

Volatiles Obtained from Whole and Ground Grain Samples by Supercritical Carbon Dioxide and Direct Helium Purge Methods: Observations on 2,3-Butanediols and Halogenated Anisoles

Larry M. Seitz,^{*,†,‡} M. S. Ram,[‡] and Ramachandran Rengarajan[‡]

USDA-ARS, U.S. Grain Marketing Research Laboratory, Grain Marketing and Production Research Center, 1515 College Avenue, Manhattan, Kansas 66502, and Department of Grain Science and Industry, Kansas State University, Manhattan, Kansas 66506

Volatile compounds were obtained from whole and ground grain samples by two methods. In the supercritical fluid extraction (SFE) method, volatiles were extracted from the grain with supercritical carbon dioxide, trapped at $-78\text{ }^{\circ}\text{C}$, and then transferred via a purge-and-trap instrument to a gas chromatograph with mass and infrared detectors (GC-MS/IR) for separation and identification. In the direct-helium-purge method (DHP), volatiles were purged directly from the grain into the purge-and-trap instrument for subsequent transfer to the GC-MS/IR system. With SFE, extraction of volatiles was favored by ground grain, low pressures ($\leq 13.8\text{ MPa}$), and high temperatures ($50\text{--}90\text{ }^{\circ}\text{C}$). DHP gave more low molecular weight compounds, while SFE was better for aldehydes, enals, 2,3-butanediols (dl and meso forms), acetic acid, and chloro- and bromomethoxybenzenes apparently associated with musty odors in sorghums. The diols were present in both musty and normal-odor sorghums.

Keywords: Grains; volatiles; odors; supercritical fluid extraction; helium purge; 2,3-butanediol; halogenated anisoles; methoxybenzenes

INTRODUCTION

Dynamic headspace (purge-and-trap) methods coupled with gas chromatography-mass spectrometry/infrared spectroscopy (GC-MS/IR) have been used to identify volatile compounds in whole grains with various odors (Seitz and Sauer, 1991, 1992, 1994; Seitz, 1995; Sauer et al., 1995). The objective of this study was to determine whether a supercritical fluid extraction (SFE) method could be used to improve analysis of volatiles from grains. In this paper, we describe results from the coupling of SFE with dynamic headspace and GC-MS/IR to determine volatiles in whole and ground grain samples. SFE pressure and temperature (thus, the fluid density) conditions were optimized for efficient extraction of volatiles from grains. Dynamic headspace (purge-and-trap) was applied (a) by direct-helium-purge of whole grains or (b) by using helium to purge volatiles from the SFE extract that was trapped at dry ice temperature. These are referred to as DHP and SFE methods, respectively.

SFE has been applied to the extraction of food and biomaterials (Rizvi, 1994), lipids in peanuts (Leunissen et al., 1996; Santerre et al., 1994), oils in seeds (Taylor et al., 1993; Walker et al., 1994; Snyder and King, 1994a), fatty acids in wheat (Artz and Sauer, 1992), soybeans (Snyder and King, 1994b; Nikolov et al., 1992), soybean oil (King, 1989), flavors and fragrances (Hawthorne et al., 1988), and ergosterol from flour (Young et al., 1993) among various other applications. The

method has also been used for the recovery of solutes from solids (McHugh and Krukoni, 1986; Castro et al., 1994; Gere et al., 1993) with described advantages as solvation of liquid solvents but with diffusivities and viscosities similar to gases. These properties provided good extraction efficiency plus the convenience of nearly instantaneous removal of solvent from the extract with minimal loss of volatile organic compounds. Also, the density and solvent strength of supercritical CO_2 can be adjusted by changes in pressure and temperature.

At the start of this work, we used an SFE method similar to that described below to obtain volatiles from a sorghum sample (G171S) with a musty odor. One interesting observation was that, on repeated extraction (under optimum conditions, $\sim 13.8\text{ MPa}$, $35\text{ }^{\circ}\text{C}$, 5.5 g of sample, and 30 mL of CO_2 each time) of the same sample of ground grain, the amounts of all volatiles except 2,3-butanediols (dl and meso forms) decreased. By the third extraction, the two peaks corresponding to the diols essentially stood out by themselves in the chromatograms from the infrared and mass detectors. Even after eight extractions the diols continued to evolve. Similar results were obtained with whole grain. During the course of this research, it became necessary to study additional questions concerning the origin of the diols, whether they were associated with musty odor, and the general effectiveness of supercritical CO_2 for extracting relatively polar volatile compounds since supercritical CO_2 has been described as a nonpolar solvent similar to hexanes.

MATERIALS AND METHODS

Samples. Grain samples analyzed in this study were selected from large groups of corn, sorghum, and wheat samples ($\sim 1\text{ kg}$ each) collected at commercial locations by

* Address correspondence to this author [telephone (785)-776-2735; fax (785)776-2792; e-mail larry@usgmrl.ksu.edu].

[†] USDA-ARS.

[‡] Kansas State University.

official grain inspectors. One sample of sorghum (~1 kg, sample FH88S) was obtained immediately after harvest from a central Kansas farm in 1988 and kept in excellent condition in our laboratory. All samples were (i) classified into odor categories by official grain inspectors and by a panel at our laboratory and (ii) kept in cold storage (~4 °C) until analyses were conducted. Ground samples were produced by grinding whole grain in a Falling Number grinder (Model KT-30; Stockholm, Sweden) to about 20 mesh.

Collection of Volatiles by SFE (SFE Method). Whole and ground grains were extracted with 30 mL of CO₂ at 26–90 °C and 10.3–41.4 MPa (1500–6000 psi) by using a 10-mL sample container in an SFX 220 system from ISCO (Lincoln, NE). About 5.5–7 g of the grain sample could be packed in a sample container, by packing it fully, but not compacted. An equilibration time of 5 min was used for most extractions, although it was extended to 1 h for two extractions. Variation in equilibration time could have an influence on extractions that are kinetically slow. A restrictor designed for a maximum flow rate of 1.5 mL/min was used. The restrictor temperature was varied from 85 to 135 °C, although the latter was used for most of the extractions to avoid plugging of the restrictors with waxy, lipid material or even ice as the restrictor was chilled by the low temperature inside the collection tube. To avoid loss of volatiles, the flow of CO₂ was kept low (0.5–1.0 mL/min) and the extract was directed into a collection tube held at –78 °C by surrounding the tube with dry ice in a dewar flask. The cold collection tube (the smaller tube that was used for direct-helium-purge) was immediately mounted on a purge-and-trap instrument for analysis of volatiles as described below.

Collection of Volatiles by Direct-Helium-Purge (DHP Method). Whole or ground grain was placed in a specially designed sample tube and placed directly on a purge-and-trap instrument for analysis of volatiles as described below. Glass tubes of two different sizes were available. The larger tube contained ~65 g of whole sorghum, while the smaller one contained ~11 g of ground sorghum. Purge gas was distributed over the entire sample by appropriately dispersing it. Different weights of the samples were used for SFE and DHP techniques to obtain reasonable detector responses and to accommodate the size of SFE extraction cartridges.

