Use of an Autosampler for Dynamic Headspace Extraction of Volatile Compounds from Grains and Effect of Added Water on the Extraction

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An autosampler attached to a purge and trap instrument was used to aid routine analyses of grain samples for volatile compounds associated with off-odors. Trapped volatiles were transferred to a gas chromatograph/mass spectrometer instrument for separation and detection. Dynamic extraction of volatiles from ∼18 g of whole grain at 80 °C was accomplished by purging helium through a sample vial with a Teflon-lined septum on each end. The autosampler automatically added internal standard to the sample before purging began, which required the addition of 1 mL of water for complete transfer of the standard to the sample. The added water enhanced extraction of 1-octen-3-ol, 1-octen-3-one, and some other compounds from soybeans but not from starchy grains such as corn and wheat. Addition of a free radical scavenger, such as citric acid, greatly diminished the recovery of 1-octen-3-ol and 1-octen-3-one from soybeans.

Keywords: *Grain volatiles; headspace autosampler; odor; soybean; 1-octen-3-ol*

INTRODUCTION

In the United States, odor is an important factor in grain grading. All samples submitted for grading are smelled according to guidelines in the Grain Inspection Handbook (U.S. Department of Agriculture, 1997). Development of objective off-odor detection and classification methodology for possible use in grain grading has stimulated research on volatile components of grains (Seitz and Sauer, 1992). In previous studies, we used dynamic headspace (purge and trap) methods to collect volatile compounds from grains, which were transferred to a gas chromatograph/mass spectrometer/infrared spectrometer for separation and identification (Seitz and Sauer, 1994; Seitz, 1995; Seitz et al., 1999). The manual procedure for handling each sample in those analyses was tedious, and the results indicated that many samples need to be analyzed to adequately identify relationships between volatiles and off-odors in grains.

Here, we describe the use of an autosampler for routine handling of grain samples for improved convenience. An important feature of the autosampler was that it automatically added an internal standard (ISTD) solution to each sample. However, consistent transfer of ISTD to the sample required the addition of at least 1 mL of water. We were concerned that the added water, and subsequent heating of the sample, could change the profile of volatiles purged from the grains.

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MATERIALS AND METHODS

Samples. A set of commercial grain samples was collected by the Grain Inspection Packers and Stockyards Administration/Federal Grain Inspection Service. The sample set consisted of 745 samples total, including 202 soybean, 210 corn, 97 sorghum, and 236 wheat. The odor of each sample was assessed by official grain inspectors and by a panel at our laboratory. Odors were classified on an intensity scale of 0−3 as ok (normal), musty, sour, insect, smoke, and COFO (commercially objectionable foreign odor, which includes insecticides, solvents, weed, and other miscellaneous off-odors). The samples were kept in cold storage (∼4 °C) until analyses were conducted.

All of these samples were analyzed by using the autosampler in the mode in which internal standard (ISTD) and 1 mL of water were added to each sample as described below. To assess the effect of added water on volatiles purged from samples, a selected set of 114 samples (33 soybean, 35 corn, 11 sorghum, and 35 wheat) representing all odors was analyzed by using the autosampler in a mode in which no water was added to the sample and the ISTD was manually injected into the sample before heating and purging began. The purges with and without added water are referred to as wet and dry purges, respectively.

Autosampler for Purging of Volatiles. An Archon autosampler (Model 5100A, Varian Associates, Walnut Creek, CA) originally designed for analyses of soil samples was used to purge the volatiles from grain samples. In addition to moving samples to and from loading postions in a given sequence, the autosampler added an internal standard, preheated the sample, and then continued heating while the sample was purged at a set flow rate for a set period of time.

Samples were loaded in 40 mL Soil-Vials with screw caps on both ends and a frit at the bottom end. The screw caps used 22 mm EPA low-bleed septa with Teflon liners. With this system, several silyl and chlorinated compounds and CS_2 were consistently detected by the GC/MS system at varying levels even after the septa had dried at 200 °C overnight, which was the best way to minimize these contaminants. The purge gas

