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Quantitation of Odor-Active Compounds in Rye Flour and Rye Sourdough Using Stable Isotope Dilution Assays

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Application of the aroma extract dilution analysis on a flavor distillate prepared from freshly ground rye flour (type 1150) revealed 1-octen-3-one (mushroom-like), methional (cooked potato), and (*E*)-2-nonenal (fatty, green) with the highest flavor dilution (FD) factors among the 26 odor-active volatiles identified. Quantitative measurements performed by stable isotope dilution assays and a comparison to the odor thresholds of selected odorants in starch suggested methional, (*E*)-2-nonenal, and hexanal as contributors to the flour aroma, because their concentrations exceeded their odor thresholds by factors >100. Application of the same approach on a rye sourdough prepared from the same batch of flour revealed 3-methylbutanal, vanillin, 3-methylbutanoic acid, methional, (*E*,*E*)-2,4-decadienal, 2,3-butanedione, and acetic acid as important odorants; their concentrations exceeded their odor thresholds in water and starch by factors >100. A comparison of the concentrations of 20 odorants in rye flour and the sourdough made therefrom indicated that flour, besides the fermentation process, is an important source of aroma compounds in dough. However, 3-methylbutanol, acetic acid, and 2,3-butanedione were much increased during fermentation, whereas (*E*,*E*)-2,4-decadienal and 2-methylbutanal were decreased. Similar results were obtained for five different flours and sourdoughs, respectively, although the amounts of some odorants in the flour and the sourdough differed significantly within batches.

KEYWORDS: Rye sourdough; rye flour; flavor; aroma extract dilution analysis; stable isotope dilution assay

INTRODUCTION

Bread manufactured from rye flour has an important marketshare among the cereal products available, in particular, in Germany and Eastern European countries (*1*). In the manufacturing process, the use of sourdough is essential to obtain the desired texture and, also, the characteristic pleasant flavor of, especially, the bread crumb (*2*). This significant influence of the sourdough fermentation on crumb flavor development has previously been established by overall sensory evaluations (*3*, *4*). The authors reported a green and cereal-like off-odor occurring in bread crumb made from chemically acidified dough, which was completely absent in the crumb of breads prepared by a traditional multiple-stage sourdough procedure.

Besides the generation of odorants during fermentation by biochemical reactions, also the flavor compounds already present in the flour may influence the overall aroma of the sourdough and, finally, of the bread itself. The first systematic studies on the composition of the volatile fraction of rye flour were carried out by Markova et al. (*5*). Thirteen volatile compounds were identified, among which ethanol, *n*-propanol, 2-methyl-1-propanol, 2-butanol, acetone, and the esters ethyl acetate and methyl formate were characterized. Investigations on rye kernels by Hougen et al. (*6*) and on rye flour by Prince and Mackey (*7*) resulted in the identification of a homologous series of *n*-alkanals (from propanal to heptanal) as well as 2-heptenal, 2-octenal, methanol, and the two methyl ketones 2-butanone and 2-pentanone. Lund et al. (*3*) later reported on the identification of 14 compounds, among which *n*-butanol, 2- and 3-methylbutanol, *n*-pentanol, *n*-hexanol as well as (*E*)-2-hexenal, benzaldehyde, 2-heptanone, 2-pentylfuran, and 2-furaldehyde were identified for the first time as volatile constituents of rye flour. It is, however, yet unclear which compounds contribute to the mild, cereal-like aroma of rye flour.

The first investigations on the composition of the volatile fraction of rye sourdough were carried out by Beccard (*8*), who characterized, in particular, acetic acid and lactic acid. Markova et al. (*5*) identified a total of 17 volatile compounds, all of which were also confirmed to be present in the flour used. Quantitative data reported by Hansen and Lund (*9*) indicated that the sourdough fermentation led to an increase of, in particular, esters such as acetates, propionates, hexanoates, lactates, and octanoates. Further comprehensive studies (*10*, *11*) were focused on the influence of temperature and the composition of the starter cultures on the production of volatiles during sourdough fermentation. In total, 36 volatiles were reported, among which

