# Adsorption of Volatile Fungal Metabolites to Wheat Grains and Subsequent Desorption

T. BÖRJESSON,[1,2,3] U. STÖLLMAN,[1] and J. SCHNÜRER[2]

## ABSTRACT

Adsorption of the volatile fungal metabolites 2-methylfuran, 3-methyl-1-butanol, and 1-octen-3-ol to wheat grains, and their subsequent desorption, were investigated. Adsorption was performed both dynamically and statically. In the dynamic system, volatile compounds in a $N_2$ flow were led through 400 g of wheat in a glass column. In the static system, 400 g of wheat was stored in an airtight glass vessel containing the volatile compounds in the atmosphere. Three desorption procedures were compared: an $N_2$ flow at 20°C, an $N_2$ flow at 50°C, and extraction with supercritical $CO_2$. 3-Methyl-1-butanol and 1-octen-3-ol were efficiently adsorbed and could also readily be desorbed to considerably higher extents

at 50°C than at 20°C. The supercritical $CO_2$ extraction was more efficient than $N_2$ desorption in extracting volatile compounds, but because of the smaller sample sizes (1 g), the amounts extracted per gram of grain were lower than the amounts extracted with $N_2$ desorption at 50°C. The adsorbed amount of each volatile compound was calculated as the difference between content in the $N_2$ stream before passage through the wheat and its content after passage. Desorption by means of a $N_2$ stream led to the recovery of about 5% of 2-methylfuran, 35% of 3-methyl-1-butanol, and 100% of 1-octen-3-ol.

Volatile metabolites are produced during fungal growth on cereal grains (Kaminski et al 1974, Börjesson et al 1989, 1992). These compounds are correlated with other indicators of fungal grain deterioration (Abramson et al 1983, Börjesson et al 1992) and can readily be detected in the headspace of grains. The compounds can be collected by leading a stream of air through a vessel containing a grain sample and then through a porous polymer adsorbent attached to the outlet, upon which the compounds are adsorbed (Börjesson et al 1989). Alternatively, intergranular air can also be pumped out of a grain sample and collected on an adsorbent attached to the tube connecting the sample and the pump (Abramson et al 1980). The adsorbent can be stored until analysis. The compounds can be desorbed either thermally (Börjesson et al 1989) or by means of solvent extraction (Sinha et al 1988). The desorbed compounds can then be separated by gas chromatography and identified by mass spectrometry. With these techniques, it may be possible to detect pockets of fungal growth in stored grains. This would make it possible to develop methods for continuously monitoring grain stores, thus diminishing the problem of taking unrepresentative samples.

In addition, there is also a need to reliably assess the microbial status of a grain shipment at delivery. Fungal contamination in grain samples is often measured by determination of the numbers of colony-forming units (Jarvis et al 1983, Schnürer and Jonsson 1992). However, this measure is poorly correlated with fungal biomass (Pitt 1984); moreover, the technique can give erroneous results because fungal growth in grains is unevenly distributed (Tuite et al 1985). This problem was demonstrated by Jarvis et al (1983), who showed that sample sizes influenced the variation between replicates. Although other methods, such as the measurement of ergosterol content and immunological techniques are

more strongly correlated with fungal biomass (Seitz et al 1979, Schwabe et al 1992, Schnürer 1993), the sampling problems remain. Desorption of volatile fungal metabolites that had earlier been adsorbed to the grains could diminish the sampling problems. Large batches of grains can be subjected to a gas stream, and desorbed compounds can be collected on a porous polymer adsorbent (Sinha et al 1988).

Volatile fungal metabolites produced in pockets of fungal growth mainly spread in the stores by means of diffusion (S. Regnér, *personal communication*), although convection may also contribute. Volatile compounds can be adsorbed to starch (Maier and Bauer 1972) as well as cellulose (Dernovaya and Eltekov 1988). The outer pericarp of wheat grains mainly consists of the polysaccharides starch (10%), cellulose (30%), and arabinoxylans (45%) (Schwarz et al 1988). Thus adsorption to wheat grains is probable. One mechanism involved in the adsorption could be hydrogen bonding between hydroxyl groups of the polysaccharides and polar groups of the volatile compounds, which is the most important mechanism by which water adsorbs to starch (Multon 1988). Similarly, polarity of volatile compounds highly influences their adsorption to starch (Maier and Bauer 1972) and cellulose (Dernovaya and Eltekov 1988). For alcohols and hydrocarbons, a positive linear relationship was found between molecular weight and degree of adsorption (Dernovaya and Eltekov 1988).