For routine DHP analyses of the large group of samples mentioned below, an Archon autosampler (Varian Associates, Walnut Creek, CA) was interfaced to the purge-and-trap instrument. Grain samples (18 g) were placed in "Soil-Vial" containers with a septum on each end for purging of helium through the sample. A known amount of standard compound along with 1 mL of water was added to the sample in the container before it was heated to 80 °C and purged for 10 min. Conditions in the purge-and-trap instrument were as described below. Use of the autosampler caused lower recovery of diols from grains as compared to recovery from nonwetted grain in a sample tube placed directly on the purge-and-trap instrument. It appears that the addition of water to the grain was part of the reason for the lowered recovery of diols because a decrease in diol recovery was observed when water was added to grain in a sample tube placed directly on the purge-and-trap instrument. Also, because of a lack of synchronization between the autosampler and the purge-and-trap instrument, there was an initial burst of flow for 1 min followed by a slow leveling off of purge flow. While these factors had minimal, and in some cases beneficial, effect on recovery of most volatiles from grains, they could have an adverse effect on some compounds that are not strongly retained on the Tenax trap.

Analysis of Volatiles. Samples (whole grains, ground grains, or SFE extracts) in U-shaped sparge tubes (without glass frit) were attached to a Hewlett-Packard (Palo Alto, CA) purge-and-trap instrument (model G1901A-60500) equipped with a sample pocket heater (model 14-5737-020) and a capillary interface module (model G1908-60500) or to a Tekmar Co. (Cincinnati, OH) purge-and-trap instrument (model LSC 2000) equipped with a sample pocket heater (model 211005) and the capillary interface module (model 2530).

Each sample was preheated to 80 °C for 3 min, then the

volatiles from the heated samples were purged with helium at 40 mL/min onto a glass-lined Tenax trap, type 1G (Tekmar). After a 10-min sample purge, a 10-min dry purge was performed to remove excess moisture from the Tenax trap. After the trap was preheated at 175 °C, the volatiles were desorbed at 200 °C for 4 min. With the capillary interface module, the desorbed volatiles were cryofocused at –140 °C (liquid N₂), and the cryofocused zone was heated at 200 °C for 0.85 min before initiation of the analytical run. The temperature of the injector zone under the capillary interface was maintained at 200 °C.

A model 5890 series II gas chromatograph (GC) coupled with a model 5965B FTIR detector (IRD) and a model 5970 mass selective detector (MSD), all from Hewlett-Packard, were used to analyze the volatiles. A BPX35 column (50 m × 0.32 mm i.d. × 0.25 μm film thickness) from Scientific Glass Engineering Inc. (Austin, TX) was used for separation. Column head pressure was 124 kPa (18 psi) at 50 °C. Carrier gas was helium at a constant flow rate of ~1.7 mL/min. Oven temperature was held at 50 °C initially for 2 min, increased to 140 °C at a rate of 7 °C/min and then to 17.5 °C/min. Effluent from the column first passed through the IR detector and then into the MS detector. The temperature and the flow cell temperatures of the IRD were maintained at 250 °C. MSD conditions were as follows: direct transfer line temperature, 280 °C; ion source temperature, 280 °C; ionization voltage, 70 eV; mass range, 33–300 amu; scan rate, 1.91 scans/s; and electron multiplier voltage, 2800 V.

Compounds were identified by comparing, with the aid of a computer and careful visual examination, experimental infrared spectra and mass spectra of compounds with standard spectra in two IR vapor phase libraries (HP 59963A EPA and HP 59964A flavors and fragrances) and in HP 59943B Wiley PBM MS database, respectively. A mass spectral database from The National Institute of Standards and Technology (NIST/EPA/NIH), PC version 4.5 (U.S. Department of Commerce) also was used when necessary. GC retention times were used in the compound identifications, and authentic standards were utilized when possible. In addition, compound identifications were based on information (GC retention times and MS and IR spectra of authentic standards) from many previous studies conducted in this laboratory on volatiles in grains and breads (Seitz, 1995; Seitz et al., 1998).

Additional Information on Purge-and-Trap Systems. Initial experiments on sorghum G171S were analyzed using the Tekmar (model 2000) purge-and-trap instrument. Most of the data reported here were collected with the newer Hewlett-Packard (HP) instrument, which was similar to a Tekmar model 3000. It was necessary to modify the HP instrument to make it function like the Tekmar model 2000 so that all the compounds of interest were transferred from the purge-and-trap instrument to the GC. The nickel transfer line that carried the volatiles from the Tenax trap in the purge-and-trap instrument to the capillary column interface module on the GC was replaced with an inert silica tubing. The moisture control device was bypassed by using a short piece of Silcosteel (Restek Corp., Bellefonte, PA) coated tubing to directly connect the top of the Tenax trap to the valve oven. The stainless steel frit on top at the trap was removed. The stainless steel tee and the fittings at top of the Tenax trap were Silcosteel (maximum inertness type) treated by Restek Corp. The Tekmar model 2000 did not have a stainless steel frit, and all metal parts in the sample path were gold coated. Furthermore, the top of the Tenax trap on the Tekmar model 2000 was actively heated, whereas it was passively heated on the HP instrument, which resulted in a "cold" spot where compounds could get trapped. The valve oven on the HP instrument had to be heated to a higher temperature than with the Tekmar model 2000 to adequately heat the top of the trap for efficient desorption of volatiles from the Tenax.

Identification of 2,3-Butanediols. A 1-mL sample of water containing ~3000 ppm of a mixture of 2,3-butanediols (Aldrich B8490-4; lot no. 04009DM, 96.7% dl, 3.3% meso) was extracted by SFE in a manner similar to that for the grains. The two forms of the diols were eluted with retention times

7.11 and 7.26 min with total ion chromatogram (TIC) areas proportional to the dl and meso forms and were at least 100-fold higher than those obtained from the SFE of grains. However, recovery of diols was not quantified. The retention times of 2,3-butanediols fluctuated by about 0.5 min depending on the amount of water.

Comparison of TIC Areas. TIC area comparisons have been made only to compare the amounts of compounds obtained by different procedures or obtained by the same procedure with different grains, assuming similar purge efficiencies among the procedures. Conclusions were drawn only when the differences were higher than the purge efficiency variation between runs. We did not compare TIC areas of different compounds because (i) the TIC area of each compound was different and (ii) purge efficiencies of different compounds within the same run could be different. This work was only a comparative study, and no quantitative calibration for each compound was made.

RESULTS

Comparison of SFE vs DHP and Ground vs Whole Grain. Table 1 provides a comparison of volatiles determined by SFE and DHP methods from whole and ground grain sorghum (G171S) with musty odor. Total ion chromatograms of volatiles obtained from ground sorghum G171S by the two methods are compared in Figure 1. Some of the compounds that appear in the chromatograms and were omitted from Table 1 were (i) aliphatic hydrocarbons; (ii) compounds that may be contaminants such as chloroform, tetrahydrofuran, benzene, and tribromomethane; (iii) isomers of aromatic hydrocarbons that occurred only as minor peaks; and (iv) compounds that gave minor peaks that could not be determined with certainty.

