JOURNAL OF
AGRICULTURAL AND
FOOD CHEMISTRY



# Important Aroma Compounds in Freshly Ground Wholemeal and White Wheat Flour—Identification and Quantitative Changes during Sourdough Fermentation

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An investigation on the odor-active compounds of wholemeal (WWF) and white wheat flour (WF 550) by aroma extract dilution analysis (AEDA) and by quantitative studies using stable isotope dilution assays (SIDA) revealed a significant number of odor-active compounds, such as (*E*)-2-nonenal, (*E,Z*)- and (*E,E*)-2,4-decadienal, (*E*)-4,5-epoxy-(*E*)-2-decenal, 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone, and vanillin, with high odor activities in both wheat flours. The amounts and, consequently, the aroma potencies of vanillin, (*E,E*)-2,4-decadienal, and 3-(methylthio)propanal were much higher in the WWF than in the WF 550 samples. Fermentation of suspensions of both flours with lactic acid bacteria did not generate new odorants; however, many compounds, such as acetic acid or 3-methylbutanal, were increased, whereas aldehydes (formed from the degradation of unsaturated fatty acids) were decreased. Comparing the odorant concentrations present before and after fermentation gave evidence that the main influence of the microorganisms on sourdough aroma is to either enhance or decrease specific volatiles already present in the flour. A comparison with literature data indicated that most of these odorants are also important for the bread crumb aroma present after baking of the dough.

**KEYWORDS: Wheat flour; sourdough; fermentation; lactic acid bacteria; odorant; aroma extract dilution analysis; stable isotope dilution assay**

## INTRODUCTION

The characteristic aromas of bread crust and crumb have been the subject of numerous investigations, and >500 volatiles have already been reported in different kinds of bread (*1*). As previously reviewed (*2*, *3*), on the basis of the results of the application of GC/olfactometry and the odor activity value concept, the most important bread aroma compounds have been successfully identified among the bulk of non-odorous volatile compounds. As a result, the overall smell of particular samples (e.g., wheat bread crust) could be mimicked in a flavor recombination experiment using a mixture of reference odorants in exactly the concentrations as they occur in bread (*4*).

Many different kinds of breads are manufactured all over the world, and it is quite obvious that different aromas are generated depending on the recipe and the parameters of the fermentation and the baking process. In general, two main processes are used in dough-making: (i) the straight dough fermentation using mainly baker's yeast for dough leavening and (ii) a prefermentation or sourdough fermentation of the flour with lactic acid bacteria, which is then used in a straight dough procedure. Typical products of the latter process are French baguettes or the German sourdough rye breads.

It has been shown by overall sensory evaluations of breads that a prefermentation of wheat doughs generates a superior aroma compared with breads made by a straight dough process (*5*). Recent studies have revealed (*6*) that a significant increase in the concentrations of, in particular, 3-methylbutanol and 2-phenylethanol in the crumbs of breads made with preferments containing baker's yeast does significantly contribute to the superior aroma of breads containing higher amounts of the two yeast metabolites. Studies on the influence of lactic acid bacteria on the aroma of wheat bread also revealed a positive influence, in particular, on the crumb aroma, and clear differences were observed in the pattern of the volatiles formed (*7*, *8*). However, no attempts were made to correlate the amounts of single odorants with these aroma differences.

Results by Hansen and Hansen (*8*) indicated that the wheat flour might be an additional factor influencing the aroma. The authors confirmed the presence of the same volatiles in flours and bread crumbs and reported that their concentrations varied in straight-grade, low-grade, and wholemeal flour over a wide range. However, an estimation of the contribution of the wheat flour volatiles to the overall wheat crumb aroma has not been addressed in these studies.

Recent investigations on rye bread crumb aroma have clearly demonstrated that rye flour already contains a significant number of odor-active compounds which, on the basis of their odor activity values (ratio of concentration to odor threshold), also clearly contributed to the crumb aroma of rye bread (*9*, *10*). The purpose of the present investigation was, therefore (i) to

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10.1021/jf020638p CCC: $22.00 
Published on Web 10/12/2002

