23*); and d-45 (*24*). C-44 was purchased from Aldrich (Steinheim, Germany).

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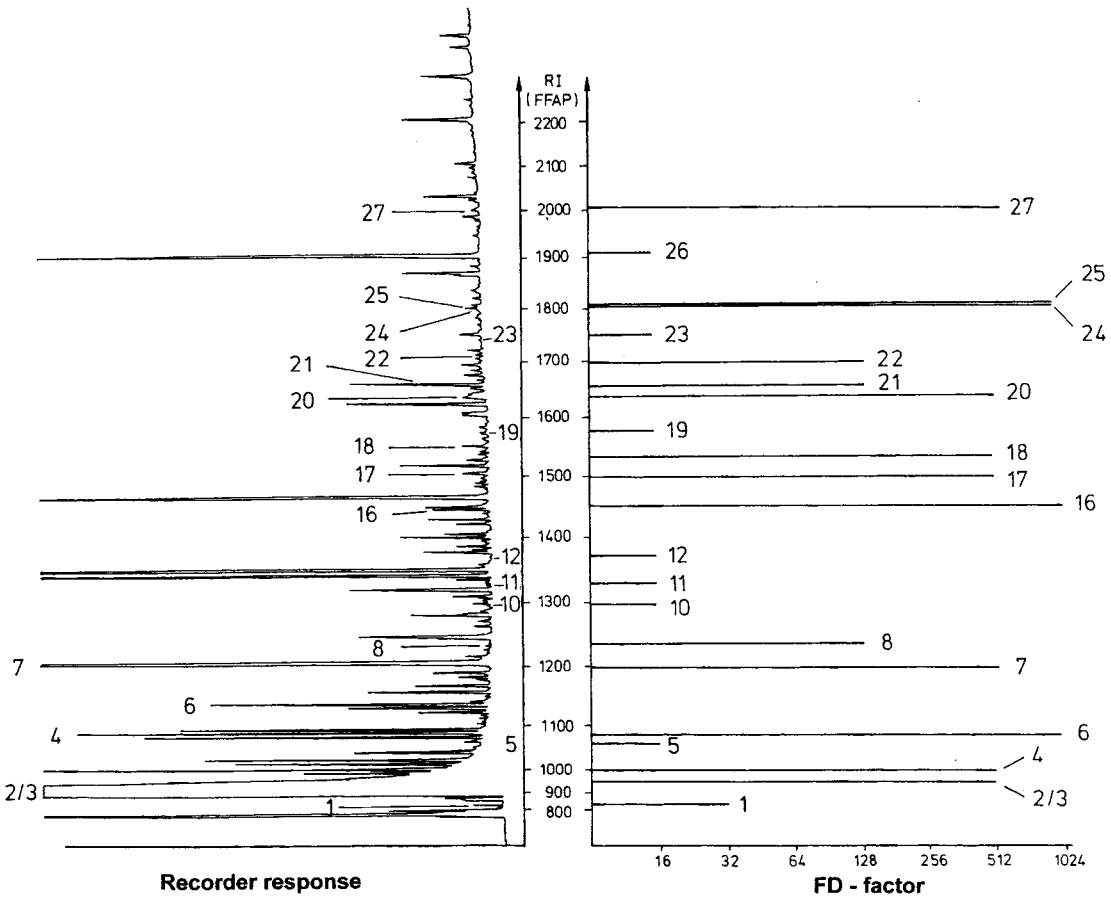

Figure 1. Flavor dilution chromatogram and gas chromatogram of the neutral−basic volatiles from rye bread crumb.

Isolation of the Crumb Volatiles for AEDA. Two hours after baking, the rye bread crumb was separated from the crust. The inner crumb material (600 g) was cut into pieces, frozen in liquid nitrogen, and then ground by means of a commercial blendor (Moulinette, Quelle, Nürnberg, Germany). The powder obtained was transferred into a Soxhlet apparatus, soaked with dichloromethane (1.8 L), and extracted for 8 h at 46 °C. The extract was concentrated to 150 mL by distilling off the solvent by means of a Vigreux column (50 cm × 1 cm). The volatile material was isolated by high-vacuum sublimation. The distillate was separated into neutral and basic compounds and acidic volatiles by treatment with aqueous Na_2CO_3 (0.5 mol/L; pH 10.9) as previously reported (*3*).