In general, with both whole and ground G171 sorghum, SFE gave more volatiles when compared to purging the grain directly with helium (Table 1 and Figure 1). In particular, SFE was better than DHP for extraction of 2,3-butanediols, acetic acid, lactones, methoxybenzenes (including 1-ethenyl-4-methoxybenzene, 1,2-dimethoxybenzene, chloro- and bromomethoxybenzenes), and the higher aldehydes (particularly from whole grain) such as octanal, nonanal, and decanal. Also, SFE obtained more of the relatively high molecular weight compounds than DHP, whereas DHP was better than SFE for volatile, low molecular weight compounds. DHP more efficiently extracted volatiles from ground grain than from whole grain, but that trend was only slight and was most significant for aldehydes such as hexanal, especially if the purge gas was forced to contact the surface of the grains efficiently by dispersing it through a frit. Further experiments with various other grain samples showed the same trend (data not shown).

Some prominent compounds observed in ground and whole grain by SFE and DHP included hexanal, 3-methyl-1-butanol, 1-pentanol, 1-hexanol, 2-pentylfuran, indene, and ethylbenzene (Table 1). Compounds that were more readily observed by DHP than SFE in both ground and whole grain included ethylbenzene, styrene, 2-methylpropanal, 2-butenal, 2-methyl-1-propanol, and 2-/3-methylbutanal (Table 1). With SFE, the volatile aldehydes and alcohol were apparently not efficiently trapped over dry ice in the glass tube used for collections of volatiles. A few compounds were observed only by SFE in ground grain, such as γ -isocapro lactone, benzyl alcohol, and 2-phenylethanol (Table 1).

SFE extracted a large amount of γ -butyrolactone from ground G167S sorghum (compound 42 in Figure 2), especially in the first extraction, whereas this lactone

was essentially not obtained from the same grain by DHP. γ -Valero- and γ -caprolactone were also extracted efficiently by SFE from G167S but not obtained by DHP.

Identification of Compounds. Mass and infrared spectra, coupled with the enhanced extraction by SFE, greatly aided the identification of several methoxybenzene compounds. Of particular interest were three isomers of chloro-1-methoxybenzene (m/z 144, **142**, 129, **127**, 101, **99**) and two isomers of bromo-1-methoxybenzene (m/z **186**, **188**, 171, 173, 143, 145) found in SFE extracts of sorghum G171S with musty odor (Table 1). The bold numbers represent masses with high intensities. The 4-halo isomers had a characteristic minor IR peak at ~ 820 cm⁻¹. The IR spectrum for the 4-chloro-1-methoxybenzene isomer had maxima at 1490 (m), 1281 (sh), 1243 (m), and 823 (w) cm⁻¹, and the spectrum for 4-bromo-1-methoxybenzene had maxima at 1482 (m), 1281 (sh), 1249 (m), and 818 (w) cm⁻¹. The peak intensities corresponding to bromo-1-methoxybenzenes were considerably lower than those of the chloro-1-methoxybenzenes (Table 1).

Relative amounts of bromo- and chloro-1-methoxybenzenes found in various grains are summarized in Table 2. Note that bromo-1-methoxybenzenes were found only in musty sorghums and not in other musty grains or sorghums with other types of odors. Even though, not as exclusive as bromo-1-methoxybenzenes, chloro-1-methoxybenzenes showed similar trends in that elevated levels were found in musty sorghums.

The compound observed at 16.45 min (Table 1) was identified as 6-methylsalicylaldehyde based on the following considerations. The MS for this compound matched the spectrum for 6-methylsalicylaldehyde from the MS library much better than spectra for the isomeric methoxybenzaldehydes. 6-Methylsalicylaldehyde was not in our IR databases. The IR spectrum of this compound had peaks at 3065 (w), 2884 (w), 1667 (vs), 1603 (m), 1455, 1301 (m), and 1190 (m) cm⁻¹. The phenolic OH group peak was at 3065 cm⁻¹ as compared with 3655 cm⁻¹ in phenol, and the aldehyde carbonyl peak was at 1667 cm⁻¹ as compared with 1730 cm⁻¹ in benzaldehyde. These shifts indicated stronger intramolecular hydrogen bonding than even in 2-hydroxybenzaldehyde, which has peaks for OH and C=O stretches at 3200 and 1680 cm⁻¹, respectively. The C=O stretching frequencies in the methoxybenzaldehydes are at 1712, 1725, and 1716 cm⁻¹ for the ortho, meta, and para isomers, respectively; thus ruling out these alternative possibilities for the structure of the compounds that were suggested by the MS spectral match information.

Two new nitro compounds, present in moderate levels, were identified by IR. One compound appeared to be 2-nitro-2-methylbutane {retention time 9.4 min; IR 2973 (m), 1569 (vs), 1453 (w), and 1382 (m) cm⁻¹}, and the other was a nitro carboxylic acid ester {retention time 15.39 min; IR 2973 (m), 1773 (s), 1573 (vs), 1498 (w), 1359 (w), 1266 (m), 1189 (m), and 1030 (m) cm⁻¹}. For these compounds, the intensity of the $m/z = 46$ peak was weak.

Comparison of Volatiles from Musty and OK Odor Sorghums. Figure 2 shows a comparison of volatiles obtained from SFE of ground G171S sorghum with a musty odor and ground G167S with an OK (normal) odor. Compounds associated with musty odors (i.e., 3-methyl-1-butanol [no. 10], 2-methyl-1-propanol [no. 4], and styrene [no. 24]) were present at much lower concentrations in sorghum G167S than in G171S.

Table 1. Volatiles Obtained from Musty Sorghum (G171S) by the SFE and DHP Methods