**Table 1.** Analytical Parameters Used in the Stable Isotope Dilution Assays

| no.[a] | compound | eluent range[b] | selected ion of analyte (*m*/*z*) | int std[c] | selected ion of int std (*m*/*z*) | calibration factor[d] |
|---|---|---|---|---|---|---|
| **1** | hexanal[e] | 1050–1090 | 83 | **[$^2H_2$]-1** | 85[g] | 0.93 |
| **2** | (*Z*)-4-heptenal[e] | 1210–1250 | 95 | **[$^2H_2$]-2** | 97 | 0.95 |
| **3** | methional[e] | 1415–1455 | 105 | **[$^2H_2$]-3** | 108 | 0.97 |
| **5** | (*E*)-2-nonenal[e] | 1500–1530 | 141 | **[$^2H_2$]-5** | 143 | 0.94 |
| **6** | (*E*,*Z*)-2,6-nonadienal[e] | 1545–1585 | 121 | **[$^2H_2$]-6** | 123 | 0.99 |
| **7** | butanoic acid[e] | | 89 | **[$^2H_2$]-7** | 91 | 0.95 |
| **8** | (*E*,*Z*)-2,4-nonadienal[e] | 1620–1700 | 139 | **[$^2H_2$]-10**[h] | 141 | 0.85 |
| **9** | 2- and 3-methylbutanoic acid[f] | | 103 | **[$^2H_2$]-9** | 105 | 0.91 |
| **10** | (*E*,*E*)-2,4-nonadienal[e] | 1620–1700 | 139 | **[$^2H_2$]-10** | 141 | 0.95 |
| **11** | pentanoic acid[f] | | 103 | **[$^2H_2$]-9**[h] | 105 | 1.30 |
| **12** | (*E*,*Z*)-2,4-decadienal[e] | 1730–1810 | 153 | **[$^2H_{3-5}$]-14**[h] | 156–158[g] | 0.82 |
| **13** | (*E*,*Z*)-2,6-nonadienol[e] | 1730–1810 | 123 | **[$^2H_2$]-13** | 125 | 1.01 |
| **14** | (*E*,*E*)-2,4-decadienal[e] | 1730–1810 | 153 | **[$^2H_{3-5}$]-14** | 156–158[g] | 0.90 |
| **21** | 3-hydoxy-4,5-dimethyl-2(5*H*)-furanone[e] | 2170–2200 | 129 | **[$^{13}C_2$]-21** | 131 | 1.00 |
| **23** | phenylacetic acid[f] | | 137 | **[$^{13}C_2$]-23** | 139 | 1.00 |
| **24** | vanillin[e] | 2530–2580 | 137 | **[$^2H_3$]-24** | 156 | 0.98 |
| **25** | 3-methylbutanal[e] | 950–1000 | 69 | **[$^2H_2$]-25** | 70–71[g] | 0.70 |
| **26** | 2-methylbutanal[e] | 950–1000 | 87 | **[$^2H_2$]-25**[h] | 70–71[g] | 1.35 |
| **29** | acetic acid[f] | | 61 | **[$^2H_3$]-29** | 64 | 0.89 |
| **30** | phenylacetaldehyde[e] | 1605–1645 | 121 | **[$^{13}C_2$]-30** | 123 | 1.00 |

[a] Numbering corresponds to **Tables 2** and **4**. [b] Retention index range (relative to *n*-alkanes) on the precolumn DB-FFAP, which was transferred onto the main column DB-5. [c] Isotopic labeling of the internal standard. [d] Calibration factors obtained by analyzing a 1+1 mixture (w/w) of the analyte and the internal standard. [e] The compound was quantified in the neutral–basic fraction using TD/HRGC/MS. [f] The compound was quantified in the acidic fraction using HRGC/MS. [g] The sum of the relative abundances of the ions was calculated. [h] The compound was quantified using the relative abundance of the respective labeled isomer.

characterize and quantify the key odorants in wholemeal and white wheat flour and (ii) to reveal changes in flour odorants occurring during wheat sourdough fermentation with lactic acid bacteria.

## MATERIALS AND METHODS

**Flour.** Wholemeal flour (WWF) and white wheat flour (WF, type 550) were obtained by grinding wheat kernels of the same batch (Ulmer Spatz, Neu-Ulm, Germany).

**Sourdough Starter.** A commercial sourdough starter was used (Böcker, Minden, Germany), which contained predominantly lactic acid bacteria.

**Sourdough Preparation.** Flour (200 g), tap water (130 g), and the commercial starter (20 g) were mixed by kneading and stored for 24 h at 28 °C in a box (relative humidity = 80%).

**Sensory Evaluation.** All sensory experiments were performed at 21 ± 1 °C in a room equipped with single booths. The sensory panel consisted of 10 experienced assessors, aged 27−40 years, 6 women and 4 men. Wholemeal samples (flour or sourdough, 10 g) were placed in glass beakers (vol = 45 mL, i.d. = 40 mm) and were presented in triangular tests in parallel with two samples of wheat flour type 550. Panelists were asked to mark the sample differing from the two others in the overall aroma. The correct answers were summed, and the significance of the result was statistically checked according to the method of Jellinek (*11*). In a second experiment, the assessors were asked to judge differences in the intensities of five odor qualities between the two flour or sourdough samples, respectively. The panel was trained using the following aqueous solutions of pure odorants (∼20 times above odor threshold; the respective odor quality is given in parentheses): acetic acid (acetic, sour), 3-methylbutanoic acid (sweaty), (*E*,*E*)-2,4-decadienal (fatty), 3-methylbutanal (malty), and 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (seasoning-like).