Enrichment by Column Chromatography/Identification Experiments. For the identification experiments, the neutral−basic fraction isolated from 2 kg of rye bread crumb was fractionated by column chromatography (*3*). The odor-active compounds perceived at the sniffing port, when analyzing each fraction, were identified by comparing the mass spectra (MS/EI; MS/CI), the retention indices on two HRGC columns of different polarity, as well as the odor quality and intensity at the sniffing port. The HRGC/MS experiments for identification were carried out described by (*5*). The determination of the odor intensity by GC/O was done the following way: aliquots of solutions of decreasing concentrations of the respective reference odorant were injected onto the GC column, and the FID signal and the odor intensity at the sniffing port were compared. A comparison of the FID signal and the odor intensity obtained at the respective retention index, when analyzing the sample, with the results for the reference solutions either confirmed or denied a similar odor potency. If no similarity was found, coelution of an overlapping non-odor-active compound occurred. In that case, sniffing and mass spectral analysis was done on a column of a different polarity.

Quantification of Odorants. Frozen, ground crumb material (5 to 1000 g, depending on the amounts of odorants present) was spiked with known amounts of the labeled internal standards (5−10 μg, dissolved in diethyl ether). The samples were soaked in diethyl ether and then extracted in a Soxhlet apparatus for 8 h. Further workup was performed as reported above (isolation of the crumb volatiles). The resulting extracts were separated by HRGC, and the ion intensities were monitored by mass chromatography (*25*) using the selected ions, capillaries, and response factors given in Table 1. The calibration factors were determined in mixtures of equal amounts of unlabeled odorants and corresponding labeled standards with ratios of 3:1 to 1:3 (by weight) by means of mass chromatography and were calculated using the equation $CF = (C_u \times I_L/C_L \times I_u)$ with C_u = concentration of the unlabeled compound, C_L = concentration of the labeled compound, I_L = intensity of the ion m/z of the labeled compound, and I_u = intensity of the ion m/z of the unlabeled compound.

For mass chromatography, the GC columns described above were coupled to an ITD-800 ion trap detector (Finnigan, Bremen, Germany), running in the chemical ionization mode with methanol as reactant gas. Mass spectra were generated at 70 eV. For the first dimension of the two-dimensional HRGC/MS (TDGC/MS), a GC (Mega 2 series, Fisons Instruments, Mainz, Germany) equipped with an FFAP capillary was used. The relevant part of the effluent, containing the analyte and the respective internal standard, was trapped in an empty, cooled, fused-silica column by means of a connected "dome"-switching technique (Fisons Instruments, Mainz, Germany), as described by Weber et al. (*26*). Chromatography was then performed by means of a type 5160 gas chromatograph (Carlo Erba, Hofheim, Germany) using either an OV-1701 capillary or a DB-5 capillary, respectively. Detection was done by an ion trap detector ITD-800 (Finnigan) coupled to the second GC.

Enzymatic Determination of Acetic Acid. Freshly prepared inner crumb material was cut into pieces, frozen in liquid nitrogen, and then ground by means of a commercial blender, described above. A 50-g portion of the powder was transferred with redistilled water (400 mL) into a 1-L volumetric flask. To remove proteins, a Carrez clarification was

Table 1. Thin Film Capillaries, Selected Ions, and Calibration Factors Used in the Stable Isotope Dilution Assays

odorant	selected ion (*m*/*z*)	internal standard[a]	selected ion (*m*/*z*)	capillary	calibration factor[b]
3-methylbutanal	69	**d-2**	70−71	DB-WAX	0.71
2-methylbutanal	87	**d-2**	70−71	DB-WAX	1.90
2,3-butandione[c]	159	**c-4**	163	SE-54	1.00
2,3-pentandione	101	**c-5**	103	SE-54	1.00
hexanal	83	**d-6**	85−87	SE-54	0.99
3-methylbutanol	71	**d-7**	73	FFAP	0.84
(Z)-4-heptenal	95	**d-8**	97	FFAP−DB-1701	0.87
methional	105	**d-16**	108	FFAP−DB-1701	1.03
(Z)-2-nonenal	123	**d-17**	125	FFAP−SE-54	0.81
(E)-2-nonenal	141	**d-18**	143	SE-54	0.76
phenylacetaldehyde	121	**c-20**	123	SE-54	1.00
(E,E)-2,4-decadienal	153	**d-24**	156−158	SE-54	0.75
(E)-β-damascenone	191	**d-25**	195−197	FFAP−SE-54	0.70
2-phenylethanol	105	**d-26**	107	FFAP	0.96
4,5-epoxy-(E)-2-decenal[d]	169	**d-27**	172−174	SE-54	0.84
butanoic acid	89	**d-37**	91−93	FFAP	1.00
3- and 2-methylbutanoic acid[e,f]	85	**d-38**	87−88	FFAP	0.73
3-hydroxy-4,5-dimethyl-2(5H)-furanone	129	**c-43**	131	FFAP−DB-1701	1.00
2-phenylacetic acid	137	**c-44**	139	FFAP−DB-1701	1.00
vanillin	153	**d-45**	156	FFAP−DB-1701	1.10

