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Determination of Geosmin, 2-Methylisoborneol, and a Musty-Earthy Odor in Wheat Grain by SPME-GC-MS, Profiling Volatiles, and Sensory Analysis

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Geosmin and 2-methylisoborneol—compounds responsible for the musty-earthy off-odor of wheat grain, were isolated by SPME and analyzed by GC-MS. Carboxen/PDMS/DVB fiber coating was selected because of its highest extraction efficiency. Concentrations of geosmin and 2-methylisoborneol as low as 0.001 μg/kg were detected in SIM mode using ion trap mass spectrometer. Apart from GC-MS determination of geosmin and 2-methylisoborneol, various methods for evaluating the musty-earthy off-odor caused by these compounds in wheat grain are presented. Sensory profile analysis differentiated wheat grain into sound and off-flavored, but the method was tedious. Similar groupings, however, were obtained using more rapid methods such as comparison of volatile profiles using SPME-fast GC with PCA projection of data and metal oxide (MOS) based electronic nose.

KEYWORDS: Electronic nose; musty-earthy off-odor

INTRODUCTION

A relatively large group of compounds, comprising 2-methoxy-3-isopropylpyrazine, some di-, tri-, tetra-, and pentachloroanisoles, octa-1,3-diene, 2-methylisoborneol, and geosmin as the main ones, are responsible for an earthy-musty off-odor (*1*). The last two have been reported as a source of musty-earthy odors in grain, which may develop during improper storage as a result of microbial growth (*2*). Geosmin was detected as a metabolite of *Penicillium vulpinum* and *Penicillium aethiopicum* and 2-methylisoborneol in cultures of *Aspergillus niger*, *Penicillium aurantiogriseum*, and also *Penicillium expansum*—strains having strong odors reminiscent of deteriorated grains (*3*, *4*). Geosmin is known also as a compound contributing to characteristic earthy red beet flavor (*5*). 2-Methylisoborneol was found in cultures of *Penicillium caseicolum* and was related to the musty-earthy notes in Brie and Camembert cheese flavor (*6*). Both geosmin and 2-methylisoborneol were identified as *Actinomycetes* metabolites (*7*). They are responsible for the musty-earthy odors in water supplies, where they are usually produced by *Streptomyces*, *Nocardia*, *Micromonospora*, *Microbispora*, *Oscillatoria*, and *Phormidium* (*8*).

Methods of analysis of these compounds are a challenge for analytical chemists because of their low odor threshold levels—0.01 μg/kg for 2-methylisoborneol (MIB) and 0.015 μg/kg for geosmin (*9*). With regard to sensitivity, olfactory detection of geosmin and methylisoborneol can compete with instrumental methods based on chromatography. However, sensory evaluation of grain samples has some important drawbacks: the subjectivity of measurements due to a "human factor" and the hazard for the inspector's health as spores of fungi can be inhaled when sniffing samples. Therefore, there is a need for the development of instrumental methods for the analysis of musty-earthy-smelling compounds, which would fulfill specific requirements: the sample preparation step should be minimized and simplified to facilitate a large sample throughput, and the method should be easy to automate and sensitive—ideally, enough to detect compounds of interest in concentrations below their odor thresholds.

Recently solid phase microextraction (SPME) proved to be a very sensitive and also low-cost, rapid method; it has been utilized for the analysis of geosmin and MIB mainly in water (*10*, *11*). Information on the quantification of these compounds by SPME in other matrices is scattered (*5*, *12*).

An alternative approach in the analysis of microbial volatile compounds is the use of the electronic nose, which mimics the human olfactory system, where an array of nonspecific sensors is exposed to vapors and the pattern of their response generated by headspace constituents is used to compare different samples. Such an approach was evaluated for the classification of spoilage fungi and grain quality (*13*−*17*).

This study was focused on the quantitation of geosmin and methylisoborneol by SPME-GC-MS in wheat grain with a musty-earthy off-odor. Additionally, we wanted to characterize fungal microflora of these samples and check two rapid instrumental methods (SPME-fast-GC-PCA and electronic nose) for their ability to discriminate between sound and off-odored samples on the basis of a comparison of their headspace.

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MATERIALS AND METHODS

Reference Compounds, Fibers, and Grain Samples. Standards of geosmin and MIB were purchased from Sigma-Aldrich and were of 99% degree of purity. SPME fibers used for method elaboration were obtained from Supelco (Bellefonte, PA). Samples of sound wheat and wheat with odor defects were of interest to verify the method's usefulness. Samples were collected from farm granaries in the vicinity of Poznań. All of them were harvested in 2001, had water content of 11−13%, and were stored in various conditions until May 2002.

