Volatile Flavor Components of Breads Made from Hard Red Winter Wheat and Hard White Winter Wheat[1]

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ABSTRACT

Volatile components of white pan bread and whole wheat bread made from hard red winter or hard white winter wheat were collected on a Tenax TA trap by dynamic headspace concentration. Bread crumb and crust were studied separately. A total of 74 compounds was separated and identified by gas chromatography–Fourier transform infrared spectrometry–mass spectrometry. Among the compounds that possessed some odor qualities, 11 of them had breadlike odors. Most of the carbonyls and pyrazines were higher in relative quantities in the crust than in the crumb. Many differences were noticed between white pan and whole wheat breads. However, many fewer differences were found between breads made from hard red winter and hard white winter wheats.

Hard red winter (HRW) wheat is the major wheat class grown in the United States, and flour made from it is the primary ingredient in most breads. Hard white wheat, a new wheat classification (May 1, 1990) is still under development by several Agricultural Experiment Stations (AES), including Kansas, Washington, Oregon, Idaho, and California (Bequette 1990). In particular, the Kansas AES is interested in developing hard white winter (HWW) wheat with above-average milling and breadbaking qualities.

Several studies have evaluated the milling quality and possible commercial uses of HWW wheats in making whole wheat hamburger buns, steamed bread, pita bread, tortillas, and couscous (Li and Posner 1989; Lang and Walker 1990; Lu and Ponte 1990; Quarooni et al 1991 a,b; Lang and Walker 1992). Chang and Chambers (1992) reported flavor differences in loaf breads made from HRW or HWW wheat. The quality of bread is judged on the basis of loaf volume, texture, color, and flavor. Flavor particularly influences acceptance by consumers, and volatile aroma components generally are regarded as the most important parameters of food flavor quality (Heath and Reineccius 1986).

The flavor compounds of bread have been studied by many researchers using different methods. Usually, more compounds are found when organic solvent extraction or steam distillation methods are used for isolation than when headspace methods are used. Von Sydow and Anjou (1969) steam-distilled samples of rye crispbread for 3.5 hr at atmospheric pressure and found 92 volatile compounds. Mulders et al (1972b) used pentane-diethyl ether (2:1) to extract bread crust and reported 30 compounds by gas chromatography (GC). Richard-Molard et al (1979) found nearly 100 compounds in French bread when methylene chloride was used as the extracting solvent. Folkes and Gramshaw (1981) isolated flavor volatiles of white bread crust by ether-extraction followed by vacuum distillation. A total of 190 compounds, including many nitrogen and sulfur compounds, were identified. Schieberle and Grosch (1985) used dichloromethane to extract bread crust and fractionated the flavor extract by column chromatography. Twenty-six compounds that were sensorially detectable in the GC effluents were identified. However, the use of solvent extraction or steam distillation allows the possibility of artifact formation or the introduction of impure solvents that increase the number of additional compounds that are identified later.

A rather simple and mild technique, direct vapor analysis, was reported by DeFigueiredo (1964), Mulders (1973), and Hironaka (1986). The possibility of artifact formation using this procedure is minimal because neither severe temperature nor organic solvents are used. Also, losses during extraction and concentration are eliminated, although other losses may occur. However, this method can collect only the most volatile compounds, and the complete picture of bread flavor may be lacking. DeFigueiredo (1964) analyzed the vapor above fresh bread by GC and found only five compounds; 15 compounds were identified by Mulders (1973) and 11 by Hironaka (1986).

Mulders et al (1972a) developed an enlarged vapor-sampling method. Purified nitrogen was passed through small bread cubes placed in a glass container for 50–75 min. The exit vapor was trapped in a stainless steel precolumn filled with uncoated glass beads, and the precolumn was cooled with dry ice. Upon vapor cumulation, the cooled precolumn was quickly heated to 120°C and connected to the GC column for analysis. Only 15 compounds were identified. The authors commented that increasing the number of compounds identified would call for other cumulation and separation techniques. However, several compounds found in that study were not observed in previous work.

Headspace analysis, by purge and trap on a porous polymer adsorbent, followed by thermal desorption or solvent elution, has been regarded as a simple and useful isolation method. The volatiles collected usually have an aroma very close to that of the original sample (Chen et al 1982). This method has been used for analysis of volatile components of peanuts (Buckholz et al 1980), passion fruit juice (Chen et al 1982), corn roots (Buttery and Ling 1985), sweet potatoes (Tiu et al 1985), crayfish tail meat (Vejaphan et al 1988), and crab meat (Matiella and Hsieh 1990). No research was found that used the dynamic headspace method to analyze bread flavor compounds.

The objectives of this study were: 1) to use the dynamic headspace method to isolate and concentrate volatile flavor components from bread, and to compare the results with those of previous studies in which other techniques were applied; 2) to separate and identify the volatile components by gas chromatography–Fourier transform infrared spectroscopy–mass spectrometry (GC-FTIR-MS); 3) to characterize odor quality of the individual components; and 4) to compare the volatile flavor differences among breads made with HWW and HRW wheats.