[a] For structures see Figure 2. [b] Calibration factors were determined in mixtures of equal amounts of unlabeled odorants and corresponding labeled standards with ratios of 3:1 to 1:3 (by weight) by means of mass chromatography. [c] Determined as quinoxaline derivative. [d] Compound 27 was determined using the MS system MAT 95 S and isobutane as the reactant gas. [e] Sum of both isomers. [f] The ratio of the 2- to 3-methylbutanoic acid was calculated from the ratio of the ions *m*/*z* 74 and *m*/*z* 60 (MS/EI).

carried out: 5 mL of Carrez-I-solution (potassium hexacyanoferrate (II) (ferrocyanide), 85 mmol/L = 3.6 g $K_4[Fe(CN)_6 \times 3 H_2O]$/100 mL) and 5 mL of Carrez-II-solution (zinc sulfate, 250 mmol/L = 7.20 g $ZnSO_4 \times 7 H_2O$/100 mL) were added and the solution was adjusted to pH 7.5−8.5 using 0.1 N NaOH. The flask was filled up to 1 L with distilled water, and the mixture was filtered. The resulting solution was subjected to the procedure given by the supplier of the enzymic test (Boehringer Mannheim GmbH, Mannheim, Germany). The principle of the procedure is as follows: Acetic acid is converted into acetyl-CoA using acetyl-CoA synthase (reaction 1). Acetyl-CoA is then transferred into citric acid by a reaction with 2-oxo-succinic acid in the presence of citrate synthase (reaction 2). The 2-oxo-succinic acid required for reaction 2 is formed from L-malate and nicotinamide-adenine dinucleotide (NAD) in the presence of L-malate dehydrogenase (reaction 3). In this reaction NAD is reduced to NADH. So, the determination is based on the formation of NADH which is measured photometrically.

High-Resolution Gas Chromatography/Olfactometry (HRGC−O). HRGC was performed by means of a Type 5160 gas chromatograph (Carlo Erba, Hofheim, Germany) using the following capillaries: FFAP (30 m × 0.32 mm i.d. fused-silica capillary, free fatty acid phase, 0.25 μm (J&W Scientific, supplied by Fisons Instruments, Mainz, Germany)); DB-5 (25 m × 0.32 mm i.d. fused-silica capillary Durabond 5, equivalent to SE-54, 0.25 μm, (Fisons Instruments, Mainz, Germany)), DB-OV-1701 (30 m × 32 mm i.d. fused-silica capillary DB 1701, 0.25 μm (Fisons Instruments, Mainz, Germany)), and CP-WAX 52 CB (50 m × 0.32 mm i.d. fused-silica capillary DB-Wax, 1.2 μm (Chrompack, Mühlheim, Germany)). The samples were applied by the cold on-column injection technique at 35 °C (SE-54, OV-1701) or 40 °C (FFAP). After 2 min, the temperature of the oven was raised at 40 °C/min to 50 °C (DB-5, OV-1701) or 60 °C (FFAP), respectively, held for 2 min isothermally, then raised at 4 °C/min (SE-54, OV-1701) or 6 °C/min (FFAP) to 240 °C, and held for 10 min. Using the CP-WAX 52 CB, the initial temperature of 35 °C was held for 5 min and then raised at a rate of 4 °C/min to 230 °C. The flow rate of the carrier gas helium was 2.5 mL/min. For GC/olfactometry and the aroma extract dilution experiments (AEDA) the effluent was split 1:1 (by volume) into a sniffing port and flame ionization detector (FID) at the end of the capillary, using deactivated but uncoated fused-silica capillary columns (30 cm × 0.32 mm i.d.). The temperature of the FID and the sniffing port was held at 220 °C. Linear retention indices (RI) of the compounds were calculated by using the retention times of *n*-alkanes as the reference (*3*).

Aroma Extract Dilution Analysis (AEDA). The flavor dilution (FD) factors of the odor-active compounds of the neutral−basic and the acidic fractions were determined separately by AEDA (*2*) using the following dilution series: The fractions (300 μL each) containing the volatiles of 600 g of rye bread crumb were stepwise diluted with dichloromethane (1:1) or diethyl ether, respectively. HRGC/olfactometry was then performed with aliquots (0.5 μL) using capillary DB-5 (neutral−basic fraction) and capillary FFAP (acidic fraction). The flavor dilution (FD) chromatogram was plotted (FD factors vs retention indices). To check the correctness of the sniffing experiments, HRGC−O of the original neutral−basic and acidic fractions, respectively, was done by six sniffers and the complete AEDA analyses of both fractions were then carried out by three experienced panelists. Recruiting of the panelists was done by selecting those who had detected the highest number of odor-active areas.