Sensory Analysis. A panel of 10 people experienced in profile sensory analysis performed analysis of wheat samples. A vocabulary of descriptors was developed for the evaluation of grain odors (*18*). The following odor descriptors were offered for examined samples: (1) grainy (resembling good-quality raw grain); (2) strawlike (straw or grain husk); (3) herbal (dried herbs); (4) malty; (5) dairy (dairy waste—unpleasant); (6) sour (acid); (7) putrid (decaying protein); (8) fungal (mushrooms); (9) musty−moldy (decaying wet wood); (10) earthy (soil, wet dirt, mud). Grain samples (100 g) were presented to panel members in glass cylinders with glass stoppers. Cylinders with samples were preheated at 40 °C to liberate volatile compounds. Grain samples evaluation was done in three sessions; that is, samples from the same lots were presented to panel members three times within 8 h. Panel members assigned the intensity of each odor descriptor on a 0−10 scale. Three hundred measurements done for all descriptors were processed using principal component analysis (PCA).

Isolation of Volatile Compounds by SPME. Volatile compounds were isolated from grain samples using SPME. Four SPME fibers were tested for their efficiency in isolating geosmin and MIB—Carboxen/PDMS, Carboxen/divinylbenzene/PDMS, divinylbenzene/PDMS, and PDMS. Isolation was performed at 50 °C for 30 min after sample preheating without fiber for 15 min. After extraction, compounds were desorbed in the injection port of a gas chromatograph at 260 °C for 5 min.

GC-MS Analysis. GC-MS analyses were performed on a Trace 2000 gas chromatograph coupled to a Finnigan PolarisQ ion trap mass spectrometer with an outer ionization source. Compounds were resolved on an HP-5 column (30 m × 0.25 mm × 0.25 μm, Agilent, Palo Alto, CA). Analyses were performed in programmed temperature from 40 to 280 °C at 8 °C/min. Inlet temperature was 260 °C, and SPME desorption time was 5 min. The PTV injector of a Trace 2000 was working as a constant temperature split/splitless injector with a purge time of 1 min. Transfer line temperature was 280 °C, whereas ion source temperature was 200 °C. For method optimization standards were run in SCAN, SIM, and MS/MS modes. SCAN was performed at a range of 40−340 amu. In SIM mode the following ions were chosen: 112, 125, and 182 for geosmin and 95, 107, and 168 for methylisoborneol. In MS/MS experiments base peaks were chosen as parent ions for further fragmentation—*m*/*z* 112 for geosmin and *m*/*z* 95 for methylisoborneol. Geosmin and methylisoborneol contents was determined in wheat samples based on external standard calibration using extracted ion intensities (*m*/*z* 112 for geosmin and *m*/*z* 95 for methylisoborneol). These analyses were run in SIM mode.

Evaluation of Fungi Present on Grain Kernels. One hundred kernels of each sample were used to evaluate microflora present in wheat samples. Ten kernels were placed on each of 10 Petri dishes with agar and incubated at 24 °C for 7 days. After that time, fungi grown on kernels were identified on the basis of morphological features using adequate identification manuals (*19*−*21*).

Fast Chromatography and PCA. A Hewlett-Packard HP 6890 gas chromatograph equipped with a split/splitless injector with a 0.75 mm liner, a flame ionization detector able to acquire data with a speed of 200 Hz, and a 10 m × 0.100 mm × 0.34 μm HP-5 column (Agilent Technologies, Palo Alto, CA) were used for analyses. Volatiles from 10 g wheat samples placed in 20 mL vials were extracted at 50 °C for 20 min using an SPME syringe. Afterward they were injected manually into the gas chromatograph at 260 °C in splitless mode in a minimum of four replicates. Hydrogen was used as a carrier gas at a flow of 1.0 mL/min; the initial oven temperature was 40 °C and was ramped at 25 °C/min to 290 °C. Total analysis time was 10 min. Chromstat software (Analyt GmbH) was used to process chromatographic data from fast GC, group them, and project them as a PCA graph.

Electronic Nose. A Fox 4000 electronic nose with 18 metal oxide sensors in three chambers was used for analyses (Alpha M.O.S., Toulouse, France). Samples of wheat (5 g) were placed in 10 mL vials, capped, and placed in a Combipal type autosampler (Alpha M.O.S.). Samples were incubated for 20 min at 50 °C, and then volatiles (2.5 mL) were transferred automatically to the electronic nose by a gastight syringe. An air flow of 150 mL/min was used to sweep samples through the electronic nose chambers. Each sample was analyzed in triplicate—three vials prepared from the same sample lot. Sensor optimization and data treatment were performed using the Alpha Soft v 0.8. software package (Alpha M.O.S.).

