Off-Odorous Compounds Produced by Molds on Oatmeal Agar: Identification and Relation to Other Growth Characteristics

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Ten *Penicillium* and *Aspergillus* species, four with a strong musty off-odor and six reference fungi without any characteristic odor, were cultivated on oatmeal agar for 5 days in cultivation vessels provided with an inlet and an outlet for air. Samples of volatile metabolites were collected on a porous polymer adsorbent attached to the outlet from day 2 through day 5 after inoculation. Adsorbed compounds were desorbed thermally and analyzed with GC/MS and a combined GC and sensory analysis, the GC sniff technique. Multivariate analysis of GC/MS and fungal odor data revealed strong associations between 6 of 65 volatile compounds and musty off-odor. The GC sniff technique showed that five of these, dimethyl disulfide, 1-octen-3-ol, 2-methylisoborneol, and two $C_{11}H_{18}$ compounds, had prominent off-odors. In addition, geosmin, 1-methoxy-3-methylbenzene, and methylphenol were produced in large amounts by some off-odorous fungi and contributed to their unpleasant odor. 3-Methylfuran, 2-methyl-1-propanol, and 3-methyl-1-butanol were much more commonly produced than the off-odorous compounds. Both odorous and other volatile metabolites could be detected after 2 days of fungal growth. The production of odorous metabolites was enhanced at the time of sporulation.

INTRODUCTION

To prevent fungal growth in cereal grains, moisture contents corresponding to water activities (a_w) of 0.65 or less must prevail during storage. During storage at elevated moisture contents, odors described as earthy, musty, or moldy arise. These descriptive terms have been used to differentiate moist wheat (a_w 0.8) stored for 1 or 2 weeks from newly moistened wheat (Zawirska-Wojtasiak et al., 1991). The presence of off-odors makes grains and grain products less palatable, thus decreasing their quality. The odor of grain samples is also used as an indicator of grain deterioration in general (*Statute Book*, 1991). This method of classifying grains has many drawbacks. For example, fungal spores may cause allergic reactions such as hypersensitivity pneumonitis (Rylander, 1986). In addition, volatile fungal metabolites may cause headache, eye, nose, or throat irritation and other disease symptoms (Samson, 1985) which may predispose for allergic reactions. Furthermore, Schnürer and Jonsson (1992) found no correlation between the content of ergosterol, a fungal-specific membrane compound, and odor in an analysis of a large number of oat and barley samples. This suggests that odor cannot be used as an indicator of the degree of fungal infection.

Some of the most frequently reported volatile metabolites produced during fungal growth on grain or grain products include 2-methyl-1-propanol, 3-methyl-1-butanol, 3-octanone, and 1-octen-3-ol (Kaminski et al., 1974; Tuma et al., 1989; Börjesson et al., 1992). The odor characteristics of these compounds generally do not correspond to the must, earthy, or moldy note of deteriorated grains. Detection of mold growth as early as possible requires that metabolites produced at an early stage of growth are measured. Production of 3-methyl-1-butanol reached its maximum earlier than other compounds during growth of *Penicillium cyclopium* and *Aspergillus flavus* on wheat (Börjesson et al., 1989). Karahadian et al. (1985) made a similar observation for *Penicillium caseicolum* growing on Czapek agar, where 3-methyl-1-butanol preceded the peaks of earthy–musty-smelling compounds. Thus the use of GC/MS technique to characterize the volatile metabolite profile should give a more reliable measure of fungal activity since even nonodorous compounds can be detected.

Table I. Odor Thresholds in Water for Off-Odorous Fungal Metabolites and Some of the Most Commonly Reported Fungal Volatile Metabolites

compound	odor threshold in water (ppb)	odor characteristics
2-methylisoborneol	0.1[a]	musty, earthy[e]
geosmin	0.015[b]	musty, earthy[e]
2-methyl-1-propanol	3000[c]	
3-methyl-1-butanol	250[c]	malty[f]
1-octen-3-ol	10[d]	raw mushrooms[g]
3-octanone	50[d]	earthy, ketonic, mushroom-like[g]

[a] Medsker et al., 1969. [b] Tyler et al., 1979. [c] van Gemert and Nettenbreijer, 1977. [d] Pyysalo and Suihko, 1976. [e] Wasowicz et al., 1988. [f] Schieberle and Grosch, 1991. [g] Karahadian et al., 1985.

The main compounds responsible for earthy, musty off-odors are geosmin and 2-methylisoborneol, which are present in small quantities in mold-damaged cereal grains (Wasowicz et al., 1988). These compounds are produced by both fungi and actinomycetes (Kikuchi et al., 1981; Wood et al., 1983; Karahadian et al., 1985; Mattheis and Roberts, 1992) and have lower odor thresholds in water than do the most commonly reported metabolites (Table I).

Substantial qualitative differences in the production of volatile metabolites between fungal species have been reported (Gallois et al., 1990; Börjesson et al., 1992), indicating that the production of odorous compounds is also highly variable. Thus, there is good reason to believe that other compounds in addition to 2-methylisoborneol and geosmin contribute to the off-odor indicative of deterioration of grains.