Sensory Evaluations. All sensory analyses were performed in a sensory panel room at 21 ± 1 °C. The test panel consisted of 10 experienced assessors, aged 25−35 years, 4 women and 6 men, who were trained to describe the flavor qualities of 60 defined odor-active chemicals (*13*). Six aroma attributes were selected for the investigations of the flavor profiles of rye bread crumb and the corresponding model mixtures. The panelists were trained with suprathreshold aqueous solutions of the reference stimuli: 3-methylbutanal for malty, 2,3-butandione for buttery, acetic acid for sour-pungent, 3-methylbutanoic acid for sweaty, (*E*)-2-nonenal for fatty, and with 3-hydroxy-4,5-dimethyl-2(5)*H*-furanone (sotolon) for the odor quality spicy. The bread crumb samples (10 g each) and the model mixtures (10 mL each) were presented in covered glass beakers (internal diameter 40 mm, total volume 45 mL) immediately after preparation. For nasal evaluation, the glass cover was removed and the sample was sniffed by the panelists.

Flavor Reconstitution. The following mixture of 20 freshly distilled reference compounds was prepared in 500 μL of ethanol: 3-methylbutanol (2800 μg); butanoic acid (2200 μg); vanillin (1200 μg); 3-methylbutanoic acid (1100 μg); 2-phenylacetic acid (750 μg); 2-phenylethanol (716 μg); hexanal (380 μg); 2,3-butandione (334 μg); phenylacetaldehyde (244 μg); 3-methylbutanal (150 μg); methional (75 μg); (*E*,*E*)-2,4-decadienal (63 μg); (*E*)-2-nonenal (49 μg); 2,3-pentandione (43 μg);

Table 2. Most Odor-Active Neutral−Basic Volatiles (FD ≥ 4) in Rye Bread Crumb

no.[a]	odorant[b]	odor quality[c]	CC[d] fraction	RI on FFAP	RI on SE-54	RI on OV-1701	FD[e] factor
1	2-methylpropanal	malty	2	821	<600	−	32
2/3	3- and 2-methylbutanal	malty	2	945	652	732	512
4	2,3-butandione	buttery	2	1000	<600	700	512
5	2,3-pentandione	buttery	2	1054	700	782	16
6	hexanal	green	2	1082	800	880	1024
7	3-methylbutanol	malty	4	1200	732	842	512
8	(*Z*)-4-heptenal	sweet, biscuit-like	2	1238	898	987	128
9	octanal	fruity, soapy	2	1280	1000	1088	4
10	1-octen-3-one	mushroom-like	2	1296	975	1067	16
11	2-acetyl-1-pyrroline[f]	roasty, popcorn-like	4	1330	922	1016	16
12	dimethyl trisulfide	cabbage-like	2	1372	965	1034	16
13	(*Z*)-1,5-octadien-3-one[f]	geranium-like	2	1377	984	1081	4
14	nonanal	fruity, soapy	2	1386	1103	1193	4
15	(*E*)-2-octenal	fatty, waxy	2	1419	1057	1164	4
16	methional	boiled potato	3	1448	904	1040	1024
17	(*Z*)-2-nonenal	green, fatty	2	1496	1149	1260	512
18	(*E*)-2-nonenal	green, fatty	2	1527	1161	1279	512
19	(*E*,*Z*)-2,6-nonadienal	cucumber-like	2	1577	1153	1269	16
20	phenylacetaldehyde	honey-like	3	1638	1042	1175	512
21	(*E*,*Z*)-2,4-nonadienal	green, fatty	2	1647	1188	1329	128
22	(*E*,*E*)-2,4-nonadienal	fatty, waxy	2	1698	1211	1345	128
23	(*E*,*Z*)-2,4-decadienal	green, fatty	2	1752	1287	1414	16
24	(*E*,*E*)-2,4-decadienal	fatty, waxy	2	1804	1317	1450	1024
25	(*E*)-*β*-damascenone	boiled-apple-like	2	1806	1386	1497	1024
26	2-phenylethanol	flowery	4	1911	1110	1281	16
27	4,5-epoxy-(*E*)-2-decenal	metallic	3	2006	1381	1558	512
28	2-methoxy-4-vinyl-phenol	clove-like	3	2194	1317	1480	8
29	unknown	fruity	−	−	853	−	4
30	unknown	soapy	−	−	900	−	4
31	unknown	fatty	−	−	915	−	16
32	unknown	mushroom−like	−	−	940	−	4
33	unknown	soapy	−	−	1091	−	16
34	unknown	fatty, waxy	−	−	1240	−	16
35	unknown	mushroom-like	−	−	1337	−	16