MATERIALS AND METHODS

Sample Preparation

HRW and HWW88 (crop year 1988) white pan breads and HRW and HWW90 (crop year 1990) whole wheat breads were prepared. The flours and procedures used for preparing these breads were described by Chang and Chambers (1992). Three

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replicates of breads were made. Each replicate was analyzed on two successive days (16- to 40-hr-old samples). Bread crust and crumb were studied separately.

Dynamic Headspace Sampling

Each sample (10 g), crust or crumb, was placed in a 25-ml Tekmar sparger of a Tekmar purge and trap instrument (model LSC 2000) equipped with a sample heater (model 211005) and a capillary interface module (model 2530) (Tekmar Co., Cincinnati, OH). Each bread sample was preheated without gas flow at 60°C for 2 min, and then the volatiles from the heated sample were purged with helium at 40 ml/min onto a Tenax trap (0.29 g, 60–80 mesh) (Tekmar). After a 12-min sample-purge time, a 12-min dry purge was performed to remove excess moisture from the Tenax trap. The collected volatiles were preheated at 175°C and desorbed at 180°C for 4 min. With the capillary interface module, the desorbed volatiles were cryofocused at −150°C by liquid nitrogen at the top of the GC column. Rapid heating of the cryofocused zone at 180°C for 0.75 min released the components in a narrow band for separation on a GC column.

GC-FTIR-MS and Descriptive Sensory Evaluation

A model 5890, series II GC, coupled with a model 5965B FTIR detector, and a model 5970 mass selective detector (MSD), all from Hewlett Packard Co. (Palo Alto, CA), were used to analyze bread volatiles. A Supelcowax 10 column (30-m × 0.32-mm i.d. × 0.25-μm film thickness) from Supelco Inc. (Bellefonte, PA) was used for separation. Column head pressure was 86.1 kPa (12.5 psi) at 50°C, which gave a helium flow rate of ~1.7 ml/min. Then the flow rate was held constant by automatically increasing pressure as oven temperature increased. Oven temperature was held at 50°C initially for 2 min, and then was increased to 140°C at a rate of 7°C/min, and to 230°C at a rate of 17.5°C/min. The temperature of the GC injector zone under the capillary interface module was maintained at 200°C. Two lengths of deactivated silica tubing (0.32-mm i.d.) were connected to the end of the GC column with a Y-shaped splitter (Restek Corp., Bellefonte, PA) to split the effluent into two nearly equal parts. The effluent from one tubing was directed out of the oven through the heated front inlet of the GC (the capillary interface module for the purge and trap instrument was placed over the rear inlet) for odor evaluation by four panel members who previously had undergone 120 hr of training in all aspects of sensory techniques and analyses. Each of the members had more than 500 hr of sensory testing experience, and all had prior experience testing bread products. An additional transfer line heater (set at 150°C) allowed extension of the line ~1 m away from the GC, where the person doing the sniffing could comfortably smell the end of the transfer line. The effluent from the other tubing first passed through the FTIRD and then into the MSD. FTIRD conditions included transfer line and flow-cell temperatures maintained at 200°C, a liquid nitrogen-cooled Hg-Cd-Te detector (750–4,000 cm^{-1}), and a spectral resolution of 16 cm^{-1} at a scan rate of 0.9 spectra/sec. MSD conditions were: 230°C direct transfer line temperature,; 250°C ion source temperature; 70 eV ionization voltage; mass/charge 33–230 a.m.u. mass range; 1.57 scans/sec scan rate; 2,600 V electron multiplier voltage.

Compound Identification

Compounds were identified by computer matching of experimental infrared spectra and mass spectra of compounds with standard spectra in two IR vapor phase libraries (HP59963A EPA and HP59964A Flavors and Fragrances) and in the HP59943B Wiley PBM MS database, respectively. All databases were from Hewlett Packard. GC retention indices from published data (Jennings and Shibamoto 1980) also were used to provide additional confirmation.

Statistical Analysis

Analysis of variance was conducted on the observed total ion abundance (Table I). To test differences in the treatments, least significant differences (LSD) were determined on the response variables. Differences in results at the 5% significance level are reported. All the statistical analyses were made with the Statistical Analysis System software package (SAS Inc., Cary, NC).

RESULTS AND DISCUSSION

The total ion chromatograms from various crust and crumb volatiles were qualitatively similar to one another. A typical total ion chromatogram of a bread sample is shown in Figure 1. Evidence was obtained for the presence of 74 compounds, including 18 alcohols, 15 aldehydes, 5 ketones, 5 esters, 1 lactone, 1 hydroxyketone (acetoin), 13 pyrazines, 2 pyrroles, 4 hydrocarbons, 2 sulfur compounds, and 7 miscellaneous compounds, and 1 unknown (possibly an acetyldihydropyridine). Sixty-three of the compounds are listed in Table II, along with their relative abundances, as determined by electronic integration of peaks in total-ion chromatograms. By using extracted-ion techniques, evidence was obtained for seven additional pyrazines and one unknown compound. The data shown in Table II should be recognized as only the approximate relative amounts of each component, because detector response factors and adsorption recoveries were not determined. Retention indices of most components, which were reported by Jennings and Shibamoto (1980), also are included in Table II. They help confirm the general elution sequence.