**Chemicals.** Reference odorants of the aroma compounds **1**−**3**, **5**−**7**, **9**−**11**, **14**, **15**, **18**, **20**, **21**, **23**−**27**, **29**, **30**, **33**, and **34** (cf. **Table 1**) and the isotopically labeled standards **[$^{13}C_2$]-23** and **[$^2H_3$]-29** (cf. **Table 1**) were from Aldrich (Steinheim, Germany). Compound **35** was from Lancaster (Mühlheim/Main, Germany). The following reference compounds and isotopically labeled internal standards (cf. **Table 1**) were synthesized according to the literature given in parentheses: no. **4** (*12*); no. **8** and **12** (*13*); no. **13** (*14*); no. **16** (*15*); **[$^2H_{2-4}$]-1**, **[$^2H_2$]-5**, **[$^2H_2$]-6**, and **[$^2H_2$]-14** (*16*); **[$^2H_2$]-2** (*17*); **[$^2H_2$]-3** (*18*); **[$^2H_2$]-7** (*19*); **[$^2H_2$]-9** (*20*); **[$^2H_2$]-10** (*21*); **[$^2H_2$]-13** (*22*); **[$^{13}C_2$]-21** (*23*); **[$^2H_2$]-24** (*24*); **[$^2H_2$]-25** (*25*); and **[$^{13}C_2$]-30** (*26*).

**Isolation of Volatiles.** A suspension of flour (130 g) and tap water (70 mL) or the respective sourdough (200 g) was immediately frozen with liquid nitrogen. The samples were homogenized with anhydrous sodium sulfate (200 g) in a commercial blender (Moulinette, Quelle, Nürnberg, Germany), and the powder was extracted with dichloromethane (1 L) for 8 h at 40 °C using a Soxhlet extractor. The extract was concentrated on a Vigreux column (40 × 1 cm) to ∼100 mL by distilling off the solvent, and the volatiles were then isolated by solvent-assisted flavor evaporation (SAFE) distillation (*27*). The distillate was extracted with an aqueous sodium carbonate solution (0.5 mol/L, 3 × 50 mL) to remove acidic volatiles. The aqueous solution was washed with dichloromethane (50 mL), and the organic phases were combined, dried over anhydrous sodium sulfate, filtered, and concentrated to ∼0.1 mL by distilling off the solvent on a Vigreux column (40 cm × 1 cm) followed by microdistillation (neutral−basic fraction).

The alkaline aqueous phase obtained above (containing the acidic volatiles) was adjusted to pH 1 with hydrochloric acid (1 mol/L) and extracted three times with dichloromethane (total volume = 150 mL). The organic phase was dried over anhydrous sodium sulfate, filtered, and concentrated to ∼0.1 mL by distilling off the solvent at a Vigreux column (40 × 1 cm) and subsequently by microdistillation (*32*) (acidic fraction).

**Aroma Extract Dilution Analysis (AEDA).** The neutral−basic and the acidic fractions were stepwise diluted with dichloromethane (1+1, v/v), and each dilution was analyzed by high-resolution gas chromatography/olfactometry (HRGC/O) to give the flavor dilution (FD) factors of odor-active compounds (*10*, *28*).

**High-Resolution Gas Chromatography/Olfactometry.** HRGC was performed (Carlo Erba 5160, Hofheim, Germany) using the fused silica capillaries DB-5 and DB-FFAP (each 30 m × 0.32 mm, 0.25 μm film thickness) (J&W Scientific, Folsom, CA). The flow rate of the carrier gas helium was 2 mL/min. The samples were applied at 40 °C by the cold on-column technique. After 2 min, the temperature was raised at a rate of 6 °C/min to 230 °C and held for 10 min. The eluate was split at the ends of the capillaries (1:1 by volume) into a flame ionization detector (FID) and a sniffing port (*29*).