[a] Numbering refers to Figure 1. [b] The compound was identified by comparing it with the reference substance on the basis of the following criteria: retention index (RI) on 3 stationary phases given in the table, mass spectra obtained by MS (EI) and MS (CI) and odor quality as well as odor intensity perceived at the sniffing port. [c] Odor quality perceived at the sniffing port. [d] The fraction refers to the column chromatography applied. [e] Flavor dilution (FD) factor determined in extracts containing the neutral−basic sourdough volatiles. [f] The obtained MS signals were too weak for an unequivocal interpretation. The compound was identified on the basis of the remaining criteria given in footnote b.

3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (6 *μ*g); 4,5-epoxy-(*E*)-2-decenal (4.6 *μ*g); (*Z*)-2-nonenal (3.4 *μ*g); (*Z*)-4-heptenal (2.7 *μ*g); and (*E*)-*β*-damascenone (0.4 *μ*g). The entire mixture and 1700 mg of pure acetic acid were added to 1 L of citrate buffer (0.01 mol/L; pH 4.25), and the mixture was stirred for 30 min (citrate buffer model).

The same amount of the ethanolic solution of the reference compounds and acetic acid was mixed with 1 kg of edible corn starch (Fixella-Remiga, Krefeld, Germany). The mixture was homogenized in a shaking machine (Type Turbula, Bachofen, Basel, Switzerland) for 30 min (starch model).

Powdered rye bread crumb material (about 1 kg) was extracted successively with dichloromethane, diethyl ether, and then acetone. After evaporation in vacuo, the material was freeze-dried. The procedure was repeated twice.

Citrate buffer (500 mL; 0.01 mol/L; pH 4.25), containing the entire set of reference compounds and acetic acid as reported above, was then added to the deodorized crumb powder (500 g). The mixture was homogenized for 30 min by means of a shaking machine (deodorized crumb model).

Flavor Profile Analyses. The intensities of the six odor qualities selected for rye bread crumb and the model systems were scored nasally and retronasally on a seven point scale from 0 to 3 (0; 0.5; 1; ...; 3.0). The values given by the panelists were averaged and differed by not more than 10% between different sessions.

Omission Tests. Citrate buffer and starch models were prepared as reported above, but by omitting one of the compounds singly. Each of these "reduced model systems" were presented to the sensory panel for comparison with the entire citrate buffer or starch model, respectively, by means of the triangle test.

RESULTS AND DISCUSSION

In a preliminary experiment, five different crumbs of rye breads available on the local market were sensorially evaluated by a group of 10 panelists. A rye bread made by a three-stage sourdough procedure was judged to elicit the most attractive flavor, with a high significance. This bread, manufactured in a traditional way, was chosen for the experiments.

Application of AEDA to an extract, prepared by solvent extraction and high vacuum sublimation, which elicited the typical crumb aroma, led to the perception of 43 odor-active regions with FD factors ranging from 4 to 2048. In Figure 1, the results of the AEDA are contrasted to the gas chromatogram of the neutral−basic crumb volatiles. Interestingly, compounds no. 16, 24, and 25 with the highest flavor dilution (FD) factors did not give a clear signal at the flame ionization detector.

The results of AEDA and the identification experiments subsequently performed are summarized in Tables 2 and 3. In the neutral−basic fraction (Table 2), the following compounds were found with the highest FD factors: hexanal (no. 6; green), methional (no. 16; boiled potato), *E*,*E*-2,4-decadienal (no. 24; fatty, waxy),

Table 3. Most Odor-Active Acidic Volatiles (FD factor ≥ 4) in Rye Bread Crumb

no.[a]	odorant[a]	odor quality[b]	RI on FFAP	FD[c] factor
36	acetic acid	sour, pungent	1444	256
37	butanoic acid	sweaty	1618	512
38/39	3- and 2-methylbutanoic acid	sweaty	1659	2048
40	pentanoic acid	sweaty	1732	512
41	hexanoic acid	sweaty	1835	512
42	unknown	spicy	2132	16
43	3-hydroxy-4,5-dimethyl-2(5*H*)-furanone	spicy	2196	2048
44	2-phenylacetic acid	sweet, honey-like	2570	512
45	vanillin	vanilla-like	2581	2048

[a] The compound was identified by comparing it with the reference substance on the basis of the following criteria: retention index (RI) on the stationary phase given in the table, mass spectra obtained by MS−EI and MS−CI, and odor quality, as well as odor intensity perceived at the sniffing port. [b] Odor quality perceived at the sniffing port. [c] Flavor dilution (FD) factor determined in extracts containing the rye bread crumb volatiles.