10.1021/jf990243e CCC: $18.00
Published on Web 09/14/1999

Table 1. Ratios of Wet-Purge to Dry-Purge Amounts for Selected Compounds

	soybean				corn				sorghum		wheat	
compound	KM 2.1[a]	ok	M 1.9	ok	B 2.3	M 2.0	S 3.0	M 3.0	B 2.8	M 2.0	MI 2.9	ok
1-chloro-4-methoxybenzene	–[b]	–	–	–	–	–	–	1.3	1.0	–	0.6	–
1,2-dimethoxybenzene	2.9	–	0.4	–	–	1.2	0.4	1.4	–	0.7	0.6	–
1-ethenyl-4-methoxybenzene	–	–	–	–	–	1.6	–	1.8	–	–	0.6	–
3-methyl-1-butanol	6.6	1.7	1.9	4.6	1.2	1.3	0.4	1.0	2.1	0.8	0.8	0.6
methoxybenzene (anisole)	–	–	–	–	–	–	–	–	–	–	0.8	–
1-octen-3-ol	44.8	12.5	10.5	24.8	1.1	1.4	0.5	1.7	1.4	0.6	0.7	0.5
3-octanone	–	–	–	–	0.7	1.4	0.6	1.1	1.9	–	0.8	0.4
geosmin	–	–	–	–	–	–	–	3.4	–	–	0.7	–
nitromethane	–	–	–	–	–	1.2	–	–	–	2.0	–	–
2-pentanol	6.4	–	1.5	4.0	1.4	1.6	–	1.2	1.5	1.9	1.1	0.5
1-pentadecene	–	–	–	–	–	–	–	–	1.0	–	0.5	–
sesquiterpene (insect)	–	–	–	–	–	–	–	–	–	–	0.6	–
2-pentanone	–	1.7	1.7	3.6	1.5	1.4	0.6	1.2	1.9	1.1	1.0	0.5
linalool	–	–	–	–	1.2	–	–	–	1.8	–	–	–
styrene	2.2	0.9	0.5	2.0	1.3	1.4	0.7	1.1	1.3	–	0.9	0.5
methyl acetate	0.3	–	0.2	1.6	0.8	0.4	0.7	–	2.2	–	0.4	2.0
ethyl acetate	–	–	–	–	1.2	–	0.4	–	–	–	–	–
methyl butanoate	2.2	–	0.4	1.2	0.5	0.5	2.1	–	2.9	0.5	–	1.7
ethyl butanoate	–	–	–	–	–	–	0.6	–	–	–	–	–
methyl pentanoate	2.6	–	0.5	–	0.4	–	–	0.2	1.0	0.3	0.2	2.2
methyl 3-methylbutanoate	–	–	–	–	1.3	–	0.6	–	–	–	–	–
ethyl pentanoate	–	–	–	–	–	–	0.6	–	–	–	–	–
methyl hexanoate	3.2	0.5	0.5	1.7	0.4	0.2	–	0.2	0.8	0.3	–	2.5
benzofuran	2.8	0.9	–	1.8	1.3	1.4	–	1.3	1.6	1.1	0.8	0.6
1*H*-indene	2.8	0.9	0.4	2.1	1.3	1.4	–	1.2	1.4	1.0	0.7	0.6
2-methylbenzofuran	2.4	–	–	–	1.4	1.4	–	1.4	1.3	–	0.7	0.7
hexylbenzene	2.2	–	–	–	–	–	–	1.0	–	1.0	–	–
2-ethylpyridine	–	–	0.4	4.7	1.3	–	–	0.7	0.3	2.8	0.6	0.6
S=P(SMe)(OMe)2[c]	–	0.9	–	–	–	–	–	1.4	–	–	0.6	–

[a] Letters indicate odor categories: B, barnyard sour; K, smoke; M, musty; S, sour; ok, ok or normal. Numbers indicate intensity level on a 0–3 scale. [b] The "–" indicates that little or no compound was present. [c] From breakdown of the insecticide Malathion.

entered at the bottom and exited at the top of the vial through a Silcosteel (Restek Co., Bellefonte, PA) treated needle with holes on the side. The inlet needle was just long enough to puncture the bottom septum and would not reach the bottom frit. The vials were filled with samples, with some headspace room left for the helium purge outlet needle, and then placed in the sample tray. Samples were automatically analyzed in sequence.

To each sample the autosampler added 1 μL of the ISTD solution from a loop on a valve through the top needle. The autosampler then rinsed the loop with at least 1 mL of water and added that to the sample vial. The sample was heated to 80 °C, equilibrated for 3 min, and then purged for 8 min. The purged volatiles were transferred to a Tenax-TA trap in a purge and trap instrument (described below) through a heated transfer line with Silcosteel (Restek Corp.) coated interior. The transfer line was kept at 130 °C to avoid condensation and decomposition and still allow efficient transfer to the Tenax trap. The trap was held at 30 °C while the sample was purged and compounds were transferred to the trap. Because all operations of the autosampler could not be exactly synchronized, the purge flow rate increased to nearly 90 mL/min during the first minute and then quickly dropped to the set value of 40 mL/min for the remainder of the 8-min purge time.

Purge and Trap GC/MS system. A Hewlett-Packard Co. (Palo Alto, CA) purge and trap (Model G1901A-60500) instrument was used with the following modifications to remove active and "cold" spots. 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 inert silica tubing. The moisture control device was bypassed by using a short piece of Silcosteel coated tubing to directly connect the top of the Tenax trap to the valve. The stainless steel frit at the top of the trap was removed. The stainless steel tee and the fittings at the top of the trap were Silcosteel treated (maximum inertness type). The top of the trap was passively heated by the valve oven, so the valve oven was heated to 225 °C to allow efficient desorption of the Tenax trap. The desorption time was increased from 4 to 6 min (4 min is usually used) because the flow rate through the GC column, which is also the flow rate through the trap when the purge and trap instrument is in the desorb mode, could not be increased much beyond 1 mL/min without shutting down the mass spectral detector. With these modifications, compounds (especially 1-pentadecene, longifolene, and 1,2-dimethoxybenzene) that were not efficiently moved through the purge and trap instrument without these modifications appeared on the total ion chromatograms at the expected intensity levels. As explained below, longifolene was used as a pseudo-standard for the unidentified sesquiterpene associated with insects.

After volatiles were collected, a 10-min dry purge was performed to remove excess moisture from the Tenax trap. The trap was preheated at 220 °C, and the volatiles were desorbed at 225 °C for 6 min. With the capillary interface module, the desorbed volatiles were cryofocused at −140 °C at the top of the GC column. The cryofocused zone was heated at 200 °C for 0.85 min before initiation of the analytical run. Volatiles were analyzed with a GC (Model 5890, Series II, Hewlett-Packard) coupled with an MS detector (Model 5971, HP). A BPX5 column (50 m × 0.32 mm i.d. × 0.25 μm film thickness, 5% phenyl-polysilphenylene-siloxane) from Scientific Glass Engineering Inc. (Austin, TX) was used for separation. Carrier gas was helium at a constant flow rate of ~1.0 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 increased to 260 °C at a rate of 17.5 °C/min.