Ventilation of bin-stored wheat resulted in a substantial decrease in desorbed amounts of fungal volatiles, indicating that volatile compounds bound to the grains were easily released (Sinha et al 1988). Maier and Bauer (1972) also found that all of the adsorbed hexane and the main part of adsorbed ethanol and acetone were desorbed from starch with 8.2% moisture in vacuum and that the desorption was temperature dependent.

They also demonstrated a strong positive correlation between water content and adsorption. It is thus probable that volatile compounds in the gas phase bind reversibly to grains, that they can be desorbed by means of a gas stream, and that the desorption is temperature dependent. The relative efficiency with which volatile compounds can be desorbed by passing a gas stream

[1]SIK–Swedish Institute for Food Research, Göteborg, Sweden.
[2]Department of Microbiology, Swedish University of Agricultural Sciences, Uppsala, Sweden.
[3]Author to whom correspondence should be addressed.

through the samples is unknown; thus comparisons with other extraction methods are needed.

Supercritical $CO_2$ is as efficient as organic solvents in extracting volatile compounds (Nielsen 1991), and it is superior to organic solvents in many other respects. Supercritical fluids combine the high solvent power of liquids and the penetrating power of gases and, at least when using $CO_2$, traces of solvents in the samples after extraction are avoided (Zadow 1988). For most volatile fungal metabolites, which are only moderately polar, pure $CO_2$ is a suitable medium (Hawthorne 1990). In terms of safety, $CO_2$ is also advantageous. It is nontoxic and its supercritical properties develop at moderately high pressures and temperatures ($31.4°C$ and $8 \times 10^6$ Pa) (Rizvi et al 1986). However, the supercritical extraction technique is not suitable for sample sizes larger than 10 g as larger samples require volumes of extraction fluid too high to permit efficient trapping afterwards (Hawthorne 1990).

This investigation had two objectives. The first was to study the adsorption of three representative volatile fungal metabolites to wheat grains. The metabolites, which differed in volatility, were 2-methylfuran, 3-methyl-1-butanol, and 1-octen-3-ol. Two different systems were compared: adsorption in a dynamic system (where the volatile compounds were carried through the wheat by flowing $N_2$) and adsorption in a static system (where wheat was stored in an atmosphere containing the volatile compounds).

The second objective was to study the extent to which these compounds can be desorbed from the grains using a $N_2$ stream at $20°C$ and $50°C$, respectively. The desorbed amounts were then compared with amounts extracted from a small portion of the grain by means of supercritical $CO_2$.

## MATERIALS AND METHODS

### Wheat

Winter wheat (cultivar Kosack) harvested in southwestern Sweden in 1991 and provided by AB AnalyCen, Lidköping, Sweden, was used. The water content of the wheat was calculated as 8.9%, based on weight loss after 3 hr at $103°C$.

### Volatile Compounds

The volatile compounds chosen for this investigation were 2-methylfuran (Purum, Fluka AG, Buchs, Switzerland), 3-methyl-1-butanol (Pro Analysi, Merck, Darmstadt, Germany), and 1-octen-3-ol (Purum, Parento, Croton-on-Hudson, NY). They represent different degrees of volatility and probable adhesive properties to grains, as indicated by their different retention times on a gas chromatogram (Börjesson et al 1992). 3-Methyl-1-butanol and 1-octen-3-ol are both commonly produced by molds growing on cereals (Kaminski et al 1974, Tuma et al 1989, Börjesson et al 1992). Instead of using 3-methylfuran, a common but very expensive fungal metabolite (Börjesson et al 1992), 2-methylfuran was chosen. It was assumed that 2-methylfuran and 3-methylfuran have similar adhesive properties as they have similar structures and the same molecular weight.

### Preparation of Solutions Used for Generating Volatile Compounds

The main interest when preparing solutions for generating volatile compounds was that the concentrations in the headspace should reach levels similar to those in a cultivation flask with growing fungi, almost 1 $\mu g/l$ of 3-methyl-1-butanol and almost 0.1 $\mu g/l$ of methylfuran and 1-octen-3-ol (Börjesson et al 1989). However, preliminary experiments showed that 2-methylfuran is adsorbed weakly by wheat, so headspace concentrations about 100 times higher were used for this compound. Equilibrium headspace concentrations of pure compounds (Weast 1971) are higher than those observed in earlier studies of fungal growth (Börjesson et al 1989, 1992). Thus the compounds had to be diluted. 3-Methyl-1-butanol and compounds similar to 2-methylfuran and 1-octen-3-ol are reported to be soluble in organic solvents (Windholz et al 1983). When using organic solvents, however, headspace concentrations of the solvent is high; this could interfere both

with the adsorption of test compounds and with the gas chromatographic analysis. To circumvent these potential difficulties, rapeseed oil of high quality (Karlshamns AB, Karlshamn, Sweden) was chosen as diluent.