Many more compounds were detected using a smaller amount of sample and a shorter time than had previously been found in other headspace analysis studies by DeFigueiredo (1964), Mulders (1973), and Hironaka (1986). However, studies using solvent extraction or steam distillation have isolated some compounds that we were not able to detect. For example, Folkes and Gramshaw (1981) isolated the compounds 2-acetylpyridine, 2-acetylpyrazine, and 2-acetylthiazoline in white bread crust and believed that they all contributed to the crust odor. Three reasons may explain why we could not detect these compounds. First, their bread formula was different and the baking time was much longer. Bread baked for a longer time has a stronger flavor than bread baked for a shorter time, because more browning reaction products are allowed to form. Second, 1 kg of bread crust was used to extract the flavor compounds in their study. Only 10 g of sample were used in our study. Thus, failure to detect components with low volatility or those present only in minor quantities was possible. In fact, during sensory evaluation of individual volatile components, panelists occasionally perceived some odors from the GC effluent, although no peaks were shown on the chromatogram. For instance, the panelists perceived a sweaty, sour, musty, chemical odor at retention time of 7.0 min; a mushroomlike, earthy, mildew odor at 8.2 min; a popcorn, crustlike, toasted odor at 10.5 min; a potatolike, crackerlike odor at 11.1 min; a peanut butter, beany, nutty odor at 14.7 min; a brown, breadlike odor at 15.2 min. No peak was detected at any one of these instances. Possibly, some compounds were present at a concentration above the detection threshold by the human nose but below the sensitivity level of the instrument. Third, the chance for artifact formation during solvent extraction and vacuum distillation in the previous study may have increased the number of compounds the researchers detected. Differences in formula, baking time, sample size, and extraction method probably also account for our failure to detect 2-acetyl-1-pyrroline

TABLE I
Analysis of Variance Design

Source of Variation	Degree of Freedom
Wheat type (W)	1
Bread type (B)	1
Crust/crumb (C)	1
W × B	1
W × C	1
B × C	1
W × B × C	1
Rep	2
Error	14

(2AP) identified by Schieberle and Grosch (1985) using a solvent-extraction procedure. The 2-acetyl-1-pyrroline was recognized by them as a character-impact compound in wheat bread crust. We were aware of the chromatographic and spectroscopic properties of 2AP and had used our instrument to detect it in other studies. At about the same time these bread samples were analyzed, and by using the same instrument configuration, Seitz et al (1993) identified 2AP in wetted millet samples with off-odor. In the bread samples, however, we could not find any evidence for 2AP, even by using extracted-ion techniques with the MS data and selected wavelength (especially looking for absorbance at 1717 cm^{-1}) with the IR data.

Schieberle and Grosch (1985) also reported the identification of 2,3,5-trimethylpyrazine, 2,5-dimethyl-3-ethylpyrazine, and 2,6-dimethyl-3-ethylpyrazine in bread. By using extracted-ion techniques, we found evidence for trace levels of seven pyrazines in addition to those listed in Table II. By searching for mass 80 (m/z), pyrazine was found at 6.85 min. Searches for masses 121 and 122 showed the presence of 2-ethyl-6-methylpyrazine at 9.77 min, 2-ethyl-5-methylpyrazine at 9.87 min, and trimethylpyrazine at 10.14 min. The latter compound was nearly coeluted with 2-ethyl-3-methylpyrazine, so some of the area values reported in Table II for 2-ethyl-3-methylpyrazine probably included a small contribution from trimethylpyrazine. Searches for masses 135 and 136 indicated the presence of ethyl-dimethylpyrazines at 10.60, 10.78, and 11.08 min.

An unknown compound that may be a heterocyclic nitrogen compound was detected at 15.36 min. Some ions in the MS of this compound were 39 (12%), 43 (15%), 51 (15%), 53 (40%), 78 (12%), 80 (100%), 123 (38%), and 124 (2.3%). The mass 123 appears to be the parent ion, which is consistent with a compound containing one nitrogen atom. The IR spectrum was weak, but it clearly showed absorbances at 1,741 (strongest), 1,505, 1,220, and 1,085 cm^{-1}. From a review of the MS spectrum of 2-acetylpyridine retrieved from the Wiley database, it appears that the MS spectrum of the unknown compound could be consistent with an acetyldihydropyridine. Furthermore, the 1,741 cm^{-1} IR band is consistent with a carbonyl in an acetyl group that is probably not conjugated with a double bond in the heterocyclic ring. This information suggests that the compound might be 2-acetyl-2,3-dihydropyridine.

Alcohols, especially ethanol, isobutanol, and 3-methyl-1-butanol, were the major components of the bread volatiles. In addition to alcohols, carbonyl compounds such as 3-methyl butanal, 2,3-butanedione, and hexanal also were quantitatively important. However, these predominant substances did not necessarily make major contributions to the flavor quality of the bread. When the GC effluents were subjected to sensory appraisal, a large number of odors were perceived, many of which had no obvious relationship to the bread flavor, although in total they may have had some influence. There were 11 compounds that had breadlike odors such as roasted, popcornlike, nutty, toasted, brown, and crustlike odors (Table II). These breadlike odors probably are sensorially important to bread flavor. The compounds responsible for these odors are 3-methyl-1-butanol, methylpyrazine, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, ethylpyrazine, 2,3-dimethylpyrazine, 2-ethyl-3-methylpyrazine, 2-furfural, 5-methyl-2-furfural, dihydro-2(3H)-furanone, and 2-phenylethyl acetate. As noted in Table II, not all compounds had an odor description. To be sensorially detectable, the chemical must have some odor quality and exist at a concentration above the sensory threshold level.