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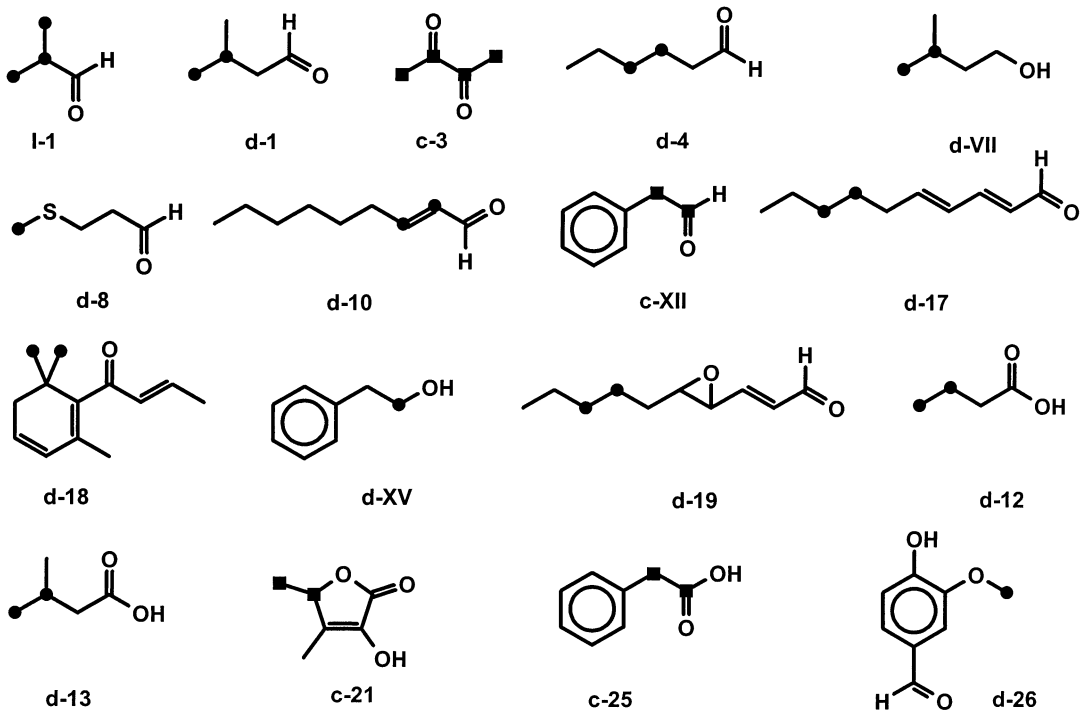

Figure 1. Structures of the labeled internal standards used in the stable isotope dilution assays: ●, deuterium label; ■, carbon-13 label.

(*E*)-2-butenal, (*Z*)-3-hexenal, nonanal, and heptadienal were characterized as sourdough constituents. Furthermore, it was found that a lower number of aldehydes was present if heterofermentative instead of homofermentative strains were used in sourdough preparation. It is, however, still unclear which compounds have a flavor impact on sourdough aroma.

The application of dilution to odor threshold techniques, such as aroma extract dilution analysis (AEDA), followed by a calculation of odor activity values (OAVs; ratio of concentration to odor threshold), allows one to differentiate between odorless volatiles and aroma compounds contributing to a given flavor (*12*). Recently, this approach has been successfully applied by us to the aroma compounds of a rye bread crumb prepared by using a three-stage sourdough procedure (*13*). The results have finally led to a rye crumb aroma recombinate containing 20 crumb odorants on deodorized crumb material as the matrix, which clearly mimicked the original crumb aroma.

The literature survey shows that it is not yet clear which compounds contributing to the rye crumb aroma stem either from the flour used or from microbial activities during fermentation. To elucidate the sources of aroma compounds in rye bread crumb, the aim of the following investigation was (i) to characterize the key odorants in rye flour by application of the AEDA and by subsequent quantitations/odor threshold correlations and (ii) to compare the data with the odorants characterized by the same approach in sourdough made by using the same batch of flour.

MATERIALS AND METHODS

Materials. Rye flour (type 1150) and a commercial starter culture (Böcker, Minden, Germany) were used in the sourdough manufacturing. In the first refreshment, flour (400 g), water (400 g), and the starter culture (80 g) were incubated for 6 h at 25−26 °C. The material (800 g) was then mixed with flour (2267 g) and water (933 g) for a second refreshment at 24−25 °C for 3 h. The sourdough (full sour) was finally prepared by using flour (2610 g), water (2150 g), and 250 g of the second refreshment and by incubating the mixture at 28−30 °C for 16 h.

Chemicals. The reference compounds of the odorants given in the tables were mostly obtained from commercial sources. Compounds that were not commercially available were synthesized following the literature: **IX** (*14*), **9** (*15*), and **19** (*16*). Compound **16** was isolated from commercial (*E*,*E*)-2,4-decadienal according to the method of ref *17*. Compounds **6** and **18** were gifts from Haarmann and Reimer (Holzminden, Germany).

The internal standards used in the stable isotope dilution assays, labeled with either deuterium (**d**) or carbon-13 (**c**) (**Figure 1**), were synthesized as described in previous studies given in parentheses: **I-1** (*18*); **d-1** (*19*); **c-3** (*20*); **d-4** (*21*); **d-VII** and **d-XV** (*22*); **d-8** (*23*); **d-10**, **d-17,** and **d-19** (*24*); **c-XII** (*25*); **d-18** (*26*); **d-12** (*27*); **d-13** (*28*); **c-21** (*29*); and **d-26** (*30*). Compound **c-25** was purchased from Aldrich, Steinheim, Germany.