Besides the above-mentioned considerations, the concentrations chosen (0.1 mg/ml of 2-methylfuran, 0.5 mg/ml of 3-methyl-1-butanol, and 1 mg/ml of 1-octene-3-ol) were based on earlier reports of partition coefficients (concentration in gas phase/concentration in liquid or solid phase) for different aldehydes (Hall and Andersson 1983) and the authors' unpublished data. The solutions were stored at $4°C$ before use in the experiments.

### Preparation of Glassware

All glassware used was cleaned both before the start of the first experiment and after use in each experiment by: 1) washing with hot water and detergent, 2) rinsing with 95% ethanol, 3) rinsing with charcoal-filtered water, and 4) oven drying ($105°C$) for at least 30 min.

### Dynamic Adsorption of Volatile Compounds

From each solution of volatile compound, 1-ml portions were added to glass dispensers (2 ml) and put in a glass vessel (generating vessel) with a gas inlet and outlet. $N_2$ (<5 ppm of $O_2$ and <5 ppm of $H_2O$, AGA AB, Lidingö, Sweden) was flushed through the vessel (10 ml/min) and then through a glass column (4 cm i.d. and 40 cm high) filled with 400 g of wheat for 24 hr (Fig. 1). Before the start of each flushing through wheat, the vessel generating volatile compound was flushed for 1 hr. To be able to calculate the amounts theoretically adsorbed by the wheat, the concentrations of test compounds in the $N_2$ stream were measured before and after passage through the wheat column. This was performed by adsorbing compounds on a porous polymer adsorbent (Chromosorb 102, Johns-Manville, Denver, CO). Glass tubes were filled with the adsorbent and attached to the Teflon tubing exiting the generating vessel or to the outlet of the wheat column. Each collection was made for 1 hr, allowing 600 ml of $N_2$ to pass through. Volatile compounds exiting the generating vessel were checked three times: before the start of the passage through wheat, after 12 hr of passage, and after completing the adsorption. Concentrations of volatile compounds exiting the wheat column were measured after 12 hr of adsorption.
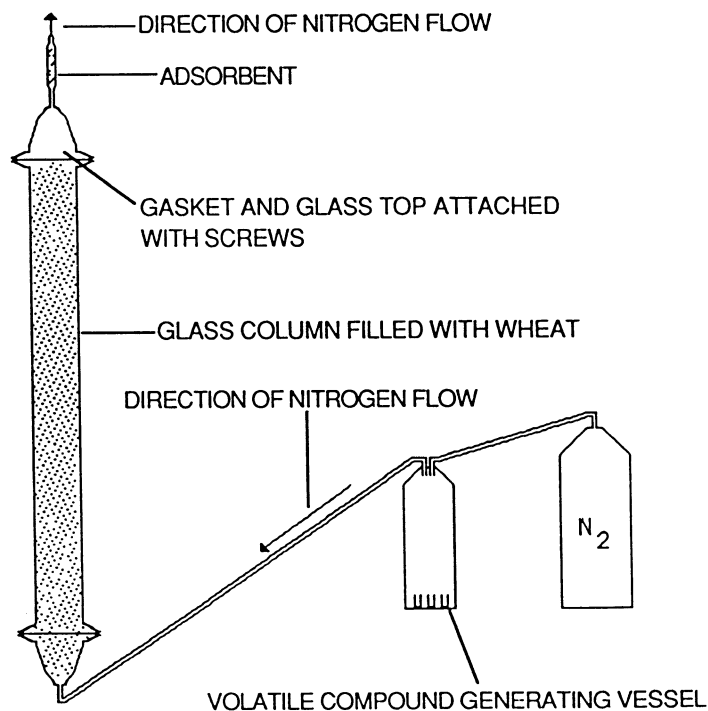


Fig. 1. Equipment used for dynamically adsorbing volatile fungal metabolites to wheat grains and for subsequent desorption.

After the adsorption, the wheat was stored in 1-L glass jars covered with aluminum foil. The jars were stored at ambient temperature for 36 hr before desorption. The experiment was repeated six times.