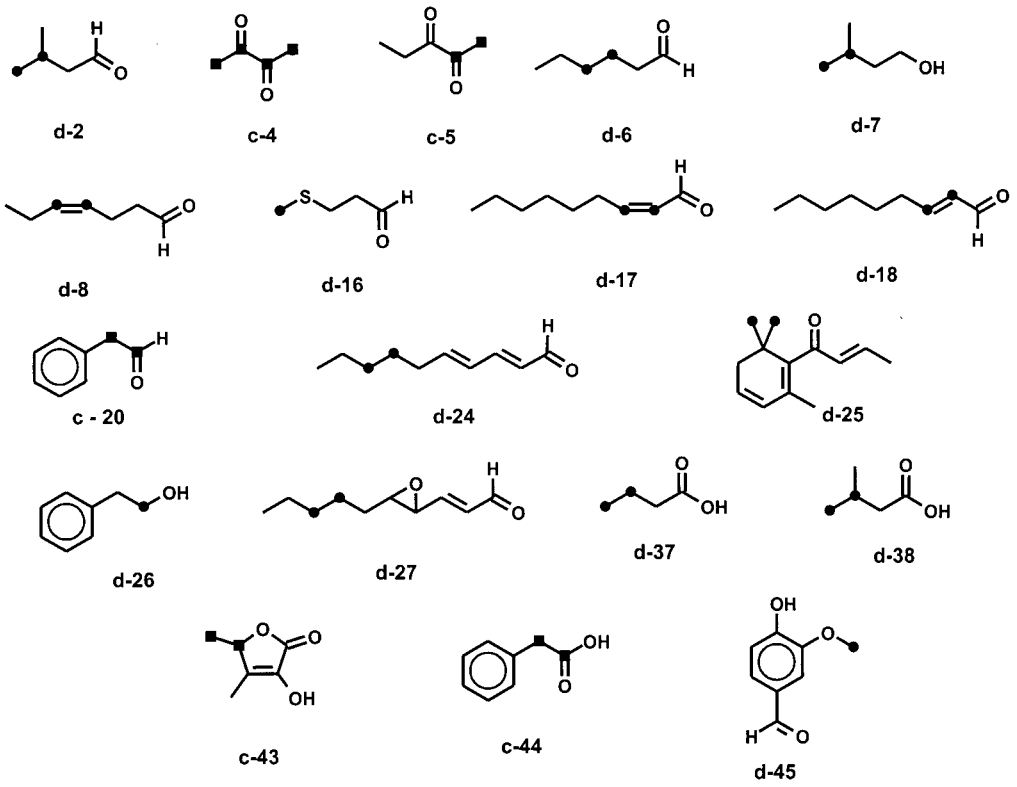

Figure 2. Structures of the internal standards used in the isotope dilution assays: (●) deuterium label; (■) carbon − 13 label.

and (*E*)-*β*-damascenone (no. 25; boiled-apple-like), followed by 3- and 2-methylbutanal (nos. 2 and 3; malty), 2,3-butandione (no. 4; buttery), 3-methylbutanol (no. 7; malty), (*E*)- and (*Z*)-2-nonenal (nos. 17 and 18; green, fatty), phenylacetaldehyde (no. 20; honey-like), and 4,5-epoxy-(*E*)-2-decenal (no. 27; metallic). In the fraction of the acidic odor-active compounds (Table 3), 3- and 2-methylbutanoic acid (sweaty), vanillin, and 3-hydroxy-4,5-dimethyl-2(5*H*)furanone (spicy) were characterized with the highest FD factors. The results confirmed data previously reported by us on a similar type of rye bread (*3*) and, thereby, corroborated the importance of the compounds identified for the crumb flavor.

A further step needed to evaluate the odor contribution of single compounds identified with high potencies in GC/O experiments is quantitation. For this reason, the 22 volatiles exhibiting FD factors >128 were determined by stable isotope dilution assays (SIDAs) using the labeled internal standards presented in Figure 2. For mass chromatography, compounds nos. 8, 16, 17, 25, and 43−45 had to be enriched by two-dimensional HRGC, because they were present in trace amounts and coeluted with interfering volatiles. Compound no. 4 was analyzed after derivatization with 1,2-benzenediamine as previously reported (*27*). The results of the quantitation are summarized in Table 4.

Acetic acid, determined by means of an enzymatic test, was present in a very high concentration of 1.7 g/kg rye bread crumb, and it was followed by 3-methylbutanol, butanoic acid, vanillin, and 2- and 3-methylbutanoic acid with values ranging between 1 and 3 mg/kg (Table 4). Slightly lower concentrations were found for phenylacetic acid and 2-phenylethanol. The oxo-compounds investigated showed values in the *μ*g/kg range, but (*E*)-*β*-damascenone was found to occur in concentrations lower than 1 *μ*g/kg rye bread crumb.