To link volatile compounds to certain odors, at least two methods can be used: The so-called GC sniff technique

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Table II. Odorous Fungi Used, Including Data on Their Odor Characteristics during Growth on MEA Petri Dishes, and Reference Fungi Used

strains with strong off-odors	odor characteristics
P. aurantigoriseum Dierckx CBS 548.77	musty, fruity, moldy, mossy
P. aethiopicum Frisvad IBT[a] 5902	moldy, fruity, pungent
P. vulpinum (Cooke & Massee) Seifert & Samson IBT 10606	wet concrete, plastic, musty, varnish
A. niger van Tieghem CBS 131.52	sulfur, musty
Reference Strains	
Penicillium brevicompactum Dierckx CBS 257.29	
Penicillium glabrum (Wehmer) Westling SLU[c] J3	
Penicillium roqueforti Thom SIK[b] 5.18.98	
Aspergillus versicolor (Vuill.) Tiraboschi CBS 111.32	
Aspergillus candidus Link CBS 102.13	
A. flavus Link CBS 569.65	

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can be used to identify odorous compounds and to describe their individual characters (Fors and Nordlöv, 1987; Wasowicz et al., 1988). In this technique, the effluents from a gas chromatograph are divided up so that one part of the sample goes to a detector while the rest ends up at a sniffing port. Alternatively, by using multivariate methods like discriminant analysis (Dravnieks et al., 1973) or partial least-squares (PLS) analysis (Martens and Martens, 1986), it is possible to link GC peaks to odor perceived when sniffing a fungal culture. These methods are less time consuming than the GC sniff technique since a sample can be judged by a panel member in just a few seconds, while it takes 30–45 min for a sample run by the GC sniff technique. It does not, however, permit the odor of each individual compound to be characterized.

For some fungi, sporulation has been reported to be associated with the onset of the production of certain metabolites, some of which are odorous (Latrasse et al., 1985; Pesis and Marinansky, 1990).

The present investigation was performed to (i) compare the production of odorous compounds with that of the most common volatile metabolites produced by 10 *Aspergillus* and *Penicillium* species during 4 days of growth, (ii) identify odorous, volatile fungal metabolites using the GC sniff technique and search for possible correlations between odor and the presence of certain volatile metabolites using multivariate data analysis, and (iii) investigate the relationship between sporulation and the production of odorous compounds.

MATERIALS AND METHODS

Fungal Cultures. In a preliminary experiment the overall character and intensity of the odors of 10 strains with noticeable odors growing on 2% malt extract agar (MEA) were estimated by three trained judges. Four strains having strong, musty odors reminiscent of deteriorated grains were selected for further studies (Table II). In addition, six strains that had been used in our previous investigation (Börjesson et al., 1992) and which frequently occur on cereal grain were used as reference fungi (Table II).

Substrate. Oatmeal agar (OMA) was used as substrate since, although it has a less disturbing background odor than whole grains, the profile of volatile fungal metabolites produced is similar to that obtained with whole grains (Börjesson et al., 1990). The OMA was prepared by adding 30 g of rolled oats to 1000 mL of distilled water, which was then boiled slowly for 1 h. After filtration through cotton cloth, 20 g of agar was added. After another 20 min of boiling, the mixture was autoclaved for 15 min at 121 °C.

Equipment for Cultivation of Fungi. Stainless steel, cylindrical cultivation vessels (180 mL) were filled with 20 mL of OMA and autoclaved at 121 °C for 15 min. After cooling, the vessels were inoculated and equipped with glass tubes filled with glass wool to prevent contaminating spores from entering or exiting the vessels.

Inoculation of Fungi. All fungi were cultivated for about 1 week on 2% MEA Petri dishes containing chloramphenicol (0.1 g/L). For each fungus a spore suspension was prepared as described elsewhere (Börjesson et al., 1992). The suspension was diluted to 10^7 spores/mL, and 12 drops (ca. 0.1 mL) were applied on the agar surface of the cultivation vessel using a sterile plastic syringe. The spore suspension was then evenly spread over the whole agar surface using a glass rod.

Each odorous fungus was used to inoculate three vessels on each of two occasions. To investigate the relation between onset of sporulation and production of odorous metabolites, the ability of *Penicillium aurantiogriseum* to sporulate was enhanced by cultivation on bran koji, prepared as follows: wheat kernels (100 g) were coarsely milled, $CaCO_3$ (15 g) and water (75 g) were added, and the mixture was autoclaved at 121 °C for 15 min. Precultivation on bran koji was used on one of the cultivation occasions prior to inoculating it on MEA Petri dishes as described above. In all other cases, refrigerated agar cultures were used as inoculum.

Two parallel vessels were inoculated with reference fungi, and one vessel was left uninoculated as a control.