## Static Adsorption of Volatile Compounds

A 400-g portion of wheat was added to each of a number of glass vessels (10 cm i.d. and 17 cm high) containing one dispenser of each volatile compound solution (Fig. 2). The dispensers were attached to a steel tray in the bottom of the vessel, and the wheat was poured into a perforated steel basket in the vessel. Each vessel was covered with an airtight glass lid attached by clamps. The glass lid had an inlet and an outlet for air that were fitted with Teflon stoppers during storage. The closed vessels had a volume of about 800 ml. The wheat was stored in these vessels for 24 hr or one week. Headspace samples were taken after 1, 12, and 23 hr of storage (in the experiment with 24-hr storage) and after one, three, five, and seven days (in the one-week storage experiment). Samples were taken by pumping 60 ml of air out of the vessels through an adsorbent attached to the outlet (Fig. 2). The inlet was also opened during the sampling to avoid altering the air pressure in the vessels. After 24 hr and one week, respectively, the wheat was transferred to glass jars and stored as described earlier. Each storage period was replicated six times.

## Desorption with $N_2$

The type of glass column (Fig. 1) used for active adsorption was also used for desorption by means of a $N_2$ stream.

From each 400-g wheat sample, 5 g was reserved for supercritical $CO_2$ extraction. The remaining 395 g was poured in the glass column and subjected to a $N_2$ flow of 50 ml/min for 3 hr (total $N_2$ volume: 9 L). Three wheat batches from each adsorption procedure were kept at 20°C and three were kept at 50°C in a thermostated chamber during the desorption. Desorbed compounds were collected as described above on adsorbents attached to the outlet and kept at ambient temperature (20–22°C) during sampling. During the heating to 50°C, water was released from the grain. To avoid water condensation on the adsorbents, which could hinder the subsequent thermal desorption, the $N_2$ exiting the glass column was led through a flask cooled in an ice bath. The amounts of volatile compounds retained in the cooled flask were measured and compensated for in the results.

## Supercritical $CO_2$ Extraction

Before the $N_2$ desorption, 5 g of the wheat from each experiment was put in small glass vials, provided with screw caps with Teflon gaskets, and stored in a freezer (−18°C) for one to three weeks before supercritical $CO_2$ extraction. One gram of the wheat was then poured in an extraction cell, consisting of a 45-mm steel tube, 6.5 mm i.d. The cell was kept at 40°C and $10^7$ Pa during flushing with $CO_2$. The $CO_2$ exiting the extraction apparatus (100 ml/min) was collected on an adsorbent as described above. A
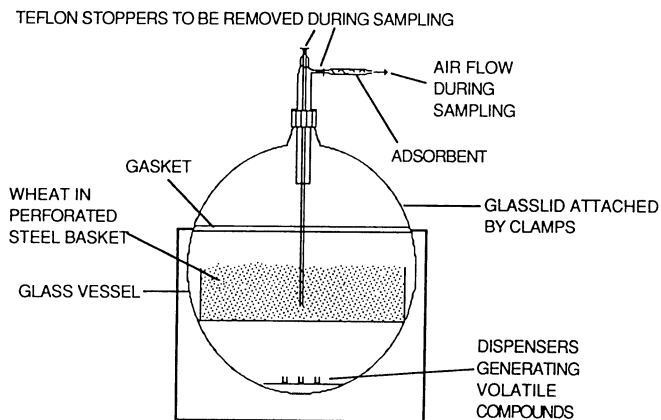
volume corresponding to 14 L of gaseous $CO_2$ was allowed to pass through the cell, which, according to the authors' unpublished data, was found to be sufficient for quantitative 1-octen-3-ol extraction. 2-Methylfuran and 3-methyl-1-butanol were also considered to be quantitatively extracted as these compounds have lower boiling points than that of 1-octen-3-ol and similar polarities (Weast 1971, Windholz et al 1983). Compounds breaking through the adsorbents were quantified by attaching a second adsorbent in series after the first one. The apparatus used was a Pickels Original Supercritical Fluid Chromatograph, model NWA-PM 101, connected to a water bath in which the cell was kept.