RESULTS AND DISCUSSION

Sensory Evaluation of Grain Samples. Figure 1 represents the PCA projection of grain samples, which were grouped into three distinctive clusters. The grouping was based on the odor profile characterization for all samples. Samples E−H, perceived as sound grain, were grouped into one cluster, characterized by an odor of good-quality raw grain and strawlike and herbal to a lesser extent. The second cluster was formed by samples D and A. These two samples were perceived by the sensory panel as the mustiest and earthiest of all and were located on the PCA graph in the region of musty-moldy (*9*) and earthy (*10*) vectors. The third group containing samples B and C was characterized by a putrid attribute and also by unpleasant dairy and sour notes. According to the *Grain Inspection Handbook* (*22*) grain failing for odor classification can be graded as musty, sour, and commercially offensive foreign odors (COFO). A musty odor can be related to mold spoilage or insect infestation. Profile sensory analysis gave a broader classification of odors providing more precise sample classification. It enabled a clear distinction between off-odored and sound wheat grain, but was tedious and time-consuming to carry out.

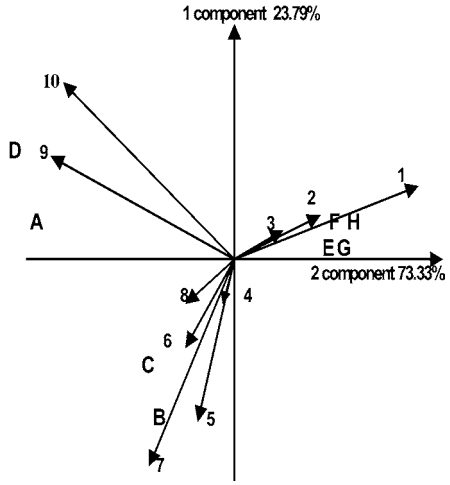

Figure 1. PCA plot of eight wheat samples characterized with different odors using profile sensory analysis: samples A and D, musty, moldy, earthy; samples B and C, putrid, sour, fungal, dairy; samples E–H, typical odor for sound grain.

Elaboration of Geosmin and Methylisoborneol Analysis Parameters. To achieve detection limits comparable to or even lower than odor thresholds for geosmin and MIB, isolation of these compounds from the matrix is crucial for the whole method's performance. Of all tested fibers, 2 cm Carboxen/divinylbenzene PDMS gave the largest responses for both geosmin and MIB (**Figure 2**). The efficiency of the divinylbenzene/PDMS fiber was the highest, taking its length into account. Divinylbenzene was a better polymer to adsorb

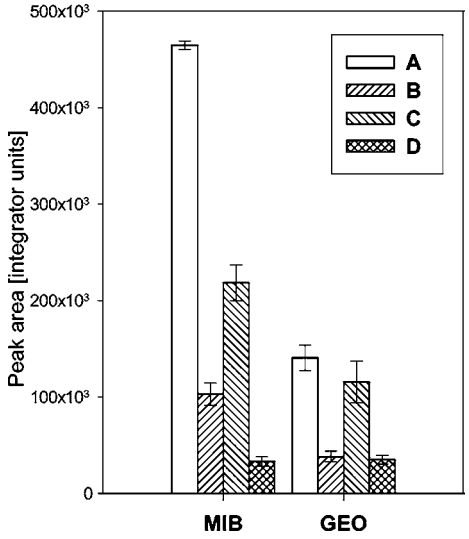

Figure 2. Peak areas of methylisoborneol (MIB) and geosmin (GEO) after extraction from wheat kernels spiked with standards using different SPME fibers: (A) 2 cm Carboxen/DVB/PDMS; (B) 1 cm Carboxen/PDMS; (C) 1 cm DVB/PDMS; (D) 1 cm PDMS.

methylisoborneol and especially geosmin than Carboxen, as can be seen from the comparison of divinylbenzene/PDMS and Carboxen/PDMS fibers. The poorest performance was observed for the PDMS fiber. Watson et al. (*11*) compared different fibers for geosmin and MIB extraction from water and observed the highest recoveries for the PDMS/DVB fiber. PDMS/Carboxen/DVB gave somewhat lower recoveries for both compounds. PA and PDMS fibers gave higher recoveries for geosmin than for MIB, but compared to porous phases fibers responses were lower.

An extraction time of 30 min was chosen in our experiments as a compromise between sensitivity and analysis time. The extraction temperature was set at 50 °C, as peak areas for geosmin and MIB were roughly 6 and 4 times higher at that temperature than at 20 °C, respectively. For analyses run in SIM mode relative standard deviations (RSD) for peak area of 1 μg/kg of geosmin and MIB were 8.13 and 8.56%, respectively, for manual injections. Linearity was measured in the range of 0.005−10 μg/kg and was expressed with R^2 values of 0.991 and 0.997, respectively, for geosmin and MIB. Limits of detection for geosmin and MIB in SIM mode were 0.001 μg/kg for both compounds. Extracted ions (*m*/*z* 112 for geosmin and *m*/*z* 95 for MIB) from three acquired for each compound were used for quantitative purposes. As qualifier ions *m*/*z* 125 and 182 and *m*/*z* 107 and 168 were selected for the confirmation of geosmin and MIB identities.