peak no.	ret. time (min)	compound	TIC area ^a			
			ground		whole	
			SFE	DHP	SFE	DHP
1	3.52	2-methylpropanal	0	6800	0	180
2	3.64	2-butenal	0	1600	0	0
3	3.82	2-butanone	9600	5800	430	350
4	4.05	2-methyl-1-propanol	6400	12400	0	69
5	4.33	3-methylbutanal	0	16400	0	250
6	4.40	2-methylbutanal	0	9000	0	130
7	4.56	acetic acid	16200	0	4800	0
8	4.80	2-butanol	1700	600	0	210
9	5.15	pentanal	2300	6900	2100	170
10	5.37	3-methyl-1-butanol	30400	43800	5100	1900
11	5.77	dimethyl disulfide	0	1500	0	220
12	5.93	1-pentanol	18200	12100	2400	320
13	5.98	toluene	1100	1500	0	260
14	6.70	hexanal	31100	91800	40600	2000
15	7.04	2,3-butanediol (dl)	11500	0	12300	0
16	7.57	2,3-butanediol (meso)	16500	0	16000	0
17	7.69	ethylbenzene	14500	44100	740	5600
18	7.84	hexanol	29800	26800	3000	520
19	7.92	methylpyrazine	1200	73	560	12
20	8.16	2-hexenal	330	1200	1800	0
21	8.34	2-heptanol	1200	1000	0	62
22	8.45	2-heptanone	2300	750	190	130
23	8.57	furan-2-carboxaldehyde	1200	0	57	0
24	8.74	styrene	8900	15300	500	1900
25	8.75	heptanal	3600	7500	1300	0
26	8.96	methyl hexanoate	1100	610	430	63
27	9.41	2-nitro-2-methylbutane	2800	4900	360	410
28	9.64	methoxybenzene (anisole)	36	91	29	3
29	9.77	dimethylpyrazine	4800	180	3700	5
30	10.01	heptanol	2700	1600	340	0
31	10.12	1-octen-3-ol	2700	1600	1300	120
32	10.16	2-pentylfuran	21800	23600	1900	620
33	10.34	3-octanol	1100	1800	1700	49
34	10.44	3-octanone	3400	1700	240	160
35	10.63	2-octanone	2900	4300	0	110
36	10.77	6-methyl-5-hepten-2-one	600	2300	570	74
37	10.79	limonene	450	2300	0	130
38	10.96	octanal	6300	2900	2300	26
39	11.12	2-ethyl-1-hexanol	1300	1700	410	38
40	11.14	methyl heptanoate	290	0	940	38
41	11.42	benzaldehyde	870	460	130	17
42	11.46	γ -butyrolactone	4000	0	790	0
43	11.82	2-methyl-2-heptenal	470	660	0	6
44	11.84	1,4-dichlorobenzene	380	270	0	17
45	11.91	polyalkylbenzene	890	1300	140	69
46	11.97	γ -valerolactone	1100	0	120	0
47	12.07	2-propenylbenzene	960	550	130	42
48	12.10	3-octen-2-one	980	2000	210	46
49	12.41	polyalkylbenzene	1600	500	260	45
50	12.49	polyalkylbenzene	560	2100	230	32
51	12.55	2-octenal	2700	210	1800	6
52	12.63	indene	16400	11900	2200	750
53	12.79	γ -isocapro lactone	3700	0	0	0
54	12.94	benzyl alcohol	870	0	0	0
55	13.14	nonanal	12400	6600	7400	71
56	13.34	benzeneacetaldehyde	5100	550	610	8
57	13.50	polyalkylbenzene	2100	910	370	78
58	13.70	3,5-octadienone + polyalkylbenzene	1400	310	0	0
59	13.78	acetophenone	1100	230	370	0
60	13.89	2-chloro-1-methoxybenzene	1100	590	190	34
61	14.02	methyl benzoate	6200	2400	210	5
62	14.06	γ -caprolactone	1200	0	190	0
63	14.20	4-chloro-1-methoxybenzene	2400	1100	330	40
64	14.33	polyalkylbenzene	550	23	130	5
65	14.55	2-methyl-1-propenylbenzene	450	650	0	3
66	14.62	nonenal	440	1300	240	0
67	14.66	2-phenylethanol	3800	0	0	0
68	14.70	3-chloro-1-methoxybenzene	35	12	2	1
69	15.16	4-ethenyl-1-methoxybenzene	17500	7800	1200	160
70	14.97	decanal	1500	0	2400	0
71	15.36	1,2-dimethoxybenzene	7500	2100	870	71
72	15.39	methyl 4-methyl-4-nitropentanoate	1700	0	460	0
73	15.59	1,4-dimethoxybenzene	640	620	0	1

Table 1 (Continued)

peak no.	ret. time (min)	compound	TIC area ^a			
			ground		whole	
			SFE	DHP	SFE	DHP
74	16.04	naphthalene	7800	3400	900	92
75	16.19	4-bromo-1-methoxybenzene	490	160	43	3
76	16.45	6-methylsalicylaldehyde	240	31	50	3
77	16.48	2-bromo-1-methoxybenzene	130	31	0	0
78	17.27	sesquiterpene (α -guiene?)	800	420	0	11
79	17.64	sesquiterpene	180	18	0	0
80	18.66	sesquiterpene	1000	370	0	26

^a TIC area/10E+03 per 10 g of grain.

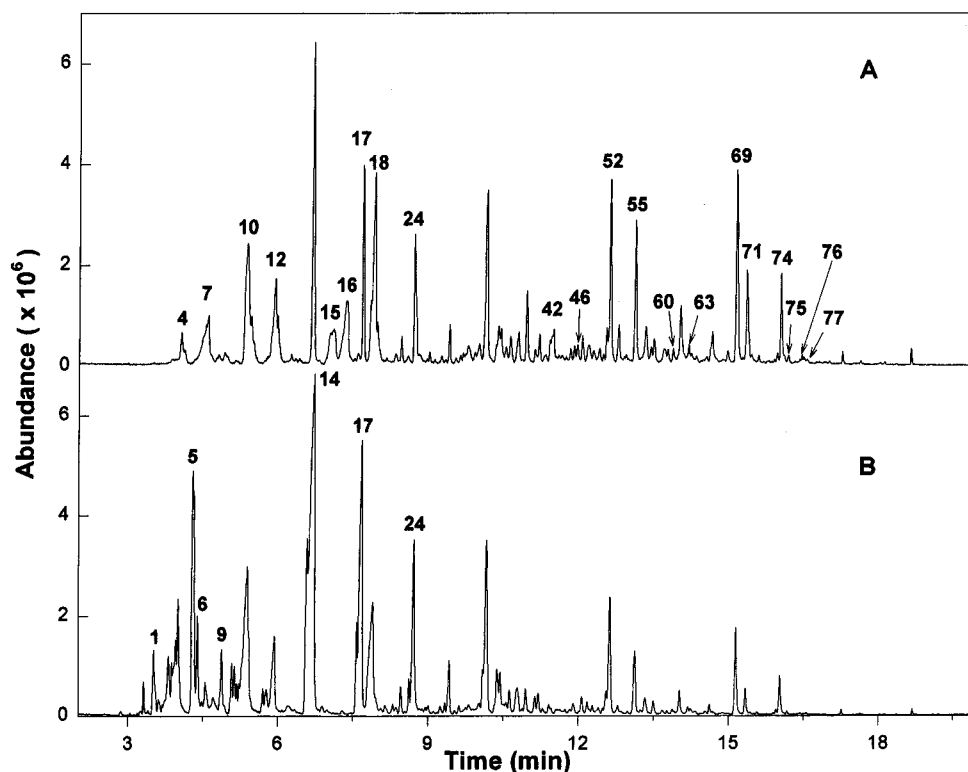


Figure 1. GC-MS total ion chromatograms to show a comparison of SFE (chromatogram A) and DHP (chromatogram B) procedures for collecting volatiles from ground sorghum (G171S) with musty odor. Abundance (*y*-axis) in chromatogram B was multiplied by 0.5 to normalize for the different weights of ground sorghum used in SFE (5.5 g) and DHP (11 g). SFE conditions were 35 °C and 17.2 MPa. SFE extract and DHP samples were purged in identical tubes at 80 °C with a helium flow rate of 40 mL/min for 10 min. Compound numbers are identified in Table 1. Compounds marked in chromatogram B (DHP) were the more volatile compounds and were more intense than those in chromatogram A (SFE). However, acetic acid and 2,3-butanediols were absent in DHP. Compounds marked in the second half of chromatogram A (SFE) were the less volatile compounds and were more intense than in chromatogram B (DHP).