Sampling of Volatile Metabolites. Air was purged through the vessels for 1 h (20 mL/min) 1 day after inoculation to make the results of the day 2 sampling more comparable with the following samplings that were preceded by a sampling the day before. Also, earlier studies (Börjesson et al., 1989) indicated a very low production of volatile fungal metabolites during the first day after inoculation. Thus, this purging would primarily diminish the background of volatile compounds emanating from the substrate. To prevent contamination of the vessels by volatile compounds from incoming air, a glass tube filled with the porous polymer adsorbent Chromosorb 102 (Johns-Manville) was attached to the air inlet of the cultivation vessel.

The vessels containing odorous fungi intended for GC/MS analysis were sampled in the following manner: On days 2–5 after inoculation, the character and intensity of the odor of the air leaving the cultivation vessels were evaluated by two trained judges. The presence of a musty off-odor was noted and the intensity of the smell, irrespective of the character, was rated from 0 = no smell to 5 = very strong smell. Thereafter, a glass tube filled with 250 mg of the porous polymer adsorbent TENAX TA (ENKA, Holland) was attached to the outlet, and air was passed through the vessel for 8 h (20 mL/min). On days 2 and 3 and days 3 and 5 after inoculation, the same procedure was performed with the reference fungi and the uninoculated control, respectively. These samples were used for GC/MS analysis and subsequent multivariate analysis. Two vessels of each of the four odorous fungi were sampled on days 4 and 5 for GC sniff analysis.

To relate the results to mold-infected grains, dried oats (100 g) with visible mold growth and with strong musty–earthy odor were poured into a glass flask and air was purged through the oats for 8 h (20 mL/min). Volatile compounds were adsorbed on TENAX TA in the same manner as for the agar cultures.

TENAX TA retains only limited amounts of water, which can otherwise cause problems during the concentration of volatile compounds in the cold trap prior to injection. In a preliminary experiment, it was found that $^4/_5$ of the ethanol and $^1/_{10}$ of 2-methyl-1-propanol entering the adsorbent passed through (data not shown). These losses were compensated for in the results given. By contrast, 3-methyl-1-butanol and 1-octen-3-ol did not pass through under the conditions stated above.

Equipment and Procedures Used for Analysis and Data Processing. The equipment and temperature conditions used for thermal desorption of adsorbed volatile compounds were described elsewhere (Börjesson et al., 1989, 1990). A Varian 3700 gas chromatograph coupled to an Incos 50 (Finnigan MAT) quadrupole mass spectrometer was used. The gas chromatographic column used was a fused silica capillary column (60 m by 0.32 mm i.d.) DB 1701, J&W. The gas chromatograph was programmed to increase the temperature from 30 to 200 °C at

Table III. Compounds Produced by Four Different Fungi during Growth on Oatmeal Agar and Found during GC Sniff Analysis To Be Off-Odorous

fungus	off-odorous compound	odor character
P. vulpinum	1-methoxy-3-methylbenzene	concrete, plastic, moldy
	geosmin	red beet, musty, earthy
P. aethiopicum	geosmin	earthy, red beet, musty
P. aurantiogriseum	1-octen-3-ol	mushroom, moldy
	2-methylisoborneol	moldy, musty
A. niger	dimethyl disulfide	sulfurous
	2-methylisoborneol	moldy, musty

Table IV. Headspace Concentrations of Metabolites Found To Be Off-Odorous in GC Sniffing Tests and Concentrations of the Most Common Volatile Metabolites in Cultivation Vessels Inoculated with Various Odorous Fungi on Oatmeal Agar (ng/L Headspace, $n = 4$)

	fungus and days after inoculation							
	P. aurantiogriseum				*P. aethiopicum*			
metabolite	2	3	4	5	2	3	4	5
3-methylfuran	1.3	6*	7*	12*		0.46**	0.36**	
2-methyl-1-propanol	9*	120*	140*	190*	8.6*	100	250	220
methyl-1-butanol[a]	90*	630*	890**	940**	6.4*	33	39	14
1-octen-3-ol[b]	100*	360**	1200**	1500**				
2-methylisoborneol[b]	2.8*	1.7*	1.9*	1.5*				
1-methoxy-3-methylbenzene[b]					0.22**	1.4	1.2*	4.5**
4-methylphenol[b]								
geosmin[b]					51*	390	410	330
dimethyl disulfide[b]								

	fungus and days after inoculation							
	P. vulpinum				*A. niger*			
metabolite	2	3	4	5	2	3	4	5
3-methylfuran	0.16**	0.21**	0.37**	0.7**	0.1**	0.75*	1.5*	0.85*
2-methyl-1-propanol	0.81**	33**	210	370		5.2*	11**	7.0**
methyl-1-butanol[a]		51**	320**	810*	2.0*	57*	16**	0.41**
1-octen-3-ol[b]						27*	19*	6.0*
2-methylisoborneol[b]					0.8	18*	6.5*	1.8*
1-methoxy-3-methylbenzene[b]	1.0**	170**	710*	910				
4-methylphenol[b]		100*	580**	990				
geosmin[b]		0.63**	0.15**	0.40**				
dimethyl disulfide[b]					13*	24*	31*	21

[a] Mixture of 2-methyl-1-butanol and 3-methyl-1-butanol. [b] Off-odorous. *Coefficient of variation ≥50%. **Coefficient of variation ≥100%.