Isolation of the Volatiles. For the isolation of the flour volatiles, a powder obtained by homogenization of a flour/water (83 g + 67 g) mixture with sodium sulfate (180 g) was extracted with dichloromethane (1.8 L) in a Soxhlet apparatus for 8 h. Full sourdough (150 g; water content = 44%) was mixed with anhydrous Na_2SO_4 (180 g) in a commercial blender. The mixture was extracted with dichloromethane (1.8 L) under the same conditions.

The extracts obtained were each concentrated to 150 mL by distilling off the solvent at 45 °C using a Vigreux column. The volatiles were separated from the nonvolatile material by high-vacuum distillation as recently reported (*31*). The distillates obtained were treated three times with an aqueous Na_2CO_3 solution (0.5 mol/L; total volume = 200 mL). The organic layer containing the neutral/basic volatiles was washed with brine (total volume = 200 mL) and then dried over anhydrous Na_2SO_4. For the isolation of the acidic volatiles, the combined aqueous solutions were carefully adjusted to pH 2.5 with hydrochloric acid (2 mol/L) and extracted three times with diethyl ether (total volume = 200 mL). After a washing with brine (total volume = 200 mL), the organic layer was dried over anhydrous Na_2SO_4.

Both extracts were finally concentrated to 300 μL at 40 °C using a Vigreux column (50 cm × 1 cm) followed by microdistillation (*32*).

Column Chromatography. For the identification experiments, the neutral/basic fraction obtained from flour (1 kg) or a freshly prepared sourdough made therefrom (1 kg) was fractionated at 10−12 °C on a water-cooled column (30 × 1.6 cm) filled with a slurry of purified (*33*) silica gel 60 in *n*-pentane. Stepwise elution was performed by using *n*-pentane with increasing amounts of diethyl ether: fraction 0

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

no.[a]	odorant	ion (*m*/*z*)	int std[b]	ion (*m*/*z*)	capillary	calibrn factor[c]
I	2-methylpropanal	73	**I-1**	80	SE-54	1.30
1	3-methylbutanal	69	**d-1**	70–71	DB-Wax	0.71
2	2-methylbutanal	87	**d-1**	70–71	DB-Wax	1.90
3	2,3-butanedione[d]	159	**c-3**	163	SE-54	1.00
4	hexanal	83	**d-4**	85–87	SE-54	0.99
VII	3-methylbutanol	71	**d-VII**	73	FFAP	0.84
8	methional[g]	105	**d-8**	108	FFAP + DB-1701	1.03
10	(*E*)-2-nonenal[g]	141	**d-10**	143	SE-54	0.76
XII	phenylacetaldehyde	121	**c-XII**	123	SE-54	1.00
17	(*E*,*E*)-2,4-decadienal	153	**d-17**	156–158	SE-54	0.75
18	(*E*)-β-damascenone[g]	191	**d-18**	195–197	FFAP + SE-54	0.70
XV	2-phenylethanol	105	**d-XV**	107	FFAP	0.96
19	4,5-epoxy-(*E*)-2-decenal[f]	169	**d-19**	172–174	SE-54	0.84
12	butanoic acid	89	**d-12**	91–93	FFAP	1.00
13	2- and 3-methylbutanoic acid[e]	85	**d-13**	87–88	FFAP	0.73
21	3-hydroxy-4,5-dimethyl-2(5*H*)-furanone[g]	129	**c-21**	131	FFAP + DB-1701	1.00
25	2-phenylacetic acid	137	**c-25**	139	FFAP + DB-1701	1.00
26	vanillin	153	**d-26**	156	FFAP + DB-1701	1.10

[a] Numbering of the compounds refers to **Tables 2** and **4**. [b] Int std = internal standards used. For structures see **Figure 1**. [c] Calibration factors were determined in mixtures of equal amounts of unlabeled odorants and corresponding labeled standards (*26*). Measurements were done by MS/CI with methanol as the reactant gas. [d] Compound **3** was determined after derivatization with 1,2-benzenediamine as reported previously (*36*). [e] Sum of both isomers. The ratio of the 2- to 3-methylbutanoic acid was calculated from the ratio of the ions *m*/*z* 74 to *m*/*z* 60 (MS/EI). [f] Compounds was quantified by MS/CI using isobutane as the reactant gas. [g] Compound was determined using TDGC/MS for enrichment.

(100 mL of *n*-pentane); fraction 1 (70 mL; 95:5, v/v); fraction 2 (160 mL; 85:15, v/v); fraction 3 (140 mL; 7:3, v/v); fraction 4 (150 mL; 2:8, v/v). The fractions were each concentrated to 200 μL and then used for high-resolution gas chromatography−olfactometry in parallel with high-resolution mass spectrometry. The fraction containing the acidic volatile compounds was used without further separation.