The ion trap detector showed its advantages in sensitivity and sample identity confirmation. Limits of detection in SCAN mode (*m*/*z* 40−340) were only 2−3 times higher than in the SIM mode. Another feature of the ion trap detector was that the MS/MS mode enabled the confirmation of compound identities. **Figure 3** shows the spectrum of geosmin, which was the prevailing musty-earthy compound in analyzed samples acquired in SCAN and MS/MS modes, and also the origin of fragments detected in the MS/MS mode. The geosmin molecule after isolation of ion *m*/*z* 112 and its subsequent collision-induced dissociation (excitation voltage = 1.0, time = 15 ms) yields an abundant ion *m*/*z* 97 and also *m*/*z* 83. This gives another confirmation of the compound's identity. Because MS/MS results in elimination of background influence to a large extent, the signal-to-noise ratio improves; however, only products of dissociation of one isolated ion are collected, so the overall signal intensity was ∼10 times less than in SIM mode.

Determination of Geosmin and 2-Methylisoborneol in Analyzed Grain Samples. All samples were subjected to geosmin and methylisoborneol determination by SPME-GC-MS. Geosmin and MIB were quantified on the basis of SIM using an external standard method. Sound wheat of 13% water content was spiked with standards of geosmin and MIB dissolved in methanol and used as a matrix. Standard samples were analyzed 1 day after spiking to allow absorption of analyte onto a wheat matrix. Geosmin was found in six of eight samples in concentrations ranging from 0.01 to 7.57 μg/kg (**Table 1**). Samples A and D, which were described as musty, moldy, and earthy in the profile sensory analysis, contained 4.02 and 7.57 μg of geosmin/kg, respectively. High levels of geosmin were also detected in samples B and C, characterized by putrid, sour, or dairy odor notes as the predominant ones (3.19 and 4.38 μg/kg, respectively). This would indicate that compounds of putrid character mask the earthy-musty odor caused by geosmin. In two samples that were perceived as sound—samples E and F—geosmin was detected at the concentrations of 0.01 and 0.06 μg/kg, respectively. MIB occurred in only three samples in trace concentrations. The highest concentration of MIB was detected in samples described as musty, moldy, and earthy—samples A (0.16 μg/kg) and D (0.14 μg/kg). The contribution of MIB to the musty-earthy odor of analyzed samples was therefore less distinct than that of geosmin.

Evaluation of Microflora Present in Grain Samples. Analyzed grain samples showed different degrees of contamination with fungi and bacteria. The prevailing fungi on evaluated grain kernels were *Alternaria*, *Fusarium*, *Mucor*, *Penicillium*, and *Aspergillus* (mainly *A. flavus* and *A. candidus*) (**Table 2**). On the basis of fungi detected on kernel surfaces, all samples could be grouped into two sections—one comprising samples A−D and the other samples E−I. The characteristic feature distinguishing these two groups was the different level of *Alternaria*, *Fusarium*, *Penicillium*, and *Aspergillus flavus* strains. Samples A−D were characterized with a low incidence of *Alternaria* and *Fusarium* fungi with a simultaneously high incidence of *Penicillium*, *A. flavus*, and *Mucor*. Also, bacteria were abundant in these samples (except sample C) compared to the remaining samples E−H. Such a proportion of microflora is characteristic for samples in which deterioration due to storage conditions occurs—the typical field fungi, *Alternaria* and *Fusarium*, were replaced with the characteristic storage microflora, *Penicillium* and *Aspergillus*. In the remaining four samples (E−H) the incidence of field microflora was high. *Alternaria* species were present in 42−90% of all kernels, whereas *Fusarium* species were present in 18−66% of kernels. *A. flavus* incidence in samples E−H was from 0 to 4% compared to 36−98% of kernels in samples A−D. On the basis of the profile of fungi, all samples could be divided into good quality (E−H) and bad quality ones. A high incidence of *Penicillium* and *A. flavus* was clear evidence of abundant growth of storage microflora in the latter group. It is also known that storage fungi are capable of producing volatile metabolites, which can be a cause of off-odor in grain and indicate the deterioration of grain due to fungal growth (*23*, *24*). Microbial analysis, although enabling characterization of the microorganism profile, was time-consuming and required an experienced microbiologist to identify fungi. Therefore, a search for fast chemical tests to replace microbiological sample assessment seems to be evident.