4 deg/min and with an initial hold for 2 min. Mass spectral reference data were taken mainly from the National Bureau of Standards (NBS) (38 500 spectra) and the *Wiley Registry of Mass Spectra* (140 000 spectra, John Wiley and Sons Inc.). Variation between replicates was described with coefficients of variation as outlined in Sokal and Rohlf (1981) and Dunn and Clark (1974).

Data on the character and the intensity of the odor of the air leaving the cultivation vessels were compared with GC/MS data, and correlations were evaluated using multivariate analysis. To find out which volatile metabolites were best at predicting odor intensity and musty odor, respectively, partial least-squares (PLS) analysis (Martens and Martens, 1986; Höskuldsson, 1988) was performed. In this analysis, odor intensity and the presence of moldy odor, respectively, were used as dependent variables, and the 65 compounds present in the highest concentrations in the cultivation vessels were treated as independent variables. The results from half the samples taken on day 3 after inoculation were used for the PLS analysis, including the uninoculated control (15 vessels). The data were standardized and log transformed before PLS analysis. The PLS model was validated using the remaining 14 inoculated vessels as an independent validation set (Martens and Martens, 1986). Although not independent, results from the uninoculated control vessel sampled on day 2 were also included in the validation set. SIRIUS software (Pattern Recognition Systems) was used for the PLS analysis.

During the GC sniffing, the odorous volatile metabolites were split between a flame ionization detector (FID) and a sniffing port after previous separation by gas chromatography using a Varian 4600 gas chromatograph. The FID–sniffing port split ratio was 1:5.1. The desorption procedure, temperatures, and column used were the same as for GC/MS analysis. For each odorous fungus, samples taken on days 4 and 5 were combined to increase the concentration of odorous metabolites. Three trained judges each sniffed for 15 min during the 45-min sample run. Two such runs were made for each odorous fungus. Between the replicates, the persons changed positions, so that the compounds from each 15-min period were sniffed by two persons. The judges were instructed to specifically find peaks having off-odors reminiscent of the off-odor of the fungal culture. For identification of odorous compounds the GC sniff chromatograms were compared with those of the GC/MS analysis.

RESULTS

Descriptions of Odors of Fungi Growing on Petri Dishes. The four odorous fungi selected had a strong overall odor and a musty note similar to that of moldy grains. The descriptive terms used by the judges to describe the odors of the fungi are presented in Table II.

Identification of Odorous Compounds by GC Sniffing. Compounds that were possible to identify mass spectrometrically and found to be off-odorous by both judges during GC sniffing are presented in Table III. The identities of geosmin, 2-methylisoborneol, and 1-octen-3-ol were confirmed by using standards. The mass spectra of other compounds in Table III were very compound specific, and it was not considered necessary to use standard compounds.

Mostly, these compounds were present at concentrations of >10 ng/L headspace (Figure 1; Table IV). Other compounds also influenced the overall odor of the fungi. Especially noteworthy in this regard were terpenoid