High-Resolution Gas Chromatography—Olfactometry (HRGC-O). HRGC was performed using a type 5160 gas chromatograph (Carlo Erba, Hofheim, Germany) equipped with the following capillaries: FFAP [30 m × 0.32 mm i.d. fused silica capillary, free fatty acid phase (FFAP), 0.25 μm; J&W Scientific, Fisons Instruments, Mainz, Germany]; DB-5 (25 m × 0.32 mm i.d. fused silica capillary column, 0.25 μm; DB-5 equals silicone SE-54); DB-1701 (30 m × 32 mm fused silica capillary DB-OV-1701, 0.25 μm); and CP-Wax 52 CB (50 m × 0.32 mm i.d. fused silica capillary, 1.2 μm) were from Chrompack, Mühlheim, Germany. The samples were applied by the cold-on-column injection technique at 35 °C (DB-5, DB-1701) and 40 °C (FFAP), respectively. After 2 min, the temperature of the oven was raised at 40 °C/min to 50 °C (DB-5, DB-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 capillary, 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 of the carrier gas helium was 2.5 mL/min. For HRGC-O, the effluent was split 1:1 (by volume) into a sniffing port and a flame ionization detector (FID) at the end of the capillary, using deactivated but uncoated fused silica capillary columns (30 cm × 0.32 mm) (*17*). The FID and the sniffing port were held at 220 °C. Linear retention indices (RI) of the compounds were calculated in relation to *n*-alkanes (*17*).

Aroma Extract Dilution Analysis (AEDA). The flavor dilution (FD) factors of the odor-active compounds were determined by AEDA (*34*) of the following dilution series: The original extract (100 μL) containing the volatiles from either rye flour (83 g) or sourdough (150 g), respectively, was stepwise diluted with dichloromethane (1 + 1). HRGC-O was then performed with aliquots (0.5 μL) of the original extract and the dilutions using either capillary DB-5 (neutral/basic fraction) or capillary FFAP (acidic fraction). In the first experiment, five experienced panelists performed three GC-O runs on the original extract and on 1:10 and 1:100 dilutions and agreed upon the odor-active areas detected. Then, the three panelists who were able to detect the highest number of odor-active compounds separately performed GC-O runs on the serial dilutions. Training of the panelists on the aroma qualities was done prior to GC-O analysis using aqueous solutions of ~60 odorants to become familiar with an "aroma language", for example, hexanal for green or 3-methylbutanal for malty. The FD chromatogram was finally drawn as FD factor versus the respective retention index of an odorant based on the averaged FD factors.

Quantitation by Stable Isotope Dilution Assays (SIDA). Either the freshly prepared sourdough (diluted 1:1 with water, 2−100 g, depending on the approximate concentration of the analyte determined in preliminary experiments) or an aqueous suspension prepared from flour and water (4 + 6; 2−100 g) was spiked with known amounts of the 17 labeled internal standards (5−10 μg each, dissolved in ethanol), and the mixture was equilibrated for 30 min. In preliminary experiments it was shown that extending the equilibration time increased the concentrations of some odorants due to microbial activities; therefore, 30 min was chosen as a compromise between "optimum" equilibration and a "prolongation" of the fermentation time. However, reproducible results were obtained in triplicate (SD = ±5%).

Anhydrous Na_2SO_4 was then added and the powder extracted with diethyl ether for 8 h in a Soxhlet apparatus. The volatiles and the internal standards were isolated by high-vacuum distillation and then separated into the neutral/basic and the acidic volatiles as described above. HRGC−mass chromatography was performed using the MS response factors, selected ions, and capillaries summarized in **Table 1**.

Mass Spectrometry (MS). For identification, mass spectrometry (MS) was performed by means of an MAT-95 S high-resolution mass spectrometer (Finnigan, Bremen, Germany) in tandem with the capillaries FFAP and DB-5. Mass spectra in the electron impact mode (MS/EI) were generated at 70 eV and in the chemical ionization mode (MS/CI) at 115 eV with isobutane as the reagent gas.

Isotope dilution assays were performed using the ion trap detector ITD-800 (Finnigan) in the chemical ionization mode using methanol as the reagent gas as described previously (*31*). For two-dimensional GC−MS (TDGC-MS) a GC (Mega 2 series, Fisons, Mainz, Germany) equipped with a capillary FFAP was used in the first dimension. After the relevant part of the effluent containing the analyte and the internal standard had been trapped by means of a connected "dome"-switching technique (*35*), the cut was rechromatographed using a second GC (type 5160 Carlo Erba, Hofheim, Germany) equipped with either the DB-OV-1701 or the DB-5 capillary, respectively (**Table 1**). Measurement of the ion intensities of the analyte and the respective internal standard was done by means of the ion trap detector ITD-800 (Finnigan).