**High-Resolution Gas Chromatography/Mass Spectrometry (HRGC/MS).** MS analyses were performed with an MAT-95S mass spectrometer (Finnigan, Bremen, Germany) in tandem with the capillaries described above. Mass spectra were generated in the electron

**Table 2.** Odor-Active Compounds (FD ≥ 32) Identified in Extracts of Wholemeal Wheat Flour (WWF) and White Wheat Flour (Type 550; WF 550)

| no. | compound[a] | odor quality | RI[b] | | FD[c] in | |
|---|---|---|---|---|---|---|
| | | | FFAP | DB-5 | WF 550 | WWF |
| **1** | hexanal | green, grassy | 1071 | 800 | 64 | 64 |
| **2** | (*Z*)-4-heptenal | biscuit-like, putrid | 1230 | 900 | 32 | 64 |
| **3** | 3-(methylthio)propanal | cooked-potato-like | 1436 | 903 | 32 | 64 |
| **4** | (*Z*)-2-nonenal[d] | fatty, tallowy | 1490 | 1151 | 64 | 256 |
| **5** | (*E*)-2-nonenal | fatty | 1516 | 1161 | 256 | 256 |
| **6** | (*E*,*Z*)-2,6-nonadienal | cucumber-like | 1566 | 1154 | 64 | 256 |
| **7** | butanoic acid | sweaty | 1612 | | 64 | 64 |
| **8** | (*E*,*Z*)-2,4-nonadienal[d] | fatty | 1643 | 1196 | 16 | 32 |
| **9** | 2- and 3-methylbutanoic acid | sweaty | 1652 | | 64 | 256 |
| **10** | (*E*,*E*)-2,4-nonadienal | deep fat fried | 1682 | 1216 | 64 | 32 |
| **11** | pentanoic acid | sweaty | 1726 | | 32 | 64 |
| **12** | (*E*,*Z*)-2,4-decadienal | fatty | 1749 | 1298 | 64 | 1024 |
| **13** | (*E*,*Z*)-2,6-nonadienol | cucumber-like | 1754 | 1168 | 8 | 32 |
| **14** | (*E*,*E*)-2,4-decadienal | deep fat fried | 1792 | 1320 | 64 | 1024 |
| **15** | hexanoic acid | sweaty | 1832 | | 64 | 64 |
| **16** | unknown | tea-like | 1863 | 1263 | 64 | 64 |
| **17** | (*E*)-4,5-epoxy-(*E*)-2-decenal | metallic | 1994 | 1382 | 256 | 1024 |
| **18** | $\gamma$-nonalacton | coconut-like | 2018 | 1365 | 64 | 64 |
| **19** | unknown | coconut-like | 2065 | 1363 | 64 | 64 |
| **20** | 4-methylphenol | faecal | 2071 | 1078 | 64 | 16 |
| **21** | 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone | spicy | 2186 | 1110 | 256 | 256 |
| **22** | unknown | coconut-like | 2218 | 1458 | 64 | 8 |
| **23** | phenylacetic acid | honey-like | 2548 | | 64 | 256 |
| **24** | vanillin | vanilla-like | 2555 | 1410 | 1024 | 4096 |

[a] The compound was identified by comparing the mass spectra (MS/EI; MS/CI), the retention indices on capillary DB-FFAP and DB-5, and the odor quality perceived during sniffing with the data obtained for the reference compound. [b] Retention index (RI) on the capillaries DB-FFAP and DB-5. [c] Flavor dilution (FD) factor determined by AEDA; compounds with an FD ≥ 32 in at least one of both samples are listed. [d] The MS signal was too weak for an unequivocal interpretation. The compound was tentatively identified on the basis of the remaining criteria given in footnote *a*.

impact mode (MS/EI) at 70 eV. MS analyses in the chemical ionization mode (MS/CI) with methanol as reagent gas were carried out by means of the Saturn 2000 mass spectrometer (Varian, Darmstadt, Germany) using the GC columns described above.

**Two-Dimensional High-Resolution Gas Chromatography/Mass Spectrometry (TD/HRGC/MS).** TD/HRGC/MS was performed by using the moving column stream switching (MCSS) system (ThermoQuest Analytical Systems, Egelsbach, Germany) (*29*) with the following modifications: The end of the main column was split into the mass spectrometer and a sniffing port, at which the odorant to be analyzed was detected by sniffing. After transferring the respective odorant onto the analytical column, mass spectra were recorded in the MS/EI and MS/CI modes.

**Quantification of Odorants.** Either flour, the sourdough starter, or the final sourdough (50 g each) was spiked with known amounts of the 15 labeled internal standards (**Table 1**) at 1−200 $\mu$g (depending on the amount of the analyte present). After 15 min of equilibration, the volatiles were extracted from the samples as described above. The neutral−basic fraction was analyzed by TD/HRGC/MS, and the acidic fraction was analyzed by HRGC/MS in the MS/CI mode. The concentrations were calculated from the relative abundances of ions selected for the analyte and the internal standard, and the result was corrected by means of a calibration factor determined in mixtures containing known amounts of the respective standard and the analyte (*10*).

## RESULTS

**Key Odorants in Wheat Flour.** In a preliminary sensory experiment, the overall aromas of freshly ground white wheat flour (type 550; WF 550) and wholemeal wheat flour (WWF) were compared. A significant difference ($\alpha < 0.001$) was found in a triangular test, because 9 of 10 panelists were able to clearly discern the WWF from the two WF 550 samples. Some panelists were also able to specify their results by judging the WWF to be more intense in certain odor qualities.