Following the odor activity concept (*2*), the odor activity values (OAVs) were then calculated on the basis of either the odor thresholds in starch or in water, respectively, because both are the main ingredients in rye bread crumb. The lowest odor thresholds in starch were determined for (*E*)-*β*-damascenone, (*Z*)-2-nonenal, and 4,5-epoxy-(*E*)-2-decenal, whereas acetic acid, butanoic acid, 2-phenylethanol, and 3-methylbutanol showed relatively high thresholds (Table 5). A calculation of the OAVs revealed the highest values for

Table 4. Concentrations of 21 Aroma Compounds in Fresh Rye Bread Crumb

odorant	concentration (μg/kg)
acetic acid	1700000
3-methylbutanol	2800
butanoic acid	2200
vanillin	1200
3- and 2-methylbutanoic acid	1100
2-phenylacetic acid	741
2-phenylethanol	716
hexanal	380
2,3-butandione	334
phenylacetaldehyde	244
3-methylbutanal	150
methional	75
(*E*,*E*)-2,4-decadienal	62
(*E*)-2-nonenal	49
2,3-pentandione	43
3-hydroxy-4,5-dimethyl-2(5*H*)-furanone	6.3
2-methylbutanal	< 5
4,5-epoxy-(*E*)-2-decenal	4.5
(*Z*)-2-nonenal	3.4
(*Z*)-4-heptenal	2.7
(*E*)-β-damascenone	0.41

Table 5. Odor Thresholds in Starch and Odor Activity Values (OAVs) of Crumb Aroma Compounds

odorant	odor threshold[a] (μg/kg)	OAV[b]
methional	0.27	278
vanillin	4.6	261
3- and 2-methylbutanoic acid	6	183
(*Z*)-2-nonenal	0.036	94
(*E*)-2-nonenal	0.53	92
acetic acid	31140	55
2,3-butandione	6.5	51
4,5-epoxy-(*E*)-2-decenal	0.19	24
(*E*,*E*)-2,4-decadienal	2.7	23
butanoic acid	100	22
hexanal	30	13
phenylacetaldehyde	28[c]	9
2-phenylethanol	125[d]	6
3-methylbutanal	32	5
2,3-pentandione	16	3
3-hydroxy-4,5-dimethyl-2(5*H*)-furanone	2.1	3
(*E*)-β-damascenone	0.2[d]	2
(*Z*)-4-heptenal	3[d]	<1

[a] Threshold values according to ref *28*. [b] The odor activity values were calculated by dividing the concentrations of the odorants by their nasal odor detection thresholds. [c] Fickert, B., personal communication. [d] Threshold value determined in cellulose.

methional, vanillin, and 2- and 3-methylbutanoic acid (Table 5). However, when odor thresholds in water were used for OAV calculation, only 3-methylbutanal remained important. In this matrix, however, (*E*,*E*)-2,4-decadienal, (*Z*)-2-nonenal, and (*E*)-β-damascenone significantly increased in odor activity (Table 6).

The quantitative results were then scrutinized by means of sensory model studies, to link the analytical results with the sensory properties of the crumb. In a first step, a trained sensory panel determined the odor profile of the fresh rye bread crumb and described the aroma as malty, sour, fatty, sweaty, spicy, and buttery (cf. Figure 3 A). On the basis of high OAVs, it can be assumed that 3-methylbutanal is responsible for the malty note, acetic acid for the sour quality, 2,3-butandione for the buttery aroma, and 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone for the spicy odor quality. On the other hand, (*E*,*E*)-2,4-decadienal and (*E*)-2-nonenal are suggested to be responsible for the green and fatty characteristics of the crumb odor.

Table 6. Odor Thresholds in Water and Odor Activity Values (OAVs) of Crumb Aroma Compounds

odorant	odor threshold[a] (μg/kg)	OAV[b]
3-methylbutanal	0.4	375
(*E*,*E*)-2,4-decadienal	0.2	310
(*Z*)-2-nonenal	0.02[c]	170
(*E*)-β-damascenone	0.004[d]	103
acetic acid	22000	77
(*E*)-2-nonenal	0.8	61
phenylacetaldehyde	4	61
vanillin	25	48
methional	1.8	42
4,5-epoxy-(*E*)-2-decenal	0.12	38
hexanal	10.5	36
2,3-butandione	15	22
3-hydroxy-4,5-dimethyl-2(5*H*)-furanone	0.3[e]	21
(*Z*)-4-heptenal	0.2	14
3-methylbutanol	1000	3
butanoic acid	1000	2
3- and 2-methylbutanoic acid	740	2
2,3-pentandione	30	1
2-phenylethanol	1000	<1
2-phenylacetic acid	10000	<1

[a] Threshold values according to ref *28*. [b] Odor activity values were calculated by dividing the concentrations of the odorants by their nasally determined detection thresholds. [c] Kerscher, R. personal communication. [d] Ref *29*. [e] Ref *30*.