RESULTS AND DISCUSSION

Odor-Active Compounds in Rye Flour. The distillate of the flour volatiles isolated by Soxhlet extraction and high-

Table 2. Odor-Active Volatiles (FD ≥ 4) Identified in a Freshly Ground Rye Flour (Type 1150)

no.	odorant[a]	odor quality[b]	retention index on FFAP	retention index on DB-5	retention index on DB-1701	FD factor[c]	earlier reported as volatile constituent in rye flour
1/2	2- and 3-methylbutanal[d]	malty	945	652	732	4	*3*, *5*, *6*
3	2,3-butanedione[d]	buttery	1000	<600	700	4	
4	hexanal[d]	green	1082	800	880	64	*3*, *6*, *7*
5	octanal	fruity, soapy	1280	1000	1088	8	
6	1-octen-3-one	mushroom-like	1296	975	1067	128	
7	(*E*)-2-octenal	fatty, waxy	1419	1057	nd[f]	8	
8	methional[d]	cooked potato	1448	904	1040	128	
9	(*Z*)-2-nonenal	green, fatty	1496	1149	1260	32	
10	(*E*)-2-nonenal[d]	green, fatty	1527	1161	1279	128	
11	(*E*,*Z*)-2,6-nonadienal	cucumber-like	1577	1153	nd	16	
12	butanoic acid[e]	sweaty	1618	nd	nd	8	
13	2- and 3-methylbutanoic acid[e]	sweaty	1659	nd	nd	8	
14	(*E*,*E*)-2,4-nonadienal	fatty, waxy	1698	1211	nd	16	
15	pentanoic acid[e]	sweaty	1732	nd	nd	8	
16	(*E*,*Z*)-2,4-decadienal	green, fatty	1752	1287	nd	8	
17	(*E*,*E*)-2,4-decadienal[d]	fatty, waxy	1804	1317	1450	32	
18	(*E*)-β-damascenone[d]	boiled-apple-like	1806	1386	1497	4	
19	4,5-epoxy-(*E*)-2-decenal[d]	metallic	2006	1381	1558	32	
20	4-vinyl-2-methylphenol	clove-like	2194	1317	nd	4	
21	3-hydroxy-4,5-dimethyl-2(5*H*)-furanone[d,e]	seasoning-like	2196	nd	nd	64	
22	unknown[e]	metallic	2253	nd	nd	8	
23	3-hydroxy-5-ethyl-4-methyl-2(5*H*)-furanone[e]	spicy	2267	nd	nd	8	
24	unknown[e]	spicy	2346	nd	nd	16	
25	2-phenylacetic acid[e]	sweet, honey-like	2570	nd	nd	8	
26	vanillin[e]	vanilla-like	2581	nd	nd	8	

[a] Compound was identified by comparing it with the reference substance on the basis of the following criteria: mass spectra (MS/EI, MS/CI), retention index (RI) on the three stationary phases given in the table, 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. [d] Only the MS/CI could be obtained. [e] Evaluation was done in the fraction containing the acidic volatile constituents. [f] nd = not determined.

vacuum distillation from a freshly milled rye flour (type 1150 = 1150 mg of minerals/kg of flour) smelled green-fatty and cereal-like when an aliquot was sensorially evaluated on a strip of filter paper. The evaluation was done by a panel consisting of 10 persons (6 females, 4 males; aged 26−32 years). The flavor impression was rated identical to a solvent extract of flour prepared without vacuum distillation by 8 of the 10 panelists. This confirmed that the distillation process did not significantly change the set of odor-active compounds. It should be stressed that the extracted flour was odorless.

By application of the AEDA on the neutral/basic and the acidic volatiles in separate GC-O runs, a total of 26 odor-active regions were detected by five panelists in the original extract (**Table 2**). Sniffing of a series of dilutions (FD factor range 1−1024) by three panelists showed that among them, compounds **6** (mushroom-like), **8** (cooked potato-like), and **10** (green-fatty) exhibited the highest FD factors followed by compounds **4** (green) and **21** with a seasoning-like odor quality. The three panelists did not differ by more than two dilution steps in their judgment, and the averaged data obtained are displayed in **Table 2**.

In total 24 of the 26 odorants sensorially detected could be identified (**Table 2**). Twenty-one of these have previously not been described as flour constituents. Within the project (duration 2 years), the AEDA was performed on about six different batches of flour. The differences in the FD factors determined were within the experimental error of the AEDA procedure.

To get an impression on their potential contribution to the rye flour aroma, six compounds that appeared with high FD factors during AEDA were quantified (**Table 3**). Furthermore, their odor thresholds were determined in wheat starch because starch is the main matrix component of the flour.

Hexanal was present in quite high amounts in the milligrams per kilogram range (**Table 3**), whereas the concentrations of, for example, 4,5-epoxy-(*E*)-2-decenal or 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone were very low. A comparison of the quantitative data with the odor thresholds revealed, in particular, methional to be present ∼900-fold above its odor threshold, so this compound surely belongs to the key odorants in rye flour.