To identify the most potent odorants, the volatile fractions from both samples were isolated, and the extracts obtained were screened by means of aroma extract dilution analyses (AEDA). A total of 24 odor-active areas were detected in both samples in the FD factor range of 32−4096 (**Table 2**). The results of the identification experiments in combination with the FD factors revealed vanillin (**24**) followed by 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (**21**), 4,5-epoxy-(*E*)-2-decenal (**17**), and (*E*)-2-nonenal (**5**) as the most odor-active among the 21 aroma compounds characterized in the WF 550 flour.

The same aroma compounds were also ranked with high FD factors in the wholemeal flour, but another six compounds, namely, (*Z*)-2-nonenal (**4**), (*E*,*Z*)-2,6-nonadienol (**13**), 2- and 3-methylbutanoic acid (**9**), (*E*,*Z*)-2,4-decadienal (**12**), (*E*,*E*)-2,4-decadienal (**14**), and phenylacetic acid (**23**), were identified as additional flavor-active compounds in the WWF. With the exception of hexanal (**1**), each odorant listed in **Table 2** is reported here for the first time as a wheat flour constituent.

In general, the results suggest that in the wholemeal flour compounds derived from lipid peroxidation, such as (*E*,*Z*)- and (*E*,*E*)-2,4-decadienal, are significantly higher. To check this assumption, 20 odorants were quantified by stable isotope dilution assays (SIDA) in both flours. The analyses confirmed the fatty-smelling odorants (*E*,*Z*)- and (*E*,*E*)-2,4-decadienal, (*E*)-2-nonenal, (*E*,*Z*)-2,6-nonadienal, and (*E*,*Z*)-2,6-nonadienol to be present in WWF in 3−5-fold higher amounts (**Table 3**). Higher concentrations were also determined for 2- and 3-methylbutanal, 2- and 3-methylbutanoic acid, phenylacetaldehyde, phenylacetic acid, 3-(methylthio)propanal, acetic acid, pentanoic acid, and vanillin. On the other hand, 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone was much lower in WWF compared to WF 550.

The data clearly show that flours already contain large amounts of odorants that have previously been identified as important contributors to the aroma of wheat bread crumbs (*2*, *3*). Consequently, it can be concluded that wheat flours are an important source of bread odorants and, therefore, the choice

**Table 3.** Concentrations (Micrograms per Kilogram of Dry Weight) of Important Odorants in White Wheat Flour (Type 550; WF 550) and Wholemeal Wheat Flour (WWF)

| compound | flour | |
|---|---|---|
| | WF 550 | WWF |
| 3-methylbutanal | 97 | 153 |
| 2-methylbutanal | 30 | 74 |
| 2- and 3-methylbutanoic acid | 342 | 629 |
| phenylacetaldehyde | 183 | 508 |
| phenylacetic acid | 142 | 418 |
| 3-(methylthio)propanal | 25 | 127 |
| hexanal | 11000 | 11200 |
| (*Z*)-4-heptenal | 14 | 20 |
| (*E*)-2-nonenal | 86 | 262 |
| (*E*,*Z*)-2,6-nonadienal | 22 | 65 |
| (*E*,*Z*)-2,6-nonadienol | <2 | 12 |
| (*E*,*Z*)-2,4-nonadienal | 11 | 18 |
| (*E*,*E*)-2,4-nonadienal | 33 | 64 |
| (*E*,*Z*)-2,4-decadienal | 389 | 1810 |
| (*E*,*E*)-2,4-decadienal | 355 | 1690 |
| acetic acid | 134000 | 218000 |
| butanoic acid | 5900 | 6980 |
| pentanoic acid | 6900 | 11600 |
| 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone | 86 | 21 |
| vanillin | 583 | 2910 |

of the flour type should clearly influence the aroma quality of wheat breads.

**Odorants in Wheat Sourdough.** For the production of French breads, wheat sourdoughs are manufactured by an overnight fermentation with lactic acid bacteria. To gain an insight into the influence of the fermentation on changes in flour odorants, WWF and WF 550 were separately fermented with the same starter culture. An overall sensory evaluation of the two fermented doughs (WWF-F and WF 550-F) using the triangular test revealed a significant difference ($\alpha < 0.001$; 9 of 10 assessors were able to detect the differing sample). The WF 500-F was judged by eight panelists to be less sour than WWF-F, and the malty and sweaty impression was also rated as more intense in WWF-F.