Sample Differentiation Based on Profiles of Volatile Compounds. Although two compounds present in low concentrations—

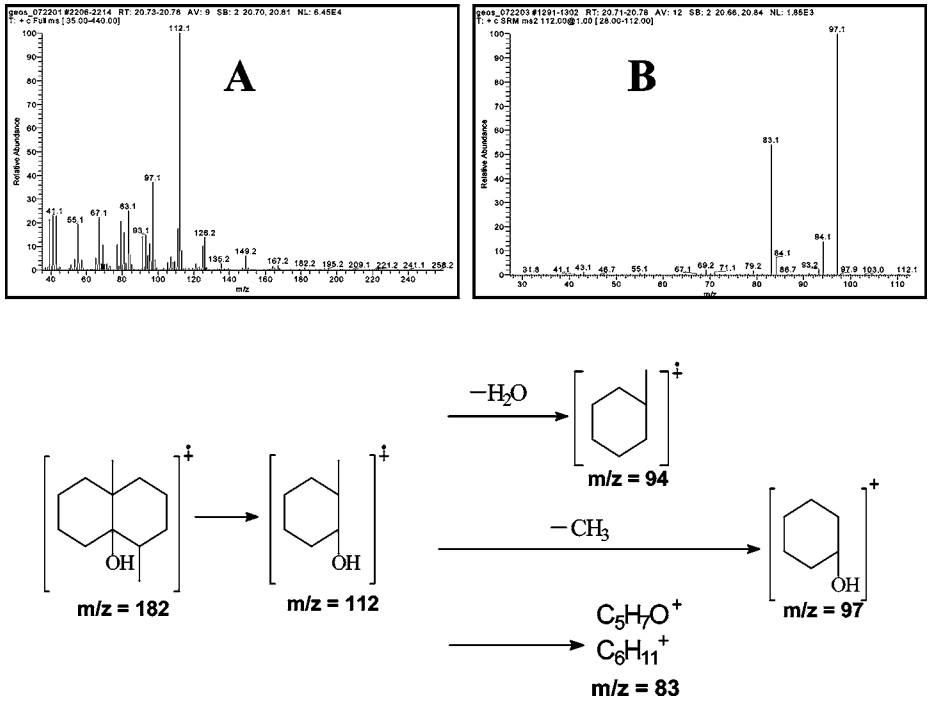

Figure 3. Mass spectra of geosmin detected in wheat samples: (A) full scan spectrum acquired on ion trap instrument; (B) MS/MS spectrum after fragmentation of isolated *m/z* 112 ion. Below are shown main fragment origins in this spectrum.

Table 1. Content of Geosmin and 2-Methylisoborneol in Analyzed Wheat Samples

sample	dominating odor characteristics	compound (μg/kg) geosmin	MIB
A	musty, moldy, earthy	4.02	0.16
B	putrid, dairy	3.19	nd
C	sour, fungal, putrid	4.38	0.04
D	musty, moldy, earthy	7.57	0.14
E	typical for good grain	0.01	nd
F	typical for good grain	0.06	nd
G	typical for good grain	nd	nd
H	typical for good grain	nd	nd

Table 2. Dominating Microorganisms Isolated from Eight Examined Samples of Wheat, A–H

	A	B	C	D	E	F	G	H
Alternaria	4	–	–	8	72	42	84	90
Fusarium	8	–	2	8	18	62	48	66
Penicillium	44	2	14	92	30	20	8	2
Aspergillus flavus	68	98	88	36	–	2	2	–
A. candidus	–	6	16	–	74	20	4	2
A. glaucus	52	2	–	2	–	–	–	–
other *Aspegillus*	8	4	10	–	–	–	–	6
Ulocladium	–	–	–	4	–	–	2	–
Drechslera	–	–	–	–	–	–	–	2
Chaetomium	–	–	–	–	2	10	–	8
Epicoccum	–	–	–	–	–	–	12	28
Mucor	74	80	78	72	6	44	–	–
other fungi	–	–	–	–	–	–	12	12
bacteria	50	30	4	38	26	10	16	10

geosmin and 2-methylisoborneol—are responsible for the musty-earthy off-odor of grain, the whole pattern of grain volatile compounds is influenced by the presence and activity of microorganisms. On the basis of this assumption, comparison of headspace phases over sound and malodorous, infected samples can be used for sample differentiation. Electronic noses and chemometrical analysis of chromatographical data have been used for these purposes (*13*−*16*, *24*, *25*).