Table 3. Concentrations, Odor Thresholds in Starch, and Odor Activity Values (OAVs) of Several Odor-Active Compounds in the Rye Flour

odorant	concn (μg/kg of dry wt)	odor threshold[a] (μg/kg in starch)	OAV[b]
hexanal	3080	30	103
methional	242	0.27	896
(*E*)-2-nonenal	98	0.53	185
(*E*,*E*)-2,4-decadienal	80	2.7	30
3-hydroxy-4,5-dimethyl-2(5*H*)-furanone	1.8	2.1	<1
4,5-epoxy-(*E*)-2-decenal	0.5	0.19	2.6

[a] Threshold values according to ref *37*. [b] OAVs were calculated by dividing the concentrations of the odorants by their orthonasal odor thresholds.

Odor-Active Compounds in Rye Sourdough. Extraction of a sourdough prepared from the same batch of rye flour followed by high-vacuum distillation gave an essence with a sour-malty aroma. The sensory penal judged the extract to be very similar to the overall odor of the sourdough when the extract was evaluated on a strip of filter paper.

After separation of the distillate into the neutral/basic and acidic volatile compounds, both fractions were subjected to AEDA. In total, 35 odor-active regions were sensorially located (**Table 4**). Compounds that were not detected in the flour are assigned by Roman numerals; aroma compounds that were present in both flour and sourdough are assigned by the same numbers as used in the flour experiment (cf. **Table 2**).

The results of the identification experiments in combination with the FD factors (**Table 4**) revealed methional (**8**) and 2- and 3-methylbutanal (**1** and **2**, respectively), followed by 4,5-epoxy-(*E*)-2-decenal (**19**), vanillin (**26**), (*E*)-β-damascenone (**18**),

Table 4. Most Odor-Active, Neutral/Basic Volatiles (FD Factor ≥ 4) Identified in a Fresh Three-Stage Rye Sourdough

no.[a]	odorant[b]	odor quality[c]	retention index on FFAP	retention index on DB-5	retention index on DB-1701	FD factor[d]	earlier reported as volatile compound in rye or wheat sourdough
I	2-methylpropanal	malty	821	<600	nd[f]	8	*5*
II	unknown	solvent-like	900	nd	nd	8	
1/2	3- and 2-methylbutanal	malty	945	652	732	512	*5*, *9–11*, *38*, *39*
3	2,3-butanedione	buttery	1000	<600	700	64	*9–11*, *38–41*
III	ethyl 2-methylbutanoate	sweet, fruity	1042	845	907	16	
IV	ethyl 3-methylbutanoate	sweet, fruity	1062	855	909	16	
4	hexanal	green	1082	800	880	64	*9–11*, *38–41*
V	unknown	musty	1095	nd	nd	16	
VI	3-methylbutyl acetate	sweet, fruity	1115	878	nd	16	*9*, *40*, *41*
VII	3-methylbutanol	malty	1200	732	842	32	*5*, *9–11*, *38*, *39*, *41*
VIII	(*Z*)-4-heptenal	sweet, biscuit-like	1238	898	987	4	
5	octanal	fruity, soapy	1280	1000	1088	8	*38*
6	1-octen-3-one[e]	mushroom-like	1296	975	1067	64	
IX	2-acetyl-1-pyrroline[e]	roasty, popcorn-like	1330	922	1016	8	
X	dimethyl trisulfide	cabbage-like	1372	965	1034	4	
XI	acetic acid	sour pungent	1444	nd	nd	8	*8*, *38*, *39*, *42*
8	methional	boiled potato	1448	904	1040	1024	
9	(*Z*)-2-nonenal[e]	green, fatty	1496	1149	1260	8	
10	(*E*)-2-nonenal	green, fatty	1527	1161	1279	16	
12	butanoic acid	sweaty	1618	nd	nd	16	*41*, *42*
XII	phenylacetaldehyde	honey-like	1638	1042	1175	16	
13	2- and 3-methylbutanoic acid	sweaty	1659	nd	nd	16	*41*, *42*
15	pentanoic acid	sweaty	1732	nd	nd	16	*41*, *42*
17	(*E*,*E*)-2,4-decadienal	fatty, waxy	1804	1317	1450	16	
18	(*E*)-β-damascenone[e]	boiled-apple-like	1806	1386	1497	64	
XIII	unknown	sweet	1853	nd	nd	8	
XIV	unknown	sweet, metallic	1887	nd	nd	32	
XV	2-phenylethanol	flowery	1911	1110	1281	8	*9*
19	4,5-epoxy-(*E*)-2-decenal	metallic	2006	1381	1558	128	
XVI	γ-nonalactone	cocoa-like	2033	1362	1573	4	
21	3-hydroxy-4,5-dimethyl-2(5*H*)-furanone	spicy	2196	nd	nd	64	
22	unknown	metallic	2253	nd	nd	16	
23	3-hydroxy-5-ethyl-4-methyl-2(5)-furanone[e]	spicy	2267	nd	nd	8	
25	2-phenylacetic acid	sweet, honey-like	2570	nd	nd	8	
26	vanillin	vanilla-like	2581	nd	nd	128	

[a] Numbering refers to **Table 2**. [b] Compound was identified by comparing it with the reference substance on the basis of the following criteria: MS/EI and MS/CI, retention index (RI) on three stationary phases given in the table. Odor quality as well as odor intensity perceived at the sniffing port. [c] Odor quality perceived at the sniffing port. [d] Flavor dilution (FD) factor determined in extracts containing the neutral/basic sourdough volatiles. [e] Mass spectral data obtained did not allow an unequivocal identification. Identification is based on the remaining criteria given in footnote *b*. [f] nd = not determined.