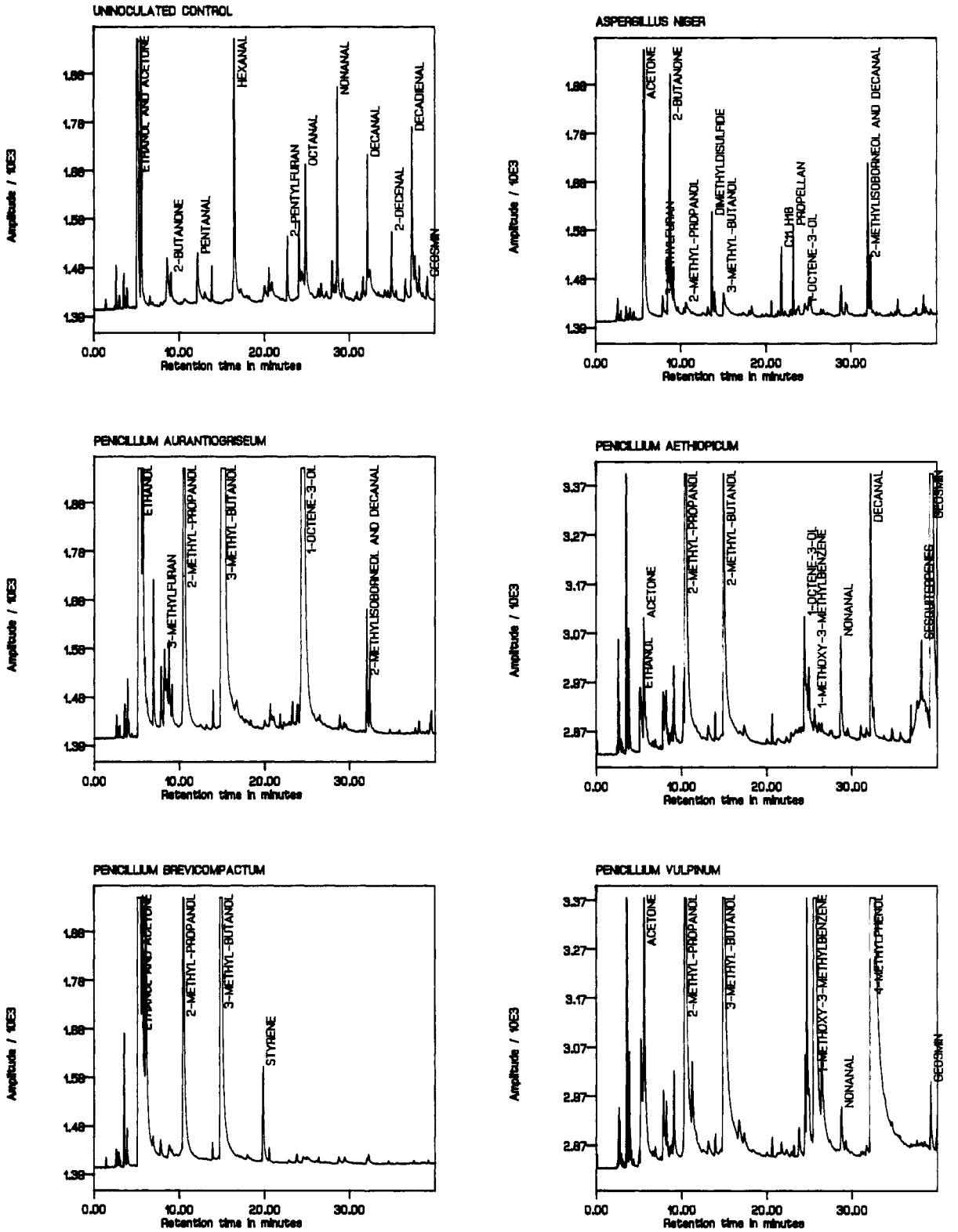

Figure 1. One chromatogram each of an uninoculated control, a reference fungus (*P. brevicompactum*), and the odorous fungi *P. aurantiogriseum*, *P. aethiopicum*, *A. niger*, and *P. vulpinum*. The samples were taken on day 3 after inoculation of the fungi. The same scale is used for all chromatograms, but because of different base-line levels, the values on the *y*-axes differ. When several important peaks appear close together, their names are written in a row starting with the peak having the shortest retention time.

compounds, which were produced by all odorous fungi, generally at concentrations below 10 ng/L headspace. Some monoterpenes had a musty, moldy, or pungent note, and sesquiterpenes were often described as mossy. These compounds seemed to be species specific. The only registered exception was a compound with the formula $C_{11}H_{18}$ possessing a pungent odor that was produced by *Aspergillus niger* and *Penicillium vulpinum*. The mass spectrum of the compound is very similar to that of propellan, synthesized by Takaishi et al. (1975). Besides 1-octen-3-ol, mushroom odor was detected from other eight-carbon compounds, e.g., octadienes. *Penicillium aethiopicum* produced 1-methoxy-3-methylbutane at the end of the experiment, which contributed a pungent smell to the overall odor of the fungus. *Penicillium vulpinum* produced 4-methylphenol in large amounts having a

Table V. Headspace Concentrations in Cultivation Vessels of Metabolites Having Off-Odors and the Most Common Volatile Metabolites Detected 3 and 5 Days after Inoculation of Reference Fungi on Oatmeal Agar (ng/L, $n = 2$)

	fungus and days after inoculation											
	P. brevi.[a]		*P. glabrum*		*P. roqueforti*		*A. versicolor*		*A. flavus*		*A. candidus*	
compound	3	5	3	5	3	5	3	5	3	5	3	5
3-methylfuran	2.5	3.8*	2.1	9.8	0.77			0.24*	1.5**	1.4*		1.2
2-methyl-1-propanol	97	200			12**	52		0.67	120	7.7**		
3-methyl-1-butanol	280	230	12	17	36*[c]	37**[c]		17	240			
2-methylisoborneol[b]			0.08**	0.62								
geosmin[b]												
1-methoxy-3-methylbenzene[b]												
dimethyl disulfide[b]			0.24**		18**							
1-octen-3-ol[b]							3.1					

[a] *Penicillium brevicompactum.* *Coefficient of variation ≥50%. **Coefficient of variation ≥100%. [b] Off-odorous. [c] Mixture of 3-methyl-1-butanol and 2-methyl-1-butanol.

plastic, pungent, or earthy odor, which highly contributed to the odor of the fungus. The sulfurous odor of *A. niger* was probably influenced by dimethyl trisulfide, which was detected in some samples. It has a lower odor threshold in air (7.3 $\mu g/m^3$) than does dimethyl disulfide (29 $\mu g/m^3$; Wilby, 1969).

The GC sniffing of moldy oats revealed that 2-methylisoborneol contributed mostly to the musty smell (data not shown). 1-Methoxy-3-methylbenzene and geosmin were also present but in lower concentrations. The compound found in the highest concentration was 2,6-dimethylpyrazine, which had a strong, pungent odor.