Application of a comparative AEDA (*28*) on both sourdoughs revealed a total number of 37 odor-active compounds with FD factors ≥32 in the wholemeal sourdough (**Table 4**). The highest FD factors were determined for the following seven aroma compounds: 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (**21**; seasoning-like) and 4,5-epoxy-(*E*)-2-decenal (**17**; metallic) followed by 3-(methylthio)propanal (**3**; cooked potato), (*E*,*Z*)-2,6-nonadienal (**6**; cucumber-like), (*E*,*Z*)-2,6-nonadienol (**13**; cucumber-like), vanillin (**24**; vanilla-like), and an unknown compound (**16**) with a tea-like aroma.

In the sourdough made from white wheat flour (WF 550-F), the highest FD factors were found for (*E*)-4,5-epoxy-(*E*)-2-decenal (metallic), 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (seasoning-like), phenylacetic acid (honey-like), and vanillin (vanilla-like). Although nearly all aroma compounds detected in WWF-F were also present in WF 550-F, the FD factors were generally much lower (**Table 4**). This is probably due to the fact that the WF 500 is lower in odorant precursors, which are converted into aroma compounds by the lactic acid bacteria.

Of the 26 odor-active compounds identified, only six, namely, hexanal, acetic acid, butanoic acid, 2- and 3-methylbutanoic acid, and pentanoic acid, have earlier been reported as wheat sourdough constituents (*8*, *30*−*32*).

To reveal the influence of the fermentation process on changes in the amounts of flour volatiles, 20 compounds were quantified by SIDA in the WWF-F and the WF 550-F sourdough. As expected from the FD factors, the concentration of almost every odorant was higher in WWF-F (**Table 5**). One of the clearest differences was that the wholemeal dough contained much more of the fatty-smelling odorants (*E*)-2-nonenal and (*E,Z*)- and (*E,E*)-2,4-decadienal as well as (*E*,*Z*)-2,6-nonadienal and its corresponding alcohol.

In total, the results clearly show that the differences found for the two flours are still present after fermentation of the corresponding sourdoughs. The stronger sour impression in WWF-F can be attributed to double the amount of acetic acid in this sample. Furthermore, the higher concentrations of 2- and 3-methylbutanal and 2- and 3-methylbutanoic acid, respectively, in the wholemeal sourdough are well correlated with the stronger malty and sweaty impressions in this sample.

**Changes Induced by Fermentation.** On the basis of the data obtained and the observations in the literature indicating that a sourdough fermentation improves the aroma quality of wheat breads (*5*), it can be assumed that the microorganisms influence the aroma (i) by either enhancing desired or eliminating undesired odorants present in the flour and (ii) by liberating aroma precursors that generate positive odorants during baking.

Because sourdough contains ∼40% water, the quantitative data given for the flour (**Table 3**) and the respective sourdough (**Table 5**) are not directly comparable. However, the results indicate that the lactic acid bacteria do not produce new flavor compounds, but obviously influence the amounts of the volatiles present in the flour in different ways. For example, aldehydes, undoubtedly generated from lipid peroxidation in the flour, were reduced, whereas some Strecker aldehydes (e.g., 3-methylbutanal) were increased.

To gain a deeper insight into the abilities of lactic acid bacteria to metabolize aroma compounds present in the flour, the odorants were also quantified in the starter culture to obtain data on odorant concentrations in all dough ingredients before fermentation. For an easier comparison, amounts per 200 g of flour or per 20 g of starter were calculated (**Table 6**) and compared to the amounts measured in the final sourdough WWF-F (350 g).

The lactic acid bacteria significantly influenced the amounts of almost every odorant investigated (**Table 6**). In particular, acetic acid, butanoic acid, phenylacetic acid, 2- and 3-methylbutanoic acid, and pentanoic acid were drastically increased during dough processing. As shown in our previous study on preferments containing baker's yeast, an amino acid degradation by the Ehrlich mechanism leading to odor-active aldehydes is an important flavor-forming reaction catalyzed by yeast. However, in the sourdough a different situation was observed, because the leucine metabolite 3-methylbutanal was increased, whereas the methionine metabolite 3-(methylthio)propanal was degraded to ∼50%.

Furthermore, the microorganisms decreased the amounts of unsaturated aldehydes. In WWF-F, the decrease of (*E*)-2-nonenal and (*E*,*Z*)-2,6-nonadienal amounted to ∼70%. About 85% of (*Z*)-4-heptenal and (*E*,*E*)- and (*E*,*Z*)-2,4-decadienal was removed during fermentation.