Halogenated methoxybenzene compounds (chloro- [nos. 60 and 63] and bromomethoxybenzenes [nos. 75 and 77]) and other methoxybenzene compounds (4-ethenyl-1-methoxybenzene [no. 69] and 1,2-dimethoxybenzene [no. 71]), which are considered to be associated with musty odors, were absent in SFE extracts of G167S or any other sorghum grain with OK odor. Two compounds with retention times identical to that of 4-ethenyl-1-methoxybenzene and 1,2-dimethoxybenzene were found in G167S also, but mass spectrometry indicated that these compounds were decanal and tridecane.

The 2,3-butanediols and acetic acid were present in both samples G167S and G171S. G167S was only one illustrated example of OK odor sorghums that showed the extraction of *dl*- and *meso*-2,3-butanediols and acetic acid by SFE but not by DHP. Thus, these results seemed to indicate that the 2,3-butanediols were not associated with molds or musty odor.

Temperature and Pressure Dependence of SFE.

Figure 3 shows the effect of temperature from 30 to 90 °C at 13.8 MPa pressure on the extraction of volatiles by SFE. High temperature generally seemed to enhance the extraction of volatiles. Note in Figure 3 that the amounts of compounds generally increased with an increase in temperature for most compounds, whereas the amounts of acetic acid and the diols decreased somewhat. High temperature especially favored the extraction of compounds like 3-methyl-1-butanol, nonanal, chloromethoxybenzenes, ethenylmethoxybenzenes, dimethoxybenzenes, enals, benzeneacetaldehyde, and 2-pentylfuran. High temperature favored only slightly the extraction of hydrocarbons such as indene, ethylbenzene, and naphthalene, possibly because the extraction of these compounds may be limited by the amounts of these compounds present. The differences in extraction of polar vs nonpolar compounds was easily observed

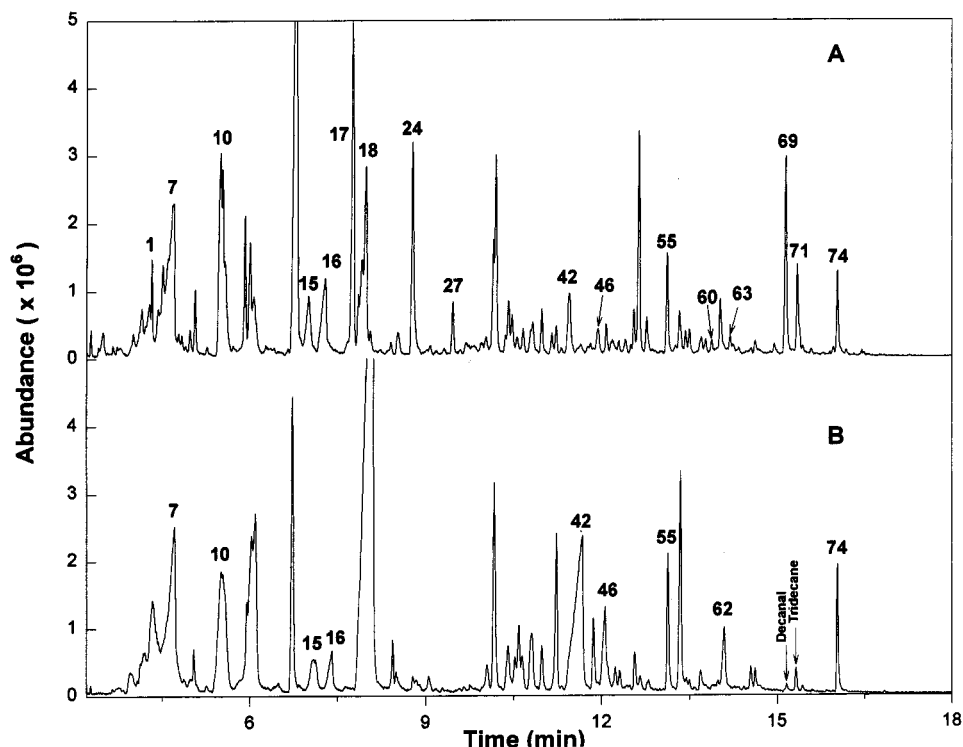


Figure 2. Volatiles obtained by SFE of ground sorghum samples with musty (G171S) and OK (G167S) odors are compared in GC-MS total ion chromatograms A and B, respectively. Compound numbers are identified in Table 1. Acetic acid and 2,3-butanediols were present in both samples. Compounds indicative of musty odor (i.e., compounds 60, 63, 69, and 71) are present in A but absent in B.

Table 2. Relative Amounts of Bromo- and Chloro-1-methoxybenzene Compounds in Selected Grain Samples

sample	grain type	odor ^b	extraction	abundance ^a				
				4-bromo-	2-bromo-	2-chloro-	4-chloro-	3-chloro-
G171S	sorghum	M 3.0	SFE ^c	4.26	0.53	8.20	16.20	1.50
H61S	sorghum	M 2.8	SFE ^d	0.19	0.39	0.10	0.57	0
H171S	sorghum	M 2.9	SFE ^d	0.54	0.67	0.15	10.70	0.30
H357S	sorghum	MS 2.2	SFE ^d	0.52	0.44	0.09	5.64	0.14
H370S	sorghum	Ms 2.3	SFE ^e	0.11	0.24	0	3.00	0
G167S	sorghum	OK	SFE ^d	0	0	0	0.15	0
FH88S	sorghum	OK	SFE ^d	0	0	0.04	0.05	0
H455S	sorghum	I 2.9 x	SFE ^e	0	0	0.31	1.05	0.09
H456S	sorghum	I 2.7 x	SFE ^d	0	0	0.35	0.88	0.07
H483S	sorghum	I 3.0 x	SFE ^d	0	0	0	0.24	0
H252C	corn	M 2.5	SFE ^d	0	0	0.04	0.37	0.04
H351C	corn	M 3.0	SFE ^d	0.04	0.05	0	0.26	0.05
H313C	corn	M 3.0	SFE ^d	0	0	0	0	0
F95C	corn	M 3.0	DHP ^f	0	0	0	0	0
H308W	wheat	M 2.9	DHP ^f	0	0	0	0	0

^a Abundance/10E+5 of *m/z* 186 and 142 for bromo and chloro compounds, respectively. Retention times are given in Table 1. ^b M, musty; I, insect; S, sour; W, weed. A lowercase letter indicates that the odor was minor. Odor intensities were judged on a 0–3 scale and are averages of six or more panelists. An x indicates that other musty-indicating methoxybenzene compounds were present, which gave evidence for mustiness even though another major odor was detected by the panelists. ^c 13.8 MPa, 90 °C, ground grain. ^d 13.8 MPa, 30 °C, ground grain. ^e 17.2 MPa, 30 °C, ground grain. ^f Whole grain.

with 2-octenal and indene, which occurred close together in the chromatogram (Figure 3). At 30 °C, the amount of indene extracted was far greater than the amount of 2-octenal, whereas at 90 °C the order was reversed.