1-octen-3-one (**6**), hexanal (**4**), and 2,3-butanedione (**3**; **Table 4**) with the highest FD factors. Further odorants suggested by AEDA as potential contributors to the sourdough aroma are listed in **Table 4**. Compared to the flour, in particular, the three esters **III**, **IV**, and **VI**, as well as 3-methylbutanol, acetic acid, phenylacetaldehyde, and phenylethanol, were increased, so that they exceeded their thresholds in air and were, consequently, detected by GC-O among the odor-active volatiles of the fermented sourdough.

The results suggested that many compounds already present in the flour also belong to the important odorants in the sourdough, for example, methional or (*E*,*E*)-2,4-decadienal.

Furthermore, a comparison with literature data on volatiles in wheat or rye sourdough showed that 18 odorants are reported here for the first time as sourdough constituents.

To evaluate their aroma contribution based on odor thresholds in a matrix, 18 of the odorants showing FD factors ≥16 were subsequently quantified in the sourdough. The results revealed that, among them, acetic acid, butanoic acid, and 3-methylbutanol were by far the most dominating volatiles, if compared on the basis of their concentrations (**Table 5**). Low concentrations in the micrograms per kilogram range were measured for (*E*)-β-damascenone and 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone.

Because the main ingredients in sourdough are starch and water, both ingredients can be suggested as a suitable matrix to study the odor thresholds of the odorants under investigation. Although it has to be stated that this is an approximation of the real situation, because the exact "binding" of aroma compounds by the matrix is not taken into account, no better models are available.

The calculation based on odor thresholds in starch (**Table 5**) revealed vanillin, 3-methylbutanoic acid, methional, butanoic acid, 2,3-butanedione, and acetic acid as potential contributors to the sourdough aroma because their concentrations exceeded their odor thresholds by factors of >100. On the basis of odor thresholds in water, in particular, 3-methylbutanal, (*E*,*E*)-2,4-decadienal, and (*E*)-β-damascenone are suggested as further flavor contributors, because their concentrations were by a factor of >100 higher than their odor thresholds.

Although these data do not yet allow a clear statement of which compounds have to be present in a sourdough yielding a superior bread flavor after baking, the compounds mentioned above are useful indicators, for example, to follow flavor production during sourdough fermentation.

The following experiments were, therefore performed to clearly indicate which portion of the flavor compounds present in the sourdough stem from the flour and which portion is formed during fermentation.

Changes Induced by Fermentation. To elucidate those odorants that are clearly changed in their concentrations during the dough fermentation, 20 aroma compounds were quantified

Table 5. Concentrations, Odor Thresholds, and Odor Activity Values (OAVs) of 18 Odor-Active Compounds Identified in Rye Sourdough

odorant	concn (μg/kg of dry wt)	odor threshold[a] (μg/kg of starch)	OAV[b] (starch)	odor threshold[a] (μg/L of water)	OAV[b] (water)
acetic acid	3140000	31140	101	22000	143
butanoic acid	21800	100	218	1000	22
3-methylbutanol	15200	nd[f]	nd	1000	15
vanillin	2790	4.6	607	25	112
3-methylbutanoic acid	2580	6	430	740	3.5
2-phenylethanol	2010	125	16	1000	2
2-methylbutanoic acid	939	24	39	540	1.7
3-methylbutanal	899	32	28	0.4	2248
2,3-butanedione	744	6.5	114	15	50
hexanal	503	30	17	10.5	48
2-methylbutanal	180	32	5.6	3.7	49
phenylacetaldehyde	170	28[c]	6	4	43
(*E*,*E*)-2,4-decadienal	78	2.7	29	0.2	390
methional	65	0.27	241	1.8	36
(*E*)-2-nonenal	40	0.53	76	0.8	50
4,5-epoxy-(*E*)-2-decenal	16	0.19	84	0.12	133
3-hydroxy-4,5-dimethyl-2(5*H*)-furanone	6.7	2.1	3.2	0.3[d]	22
(*E*)-β-damascenone	0.9	0.2	4.5	0.004[e]	225

[a] Threshold values according to ref *37*. [b] OAVs were calculated by dividing the concentrations of the odorants by their orthonasal odor thresholds. [c] Threshold value according to ref *43*. [d] Threshold value according to ref *44*. [e] Threshold value according to ref *45*. [f] nd = not determined.