Sniffing Air Exiting Cultivation Vessels and GC/MS Analysis. For the four odorous fungi, the main odorous compounds were generally detected by GC/MS 1 day before their odors became discernible in the air leaving the cultivation vessels. The composition of volatile metabolites showed little change during the 4 days of sampling.

The less odorous compounds, 3-methylfuran, 2-methyl-1-propanol, and methylbutanols, were produced more frequently than the more odorous ones (Table IV). Odor intensities in cultivation vessels generally peaked when musty off-odors were most frequently noted (Table VI). For *A. niger*, sulfurous odor was detected by day 2, before the musty smell appeared. This agrees with the GC data, which show that the production of dimethyl disulfide and dimethyl trisulfide precedes that of 2-methylisoborneol.

Reference fungi produced 3-methylfuran, 2-methyl-1-propanol, and 3-methyl-1-butanol more frequently than they produced off-odorous compounds (Table V). The odor intensities for reference fungi were generally lower than those for the odorous fungi, and musty notes were seldom registered for the former (Table VI). Sweetish notes were sometimes registered. These may have been due to the presence of 3-methyl-1-butanol, which was described as having a sweet smell during the GC sniffing.

Of the volatile fungal metabolites reported, only geosmin and 1-octen-3-ol were detected in the uninoculated control vessel. On average for days 2 and 3, 2.1 ng/L headspace of geosmin and 12 ng/L of 1-octen-3-ol were found. These amounts were therefore subtracted in the amounts given in Tables IV and V.

The production of odorous metabolites seemed to increase just prior to sporulation (Table VII). However, no qualitative change in the profile of odorous metabolites was noted in connection with sporulation. For *P. aethiopicum* and *P. vulpinum* a slight difference in time of sporulation onset was observed between the two inoculation occasions, although no precultivation on bran koji was performed.

Table VI. Odor Characteristics and Intensities Recorded by Two Judges after Sniffing Air Emerging from Cultivation Vessel Outlets[a]

		days after inoculation			
fungus	inoculation	2	3	4	5
P. aurantiogriseum	1	3.5	3.5m	2.5m	3.0
P. aurantiogriseum	2	2.5	3.5m	3.0m	3.5m
P. aethiopicum	1	0.7	1.8m	2.0m	1.8
P. aethiopicum	2	0.5	1.8m	2.6m	2.8m
P. vulpinum	1	0.0	0.0	0.8	1.4
P. vulpinum	2	0.0	0.7	2.9m	2.8m
A. niger	1	3.0	2.5m	2.3m	2.8
A. niger	2	1.0	1.8m	2.0m	3.1m
A. flavus	1	nd[b]	1.2	nd	0.8
A. versicolor	1	nd	1.0	nd	1.5m
A. candidus	1	nd	0.5	nd	0.9
P. brevicompactum	1	nd	1.5	nd	1.0
P. glabrum	1	nd	0.0	nd	0.8
P. roqueforti	1	nd	1.1	nd	0.9
uninoculated control	1	1.0	0.8	nd	nd

[a] Mean odor ratings for 2 replicates and 2 judges where 0 = no odor and 5 = very strong odor; $n = 2$ except for uninoculated control where $n = 1$. m = musty odor detected by at least 1 judge in both replicates. [b] nd = not determined.

In moldy oats, 3-methylfuran, 2-methyl-1-propanol, 3-methyl-1-butanol, and other less odorous compounds were present in addition to the odorous ones mentioned above.

PLS Evaluation. *Relating GC Peaks to Total Odor Intensity.* When estimating PLS components (latent variables), it was found that the first two components represented 79% and 13% of the total variation in odor intensity, respectively. The similarities between variables (their covariance) can be visualized in a variable loading plot (Figure 2a). When a line is drawn through the origin and the dependent variable (designated by the numeral 3 in the figure), the variables strongly correlated with the dependent variable will be close to the line but far away from the origin (Hämäläinen and Albano, 1992). The compounds fulfilling these conditions, i.e., those found to be most strongly correlated with odor intensity, were 1-octen-3-ol, 3-methyl-1-butanol, octadienes, 2-methylpropanal, 3-methylfuran, 2-methyl-1-propanol, styrene, acetone, and ethanol (see encircled area in Figure 2a). Another way of assessing the degree to which individual compounds contribute to the total odor intensity is to compare their PLS regression coefficients. This gives an estimation of which variables are best at predicting odor intensity (Martens and Martens, 1986). Except for acetone and ethanol, the compounds mentioned above were among the 10 compounds having the highest positive regression coefficients.