In general, the relative quantities of carbonyls and pyrazines were higher in the crust than in the crumb of bread. This was expected because the temperature reached in the crust during baking was higher than in the crumb, which allowed more browning reaction products to form. Ethyl acetate, 3-methylbutanal, 2,3-butanedione, 3-methylpentanone, dimethyldisulfide, isobutanol, heptanal, 2-methylpyrazine, 2,5-dimethylpyrazine, ethylpyrazine, 2,3-dimethylpyrazine, dimethyltrisulfide, 2-furfural, 1H-pyrrole, and 2-furanmethanol were all significantly higher in the crust than in the crumb ($P < 0.05$). The trace levels of ethyl-dimethylpyrazines discussed above were about 17 times higher in crust than in crumb, as indicated by summing areas representing these compounds in mass 135 extracted-ion chromatograms for all crumb and crust samples. By using similar extracted-ion procedures with mass 80 from the unknown compound discussed above, it was ~9 times more concentrated in crust than in crumb.

Many differences were noticed between white pan and whole wheat breads. Fifteen compounds were significantly higher in relative quantities in whole wheat breads than in white pan breads, including ethyl acetate, 3-methylbutanal, ethanol, 1-propanol, hexanal, isoamyl acetate, 1-butanol, heptanal, 1-pentanol, 2-octanone, 1-hexanol, ethyl octanoate, 1-octen-3-ol, 1-heptanol, and 2-furfural ($P < 0.05$). Two compounds were higher in relative quantities in white pan breads: dimethyldisulfide and 1-(2-

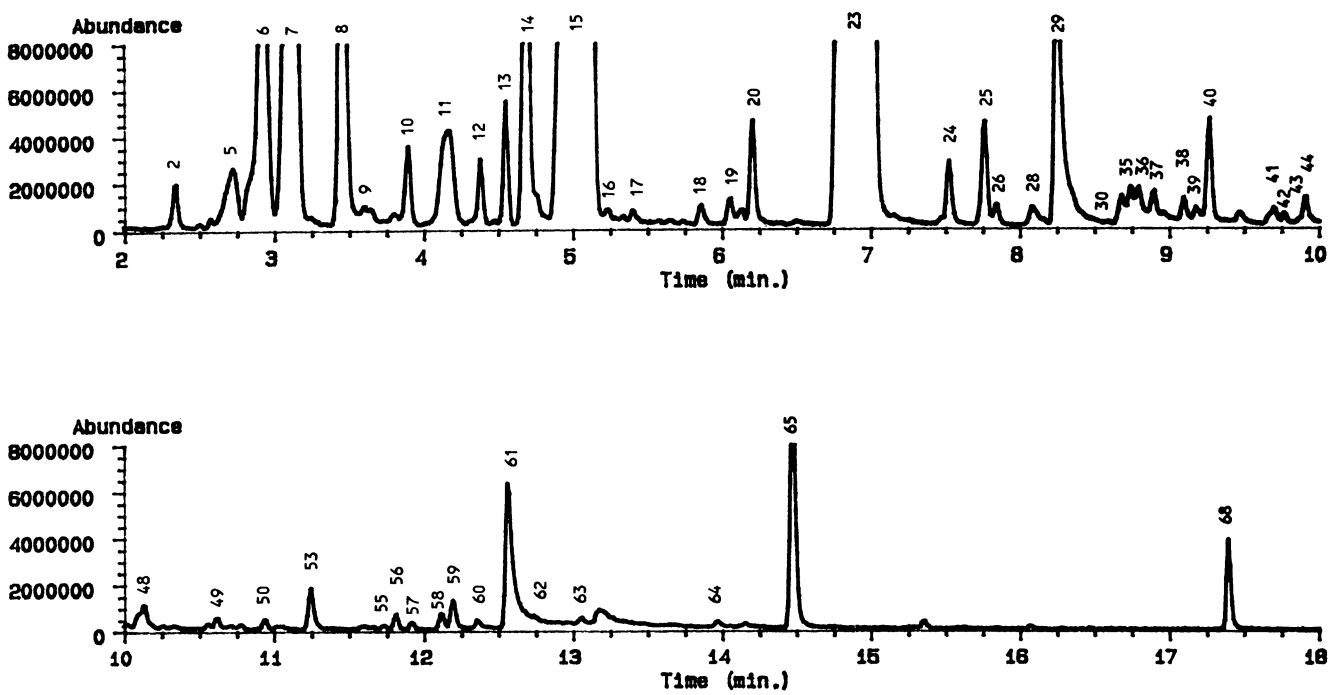

Fig. 1. Total ion chromatogram of HWW88 white pan bread crust (from replicate 2).

furanyl)-ethanone ($P < 0.05$).