Figure 4 shows the effect of pressure from 10.3 to 41.4 MPa at 35 °C on the extraction of volatiles by SFE. Unexpectedly, the best collection of volatiles was achieved at low pressures, and this was true over the entire temperature range. Even at 90 °C when volatiles were collected at 13.8 and 41.4 MPa the same trend was observed in that the higher pressure gave considerably lower amounts of volatiles than the lower pressure. On the other hand, extraction of oil and other relatively nonvolatile lipids was enhanced by SFE at high pres-

ures. The amount of these lipids collected was quite noticeable. When 6 g of ground sorghum was extracted at 10.3 MPa and 35 °C, an unmeasurable amount of oil was obtained as determined by difference in weight of the collection tube before and after the extraction. However, when the pressure was increased to 41.4 MPa at 35 °C, about 70 mg of oil was obtained. At 90 °C at 41.4 MPa, about 100 mg of oil was collected. These nonvolatile lipids were not carried through the purge-and-trap system, so they were not seen in the chromatogram.

Repeated Extractions. Figure 5 shows the amounts of some typical volatiles along with the 2,3-butanediols and acetic acid as a function of the extraction number

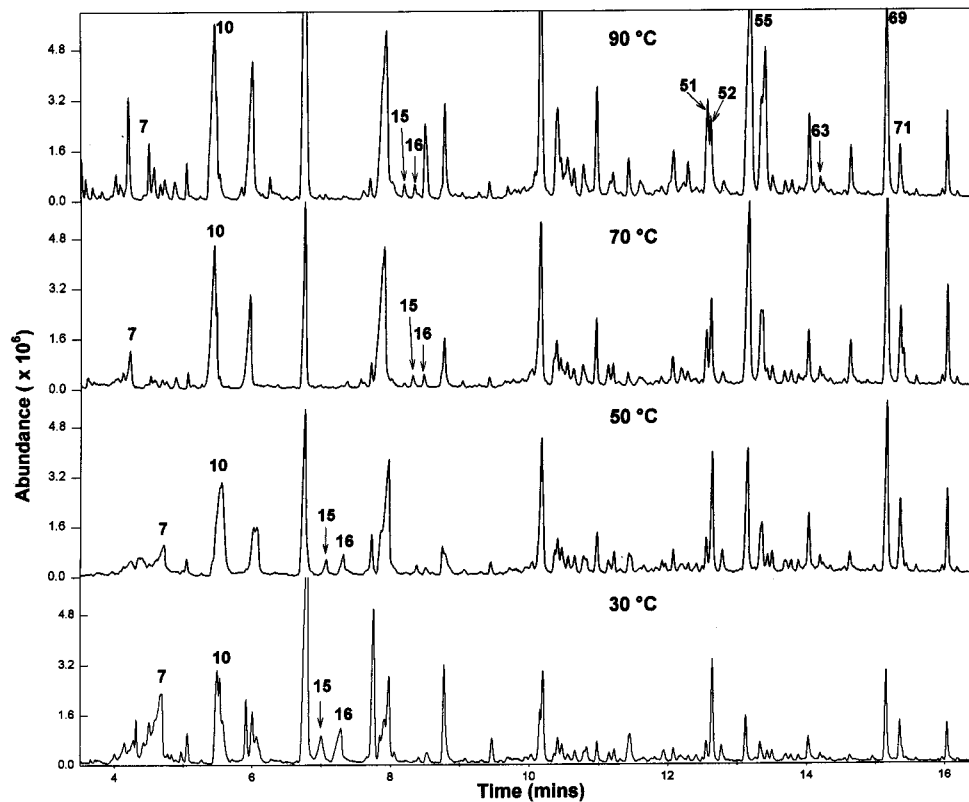


Figure 3. GC-MS total ion chromatograms showing the effect of temperature (30, 50, 70, and 90 °C) on the SFE extraction of ground sorghum (musty, G171S) at 13.8 MPa. Compound numbers are identified in Table 1. The aldehydes 2-octenal (no. 51) and nonanal (no. 55) were particularly enhanced at higher temperatures, while indene (no. 52) intensity was nearly constant. Recovery of 2,3-butanediols was however adversely affected at high temperatures.

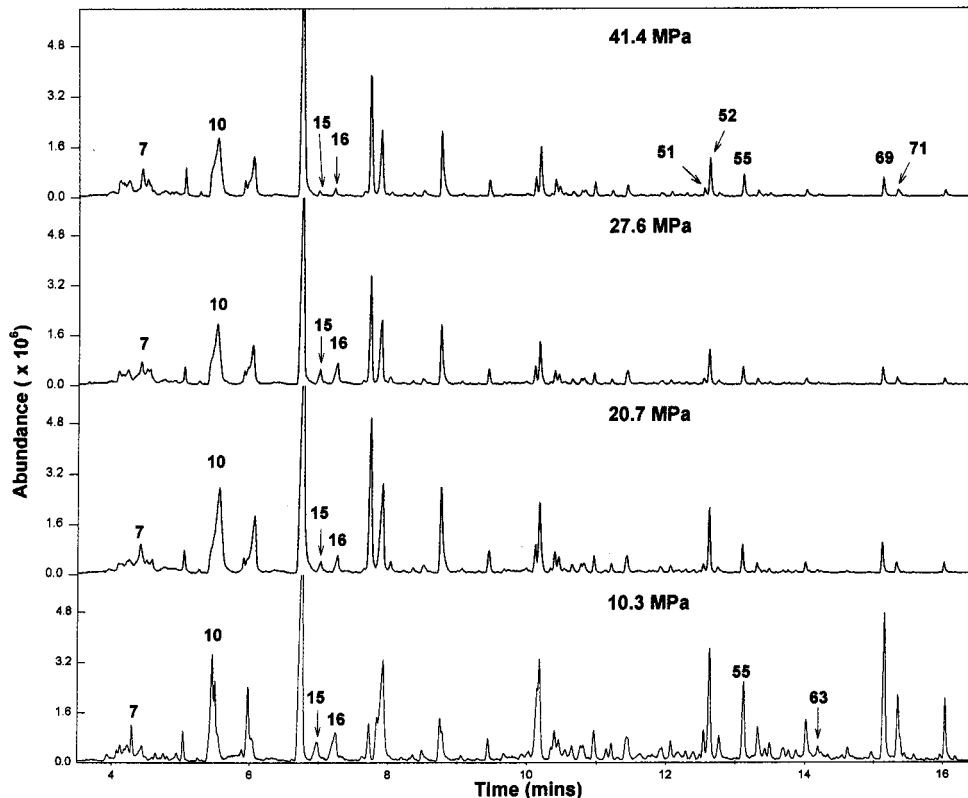


Figure 4. GC-MS total ion chromatograms showing the effect of pressure (13.8, 20.7, 27.6, and 41.4 MPa) on the SFE extraction of ground sorghum (musty, G171S) at 35 °C. Compound numbers are identified in Table 1. High pressures were not favorable for the extraction of volatiles.