Effluent from the GC column was delivered directly to the MS detector with the transfer line temperature set at 280 °C. Electron impact energy of the ion source was 70 eV and masses were scanned over the range 33–250 amu. The mass spectrometer was tuned using the high sensitivity tune procedure provided by the HP Chemstation software, which enhanced sensitivity severalfold over that provided by the standard tune procedure.

Compounds were identified by comparing, with the aid of a computer and careful visual examination, experimental mass spectra of compounds with standard spectra in the HP 59943B Wiley PBM MS database. GC retention times were considered in the compound identifications, and authentic standards were

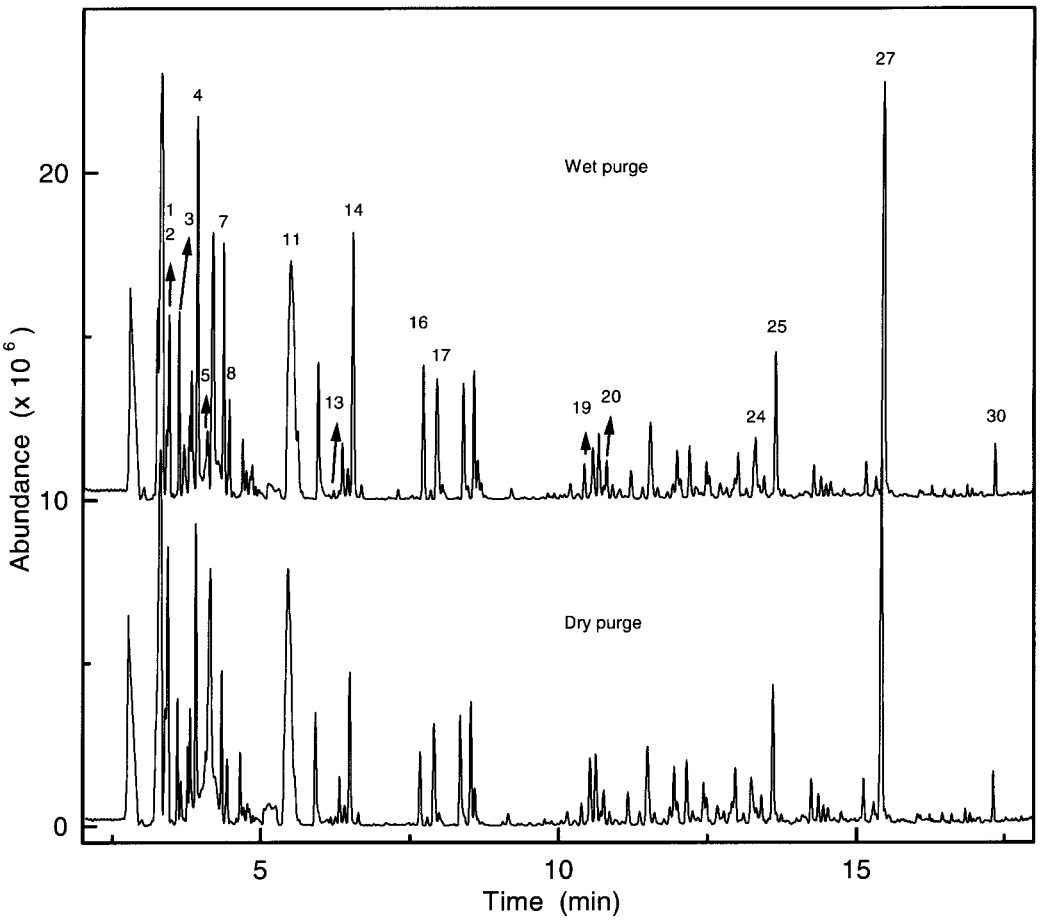


Figure 1. Total ion chromatograms for wet and dry purges of a corn sample with sour odor. Peak numbers are identified in Table 2.

utilized when possible. Compound identifications were also based on some standards run in previous studies conducted in this laboratory concerning volatiles in grain samples [see Seitz (1995)].

Target Analysis. Target analysis of selected compounds was performed by using Environmental Data Analysis (EDA) software from Hewlett-Packard Co. Ethylbenzene-d_{10} was chosen as the ISTD. The recovery of this compound from all of the grains was complete, which was not true when 4-bromo-1-fluorobenzene and naphthalene-d_8 were tried as standards. Furthermore, the target mass of *m/e* 116 selected for quantitative detection of ethylbenzene-d_{10} had no interference from other compounds, and the compound itself does not occur naturally.

Consistency in delivering the ISTD to an empty sample vial and subsequent recovery from the vial were checked by conducting 19 repeated analyses for the ISTD with the autosampler automatically adding the ISTD and water according to the usual wet-purge protocol. To each vial the autosampler added 1 μL of ISTD in methanol (50 ng of ethylbenzene-d_{10}) followed by the required 1 mL of water to ensure complete transfer of ISTD to the vial. Highest variation in ISTD response was only 15% of the average, and relative standard deviation (RSD) was 9.0%. When 1 μL of ISTD solution was manually added to an empty vial, and no water was added, the ISTD response obtained was essentially the same as the average response for the 19 analyses described above.