When the PLS model was validated on the basis of the first two components with data not used to create the

Table VII. Headspace Concentrations (ng/L Headspace) of Odorous Metabolites in Cultivation Vessels Inoculated with Three Different Odor-Producing Fungi on Two Occasions on Oatmeal Agar (n = 2)[a]

fungus	occasion	odorous compound	days after inoculation 2	3	4	5
P. aurantiogriseum	1	2-methylisoborneol	4.6	2.3s	3.0s	2.1s
P. aurantiogriseum	2	2-methylisoborneol	0.98	1.1	0.77	0.84*s
P. vulpinum	1	1-methoxy-3-methylbenzene	1.4**	300**	700*s	650s
P. vulpinum	2	1-methoxy-3-methylbenzene	0.68	10	340	910s
P. aethiopicum	1	geosmin	74	400s	310s	270s
P. aethiopicum	2	geosmin	33	500	510s	320s

[a] s = sporulation. *Coefficient of variation ≥50%. **Coefficient of variation ≥100%.

model (the 14 replicate cultivation vessels and uninoculated control), a correlation coefficient of 0.42 ($p > 0.05$) between predicted odor intensity and measured intensity was obtained. However, in one vessel with a high odor intensity score, 80% of the total volatiles consisted of undecene. With reference to the results of the other vessels, this seems to be an erroneous result. Thus, after this outlier was excluded, the correlation coefficient increased to 0.74 ($p < 0.01$).

Relating GC Data to the Occurrence of Musty Odor. The same criteria as for the total odor intensity was studied when relating GC peaks to musty odor. When two components, explaining 71% and 21%, respectively, of the variation in musty odor were estimated, a group of six compounds could be distinguished as being more strongly correlated to musty odor than the others (Figure 2b). These compounds were 2-methylisoborneol, 1-octen-3-ol, dimethyl disulfide, 2-butanone, an unidentified terpenoid compound ($C_{11}H_{18}$) produced by *A. niger*, and propellan. All of them were among the 10 compounds with the highest positive PLS regression coefficients. When the PLS model based on two components was validated with data from the replicates, the correlation coefficient between the predicted and the measured presence of musty odor was 0.56 ($p < 0.05$). After the outlier mentioned above, which may have erroneously been designated as having a musty odor, was excluded, the correlation coefficient increased to 0.96 ($p < 0.001$).

Comparison of the Results of the GC Sniff Technique and the PLS Analysis Concerning Musty Compounds. Of the six compounds indicated by PLS analysis as having the highest correlations with musty odor, five were considered to have a musty or similar odor in the GC sniff analysis. These compounds were 2-methylisoborneol, having a moldy or musty odor; dimethyl disulfide with a sulfurous note; 1-octen-3-ol having a mushroom or moldy odor; and the $C_{11}H_{18}$ compound and propellan, which were described as pungent.

Of the compounds described as having a musty or similar odor using the GC sniff technique, 1-methoxy-3-methylbenzene and geosmin did not seem to be strongly correlated with musty odor when using the PLS analysis.

DISCUSSION

The results of this investigation show that the onset of production of odorous compounds coincides closely with that of other less volatile metabolites. Sporulation seems to coincide with an increase in the production of several volatile compounds of which some are odorous, indicating that odor intensity should be positively related to numbers of colony forming units (CFU). This implies that odors may not be good indicators of fungal growth measured as fungal biomass since correlations between CFU and fungal biomass may be weak (Schnürer, 1993). This agrees with findings of Schnürer and Jonsson (1992), who found that odors were not correlated with ergosterol levels, and thus not with fungal biomass.

With PLS analysis we were able to pick out a group of compounds that were well correlated with off-odor. Generally, these compounds were also identified as being off-odorous in the GC sniff analysis. It was possible to pick out compounds suspected to be malodorous rapidly with PLS analysis with a limited need for judges, indicating that PLS analysis should be valuable in the first step of a search for off-odorous compounds.

Using the GC sniff technique, it was possible to identify off-odorous compounds and describe their individual characteristics. This procedure revealed considerable differences between the fungi examined. With the GC sniff technique it was possible to detect compounds with low odor thresholds, which were present in amounts too low to allow detection by the GC detector. By concentrating the samples, an identification would be possible. Thus each of the methods offer unique advantages, making them both valuable tools for use in searching for off-odors.

As found in earlier studies (Wasowicz et al., 1988) 2-methylisoborneol and geosmin contribute strongly to the off-odors produced by fungi commonly found in cereals. 2-Methylisoborneol was produced by two odorous fungi and one reference fungus and was also found in moldy oats. This compound had a typical musty character, indicated by PLS analysis and confirmed by GC sniffing, and was apparently the most important contributor of musty odor in this investigation. 2,6-Dimethylpyrazine, described as pungent by Fors and Olofsson (1986), made a contribution to the off-odor of deteriorated oats and has been found in higher quantities in moldy than in sound grains (Wasowicz et al., 1988). Liardon and Ledermann (1980) suggested that it is produced by molds. 1-Octen-3-ol, which has a mushroom-like smell, has frequently been reported as a metabolite of fungi growing on cereals (Kaminski et al., 1974; Harris et al., 1986). In this investigation, it was produced by 3 of the 10 fungi investigated, and the PLS analysis indicated that it made important contributions to both the total odor intensity and to the musty character of the odor. Although not reported earlier from cereals, 1-methoxy-3-methylbenzene was produced by at least two fungal species on oatmeal agar, and it was also present in mold-damaged oats. The vessels inoculated with *P. vulpinum*, the main producer of this compound, were not characterized as musty 3 days after inoculation, indicating that its odor is of another character (Table VI). Odorous compounds with the formula $C_{11}H_{18}$ as well as sulfur-containing compounds also contributed substantially to the total odor of the fungi.