for repeated SFE of the same sample of ground sorghum G171S. Note that, as the extraction number increased,

the amounts of volatile compounds decreased except for 2,3-butanediols and acetic acid. The total amount of 2,3-

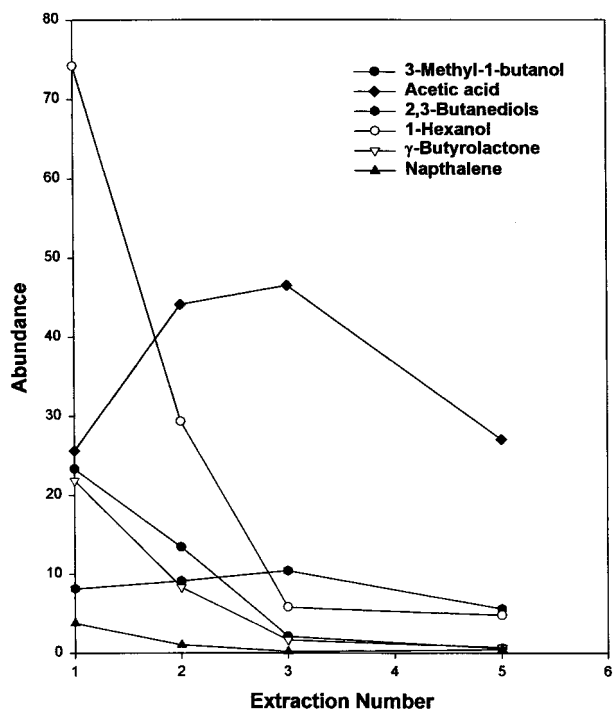


Figure 5. Peak areas (abundance) from GC-MS total ion chromatograms vs extraction number for selected compounds repeatedly extracted by SFE (13.8 MPa and 35 °C) from a ground sorghum (G167S) sample. Concentrations of most volatiles decreased greatly by extraction no. 3, but the 2,3-butanediols and acetic acid continued to evolve considerably at extraction no. 5.

butanediols and acetic acid present in the grain appeared to be vast (as indicated by adding up all of the amounts from the repeated extractions) in proportion to that of other volatiles. Because these compounds continued to evolve at a relatively high rate, it appeared that they may be part of the grains, perhaps in some type of bound form. These observations were made several times with repeated extractions of whole and ground samples of sorghum G171S and were especially noticed in the initial phase of this work when up to eight repeated extractions of the same sample continued to give relatively high amounts of 2,3-butanediol and acetic acid while amounts of most of the other compounds decreased.

Extraction number dependence similar to that observed for G171S was observed with the OK odor ground G167 sorghum samples. Even the γ -butyrolactone, which appeared to be present in very large amounts in the first SFE extraction, fell off sharply with repeated extraction, but the 2,3-butanediols and acetic acid were still evolving at a nearly steady rate at repeated extraction, up to extraction no. 5. Again, diols were not obtained by direct-helium-purge of G167S.

DISCUSSION

Selectivity in SFE of Volatiles vs Lipids: Density, Temperature, and Pressure Dependence. The density of CO₂ at 13.8 MPa decreases from about 0.80 g/mL at 35 °C to about 0.33 g/mL at 95 °C, and at 41.4 MPa the density changes from 0.96 to 0.75 g/mL for the same temperature change. The density of CO₂ is increased as pressure is increased, and near the supercritical temperature it shows a steep sigmoidal behavior before substantially leveling off in the supercritical

range. Generally, the solubility parameter shows the same trend, i.e., the solubility sharply increases near supercritical conditions and the critical point is near the midpoint of this sharp increase on the solubility-pressure diagram. Because the solubility of compounds in fluid solvents increases with density and temperature, one expects a more efficient extraction of components by SFE. These SFE conditions favored the extraction of oils from the grains, as evidenced by visible oil and the weight of the extracted material in the sample tube.

We found that volatiles from grain samples were nearly selectively extracted in the pressure range 10.3–13.8 MPa. Although volatiles were extracted with liquid CO₂ (i.e., $T < 31.5$ °C), the efficiency of extraction increased with temperature, and SC-CO₂ was better than liquid CO₂ even for volatiles. However, when we increased the pressure to greater than ~13.8 MPa, the amount of oils extracted from sorghum increased greatly. Since we observed reduced amounts of volatiles with extractions at pressures greater than ~13.8 MPa, it was possible that the extraction of the oil may have adversely affected the extraction of volatiles from the grain or that the volatiles were extracted from the grain but not efficiently purged from the oil in the sample tube on the purge-and-trap instrument.

These results appeared to be similar to the findings reported for extraction of volatile flavor components in roasted peanuts by SFE, in that volatiles were selectively extracted over lipids at low SC-CO₂ pressures (SC-CO₂ density about 0.35 g/mL) (Leunissen et al., 1996). We found that low pressures of SC-CO₂ were more important than high temperatures of SC-CO₂. This was true probably because at low temperatures the solubility of volatile compounds could be increased with SC-CO₂ density, whereas at the high temperatures the solubilities of volatile compounds in SC-CO₂ were so sufficiently heightened that even low-density SC-CO₂ was enough to get a good extraction.

Snyder and King (1994b) reported on trapping lipids and volatile compounds with a Tenax column from oil samples by SFE at 55.2 MPa and 50 °C. When they desorbed the Tenax trap thermally at 150 °C with helium flow, some oil presumably decomposed to give higher amounts of the volatiles pentane, propanal, and pentanal. When the trap was desorbed at 50 °C with SC-CO₂ at 13.8 MPa, Snyder and King observed more of the high molecular weight compounds than at 150 °C. They observed 1-octen-3-ol and 2-octenal as two of the degradation products of oils on desorption of Tenax by SFE at a temperature of 150 °C, but not at 50 °C. This may be similar to our observation that the oil in soybeans seemed to decompose to give 1-octen-3-ol when purged with added water at 80 °C (Ram et al., 1997). The increase in 2-octenal with an increase in temperature in our experiments (Figure 3) may be due to some lipid degradation at the elevated temperatures. The pathways leading to 2-octenal by retro-aldol reaction of 2,4-decadienal produced from oxidation of linoleate and to 1-octen-3-ol from oxidation of linoleate have been described (Ho and Chen, 1994; Hwang et al., 1994; Assaf et al., 1995). However, while we agree that oil could decompose in the presence of some solvents such as SC-CO₂ or H₂O, we have not obtained evidence of decomposition of any volatile compounds upon thermal desorption of the Tenax trap even at 225 °C.