With 43 grain samples (14 corn, 2 sorghum, 11 soybean, and 16 wheat) analyzed by using the same autosampler protocol, average ISTD response was ~75% of that observed for ISTD added to empty vials. Adding water to a grain sample had no effect on ISTD response, as evidenced by the fact that the mean ISTD response from analyses of 114 samples (representing all grain types and various odors) by the wet-purge method was not significantly different (*t* test, 95% confidence level) from the average ISTD response when the same samples were analyzed by the dry-purge method; that is, ISTD area counts ($\times 10^4$) were 1209 (RSD = 20%) and 1150 (RSD = 29%) for wet- and dry-purge methods, respectively. The RSD for ISTD response in the wet-purge method in which ISTD was added automatically was slightly lower than that for the dry-purge method in which ISTD was added manually.

Compounds selected for target analysis, listed in Table 1, are possibly associated with off-odors in grains (Seitz and Sauer, 1992, 1994; Seitz, 1995; Seitz et al., 1999). Except for two compounds mentioned below, authentic standards were used to set up the target analysis parameters for each compound, that is, the GC retention time, quantifying target ion, and up to three qualifier ions. Sesquiterpene (insect) was quantified by using longifolene as a pseudo-standard, and P=S(SMe)$(OMe)_2$ was analyzed with reference to a particular wheat sample having this compound at an arbitrarily set value of 100. A calibration plot consisting of four to eight data points was obtained for each compound. To correct for differences in purging and recovery among different runs, the ratio of response of the target compound to that of the ISTD was plotted against the ratio of the concentration of the target compound to that of the ISTD. Calibration plots were generally linear in the range of 10−200 ng. A linear fit with zero intercept was used in quantifying the target compounds with the EDA software. Peaks identified as target compounds were further reviewed for correct compound identification and integration of the quantifying target ion response.

Effect of Citric Acid. The effect of added citric acid on 1-octen-3-ol produced from soybeans was determined by conducting three separate experiments with different soybean samples. In each experiment, a soybean sample (18 g) was thoroughly mixed with 1 mL of water for one analysis and with a solution of 500 mg of citric acid dissolved in 1 mL of water

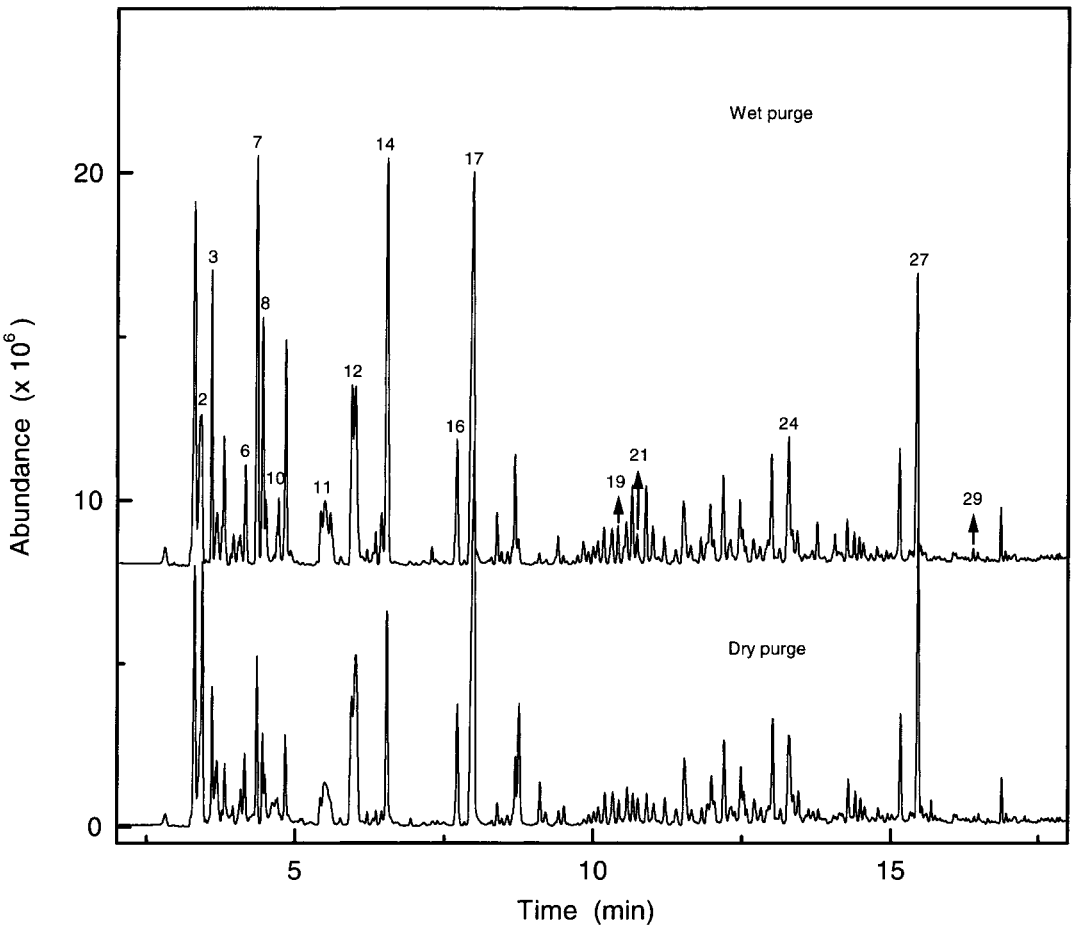


Figure 2. Total ion chromatograms for wet and dry purges of a wheat sample with normal odor. Peak numbers are identified in Table 2.

in another. The solution of citric acid was used to make sure that citric acid made contact with all of the soybean seeds. Prior to purging each of these samples, the autosampler added another 1 mL of water along with 1 μL of ISTD solution as usual.