## DISCUSSION

A comparison of the key odorants identified in wheat flour with our previous results on rye flour odorants (*9*) indicated significant differences in the qualitative and quantitative compositions of odor-active compounds between the two cereals. For example, compounds **10**, **13**, **15**, **18**, and **20** were not detected as contributors to the aroma of rye flour, whereas 2,3-butanedione, octanal, (*E*)-2-octenal, 4-vinyl-2-methoxyphenol,

**Table 4.** Odor-Active Compounds (FD ≥ 64) in Wholemeal Sourdough (WWF-F) and Sourdough Made from White Wheat Flour (WF 550-F)

| | | | RI[b] | | FD[c] | |
|---|---|---|---|---|---|---|
| no. | compound[a] | odor quality | FFAP | DB-5 | WF 550-F | WWF-F |
| **25** | 3-methylbutanal | malty | <1000 | 646 | 1 | 64 |
| **26** | 2-methylbutanal | malty | <1000 | 654 | 1 | 64 |
| **1** | hexanal | green, grassy | 1071 | 800 | 8 | 64 |
| **27** | hexanoic acid ethyl ester | fruity | 1225 | 858 | 64 | 16 |
| **2** | (*Z*)-4-heptenal | biscuit-like, putrid | 1230 | 900 | 4 | 256 |
| **28** | unknown | fatty | 1384 | | 1 | 64 |
| **3** | 3-(methylthio)propanal | cooked-potato-like | 1436 | 903 | 16 | 1024 |
| **29** | acetic acid | acetic-like, sour | 1443 | | 8 | 64 |
| **4** | (*Z*)-2-nonenal[d] | fatty | 1490 | 1151 | 64 | 512 |
| **5** | (*E*)-2-nonenal | fatty | 1516 | 1161 | 64 | 512 |
| **6** | (*E*,*Z*)-2,6-nonadienal | cucumber-like | 1566 | 1154 | 64 | 1024 |
| **7** | butanoic acid | sweaty | 1612 | | 64 | 64 |
| **30** | phenylacetaldehyde | honey-like | 1624 | 1045 | 4 | 256 |
| **8** | (*E*,*Z*)-2,4-nonadienal | fatty | 1643 | 1196 | 16 | 64 |
| **9** | 2- and 3-methylbutanoic acid | sweaty | 1652 | | 16 | 256 |
| **31** | unknown | fatty | 1686 | | 16 | 256 |
| **10** | (*E*,*E*)-2,4-nonadienal | deep fat fried | 1682 | 1216 | 32 | 512 |
| **32** | unknown | minty | 1714 | 1267 | 16 | 64 |
| **11** | pentanoic acid | sweaty | 1726 | | 16 | 64 |
| **12** | (*E*,*Z*)-2,4-decadienal | fatty | 1749 | 1298 | 8 | 64 |
| **13** | (*E*,*Z*)-2,6-nonadienol | cucumber-like | 1754 | 1168 | 64 | 1024 |
| **14** | (*E*,*E*)-2,4-decadienal | deep fat fried | 1792 | 1320 | 16 | 512 |
| **16** | unknown | tea-like | 1862 | 1274 | 16 | 1024 |
| **17** | *trans*-4,5-epoxy-(*E*)-2-decenal | metallic | 1994 | 1382 | 256 | 4096 |
| **18** | $\gamma$-nonalactone | coconut-like | 2020 | 1368 | 64 | 512 |
| **33** | octanoic acid | sweaty | 2046 | | 8 | 64 |
| **34** | 3-ethylphenol | musty | 2171 | 1172 | 16 | 256 |
| **35** | 4-vinyl-3-methoxyphenol | clove-like | 2177 | 1322 | 16 | 512 |
| **21** | 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone | spicy | 2186 | 1110 | 256 | 4096 |
| **22** | unknown | coconut-like | 2218 | 1458 | 64 | 64 |
| **36** | unknown | citrus-like | 2313 | | 16 | 64 |
| **37** | unknown | coconut-like | 2338 | | 16 | 64 |
| **38** | unknown | coconut-like | 2455 | | 16 | 64 |
| **23** | phenylacetic acid | honey-like | 2548 | | 256 | 256 |
| **24** | vanillin | vanilla-like | 2555 | 1410 | 256 | 1024 |

[a] Numbering refers to **Table 2**. [b] The compounds were identified by comparing the mass spectra (MS/EI; MS/CI), the RI on capillary DB-FFAP and DB-5, and the odor quality perceived during sniffing with data obtained for the reference compound. [c] Retention index (RI) on the capillaries DB-FFAP and DB-5. [d] Flavor dilution (FD) factor determined by AEDA; compounds with an FD ≥ 32 in at least one of both samples are listed. [e] The MS signal was too weak for an unequivocal interpretation. The compound was identified on the basis of the remaining criteria given in footnote *a*.