## Analysis of Volatile Compounds

The adsorbed volatile compounds were desorbed thermally (140°C) and concentrated in a cold trap before injection onto a gas chromatographic column (Börjesson et al 1989). A Hewlett-Packard 5890 series 2 gas chromatograph connected to an Incos 50 quadrupole mass spectrometer was used. Electron-impact ionization (70 eV) was used without selected-ion monitoring and the ion source temperature was set at 150°C. The capillary column was fused silica (60 m × 0.32 mm i.d.; DB 1701, J&W) with chemically bonded phenyl-cyanopropyl-methyl and a film thickness of 1 $\mu$m. The oven of the gas chromatograph was programmed to increase the temperature from 30°C, which was held for 2 min, to 150°C at a rate of 6°C per min. Data were collected using a Hewlett-Packard laboratory automation system, HP3350.

## RESULTS

### Dynamic Adsorption of Volatile Metabolites

*Concentration of volatile compounds in $N_2$ stream before and after passage through wheat.* The concentrations in the $N_2$ flow of 2-methylfuran, 3-methyl-1-butanol, and 1-octen-3-ol, which had been measured before starting the experiments, after 12 hr of adsorption, and after each experiment, varied somewhat with time. Concentrations of 2-methylfuran diminished, whereas those of 3-methyl-1-butanol and 1-octen-3-ol increased. The volatile fungal metabolites showed different degrees of adsorption to the wheat in the column (Table I). Based on these figures and on the changes in ingoing concentrations of the volatile compounds during the experiment, the amounts adsorbed to the grain were calculated. The percentages of volatile compounds retained were not quite constant over time, but these differences were not large enough to influence the calculations to a significant extent. A separate study had indicated that the concentrations of volatile compounds in the ingoing $N_2$ varied almost linearly. According to these calculations, 30 ng of 2-methylfuran, 150 ng of 3-methyl-



Fig. 2. Equipment used for statically adsorbing volatile fungal metabolites to wheat grains.

**TABLE I**
Volatile Compounds in the Nitrogen Stream Exiting the Generating Vessel and the Wheat Column After-12 hr Adsorption[a]

| Compound | Exiting Generating Vessel ($\mu$g/L) | Exiting Wheat Column ($\mu$g/L) |
|---|---|---|
| 2-Methylfuran | 2.4 ± 0.5 | 1.8 ± 0.6 |
| 3-Methyl-1-butanol | 4.9 ± 1.1 | 0.19 ± 0.07 |
| 1-Octen-3-ol | 0.32 ± 0.12 | BDL[b] |

[a] $n = 6$, mean values ± standard deviations.
[b] Below detection limit.

**TABLE II**
Nitrogen Desorption of Volatile Compounds Dynamically Adsorbed to Wheat[a]

| Compound | Desorption Temperature | |
| | 20° C (ng/g of wheat) | 50° C (ng/g of wheat) |
|---|---|---|
| 2-Methylfuran | 0.17 ± 0.06 | 1.4 ± 0.1 |
| 3-Methyl-1-butanol | 5.5 ± 3.8 | 54 ± 7 |
| 1-Octen-3-ol | 0.24 ± 0.25 | 11 ± 3 |

[a] $n = 3$, mean values ± standard deviations.

1-butanol, and 11 ng of 1-octen-3-ol were retained per gram of wheat.

According to the authors' unpublished results, about half of the 3-methyl-1-butanol and two-thirds of the 1-octen-3-ol were adsorbed to the glass and gaskets used. However, in grain-filled columns, the total surface area of the grain is very large, enabling it to compete effectively with the glass and gaskets in adsorbing volatile compounds. This was confirmed by the finding that almost all of the 1-octen-3-ol that passed through the column could be extracted from the grains during $N_2$ desorption. Consequently, the background adsorption was considered to be of minor importance and was not taken into account.

*Desorption using a $N_2$ stream.* The amounts desorbed from the grains varied depending on the desorption temperature and volatile compound used (Table II). Heating the grains to 50°C increased the amounts desorbed for all studied compounds as compared with the 20°C desorption. In the 50°C treatment, about 5% of the calculated adsorbed 2-methylfuran, 35% of the adsorbed 3-methyl-1-butanol, and 100% of the adsorbed 1-octen-3-ol were recovered. Data presented from the 50°C desorption temperature in Table II were compensated for percentages of desorbed compounds that were retained in the cooled flask: 0% of the desorbed 2-methylfuran, 50% of the desorbed 3-methyl-1-butanol, and 90% of the desorbed 1-octen-3-ol. Amounts of compounds desorbed at 50°C from untreated wheat (0.17 ng of 2-methylfuran, 9 ng of 3-methyl-1-butanol, and 8 ng of 1-octene-3-ol per gram) were subtracted from the 50°C desorption figures. At the 20°C desorption temperature, amounts of the compounds desorbed from untreated wheat were less than 5% compared with values in Table II, and they were not taken into account. Amounts breaking through the adsorbents were always insignificant (less than 1%).