RESULTS AND DISCUSSION

Effect of Added Water on Volatiles Purged from Grains. Ratios of amounts of target compounds obtained by wet- and dry-purge methods from soybean, corn, sorghum, and wheat samples with several different types of odors are given in Table 1. With the exception of 1-octen-3-ol in soybeans (discussed below), most of the entries lie between 0.5 and 2.0, indicating slight enhancement by one or the other purge method. In general, however, most volatiles were either not affected or slightly enhanced by the wet-purge method with all grain and odor types. Methyl acetate and methyl hexanoate, but not the other esters on the target list, were slightly enhanced in the dry-purge method.

Figures 1 and 2 show typical total ion chromatograms obtained with wet and dry purges of starchy cereals such as corn and wheat. Peaks marked by numbers are identified in Table 2. Apart from the minor differences between chromatograms mentioned below, added water did not greatly enhance or suppress extraction of any of the compounds in corn or wheat samples. Similar results were found for wet and dry purges of sorghum grain (chromatograms not shown).

With the corn sample, 3-methylbutanal (peak 7), 2-methylbutanal (peak 8), and hexanal (peak 14) were slightly enhanced in the wet-purge method (Figure 1). Compounds potentially associated with sour odor [ethyl acetate (peak 4), acetic acid (peak 5), butanoic acid (peak 13), and ethyl hexanoate (peak 20)] and musty odor [4-ethyl-1-methoxybenzene (peak 25) and 4-ethyl-1,2-dimethoxybenzene (peak 30)] were essentially not affected by the type of purge. Naphthalene (peak 27), which was most commonly found in corn and wheat, was not greatly affected by the addition of water. 4-Ethyl-1,2-dimethoxybenzene (peak 30, apparently associated with musty odor) and dimethyl sulfide (peak 1) were generally more prevalent in corn than in the other grains. Dimethyl sulfide was probably generated from methional ($CH_3SCH_2CH_2CHO$) when heated with water.

Table 2. Compounds Identified in Figures 1−3

peak	compound	peak	compound
1	dimethyl sulfide	16	d_{10}-ethylbenzene (ISTD)
2	methyl acetate	17	hexanol
3	2-methylpropanal	18	methyl hexanoate
4	ethyl acetate	19	1-octen-3-ol
5	acetic acid	20	ethyl hexanoate
6	2-methyl-1-propanol	21	octanal
7	3-methylbutanal	22	2-ethyl-1-hexanol
8	2-methylbutanal	23	2-octenal
9	1-penten-3-ol	24	nonanal
10	pentanal	25	4-ethyl-1-methoxybenzene
11	3-methyl-1-butanol	26	2-nonenal
12	1-pentanol	27	naphthalene
13	butanoic acid	28	2-decenal
14	hexanal	29	(iso)piperitone
15	2-hexenal	30	4-ethyl-1,2-dimethoxybenzene