Few differences were detected between breads made with HWW and HRW wheat flours. Only ethyl acetate, ethanol, 2-ethyl-3-methylpyrazine, and ethyl octanoate were higher in relative quantities in HRW breads than in HWW breads, and 2-butoxyethanol and 2-furfural were more abundant in HWW breads ($P < 0.05$). The small differences in flavor found between those breads by Chang and Chambers (1992) may be partly related to those compounds, especially, the differences in "brown" and "toasted" flavor characteristics. Other differences in flavor, such as bitter, probably are related to nonvolatile compounds not examined in this study.

TABLE II
Volatile Components of Breads Made from Hard White Winter Wheat and Hard Red Winter Wheat

Peak	Compound	Retention Time (min)	Index[a]	Identification[b]	Odor Description
1	Heptane	2.13	700	MS	
2	Octane + 2-methylpropanal	2.17	800	MS	
3	1-Heptene	2.20		MS	
4	Butanal	2.53		MS	
5	Ethyl acetate	2.74	872	MS, IR	Fruity
6	3-Methylbutanal	2.95	937	MS, IR	Sweet, corn flakes, fermented, dirty socks (sweaty), sour
7	Ethanol	3.05		MS, IR	Alcohol
8	2,3-Butanediol + Pentanal	3.53	963	MS, IR	Buttery, cheezy
9	Decane	3.64	1,000	MS	
10	Chloroform + tetrachloroethylene	3.91		MS, IR	Woody, cedery
11	1-Propanol	4.20	1,002	MS, IR	Butter rum
12	2,3-pentanedione	4.38	1,044	MS, IR	Butter scotch, fruity, sweet
13	Dimethyldisulfide	4.44	1,081	MS, IR	
14	Hexanal	4.59	1,084	MS, IR	Green, cut grass
15	Isobutanol	4.77	1,054	MS, IR	Sour, yeasty
16	Isoamyl acetate	5.26	1,110	MS	Sweet, fruity, banana-like
17	1,2-Dimethylbenzene	5.40		MS	
18	1-Butanol	5.85	1,113	MS, IR	Fermented, dirty feet (sweaty), putrid
19	1-Penten-3-ol	6.11	1,130	MS	Green
20	Heptanal	6.17	1,186	MS, IR	Weeds, green, sour, sweaty
21	2-pentylfuran	6.66	1,229	MS	Rubbery (shoe rubber)
22	Ethyl hexanoate	6.80	1,223	MS, IR	Fruity, melony, sweet
23	3-Methyl-1-butanol	6.82	1,184	MS, IR	Roasted, fresh bread
24	1-Pentanol	7.53	1,213	MS, IR	Fruity, sweet
25	Trimethylbenzene	7.80		MS	
26	Methylpyrazine	7.84	1,251	MS, IR	Popcorn, cut-corn, green
27	Octanal	7.96	1,278	MS	Lime, grassy, citrus
28	2-Octanone	8.11	1,304	MS	Grassy, floral, fruity, lemon, lime
29	3-Hydroxy-2-butanone (acetoin)	8.32		MS, IR	
30	2,3-Octanedione	8.60		MS	
31	2-Heptenal, (E) or (Z)	8.71		MS, IR	
32	2,5-Dimethylpyrazine	8.74	1,306	MS	Hay barn (musty), sour, popcorn
33	2,6-Dimethylpyrazine	8.84	1,325	MS	Nutty
34	Ethylpyrazine	8.93		MS	Musty, nutty
35	2,3-Dimethylpyrazine	9.15	1,330	MS	Dirty leather, popcorn, toasted
36	2-Hydroxy-2-methyl-propanal[f]	9.22		MS	
37	1-Hexanol	9.32	1,316	MS, IR	Grassy
38	Dimethyltrisulfide	9.64		MS	Dirty socks, sour clothes (sweaty, sour, putrid)
39	1,2-diethoxyethane	9.73		MS, IR	
40	2-Hexen-1-ol, (Z) or (E)	9.83	1,368	MS	
41	Nonanal	9.89	1,382	MS, IR	Rubbery, beany
42	2-Ethyl-3-methylpyrazine	10.12		MS	Toasted, nutty, brown, burnt peanut, crust-like
43	2-Butoxyethanol	10.17		MS, IR	
44	3-Octen-2-one, (E)	10.20		MS	
45	Ethyl octanoate	10.59	1,423	MS, IR	Fruity, sweet
46	1-Octen-3-ol	10.95	1,420	MS	Sour, yeasty
47	1-Heptanol	11.04	1,419	MS	
48	2-Furfural	11.26	1,449	MS, IR	Brown
49	2-Ethyl-1-hexanol	11.61		MS	Brewery hop
50	Decanal	11.71	1,485	MS	
51	1-Nitrohexane	11.77		MS, IR	
52	1-(2-Furanyl)ethanone	11.93	1,491	MS, IR	Sharp, chemical
53	1H-Pyrrole	12.12	1,524	MS	
54	Benzaldehyde	12.21	1,502	MS, IR	
55	2-Nonenal, (E)-	12.33	1,540	MS	
56	2,3-Butanediol	12.60		MS, IR	
57	1-Octanol	12.75	1,519	MS	Musty, cardboard-like, burnt hay
58	5-Methyl-2-furfural	13.07	1,563	MS, IR	Toasted, dry corn husk
59	Dihydro-2(3H)-furanone (-butyrolactone)	13.99	1,632	MS, IR	Brown, toasted, roasted peanuts
60	2-Furanmethanol (furfuryl alcohol)	14.48		MS, IR	Burnt
61	2-Phenylethyl acetate	16.47	1,785	MS	Brown, grainy, toasted
62	N-Furfuryl pyrrole	16.63		MS	Mildew, decayed vegetable
63	Benzeneethanol (2-Phenylethanol)	17.40	1,859	MS, IR	Green, corn cob, sweet, corn flakes