The PLS analysis revealed that total odor intensity was most strongly correlated with compounds indicating mold growth in general that lacked a characteristic musty odor, e.g., 2-methyl-1-propanol, 3-methyl-1-butanol, and 3-methylfuran. 1-Octen-3-ol and octadienes which have a mushroom-like aroma were also of strong predictive value.

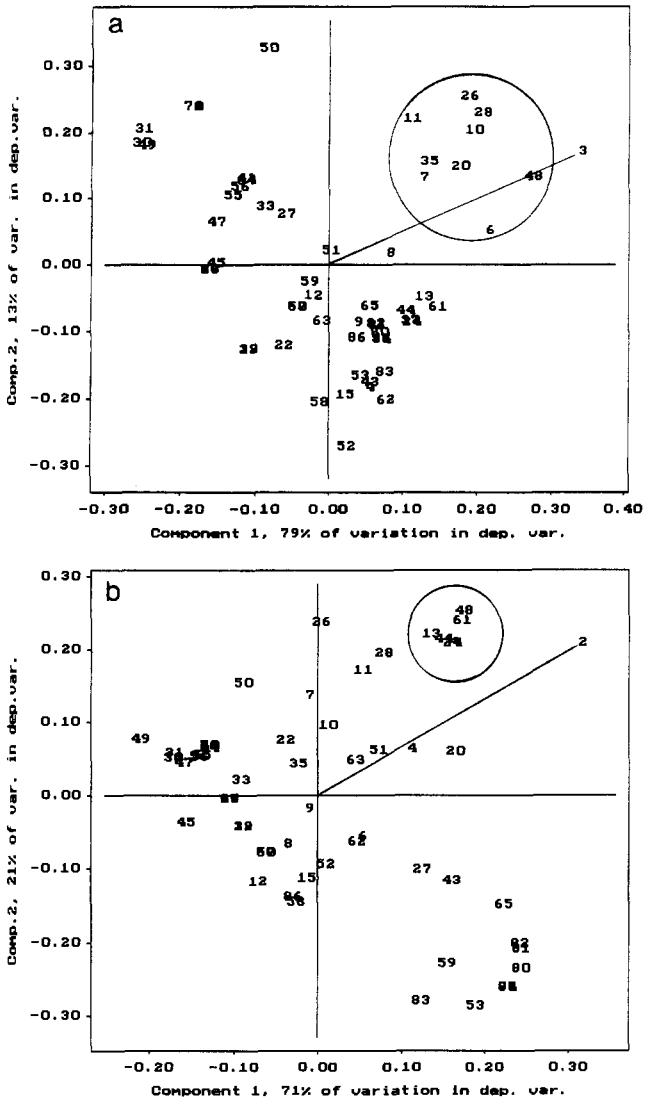

Figure 2. (a) Loading plot of the first two PLS components when using odor intensity as the dependent variable and 65 volatile compounds as the independent variables. Data from 15 samples taken 3 days after inoculation: two were taken from each odorous fungus, one from each reference fungus and one from an uninoculated control. Variables are designated by numbers, and the numerical designations of the dependent variable and encircled independent ones are as follows: (3) odor intensity; (6) ethanol; (7) acetone; (10) 2-methylpropanal; (11) 3-methylfuran; (20) 2-methyl-1-propanol; (26) 3-methyl-1-butanol; (28) octadiens;** (35) styrene; (48) 1-octen-3-ol. **Mixture of 1,3-octadiene and an isomere. (b) Loading plot of the first two PLS components when using musty odor as dependent variable and 65 volatile compounds as independent variables. Data from 15 samples taken 3 days after inoculation: two were taken from each odorous fungus, one from each reference fungus and one from an uninoculated control. Variables are designated by numbers, and the numerical designations of the dependent variable and encircled independent ones are as follows: (2) musty odor; (13) 2-butanone;* (24) dimethyl disulfide; (37) unidentified $C_{11}H_{18}$ compound;* (44) propellan;* (48) 1-octen-3-ol; (61) 2-methylisoborneol. *Members in cluster underneath 61.

Thus, compounds having musty smells tended not to produce the strongest overall odors.

2-Methyl-1-propanol, 3-methyl-1-butanol, and 3-methylfuran were the most frequently produced compounds among the fungi. This is in agreement with results of Gallois et al. (1990) who found that 4- and 5-carbon alcohols were among the most common volatile metabolites produced by 29 strains of basidiomycetes.

The results show that although off-odorous compounds are produced in high concentrations by certain fungal species, most fungi found in grain samples do not produce these metabolites. Thus, it is unlikely that the odor of a grain sample can be used to correctly assess its microbiological status. However, off-odors are in themselves a sign of bad quality. On the other hand, 2-methyl-1-propanol, 3-methyl-1-butanol, and 3-methylfuran were produced by almost all the examined fungi, although in different quantities. Thus an instrumental analysis of this group of compounds should give a more reliable measure of fungal growth in cereals than would an odor assessment.

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