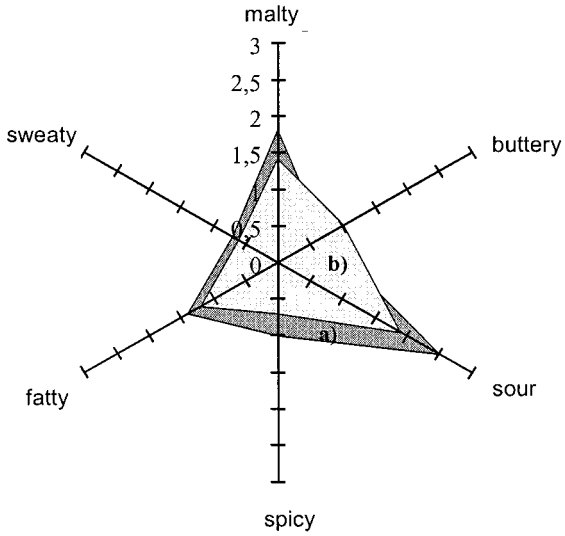

Figure 3. Orthonasal flavor profiles of the fresh rye bread crumb (a) and a flavor model (DOC) based on deodorated crumb material (b).

To prove this assumption, crumb flavor recombinates were prepared based on the concentrations determined in the crumb. The mixture contained the entire set of the 20 odorants quantitatively measured. In a first set of experiments, citrate buffer, adjusted to the pH of the rye bread crumb, and starch were used as the matrixes. Additionally, a 1:1 mixture of citrate buffer and deodorized, dried crumb material was used to achieve a better approximation to the complex crumb matrix. The odor profiles of the model systems were judged consecutively, each in comparison to the fresh bread crumb.

Each of the model systems showed a typical malty and sour aroma, satisfactorily mimicking the crumb odor. However, clear differences existed between the three models, with the deodorized crumb model giving the best agreement with the crumb flavor (Table 7 and Figure 3). By contrast, the citrate buffer and the starch model showed differences in intensities of single odor

Table 7. Nasal Flavor Profiles of Rye Bread Crumb and of Three Aroma Models: Citrate Buffer, Starch, and Deodorized Crumb/Citrate Buffer

	odor intensity[a]			
		model		
flavor attribute	bread crumb	citrate buffer	starch	deodorized crumb
malty	1.8	1.7	1.4	1.4
buttery	0.7	0.6	0.9	1
sour	2.5	1.6	1.0	1.9
spicy	1	0.6	0.7	0.7
fatty	1.4	2	1.4	1.2
sweaty	0.7	0.9	0.4	0.6

[a] The intensity of the attributes was scored to a seven point scale from 0: absent to 3: strong.

Table 8. Flavor of Model Systems as Effected by the Omission of One Compound[a]

odorant omitted from the model system	number of panelists (out of 10) detecting an odor difference
(*Z*)-2-nonenal	4
(*E*)-*β*-damascenone	6
4,5-epoxy-(*E*)-2-decenal	6
(*Z*)-2-nonenal[b]	2
(*E*)-*β*-damascenone[b]	5
4,5-epoxy-(*E*)-2-decenal[b]	5

[a] Experiments were performed in citrate buffer. [b] The citrate buffer was replaced by starch.

qualities as effected by the different odor thresholds of certain odorants in water and starch, respectively.

In further experiments, the contributions of (*Z*)-2-nonenal, (*E*)-*β*-damascenone, and 4,5-epoxy-(*E*)-2-decenal were singly evaluated by means of omission tests (Table 8). A clear differentiation between the model systems singly lacking in one of the three compounds and the full recombinates was not possible. Therefore, (*Z*)-2-nonenal, (*E*)-*β*-damascenone, and 4,5-epoxy-(*E*)-2-decenal are possibly less important for the overall flavor of rye bread crumb.

In general, the application of the odor activity concept followed by sensory model studies was proven to be a useful tool to identify the key odorants in rye bread crumb. These odorants can now serve as indicators to analytically establish influences on bread flavor caused by the processing conditions or the raw materials. Analysis of these character impact odorants is suggested to support product development and quality control in large-scale bread manufacturing.

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