[a] Retention indices used to provide additional confirmation (Jennings and Shibamoto 1980).
[b] MS = mass spectroscopy; IR = infrared.
[c] Mean values of peak areas from three replicates of MS total ion chromatograms are reported. Numbers in parentheses are their standard deviations. Mean values in the same row with the same letter are not different at the 5% significance level. WPB/WPT = HWW88 white pan bread crumb/crust: RPB/RPT = HRW white pan bread crumb/crust: WWB/WWT = HWW90 whole wheat bread crumb/crust: RWB/RWT = HRW whole wheat bread crumb/crust.
[d] Compound was found in only one of the three replicates.
[e] Compound was found in only two of the three replicates.
[f] Compound was tentatively identified by mass spectrometry.

CONCLUSIONS

Compared with other headspace methods used by DeFigueiredo (1964), Mulders (1973), and Hironaka (1986), this dynamic headspace technique demonstrated more efficient isolation and concentration of bread volatiles without a laborious sample preparation procedure. Although some compounds found in research using other methods were not found in this study, a dynamic headspace technique may be one method of choice for the study of bread flavor in a manner that most resembles what the human nose perceives. The chemistry of bread odor is complicated, and many trace compounds present in bread volatiles probably contribute to bread aroma. Many quantitative differences in volatile components were noticed between crumb and crust and between white pan breads and whole wheat breads. Few differences between breads made with HWW and HRW wheats were found.