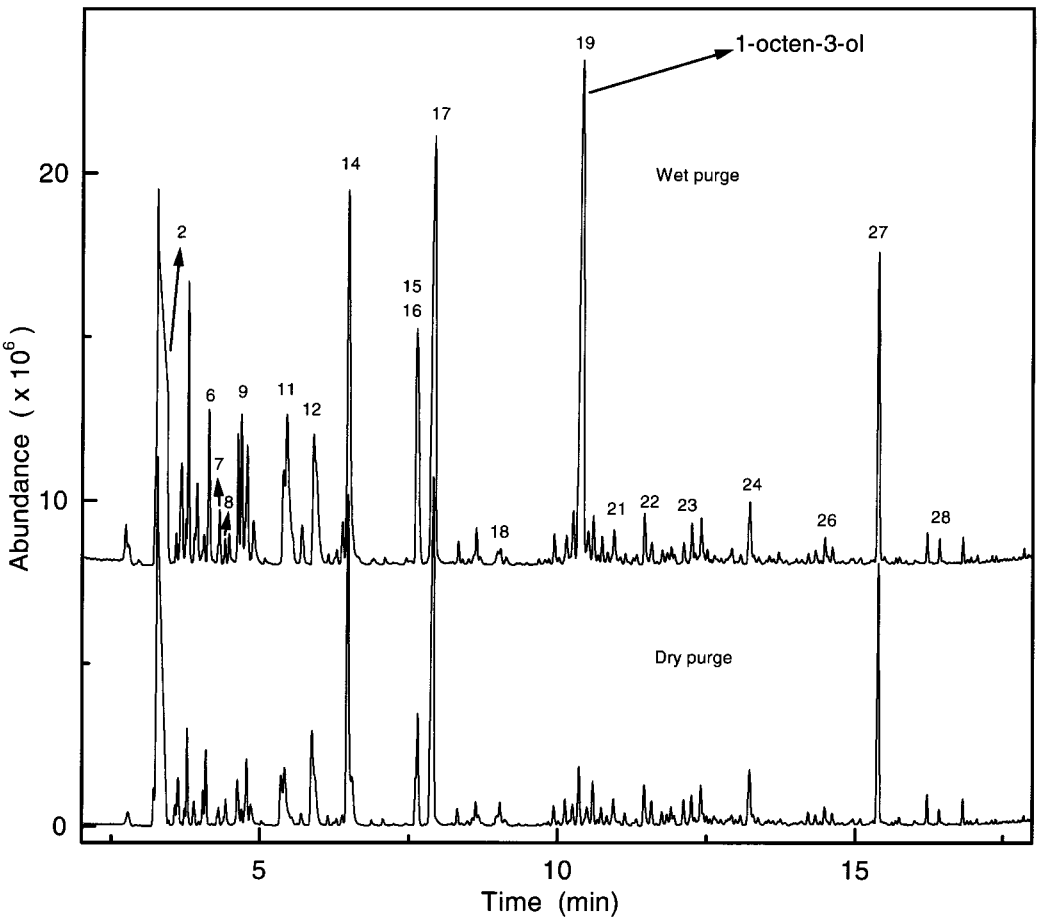


Figure 3. Total ion chromatograms for wet and dry purges of a soybean sample with normal odor. Peak numbers are identified in Table 2.

In chromatograms representing wheat, alcohols [2-methyl-1-propanol (peak 6), 3-methyl-1-butanol (peak 11), and 1-pentanol (peak 12)] and aldehydes [2-methylpropanal (peak 3), 3-methylbutanal (peak 7), 2-methylbutanal (peak 8), and hexanal (peak 14)] were slightly enhanced in the wet-purge method (Figure 2). However, methyl acetate (peak 2) was highest in the dry-purge method. Wheat generally had higher amounts of aldehydes (peaks 3, 7, 8, 10, and 14) and alcohols (peaks 12 and 17) than the other grains. 2-Isopropyl-5-methylcyclohex-2-en-1-one (peak 29), which is an isomer of piperitone, was found in all nondurum wheat samples. This compound had ν_{CO} = 1688 cm^{-1} and ν_{CH} = 2936 cm^{-1} in the IR and masses at *m/e* 109, 110, 137, and 152 in the MS.

With soybeans, the biggest difference between wet- and dry-purge results was the greatly enhanced amounts of 1-octen-3-ol (peak 19), 1-octen-3-one, 1-penten-3-ol (peak 9), and 1-penten-3-one extracted by the wet-purge method (Figure 3). 1-Octen-3-one and 1-penten-3-one were not readily evident in chromatograms such as that shown in Figure 3 because (1) they were minor compared to the corresponding alcohols and (2) 1-octen-3-one eluted at the front edge of the 1-octen-3-ol peak and 1-penten-3-one nearly coeluted with 2-pentanone. However, by using extracted ion chromatograms to quantify intensities of masses associated with 1-octen-3-one (*m/z* 55 and 70) and 1-penten-3-one (*m/z* 55 and 84) in a few representative samples, it was clear that these compounds were most prevalent in soybeans and that wet- to dry-purge ratios in soybeans ranged from 4 to 10 (data not shown). Other compounds that showed slightly enhanced extraction in the wet-purge method were aldehydes [2-methylpropanal (peak 3), 3-methylbutanal (peak 7), 2-methylbutanal (peak 8), and hexanal (peak14)] and alcohols [2-methyl-1-propanol (peak 6), 3-methyl-1-butanol (peak 11), 1-pentanol (peak 12), and hexanol (peak 17)]. Enals [2-hexenal (peak 15), 2-octenal (peak 23), 2-nonenal (peak 26), and 2-decenal (peak 28)], enols [1-penten-3-ol (peak 9) and 1-octen-3-ol (peak 19)], and methyl acetate (peak 2) generally were higher in soybeans than in other grains. However, extraction of other compounds represented in the chromatogram was normally unaffected by the addition of water in the wet-purge method.

With the wet-purge method, the amount of 1-octen-3-ol obtained from soybean samples was commonly high, and it was generally much greater than that observed for corn, sorghum, or wheat. This is illustrated in Figure 4 for 248 samples as a representation of the total 745 samples analyzed. For essentially all of >200 soybean samples analyzed by the wet-purge method, amounts of 1-octen-3-ol were elevated as represented in Figure 4. Also, for 39 samples of soybeans analyzed by both the wet- and dry-purge methods, strong enhancement of 1-octen-3-ol in the wet-purge was consistent, regardless of the odor category. This is shown in Figure 5 for a representative group of samples with various odors. With all 39 samples, the amount (nanograms) of 1-octen-3-ol per sample averaged 29 (RSD = 62%) and 328 (RSD = 41%) for samples analyzed by dry- and wet-purge methods, respectively, and amount ratios of wet/dry (calculated for each individual sample) averaged 20 (RSD = 110%). The RSD values were fairly high because