*Supercritical extraction.* Data on amounts of volatile compounds extracted by supercritical $CO_2$ are presented in Table III. Twenty percent of extracted 2-methylfuran passed through the first adsorbent, which is compensated for in Table III. The amounts desorbed per gram of grain by supercritical $CO_2$ from

### TABLE III
Supercritical Carbon Dioxide Extraction of Volatile Compounds[a]

| Compound | Dynamic, 24 hr (ng/g of wheat) | Static, 24 hr (ng/g of wheat) | Static, One Week (ng/g of wheat) |
|---|---|---|---|
| 2-Methylfuran | 3.6 ± 2.7 | 7.5 ± 2.5 | 15 ± 3 |
| 3-Methyl-1-butanol | 21 ± 10 | 66 ± 32 | 220 ± 80 |
| 1-Octen-3-ol | 3.3 ± 2.2 | 10 ± 4 | 51 ± 16 |

Header for the table columns: Adsorption procedure

[a] Dynamically adsorbed to wheat for 24 hr and statically adsorbed for 24 hr and one week, respectively. n = 6, mean values and standard deviations.

1-g samples after storage in a freezer was mostly in between the figures for 20 and 50°C $N_2$ desorption of 395-g samples. According to the authors' unpublished data, freezing did not influence the amounts of volatile compounds desorbed from the wheat. The variation between replicates was higher for supercritical extraction than it was for $N_2$ desorption.

### Static Adsorption
*Headspace concentrations of volatile compounds.* The concentrations of volatile compounds in the headspace of the static adsorption vessels were generally similar to those in the $N_2$ stream during dynamic adsorption (Table IV). The only exception was 2-methylfuran, which had higher concentrations in the static adsorptions. Except for the first sampling of the 24-hr storage experiment, headspace concentrations were similar, irrespective of storage time.

*Desorption of volatile compounds from the grains using a $N_2$ flow.* Higher amounts of volatile metabolites were desorbed from grain stored in the static adsorption vessels than from grain subjected to active adsorption (Tables II, V). Data in Table V were subjected to the same type of corrections as data from the dynamic adsorption. For all compounds, larger amounts were desorbed from material stored for one week than for material stored for 24 hr. However, the desorbed amounts were not linearly related to storage time, indicating that adsorption was higher in the beginning of the storage period than it was toward the end. As was the case for dynamic adsorption, amounts desorbed at 50°C were substantially higher than those desorbed at 20°C.

To make a comparison with the supercritical $CO_2$ extraction, two 1-g samples stored in a freezer were desorbed thermally at 50°C in the cells normally used for supercritical extraction. The flow of $N_2$ was 100 ml/min, and a total of 14 L was led through the cells. On average, 15% of the amounts extracted during supercritical $CO_2$ extraction was desorbed.

*Supercritical $CO_2$ extraction.* As was the case for dynamic adsorption, supercritical $CO_2$ extraction of 1-g samples stored in a freezer released lower amounts of all studied volatile compounds per gram of wheat compared with the 50°C $N_2$ desorption of 395-g samples, but higher amounts when compared with the 20°C desorption (Table III).

### DISCUSSION

In dynamic as well as static adsorption experiments, the headspace concentrations of test compounds reached fairly stable levels within a few hours. Concentrations of 1-octen-ol slowly and continuously increased throughout the one-week storage experiment, indicating that this compound slowly diffuses in grain stores. For 2-methylfuran, a slight decrease was observed that may have been due to losses during sampling of the headspace. In most

### TABLE IV
Changes in Headspace Concentrations ($\mu$g/L of air) of Volatile Compounds in Static Adsorption Vessels[a]

| Compound | 24-hr Storage | | | One-Week Storage | | | |
|---|---|---|---|---|---|---|---|
| | 1 hr | 12 hr | 23 hr | 1 day | 3 days | 5 days | 7 days |
| 2-Methylfuran | 15 ± 3 | 24 ± 5 | 29 ± 2 | 31 ± 6 | 33 ± 6 | 26 ± 6 | 21 ± 10 |
| 3-Methyl-1-butanol | 0.57 ± 0.15 | 3.3 ± 0.5 | 4.7 ± 0.5 | 4.0 ± 1.2 | 7.4 ± 1.5 | 7.1 ± 1.3 | 7.8 ± 1.6 |
| 1-Octen-3-ol | 0.020 ± 0.035 | 0.071 ± 0.071 | 0.099 ± 0.061 | 0.065 ± 0.056 | 0.18 ± 0.07 | 0.27 ± 0.13 | 0.40 ± 0.13 |

[a] n = 6, mean values ± standard deviations.