Relative Abundance[c] (Standard Deviations)							
WPB	**RPB**	**WWB**	**RWB**	**WPT**	**RPT**	**WWT**	**RWT**
0.00 a (0.00)	0.00 a (0.00)	0.41 a[d] (0.72)	0.37 a[d](0.65)	0.42 a[d] (0.73)	0.53 ab[d] (0.92)	0.86 ab[d] (1.48)	1.48 b[c] (1.48)
5.29 a (1.09)	7.08 a (0.71)	9.54 ab (2.74)	8.56 ab (4.40)	7.84 ab (6.20)	9.49 ab (7.36)	10.58 ab (3.74)	13.15 b (4.80)
0.00 (0.00)	0.00 (0.00)	Trace[d]	13.08[d] (22.65)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	Trace[d]
0.66 a (0.96)	0.00 a (0.00)	0.00 a (0.00)	0.48 a[d] (0.84)	0.00 a (0.00)	0.00 a (0.00)	0.00 a (0.00)	0.00 a (0.00)
2.64 a (0.90)	4.03 a (2.06)	16.33 bc (4.47)	30.73 de (7.57)	11.74 ab (3.95)	24.41 cd (6.86)	35.90 de (15.85)	42.04 e (10.46)
30.37 ab (23.77)	26.88 a (20.53)	26.77 a (5.03)	51.89 ab (22.46)	49.45 ab (16.56)	48.42 ab (21.09)	58.12 bc (17.17)	84.71 c (12.22)
97.68 a (3.29)	104.66 ab (11.18)	136.72 b–d (10.42)	147.33 cd (27.95)	98.95 a (30.13)	127.78 a–d (17.62)	117.78 a–c (8.87)	154.27 d (28.72)
44.77 ab (5.72)	38.70 a (14.24)	47.88 ab (9.57)	49.94 ab (15.02)	51.02 ab (8.70)	60.27 ab (22.19)	61.76 b (16.56)	62.31 b (8.00)
3.22 a (1.24)	2.64 a (0.82)	3.69 a (2.12)	2.92 a (2.44)	4.86 a (2.16)	3.47 a (0.65)	4.67 a (1.41)	4.06 a[c] (3.57)
8.52 ab (3.57)	6.89 ab (6.14)	10.37 ab (8.95)	5.19 a (4.23)	9.00 ab (0.83)	5.67 ab (4.49)	9.80 ab (4.78)	12.16 b (4.21)
21.95 a (3.26)	25.78 ab (3.91)	32.64 bc (8.34)	34.28 bc (6.94)	29.37 ab (8.37)	28.83 ab (6.28)	32.84 bc (8.79)	40.35 c (14.83)
3.31 a[c] (2.88)	3.36 a (0.35)	6.19 ab (2.08)	5.42 ab (2.70)	7.94 b (2.95)	5.47 ab (1.29)	6.47 b (1.10)	6.86 b (2.16)
4.80 a–c (2.18)	5.32 bc (3.15)	2.83 ab (1.13)	2.18 a (0.99)	9.65 d (2.69)	6.69 c (2.59)	5.28 bc (0.45)	5.64 c (1.71)
43.36 a (8.64)	42.05 a (10.66)	92.45 b (10.38)	85.30 b (18.34)	56.00 a (2.38)	53.69 a (14.73)	100.93 b (25.24)	85.63 b (5.08)
214.85 ab (21.10)	200.33 a (50.43)	273.52 a–c (32.44)	279.35 bc (44.18)	261.12 a–c (48.91)	314.64 c (32.83)	281.36 bc (59.39)	290.25 bc (73.00)
0.38 ab[c] (0.64)	Trace	1.45 ab (1.17)	1.14 ab (1.96)	Trace	0.00 a (0.00)	1.80 ab (0.90)	2.09 b (1.71)
Trace	0.86 a (1.32)	1.52 a[d] (2.63)	1.71 a (2.96)	2.28 a (2.26)	1.94 a (1.61)	0.00 a (0.00)	2.02 a[d] (3.50)
0.00 a (0.00)	0.00 a (0.00)	4.85 bc (1.22)	4.60 bc (5.24)	4.73 bc (2.65)	1.70 ab (0.43)	3.07 a–c (0.58)	5.92 c (2.10)
1.55 a (1.28)	3.28 a (3.00)	3.70 a (2.43)	2.64 a (0.97)	2.89 a (2.80)	1.95 a (0.84)	4.39 a (1.88)	4.35 a (2.48)
4.62 a (2.41)	4.70 a (1.78)	16.61 b (3.12)	16.50 b (6.80)	12.01 b (1.03)	6.28 a (1.67)	16.80 b (1.22)	23.09 c (2.51)
5.00 a[c] (4.43)	1.36 a[c] (2.27)	0.00 a (0.00)	6.36 a[d] (11.01)	0.00 a (0.00)	3.13 a[c] (2.74)	10.35 a[d] (17.93)	0.00 a (0.00)
1.50 a[d] (2.59)	0.72 a[c] (1.16)	0.00 a (0.00)	5.09 a[d] (8.81)	0.00 a (0.00)	2.12 a[c] (2.14)	3.64 a[d] (6.30)	0.00 a (0.00)
416.60 ab (38.14)	369.82 a (50.00)	494.19 ab (90.33)	509.76 ab (88.60)	474.00 ab (113.90)	562.23 b (101.51)	528.66 b (116.58)	455.54 ab (56.11)
6.31 ab (1.20)	5.19 a (1.87)	11.70 b (1.99)	12.16 b (4.76)	6.66 ab (1.91)	9.53 ab (6.00)	11.77 b (4.19)	11.57 b (3.37)
0.00 a (0.00)	1.92 ab (1.64)	1.31 ab (2.25)	0.54 ab[c] (0.93)	4.50 b[c] (6.33)	1.77 ab[c] (1.68)	0.00 a (0.00)	0.00 a (0.00)
2.36 ab (1.13)	0.61 a[c] (0.97)	1.97 ab (1.65)	3.09 ab (2.05)	6.11 cd (3.38)	3.84 bc (1.77)	3.86 bc (1.93)	7.59 d (0.95)
1.95 ab[c] (2.05)	0.99 ab[c] (1.64)	0.00 a (0.00)	0.00 a (0.00)	0.00 a (0.00)	2.55 b[c] (2.60)	0.00 a (0.00)	0.00 a (0.00)
0.90 a[d] (1.55)	1.33 ab[c] (1.52)	3.48 b–d (1.53)	2.73 a–d (2.30)	3.19 a–d (0.38)	1.56 a–c (1.27)	3.78 cd (1.08)	4.12 cd (1.28)