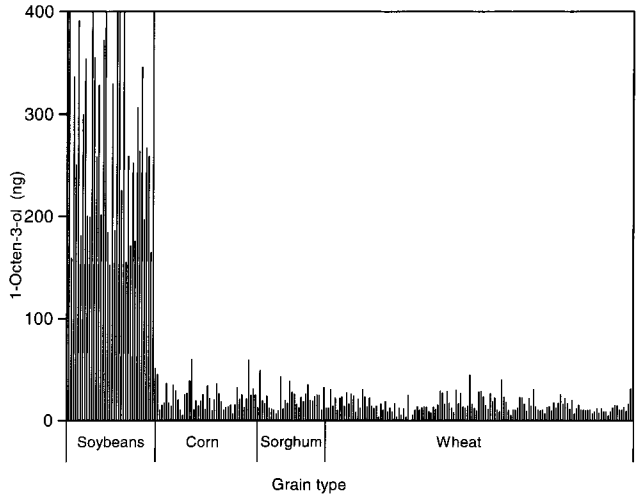


Figure 4. Amount of 1-octen-3-ol extracted from different grains by the wet-purge method. Each line represents one wet-purge analysis of one sample of grain. A total of 248 samples are represented, including 38 soybean, 44 corn, 31 sorghum, and 135 wheat. The results shown typically represent the results obtained from the entire set of 745 samples analyzed.

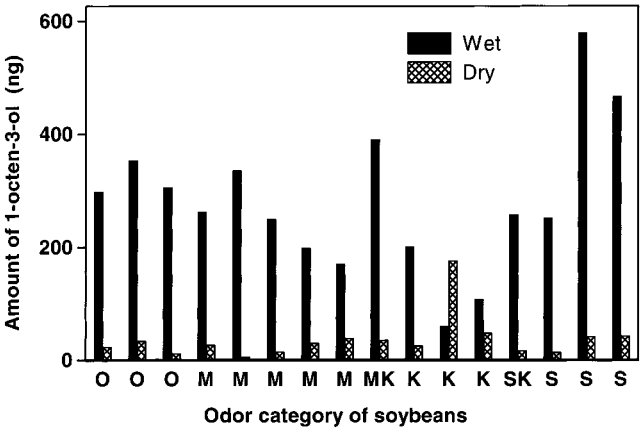


Figure 5. Amount of 1-octen-3-ol extracted by wet- and dry-purge methods from soybeans with different odors (O, OK; M, musty; K, smoke; S, sour; MK, musty and smoke). Note that the amount of extracted 1-octen-3-ol was enhanced by the wet-purge method for all soybean samples regardless of odor type, except in one sample with a distinct smoke odor that appeared to be severely damaged.

different samples of soybeans were involved. However, the enhancement in the amount of 1-octen-3-ol to wet purge was much greater than the variation from different soybean samples. Increased RSD in the ratios is a composite of variation in wet- and dry-purge analyses individually. Higher RSD values for dry-purge runs are possibly due to varying moisture contents of soybeans and a lower amount of 1-octen-3-ol in dry-purge than in wet-purge analyses. Ratio results for a few representative individual samples are given in Table 1.

Further Observations on 1-Octen-3-ol. The amount of 1-octen-3-ol obtained from soybeans increased as the amount of water added to a sample was increased (Figure 6A), but it did not depend on the moisture content of the soybeans before analysis (Figure 6B). It appeared that formation of 1-octen-3-ol, as discussed below, may occur at the surface of the soybeans after the addition of water in the wet-purge method. Also, with repeated purges of a wet soybean sample, 1-octen-3-ol continued to be purged in nearly undiminished amounts, which indicates a fairly large reservoir of

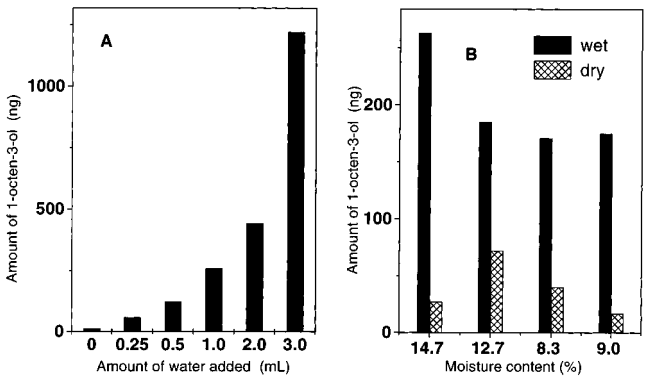
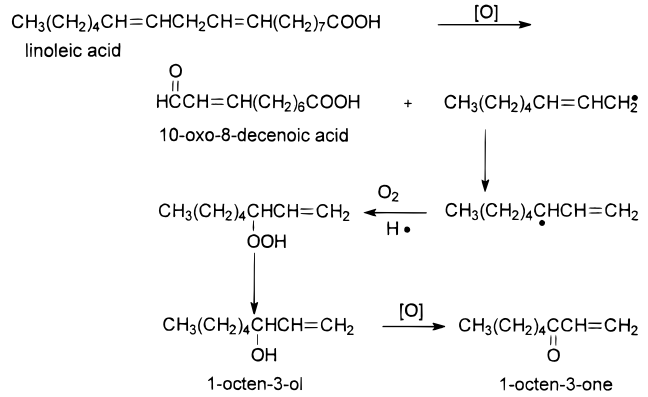


Figure 6. (A) Amount of 1-octen-3-ol extracted from soybeans as a function of the amount of water added to the sample. 1-Octen-3-ol values shown for 0, 1.0, and 2.0 mL were averages of triplicate (SD 3), duplicate (SD 18), and duplicate (SD 62) analyses, respectively; all others were single analyses. (B) Amount of 1-octen-3-ol extracted from soybean samples with various moisture contents.

Scheme 1. Possible Mechanism for Formation of 1-Octen-3-ol and 1-Octen-3-one from Linoleic Acid



1-octen-3-ol or its precursors (possibly, unsaturated fatty acids).