The profile of volatile compounds isolated from analyzed wheat samples using SPME is shown in **Figure 4**. To differentiate profiles of volatiles no identification of compounds is required when an array of nonspecific sensors is used. Two fast methods based on this principle were tested: fast chromatography with FID and PCA treatment of chromatographic data and an electronic nose. In fast-GC-PCA a chromatographical column was treated as an unspecific sensor generating response patterns depending on the number and amounts of volatile compounds. SPME and the subsequent fast chromatography of isolated compounds allowed rapid differentiation of samples (**Figure 5**). To obtain reliable results using this method four to six replicates of every sample were required; therefore, fast chromatography allowed reduction of total analysis time. Ten minute runs were "sliced" by Chromstat software into time segments of different intensities. Data were normalized, so each peak was divided by total area. Projection was done in supervised geometric display. Different numbers of time ranges were tested to enhance the discrimination between groups of samples. When the total time range number was limited to 50, the separation of samples was insufficient. Finally, 20 ranges (scope) were selected from a total of 200 to discriminate between samples. Chromatographical data transformed by Chromstat software installed on the computer used for data acquisition allowed direct and rapid sample grouping using the PCA method. Samples E−H formed one group (**Figure 5**). Samples B and C formed another cluster, and samples D and A formed a third one. When the PCA graph resulting from fast chromatography was compared to the PCA graph of sensory data (**Figure 1**), evident similarities were observed. In both graphs samples of sound grain formed a well-isolated group, whereas samples with musty and earthy off-odor were located far from sound samples on the sensory PCA graphs as well as on the graph resulting from fast-GC-PCA. No data in the literature were found on the application of the described method for the rapid differentiation of grain samples based on volatile compounds.

A Fox 4000 electronic nose was used as another tool for wheat sample differentiation based on headspace composition.

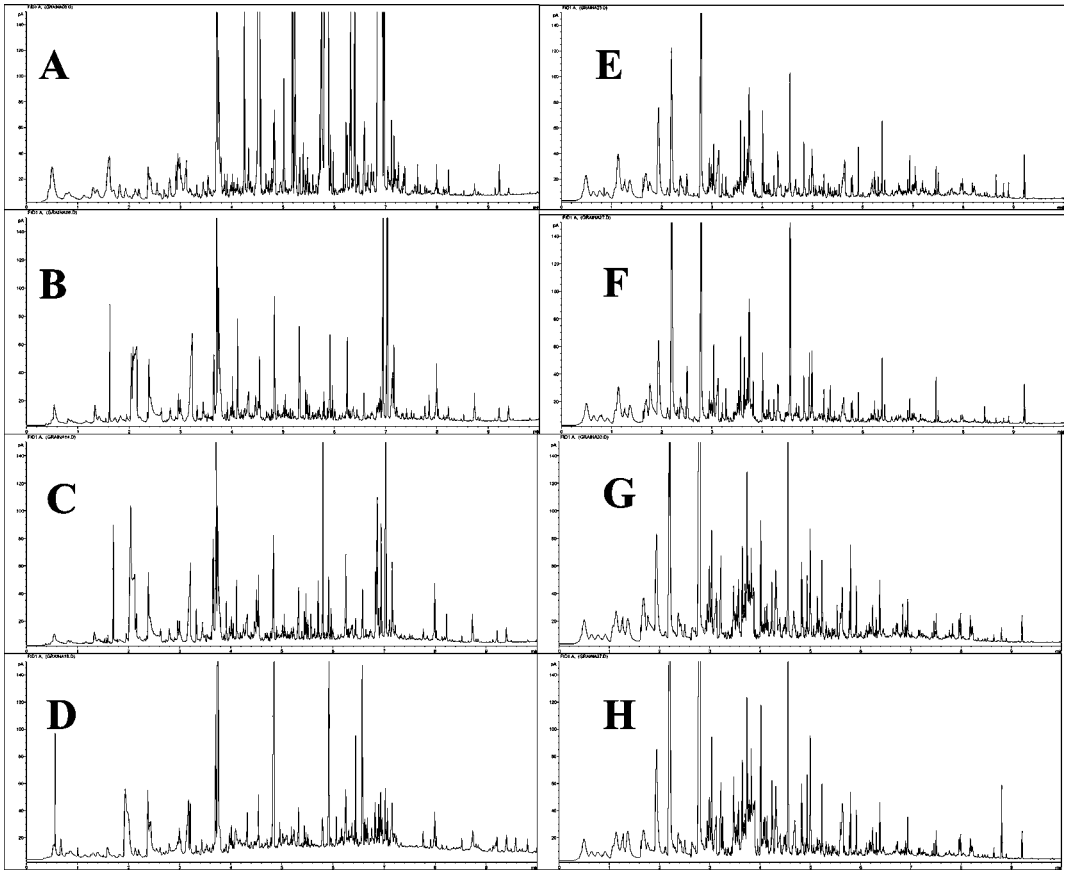

Figure 4. Profiles of volatile compounds extracted using SPME from wheat grain samples. Sample A–H descriptions are the same as specified in **Figure 1**.