Table 6. Comparison of the Concentrations of 20 Odor-Active Compounds Characterized in Rye Flour as well as in Rye Sourdough Made Therefrom

odorant	concn (μg/kg of dry wt)[a] in flour	concn (μg/kg of dry wt)[a] in sourdough
2-methylpropanal	15	20
3-methylbutanal	265	899
2-methylbutanal	205	180
2,3-butanedione	55	744
hexanal	3080	503
3-methylbutanol	76	15200
methional	242	65
(*E*)-2-nonenal	98	40
phenylacetaldehyde	121	170
(*E*,*E*)-2,4-decadienal	80	78
(*E*)-β-damascenone	<0.1	0.9
2-phenylethanol	207	2010
4,5-epoxy-(*E*)-2-decenal	0.5	16
acetic acid[b]	5.0×10^4	3.1×10^6
butanoic acid	5100	21800
3-methylbutanoic acid	920	2580
2-methylbutanoic acid	492	939
3-hydroxy-4,5-dimethyl-2(5*H*)-furanone	1.8	6.7
2-phenylacetic acid	2260	4990
vanillin	1270	2790

[a] Mean values of triplicates. The data differed to not more than ±5%. [b] Determination was carried out using a commercial enzyme kit (Boehringer, Mannheim, Germany).

by SIDAs in the rye flour and the sourdough made therefrom. The results of the quantitative experiments are contrasted in **Table 6**.

The largest increase in concentration was determined for 3-methylbutanol, which was increased by a factor of 200. Further significant changes were observed for acetic acid and 2,3-butanedione, which were by factors of 62 or 15, respectively, higher in the sourdough. In addition, 2-phenylethanol, 3-methylbutanal, butanoic acid, and 3-methylbutanoic acid were significantly higher after dough fermentation. On the other hand, some other odorants, for example, hexanal or (*E*)-2-nonenal, but also methional, were decreased during fermentation.

Depending on the source and/or the age of the flour used in commercial bread-making, the concentrations of the volatiles present may vary. To get some insight into the extent of such variations, selected volatiles were quantified in five different batches of rye flours (type 1150), which were purchased in local bakeries. The results of the quantitative studies showed (**Table 7**) that for some compounds the concentrations in the different flours varied significantly. Consequently, also in the sourdoughs the concentrations of some odorants, for example, 2-methylbutanal or butanoic acid, showed significant variations, indicating that the biosynthetic generation of flavor compounds also may vary significantly in different batches, even if the same starter culture is used as in our experiments.

Table 7. Range of Concentrations of 10 Selected Odorants Determined in Five Different Batches of Rye Flour and the Sourdoughs Made Therefrom

odorant	concn[a] (μg/kg of dry wt) rye flour	concn[a] (μg/kg of dry wt) sourdough
3-methylbutanal	265–509	405–2700
2-methylbutanal	205–385	180–503
2,3-butanedione	44–55	434–732
3-methylbutanol	65–135	7780–28000
methional	54–242	38–93
phenylacetaldehyde	121–357	130–288
2-phenylethanol	185–416	2010–9100
hexanal	3080[b]	503–749
butanoic acid	5100[b]	3400–21800
vanillin	1270[b]	2790–2940

[a] Each sample was analyzed in triplicate. These values differ to not more than ±5%. [b] Data obtained for only one batch of flour.

However, in each of the batches analyzed, compounds that are influenced by the fermentation procedure, for example, acetic acid, 3-methylbutanol, or 2,3-butanedione, were clearly identified by an increase compared to the amount present in the flour, whereas, for example, hexanal was always decreased. These data confirm that the first three mentioned compounds are metabolites of the lactic acid bacteria; however, a certain portion of the odorants is already present in the flour used for dough preparation.

In summary, the results show that significant portions of some of the flavor compounds present in rye sourdough and, finally, also in the bread itself stem from the flour used for the dough preparation, for example, 2-methylbutanal or phenylacetalde-

hyde. During fermentation biochemical reactions initiated by the starter cultures then increase several flavor compounds, for example, 2,3-butanedione, acetic acid, 3-methylbutanol, and, also, 3-methylbutanal. The latter compound is known as an enzymic degradation product of the amino acid leucine following the so-called Ehrlich mechanism (*22*). It is, however, interesting to note that other odorants, for example, methional and 2-methylbutanal, which could also be formed by the Ehrlich pathway from methionine or isoleucine, respectively, were decreased during fermentation.

Some aldehydes that are already present in the flour and undoubtedly stem from the peroxidation of flour lipids, for example, hexanal or (*E*)-2-nonenal, are clearly degraded during the fermentation process. Such reduction processes caused by microorganisms have also been observed in our previous studies on buttermilk aroma (*46*, *47*).

These data are the basis for further studies, for example, on the differences in sourdough aroma compounds caused by different starter cultures. However, as already shown for some bread crumb odorants (*48*), in addition to the starter cultures, flour has to be regarded as an important source of bread flavor compounds.

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