Oxidative formation of 1-octen-3-ol in soybean oils and soybean milk that lack lipoxygenase has been described (Shen et al., 1996; Warner et al., 1989; Kobayashi et al., 1995). Biosynthesis studies indicate that 1-octen-3-ol is formed from linoleic acid directly, rather than through an intermediary such as 13-hydroperoxylinoleate (Assaf et al., 1995; Belinky et al., 1994; Takeoka et al., 1996). The *R* (−) form of 1-octen-3-ol is obtained from soybean milk and mushrooms (Kobayashi et al., 1995; Wurzenberger and Grosch, 1984a,b). Similarly, 1-octen-3-ol and 1-octen-3-one may be produced from linoleic acid in soybeans according to Scheme 1. In addition, linolenic acid (9,12,15-octadecatrienoic acid) could be degraded to 13-oxo-9,11-tridecadienoic acid, 1-penten-3-ol, and 1-penten-3-one by pathways analogous to that in Scheme 1 (Gardner et al., 1996).

In all thee experiments conducted, citric acid suppressed the amount of 1-octen-3-ol produced from soybeans, but it did not affect the purge of 1-octen-3-ol from a standard solution in water. Representative results from one of the experiments is shown in Figure 7. Thus, citric acid appeared to inhibit the formation of 1-octen-3-ol from soybeans rather than directly suppressing the purge of 1-octen-3-ol from the seeds. A scheme involving free radicals in the formation of 1-octen-3-ol and 1-octen-3-one from autoxidation of arachidonoic acid has been described (Ho et al., 1994). Citric acid, a commonly used antioxidant, probably acted

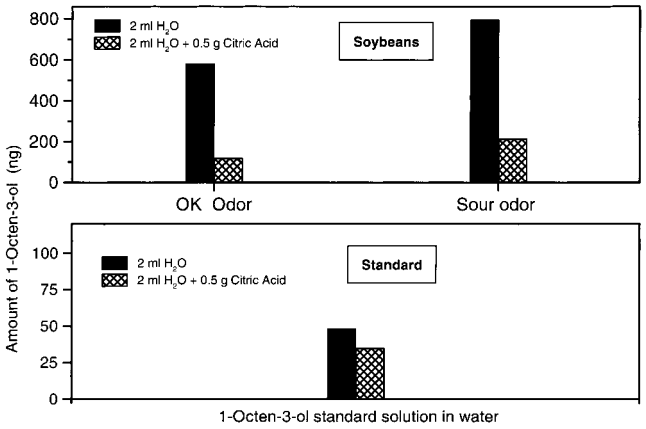
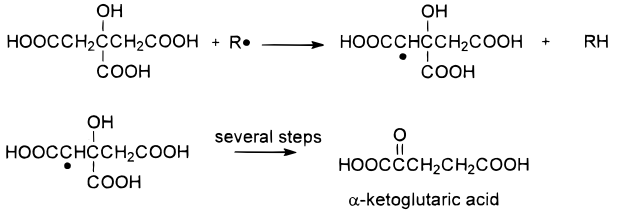


Figure 7. Effect of added citric acid on the extraction of 1-octen-3-ol from soybeans and from a standard solution of 1-octen-3-ol in water.

Scheme 2. Possible Mechanism for Scavenging Free Radicals by Citric Acid in Its Role of Inhibiting 1-Octen-3-ol Formation in Soybeans



as a free radical scavenger in the autoxidation of linoleic acid, as represented in Scheme 2.

Conclusions. The autosampler provided useful methodology for routine purging of volatiles from grain samples and aided quantification of volatiles by automatically adding a known amount of a compound to be used as an internal standard. The added water needed for complete transfer of the standard to the sample had relatively little effect on the extraction of most volatiles from starchy grains such as corn, wheat, and sorghum. With soybeans, however, the added water greatly enhanced extraction of 1-octen-3-ol by as much as 20-fold in some cases. In general, with all grains, added water slightly enhanced the recovery of polar compounds such as alcohols and aldehydes, whereas nonpolar compounds were mostly unaffected.

1-Octen-3-ol has been described as having a mushroom-like odor (Mau et al., 1993; Rapior et al., 1997), and it has been associated with musty odors in grains (Seitz and Sauer, 1992, 1994; Seitz, 1995; Seitz et al., 1999). From our results shown above, it was apparent that 1-octen-3-ol and 1-octen-3-one could not be used as indicators of off-odor (musty) in soybeans.

A possible disadvantage of the autosampler was the relatively small amount of sample (18−20 g) held in the Soil-Vial sample tubes compared to the 30−60 g held in sample tubes that are manually mounted directly on the purge and trap concentrator. However, with the heating of the tubes and excellent dispersion of gas through the sample, adequate amounts of volatiles for analysis by the GC/MS system were easily obtained from whole-grain samples.

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Received for review March 11, 1999. Revised manuscript received June 15, 1999. Accepted July 6, 1999. Contribution 99-265-J, Department of Grain Science and Industry, Kansas State Agricultural Experiment Station, Manhattan, KS. Mention of firm names or trade products does not constitute endorsement by the U.S. Department of Agriculture over others not mentioned. The U.S. Department of Agriculture, Agricultural Research Service, Northern Plains Area, is an equal opportunity/affirmative action employer and all agency services are available without discrimination.

JF990243E