### TABLE V
Nitrogen Desorption of Volatile Compounds Statically Adsorbed to Wheat[a]

| Compound | 24-hr Storage, ng/g of wheat | | One-Week Storage, ng/g of wheat | |
|---|---|---|---|---|
| | 20°C | 50°C | 20°C | 50°C |
| 2-Methylfuran | 1.3 ± 0.7 | 11 ± 2 | 2.6 ± 0.1 | 22 ± 5 |
| 3-Methyl-1-butanol | 14 ± 3 | 140 ± 22 | 37 ± 5.7 | 450 ± 35 |
| 1-Octen-3-ol | 1.1 ± 0.1 | 42 ± 5 | 4.7 ± 0.8 | 260 ± 50 |

[a] n = 3, mean values ± standard deviations.

cases, headspace concentrations in the dynamic system were similar to those in the static one. The lower amounts of 2-methylfuran in the dynamic system were probably due to the rapid $N_2$ flow rate, which may have been too high to allow concentrations of this compound to reach equilibrium in the generating vessel.

Large variations in adsorption between different compounds were found. 1-Octen-3-ol and 3-methyl-1-butanol were much more readily adsorbed than was 2-methylfuran. Storage time influenced the amounts adsorbed, although the accumulation over time was not linear. By contrast, Maier and Bauer (1972) reported linear accumulation with starch where ethanol contents increased continuously during 100 days of storage. Adsorption to grain was apparently greater in the static system than in the dynamic one, which indicates that aeration could influence the results of a desorption experiment.

Larger amounts of volatile compounds were desorbed at 50°C than at 20°C, which is consistent with findings of Maier and Bauer (1972). Large amounts of 1-octen-3-ol and 3-methyl-1-butanol were desorbed from the grain, while only little of the calculated adsorbed 2-methylfuran was desorbed. This discrepancy may be attributable to losses of 2-methylfuran during the handling of the grain before desorption.

A comparison between $N_2$ desorption at 50°C and supercritical extraction showed that the latter procedure was the more efficient one. However, losses of volatile compounds during handling were probably greater from the small sample used for the supercritical extraction than from the sample used for $N_2$ desorption. Concentration of volatile compounds emanating from grains is likely to be higher in the air surrounding the kernels in a batch of grain than in the open air. According to the partition coefficients between gas phase and solid or liquid phase (Hall and Andersson 1983), higher amounts of volatile compounds are retained in a liquid or on a surface when the concentration of the compound in the gas phase is high.

In a batch of grains, the amounts retained on the surface of individual kernels should therefore be higher than when the kernels were stored individually in the open air. The size of the batch should thus influence the degree to which volatile compounds are retained on the grain kernels. The smaller sample sizes used during supercritical $CO_2$ extraction also resulted in larger amounts of variation between replicates.

It can be concluded that 3-methyl-1-butanol and 1-octen-3-ol were efficiently adsorbed to grains, as indicated by the calculated partition coefficients in the static system and the high adsorption found in the dynamic system. These compounds can thereafter be desorbed effectively and with good reproducibility using a $N_2$ flow at elevated temperature.

The concentrations of these compounds in the gas phase were similar to those in vessels where molds were growing (Börjesson et al 1989). Thus, it is probable that these compounds and other similar volatile fungal metabolites can be desorbed from grains and used as indicators of mold growth in grain stores.

## ACKNOWLEDGMENTS

## LITERATURE CITED

ABRAMSON, D., SINHA, R. N., and MILLS, J. T. 1980. Mycotoxin and odor formation in moist cereal grain during granary storage. Cereal Chem. 57:346-351.

ABRAMSON, D., SINHA, R. N., and MILLS, J. T. 1983. Mycotoxin and odor formation in barley stored at 16 and 20% moisture in Manitoba. Cereal Chem. 60:350-355.

BÖRJESSON, T., STÖLLMAN, U., ADAMEK, P., and KASPERSSON, A. 1989. Analysis of volatile compounds for detection of molds in stored cereals. Cereal Chem. 66:300-304.

BÖRJESSON, T., STÖLLMAN, U., and SCHNÜRER. J. 1992. Volatile metabolites produced by six fungal species compared with other indicators of fungal growth on cereal grains. Appl. Environ. Microbiol. 58:2599-2605.