The temperature dependence of 2,3-butanediols and

acetic acid gave an opposite relationship to that of other volatiles. The extraction of 2,3-butanediols and acetic acid may have been influenced by hydrogen bonding to CO₂, perhaps through two hydrogen bonds forming a cyclic structure similar to the well-known cyclic carbonate esters. Such hydrogen bonding could be weakened at high temperatures and thus make the extraction of the diols into SC-CO₂ less favorable.

Repeated SFE and the Abundance of 2,3-Butanediols. Repeated extraction of ground sorghum (Figure 5) continued to give 2,3-butanediols and acetic acid. The extraction of these compounds did not appear to be limited by the solubility in SC-CO₂ because results from an extraction of about 3000 ppm diols in 1 mL of water showed at least a 100-fold increase in peak size as compared to the amount from the grains. It appeared as though the diols and acetic acid were bound to the grain matrix and that SC-CO₂ was able to extract only equilibrium amounts of these compounds each time. It was apparently not a kinetic phenomenon because equilibration times of 5 min or 1 h yielded the same amount of diols.

Because the diols in nearly undiminished amounts were obtained on repeated extractions, the total amount of diols present in the grains appeared to be vast and they may even be part of the grain (perhaps bound to the lipids or aldehydes as cyclic acetals). In addition, both musty and OK odor sorghums exhibited this behavior. Although not as extensively studied, several other grains examined also gave diols with repeat extractions by SFE. In another study on wheat samples from different locations, we have found that 2,3-butanediols extracted by SFE gave greater amounts than reported here (Seitz et al., 1997).

Significance of Chloro- and Bromomethoxybenzenes and Their Relevance to Musty Odor. The occurrence of chloro- and bromo-1-methoxybenzenes in grains was unexpected. The presence of these compounds in grains had not been previously reported. During the analyses of many grain samples, we have occasionally found trace amounts of chlorobenzene, bromobenzene, 1,4-dichlorobenzene, and chloropentane, but they did not contribute to any off-odor. However, from our observations (Tables 2 and 3), we believe that the halo-1-methoxybenzenes were associated with musty odors in grains. Other methoxybenzene and substituted methoxybenzenes, such as 1,2-dimethoxybenzene and 4-ethenyl-1-methoxybenzene, are known musty odor indicators (Seitz and Sauer, 1991, 1994). Chloroanisole was associated with a musty taint in rice when its concentration exceeded 5 ppb (Maarse, 1987). Also, it has been shown that trichloroanisole (TCA) (Buser et al., 1982; Maarse et al., 1985, 1988) and tribromoanisole (TBA) (Whitfield et al., 1997) are potent musty odor compounds. They indicated that the odor threshold of TBA was as low as 2×10^{-5} µg/L in water. TCA and TBA are known to be formed by molds growing in nongrain materials such as fiberboard packing materials (Allard et al., 1987; Boyle et al., 1992; Jerina et al., 1968; Sheikh et al., 1985; Tindale, 1987; Tindale et al., 1989). These compounds diffuse through polyethylene and other packing materials to cause musty odor in dried fruits such as raisins (Aung et al., 1996). Other polyhalogenated anisoles such as di-, tetra-, and pentachloroanisoles have also been associated with musty odor (Whitfield et al., 1985, 1986). Exactly how the monochloro- and monobromoanisoles are formed in

Table 3. Off-Odors in the H-series Set of Samples That Had the Highest Concentrations of 4-Chloro-1-methoxybenzene Determined by DHP Using the Archon Autosampler

sample	grain type	odor ^a	amount
H45W	wheat	MI 2.9	2.54
H171S	sorghum	MI 3.0	1.82
H370S	sorghum	Ms 2.3	1.82
H357S	sorghum	MS 2.2	1.78
H706S	sorghum	MS 2.2	1.58
H359S	sorghum	M 1.3	1.54
H352W	wheat	I 2.9 x	1.39
H350S	sorghum	MS 2.2	1.39
H371S	sorghum	M 2.2	1.21
H217S	sorghum	SM 2.7	1.19
H70S	sorghum	S 2.0 x	0.91
H149S	sorghum	M 1.7	0.89
H209W	wheat	I 2.6 x	0.85
H132S	sorghum	Ms 1.3	0.84
H218W	wheat	I 2.7 x	0.83
H314S	sorghum	S 2.0 x	0.47
H717S	sorghum	M 2.1	0.47
H47W	wheat	IW 2.7 x	0.45

^a M, musty; I, insect; S, sour; W, weed. A lowercase letter indicates that the odor was minor. Odor intensities were judged on a 0–3 scale and are averages of six or more panelists. An x indicates that other musty-indicating methoxybenzene compounds were present, which gave evidence for mustiness even though another major odor was detected by the panelists.

grains is not known, but it is reasonable to consider that molds may be involved and that the biochemical pathways may be similar to those reported for other materials. Because other methoxybenzenes (such as anisole, 4-ethenylanisole, and 1,2-dimethoxybenzene) are associated with molds and musty odor, the chloro- and bromoanisoles may also be associated with musty odor (Whitfield et al., 1987). They may have odor thresholds lower than 1-ethenyl-4-methoxybenzene and 1,2-dimethoxybenzenes but perhaps not as low as TBA or TCA.

A large group of samples (H-series, ~750 samples representing various odors in corn, sorghum, soybeans, and wheat) was analyzed by direct-helium-purge of grains with an Archon (Varian Associates) autosampler for rapid and consistent analysis of volatile organic compounds. Data on 18 samples having the highest concentrations of 4-chloro-1-methoxybenzene are summarized in Table 3. Thirteen of these samples were sorghum and five were wheat. Chloro-1-methoxybenzenes were preferentially found in sorghum, and bromo-1-methoxybenzenes seemed to be even more exclusively associated with musty sorghums. We do not know the reason for these apparent associations.

CONCLUSIONS

In general, the SFE method was more effective than the DHP method for obtaining volatiles from grains. DHP was better for highly volatile compounds. Most volatiles were readily extracted from the grains by SFE, while others, especially 2,3-butanediols and acetic acid, continued to be extracted from a sample even after many repeated extractions. The 2,3-butanediols were obtained by SFE of many other grains, including OK odor wheat and sorghum samples, and thus were not associated with off-odors in grains. Monochloro- and monobromo-1-methoxybenzenes were present in SFE extracts of musty odor sorghums and were absent in OK odor grains. Ground samples generally yielded higher amounts of volatiles than whole grains, but

grinding was not necessary for detection of volatiles associated with off-odors. As expected, higher SFE temperatures improved the extraction of many compounds. Unexpectedly, higher SFE pressures resulted in lower amounts of most volatiles detected by the GC-MS/IR system.

In further preliminary work, we have found the SFE method useful for extracting various other types of compounds from grains with different odors, i.e., insect metabolites from corn, sorghum, and wheat with insect type odors; geosmin and related compounds from corn with earthy-musty odors. The SFE method gave efficient extraction of chloro- and bromomethoxybenzenes that may be associated with musty odor in sorghum grain. Further SFE work on a large number of grains is needed to substantiate these findings.

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