10.85 a[d] (18.79)	14.43 a[c] (14.12)	20.39 a (6.29)	7.99 a (9.00)	39.52 b (12.09)	9.32 a (7.93)	16.43 a (14.61)	26.36 ab (3.21)
Trace[c]	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.66 (0.88)	0.00 (0.00)
1.71 a (0.24)	1.57 a (1.54)	3.18 a (0.75)	4.22 a (3.24)	2.29 a (0.18)	6.71 b (6.61)	2.99 a (1.06)	3.03 a (1.18)
Trace[d]	0.00 a (0.00)	0.00 a (0.00)	0.00 a (0.00)	4.11 b (3.21)	Trace	Trace	1.09 a[c] (1.88)
Trace[d]	0.00 a (0.00)	2.63 b (0.74)	2.07 ab[c] (2.23)	3.48 b (2.66)	1.34 ab (1.08)	1.68 ab (1.37)	2.94 b (0.62)
0.00 a (0.00)	Trace[c]	0.00 a (0.00)	0.00 a (0.00)	3.56 b (1.18)	2.20 b (1.94)	1.95 ab (1.78)	3.71 b (1.29)
Trace	0.00 a (0.00)	0.00 a (0.00)	0.00 a (0.00)	1.77 b (1.39)	Trace	Trace	0.86 ab (0.63)
0.00 a (0.00)	0.00 a (0.00)	Trace	0.62 ab[d] (1.07)	0.78 ab[d] (1.35)	0.00 a (0.00)	0.54 cab (0.86)	1.58 b (0.68)
21.18 ab (2.25)	15.33 a (4.02)	44.17 c (9.18)	34.92 bc (12.46)	13.09 a (3.80)	17.59 a (7.53)	32.60 bc (13.95)	23.56 ab (7.45)
Trace	Trace	0.83 a–c (0.84)	0.00 a (0.00)	1.47 b (0.98)	0.35 ab (0.43)	Trace	1.27 bc (0.94)
Trace[d]	1.25 a–c[c] (1.18)	1.29 bc (0.69)	0.69 a–c[c] (1.11)	1.34 c (1.45)	Trace[c]	1.22 a–c (1.02)	0.00 a (0.00)
0.00 a (0.00)	0.00 a (0.00)	0.43 a (0.57)	0.00 a (0.00)	0.00 a (0.00)	0.00 a (0.00)	0.00 a (0.00)	2.14 b (0.45)
6.20 a (2.85)	5.31 a (1.12)	6.36 a (2.02)	5.61 a (1.32)	4.62 a (2.18)	5.47 a (1.69)	4.08 a (1.28)	4.67 a (1.50)
0.00 a (0.00)	0.00 a (0.00)	0.00 a (0.00)	1.71 bc (1.72)	0.00 a (0.00)	0.73 ab[c] (0.86)	Trace[d]	2.39 c (0.10)
3.19 cd (0.56)	1.44 ab (1.17)	3.07 b–d (1.94)	2.25 a–d (1.00)	3.36 d (0.82)	0.86 a (0.99)	3.50 d (0.64)	1.70 a–c (0.41)
0.00 a (0.00)	Trace[d]	0.00 a (0.00)	0.00 a (0.00)	0.00 a (0.00)	0.00 a (0.00)	0.42 a[d] (0.73)	1.38 b (0.18)
4.46 ab (1.08)	3.21 ab (0.83)	8.99 bc (1.10)	22.85 d (7.70)	1.90 a (1.04)	2.65 a (1.16)	6.24 a–c (4.38)	11.07 c (2.63)
0.68 ab (0.31)	0.52 a (0.73)	1.77 b (0.63)	1.63 ab (1.08)	0.98 ab (0.41)	1.36 ab (1.50)	1.39 ab (0.43)	1.50 ab (0.32)
0.50 ab (0.00)	0.00 a (0.00)	2.00 c (0.89)	1.70 bc (1.10)	Trace	0.54 ab (0.76)	1.16 a–c (0.95)	0.95 a–c (0.78)
0.65 ab (0.64)	Trace	1.63 bc (0.37)	1.73 c (0.40)	3.64 d (1.16)	1.27 bc (0.67)	4.77 e (1.00)	4.71 e (0.15)
0.00 (0.00)	Trace[c]	Trace	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	Trace	0.00 (0.00)
Trace	Trace[c]	Trace	0.00 (0.00)	Trace	0.95 (1.47)	Trace	Trace
1.75 ab (0.44)	0.00 e (0.00)	1.98 a (1.15)	0.91 b–d (0.72)	1.26 a–c (0.16)	0.43 c–e (0.57)	1.21 a–c (0.62)	Trace
0.00 (0.00)	0.00 (0.00)	Trace	0.00 (0.00)	Trace	Trace	Trace	Trace
Trace[c]	Trace	0.00 (0.00)	0.00 (0.00)	1.25 (0.89)	0.95 (0.62)	Trace[c]	Trace[c]
3.09 a (0.50)	2.20 a (0.71)	3.70 a (0.56)	2.54 a (0.61)	3.20 a (0.57)	3.16 a (1.42)	3.92 a (1.87)	3.11 a (0.76)
0.17 a (0.28)	Trace	0.75 a (0.44)	0.89 a (0.68)	0.44 a (0.59)	0.77 a (0.74)	0.56 a (0.80)	0.94 a (0.41)
0.90 a (1.55)	6.70 ab[d] (11.60)	3.45 a[d] (5.95)	4.44 a[d] (7.70)	17.58 b[c] (15.32)	0.00 a (0.00)	2.97 a[c] (3.61)	8.95 ab (10.11)
Trace	0.52[c] (0.90)	Trace	0.66 (0.94)	0.00 (0.00)	Trace	Trace	0.00 (0.00)
Trace[d]	Trace[c]	0.00 (0.00)	0.00 (0.00)	Trace	Trace[c]	Trace	Trace
0.00 a (0.00)	Trace[d]	0.00 a (0.00)	0.00 a (0.00)	Trace[c]	0.00 a (0.00)	0.00 a (0.00)	0.48 b (0.66)
0.80 a (1.21)	0.74 a (1.11)	0.61 a (0.89)	1.66 ab (1.32)	18.88 c (10.11)	6.21 ab (5.59)	3.24 ab (2.89)	8.07 b (1.49)
Trace	0.00 (0.00)	0.00 (0.00)	Trace[c]	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	Trace[d]	0.63[d] (1.09)
2.49 a (4.13)	4.28 ab (3.48)	4.76 ab (1.74)	2.40 a (1.27)	6.88 b (3.59)	2.07 a (1.71)	2.91 ab (2.46)	3.23 ab (1.85)

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