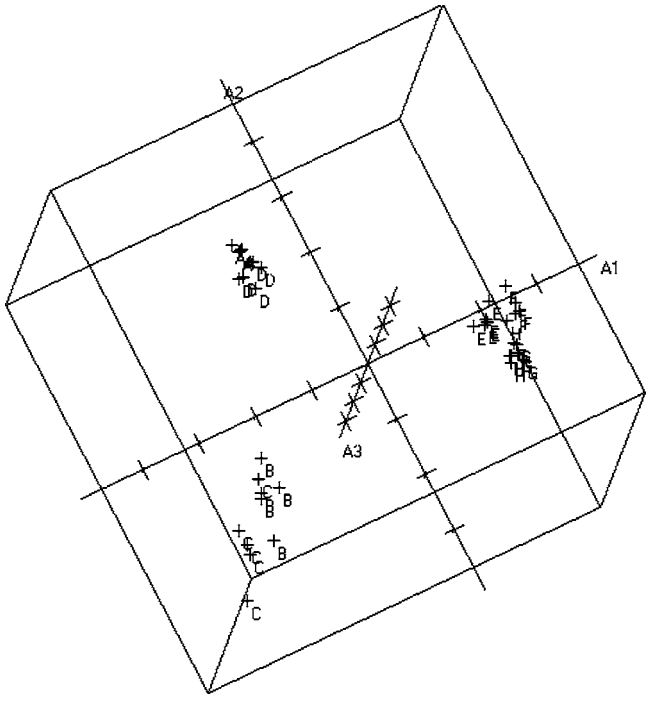

Figure 5. PCA plot of eight wheat samples characterized with different odors obtained after PCA treatment of HS-SPME-fast GC data. Sample descriptions are the same as specified in **Figure 1**.

Metal oxide sensors of two types, T and P, differing in geometry, based on tin oxide (SnO_2), were used in two chambers, whereas LY sensors based on chromium titanium oxide ($Cr_{2-x}Ti_xO_{3+y}$) and tungsten oxide (WO_3) were used in the third chamber. For the SnO_2 and WO_3 based sensors, which are n-type semiconductors in the presence of a reducing gas, their conductance increases so their resistance decreases, whereas for $Cr_{2-x}Ti_xO_{3+y}$ sensors, which are p-type semiconductors, the opposite situation takes place. Combining sensors of different types into arrays provided a broad spectrum of detectable volatile substances. In the sample acquisition, responses of all 18 sensors were recorded. After sensor optimization, five sensors providing the best sample groupings were selected: SY/LG, SY/AA, P40/2, P30/2, and T40/1. According to the manufacturer P40/2 sensors are particularly sensitive to heteroatoms, chloride, and aldehydes; P30/2 sensors are sensitive to alcohols and are used for combustion gas monitoring. T40/1 sensors are sensitive to chlorinated compounds. Sensors SY/AA are used for alcohol detection, and SY/LG sensors are especially sensitive to chlorine, fluorine, nitrogen oxide, and ozone (*26*). After elimination of one outlier, the relative standard deviation for eight groups of samples and for each sensor did not exceed 12% and generally was <7%; the RSD was highest for the P40/2 sensor. To evaluate whether differentiation exists in the wheat grain data set, unsupervised pattern recognition analysis (PCA) was done. The first PC described 73.51% of the variation, the second (C2) PC described 18.06%, and the third (C3), 7.31%. A discrimination index of 96.0% was achieved for examined samples. PCA correlation with sensory panel descriptors was performed. To correlate the results of the sensory analysis with electronic sample recognition a partial least-squares (PLS) method was applied. Means of descriptor scores after panel evaluation of samples were used for this purpose. Correlation with the grainy descriptor was 0.9484, that with musty was 0.9497, and that with earthy, 0.9102.