DERNOVAYA, L. I., and ELTEKOV, Y. A. 1988. Retention and heats of adsorption of hydrocarbons and aliphatic alcohols on cellulose. J. Chromatogr. 455:263-269.

HALL, G., and ANDERSSON, J. 1983. Volatile fat oxidation products. II. Influence of temperature on volatility of saturated, mono- and diunsaturated aldehydes in liquid media. Lebensm. Wiss. Technol. 16:362-366.

HAWTHORNE, S. B. 1990. Analytical scale supercritical fluid extraction. Anal. Chem. 62:633-642.

JARVIS, B., SEILER, D. A. L., OULD, A. J. L., and WILLIAMS, A. P. 1983. Observations on the enumeration of moulds in food and feedingstuffs. J. Appl. Bacteriol. 55:325-336.

KAMINSKI, E., STAWICKI, S., and WASOWICZ, E. 1974. Volatile flavour compounds produced by molds of Aspergillus, Penicillium and fungi imperfecti. Appl. Microbiol. 27:1001-1004.

MAIER, H. G., and BAUER, A. 1972. Die bindung flüchtiger aromastoffe an stärke. Starch/Staerke 24:101-107.

MULTON, J. L. 1988. Preservation and storage of grains, seeds and their by-products. Cereals, Oilseeds, Pulses, and Animal Feed. Lavoisier: New York

NIELSEN, T. J., JÄGERSTAD, I. M., ÖSTE, R. E., and SIVIK, B. T. G. 1991. Supercritical fluid extraction coupled with gas chromatography for the analysis of aroma compounds adsorbed by low-density polyethylene. J. Agric. Food Chem. 39:1234-1237.

PITT, J. I. 1984. The significance of potentially toxigenic fungi in foods. Food Technol. Aust. 36:218-219.

RIZVI, S. S. H., BENADO, A. L., ZOLLWEG, J. A., and DANIELS, J. A. 1986. Supercritical fluid extraction: Fundamental principles and modeling methods. Food Technol. 40(6):55-65.

SCHNÜRER, J. 1993. Comparison of methods for estimating the biomass of three foodborne fungi differing in growth morphology. Appl. Environ. Microbiol. 59:552-555.

SCHNÜRER, J., and JONSSON, A. 1992. Ergosterol levels and mould colony forming units in Swedish grains of food and feed grade. Acta Agric. Scand. sect. B, Soil and Plant Sci. 42:240-245.

SCHWABE, M., KAMPHUIS, H., TRÜMNER, U., OFFENBÄCHER, G., and KRÄMER, J. 1992. Comparison of the latex agglutination test and the ergosterol assay for the detection of moulds in foods and feedstuffs. Food Agric. Immunol. 4:19-25.

SCHWARZ, P. B., KUNERTH, W. H., and YOUNGS, V. L. 1988. The distribution of lignin and other fiber components within hard red spring wheat bran. Cereal Chem. 65:59-64.

SEITZ, L. M., SAUER, D. B., BURROUGHS, R., MOHR, H. E., and HUBBARD, J. D. 1979. Ergosterol as a measure of fungal growth. Phytopathologia 69:1202-1203.

SINHA, R. N., TUMA, D., ABRAMSON, D., and MUIR, W. E. 1988. Fungal volatiles associated with moldy grain in ventilated and nonventilated bin-stored wheat. Mycopathologia 101:53-60.

TUITE, J., KOH-KNOX, C., STROSHINE, R., CANTONE, F. A., and BAUMAN, L. F. 1985. Effect of physical damage to corn kernels on the development of Penicillium species and Aspergillus glaucus in storage. Phytopathology 75:1137-1140.

TUMA, D., SINHA, R. N., MUIR, W. E., and ABRAMSON, D. 1989. Odor volatiles associated with microflora in damp ventilated and nonventilated bin-stored bulk wheat. Int. J. Food Microbiol. 8:103-119.

WEAST, R. C., ed. 1971. Handbook of Chemistry and Physics. A Ready-Reference Book of Chemical and Physical Data. The Chemical Rubber Co.: Cleveland, OH.

WINDHOLZ, M., BUDAVARI, S., BLUMETTI, R. F., and OTTERBEIN, E. S., eds. 1983. The Merck Index, An Encyclopedia of Chemicals, Drugs, and Biologicals. Merck & Co.: Rahway, NJ.

ZADOW, J. G. 1988. Supercritical fluid extraction–New technology for the food industry. CSIRO Food Res. Q. 48:25-31.