**Table 5.** Concentrations (Micrograms per Kilogram of Wet Weight)[a] of Important Odorants in Sourdoughs Made from Wholemeal Wheat Flour (WWF-F) and White Wheat Flour (WF 550-F)

| compound | WF 550-F | WWF-F |
|---|---|---|
| 3-methylbutanal | 105 | 452 |
| 2-methylbutanal | 54 | 96 |
| 2- and 3-methylbutanoic acid | 524 | 1030 |
| phenylacetaldehyde | 144 | 325 |
| phenylacetic acid | 327 | 852 |
| 3-(methylthio)propanal | 12 | 41 |
| hexanal | 5600 | 5900 |
| (*Z*)-4-heptenal | 2.9 | 2.4 |
| (*E*)-2-nonenal | 30 | 56 |
| (*E*,*Z*)-2,6-nonadienal | 5.3 | 11 |
| (*E*,*Z*)-2,6-nonadienol | 6.4 | 22 |
| (*E*,*Z*)-2,4-nonadienal | 4 | 2.8 |
| (*E*,*E*)-2,4-nonadienal | 13 | 16 |
| (*E*,*Z*)-2,4-decadienal | 65 | 148 |
| (*E*,*E*)-2,4-decadienal | 82 | 136 |
| acetic acid | 601000 | 1220000 |
| butanoic acid | 13900 | 13400 |
| pentanoic acid | 12100 | 13700 |
| 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone | 1 | 2.4 |
| vanillin | 265 | 823 |

[a] The sourdough contained 40% water.

**Table 6.** Comparison of the Amounts of 14 Odorants in Wholemeal Flour, the Sourdough Starter, and the Fermented Sourdough

| | amount ($\mu$g) | | amount in sourdough ($\mu$g) | |
|---|---|---|---|---|
| compound | flour[a] | starter[b] | calcd[c] | measd[d] |
| 3-methylbutanal | 31 | 20 | 51 | 158 |
| 2-methylbutanal | 15 | 5.4 | 20 | 33 |
| 2- and 3-methylbutanoic acid | 126 | 12 | 138 | 361 |
| 3-(methylthio)propanal | 25 | 2.2 | 27 | 14 |
| hexanal | 2240 | 14 | 2254 | 2070 |
| (*Z*)-4-heptenal | 4 | 0.1 | 4.1 | 0.8 |
| (*E*)-2-nonenal | 52 | 1 | 53 | 20 |
| (*E*,*Z*)-2,6-nonadienal | 13 | 0.2 | 13 | 3.8 |
| (E,*Z*)-2,4-decadienal | 362 | 0.5 | 363 | 52 |
| (*E*,*E*)-2,4-decadienal | 338 | 1 | 339 | 48 |
| acetic acid | 43600 | 5400 | 49000 | 427000 |
| butanoic acid | 1400 | 110 | 1510 | 4690 |
| pentanoic acid | 2320 | 120 | 2440 | 4800 |
| vanillin | 582 | 10 | 592 | 288 |

[a] Amount in 200 g of flour. [b] Amount in 20 g of sourdough starter. [c] Calculated amount present before fermentation of a mix of 200 g of flour, 20 g of starter culture, and water (130 mL). [d] Amount determined in 350 g of fermented sourdough.

and 3-hydroxy-5-ethyl-4-methyl-2(5*H*)-furanone were characterized in rye flour but were sensorially not detected in wheat flour (**Table 2**). Furthermore, clear differences in the FD factors and concentrations of some odorants were also measured. Therefore, the set of odorants already present in the flour has to be regarded as one cause for flavor differences between different types of bread, which has not really been addressed in the literature.

A comparison with our recent investigation on odorant concentrations in rye sourdough using the same starter culture (*9*) showed nearly identical formation and degradation rates for 2-/3-methylbutanal, 2-/3-methylbutanoic acid, or (*E*)-2-nonenal. However, different rates were observed for other odor-active compounds such as acetic acid, (*E*,*E*)-2,4-decadienal, vanillin, or hexanal. Although the fermentation conditions were different, the results indicate that rye flour may have a different set of aroma precursors generating the odorants mentioned above.

Because sourdough fermentation of wheat flour as well as of rye flour causes a large shift in the amounts of key odorants already present in the flour, the type of flour used is undoubtedly an important factor to modify and/or improve the aroma quality of breads, in addition to the type of lactic acid bacteria and the fermentation conditions.

## ACKNOWLEDGMENT

We thank P. Bail and G. Laskawy for skillful technical assistance.

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**Received for review June 6, 2002. Revised manuscript received August 26, 2002. Accepted August 27, 2002. This work was supported by the FEI (Forschungskreis der Ernährungsindustrie e.V., Bonn), the AiF (Arbeitskreis industrieller Forschungsvereinigungen "Otto von Guericke" e.V.), and the Ministry of Economics; Project 11771N.**

JF020638P