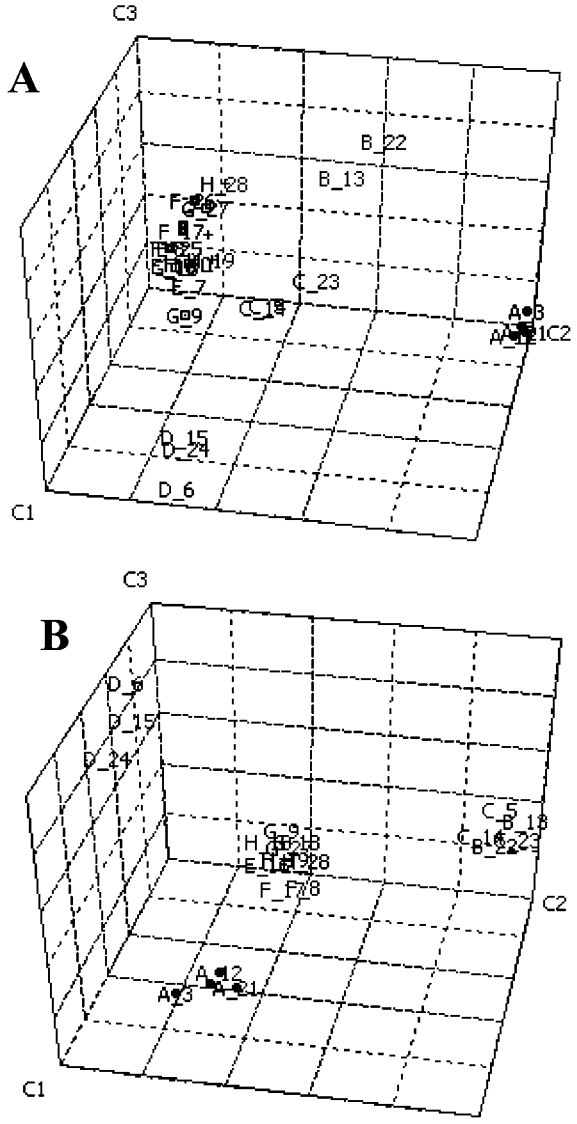

Figure 6. Classification of wheat samples characterized with different odors obtained after PCA (A) and DFA (B) procesing of electronic nose sensor data. Sample descriptions are the same as specified in **Figure 1**.

When a group of sound samples was created (E−H), samples B and C were grouped together, and samples A and D were classified with remaining groups, a discrimination index of 79% was achieved. The discrimination index gives the discrimination quality through an indication of the surfaces between groups. When groups are distinct, the discrimination index defined as $D_i = 100 \times [1 - [\text{surface (A)} + \text{surface (B)} + ... + \text{surface } (n)/\text{total surface}]]$ is positive; when groups overlap each other, the discrimination index defined as $D_i = -(\sum \text{intersection surface/total surface}) \times 100$, is negative. Euclidean distances between a group of sound samples and musty-earthy samples (A, D) were 0.041 and 0.069 and were higher than for the (B, C) group (0.018). Discriminant factorial analysis (DFA) was used to assign samples into groups mentioned in PCA classification. After cross-validation of the model, a percentage of recognition of 95% was achieved. **Figure 6** shows three-dimensional sample separation using PCA and sample classification done with DFA.

The analysis step, which influences PCA discrimination most, is a process of volatile sampling—SPME extracts much higher number and more compounds than can be isolated by static headspace used for electronic nose; therefore, to compare the performances of fast-GC-PCA and the electronic nose and their ability to classify samples, the latter one should be equipped with a heated injection port enabling SPME injections.

Electronic noses have been used to monitor the quality of grain for several years. Detailed studies indicated that grain classification based on electronic noses parallels sensory grading. Moreover, an interesting correlation was found between ANN predictions and ergosterol and bacterial or fungal CFU (*25*). Börjesson and co-workers (*15*) used an electronic nose based on MOSFET sensors, SnO_2 semiconductors, and an infrared detector for monitoring CO_2 for odor classification of grains. When wheat, barley, and oats samples were classified by grain inspectors into four classes, moldy/musty, acid/sour, burnt, and normal, the electronic nose classified correctly 75% of samples. When two categories, good and bad, were used, 95% of samples were correctly classified by the electronic nose. The electronic nose proved to be as effective as the sensory panel in differentiation of wheat samples infested by *Tilletia caries*, which produces a strong off-odor in cereals (*17*). Keshri et al. demonstrated the ability of the electronic nose to differentiate between germinating spores of different xerophilic grain and food spoilage fungi grown on wheat agar within 48 h, prior to visible growth of mycelium (*13*). Jonsson et al. (*25*) used an electronic nose for differentiation of oats, barley, and rye of different qualities. Good correlations between electronic nose and grain inspectors' scores for musty, moldy oats were observed. Olsson et al. (*27*) proved the ability of the electronic nose and GC-MS to classify naturally contaminated sample odor classes and predict levels of ergosterol and CFU on the basis of volatiles. Volatile compounds responsible for sample differentiation were also identified.

There are emerging applications for electronic noses focused on detection of mycotoxins in grains based on fungal volatile metabolites. Although specific volatile intermediates were reported for some mycotoxins—trichodiene for trichothecenes produced by *Fusarium* (*28*) or aristolochene emitted in the process of PR-toxin formation by *Penicillium roqueforti* (*29*)—volatile compounds not directly involved in the biosynthesis of mycotoxins can be used for sample differentiation if multivariate data analysis (MVDA) methods are applied. Recently, Olsson et al. (*30*) indicated that this approach can be helpful in the classification of barley with different levels of ochratoxin A (OA) and deoxynivalenol (DON). They were able to predict DON levels in naturally contaminated barley using volatile compounds. They showed also that the GC-MS system predicted OA concentration with an accuracy higher than that of the electronic nose.

Analysis of compounds responsible for specific odors relies on their isolation and separation followed by sensitive detection by mass spectrometry. To discriminate normal and off-odor grain samples on the basis of headspace analysis, arrays of sensors used in electronic noses or such combinations as fast-GC-PCA can be used for this purpose. As a result it can aid profile sensory analysis.

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