ELSEVIER

International Journal of Food Microbiology 59 (2000) 167–178

INTERNATIONAL JOURNAL OF
Food Microbiology

www.elsevier.nl/locate/ijfoodmicro

Volatiles for mycological quality grading of barley grains: determinations using gas chromatography–mass spectrometry and electronic nose

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Received 19 March 2000; accepted 2 May 2000

Abstract

The possibility of using an electronic nose or gas chromatography combined with mass spectrometry (GC–MS) to quantify ergosterol and colony forming units (CFU) of naturally contaminated barley samples was investigated. Each sample was split into three parts for (i) ergosterol and CFU analysis, (ii) measurements with the electronic nose and (iii) identification of volatiles collected on an adsorbent with a GC–MS system. Forty samples were selected after sensory analysis to obtain 10 samples with normal odour and 30 with some degree of off-odour. The data set of volatile compounds and the data collected from the electronic nose were evaluated by multivariate analyse techniques. SIMCA classification (soft independent modelling of class analogy) was used for objective evaluation of the usefulness of the data from the GC–MS or electronic nose measurements for classification of grain samples as normal or with off-odour. The main volatile compounds of grain with normal odour were 2-hexenal, benzaldehyde and nonanal, while 3-octanone, methylheptanone and trimethylbenzene were the main volatile compounds of grain with off-odours. Using data from the electronic nose three samples of 40 were misclassified, while data analysis of the volatile compounds detected with the GC–MS, led to six misclassified samples. Regression models (partial least-squares, PLS) were built to predict ergosterol- and CFU-levels with data from the GC–MS or electronic nose measurements. PLS models based on both GC–MS and electronic nose data could be used to predict the ergosterol levels with high accuracy and with low root mean square error of prediction (RMSEP). CFU values from naturally infected grain could not be predicted with the same degree of confidence.

Keywords: CFU; Ergosterol; Fungi; Kernel; Multivariate data analysis; Volatile compounds

1. Introduction

Cereal grains is the single most important food commodity for the worlds population and can in

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0168-1605/00/$ – see front matter
PII: S0168-1605(00)00355-X

some cultures represent up to 80% of the diet (Maga, 1978). Moulds are the most important spoilage organisms of cereal grains. Mould contamination can reduce the nutritional value, the technical quality, decrease germination, cause dry matter loss, heating, off-odours and form mycotoxins and allergenic spores, harmful to animals and humans (Chelkowski, 1991; Schnürer et al., 1999).

The most common laboratory method to analyse mould contamination is determination of the number of colony-forming units (CFU) but determination of ergosterol, a fungal-specific membrane sterol, is increasingly being used (Schnürer et al., 1999). Since both these methods are labour intensive and time consuming, trained grain inspectors use odour evaluation for rapid quality estimation at granaries (Börjesson et al., 1996; Magan, 1993; Stetter et al., 1993). However, smelling of grain for estimation of quality constitute a potential health hazard through inhalation of mould spores and mycotoxins. In addition, the determinations of off-odours are subjective (Jonsson et al., 1997; Stetter et al., 1993). There is therefore a need for new methods that accurately quantify mould contamination of grains in a couple of minutes. Sensor technology, e.g., the electronic nose, offers new possibilities for quality classification of grain (Börjesson et al., 1996; Börjesson and Johnsson, 1998; Jonsson et al., 1997; Stetter et al., 1993). Electronic noses have also been used to classify bacteria (Holmberg et al., 1998) and to estimate biomass and specific growth rate for *Escherichia coli* in a batch cultivation process (Bachinger et al., 1998).

The electronic nose consists of a chemical gas sensor array combined with a computer for collecting and analysing the data. So far, sensor arrays have mostly been constructed using sensors within a given category, e.g., metal oxide sensor arrays, conductive polymer sensor arrays, piezoelectric sensor arrays or metal oxide semiconductor field effect transistor devices (MOSFETs). However, combinations of sensors from different sensor categories can extend the analytical range of the sensor arrays (Dickinson et al., 1998).

We have previously shown that an electronic nose can be used to predict CFU and ergosterol levels in grain that was inoculated with *Penicillium roqueforti* (Schnürer et al., 1999). In the present study we used 40 naturally contaminated barley samples of different odour classes, determined their mould content as CFU and ergosterol and evaluated them using an electronic nose. Gas chromatography combined with mass spectrometry (GC–MS) was used to identify and quantify the volatile compounds that were desorbed from the grain samples and detected with the electronic nose.

The main objective of this study was to identify volatile compounds that correlates to odour class, CFU and ergosterol and to investigate if the electronic nose could be used for quantification of mould growth in naturally contaminated grain.

2. Materials and methods

2.1. Grain samples

A total of 40 barley samples of different odour classes were received from granaries in south central of Sweden. Among these, 10 samples were described as having normal odour, while 30 were described as having any kind of off-odour (Table 1). The off-odours were described as either mouldy, musty, sour or acid and were graded as weak (A), pronounced (B) or strong (C). The samples were stored at $+2$°C.

Each sample was split into three 100-g parts for analyses of (i) water content, fungal CFU, ergosterol levels, (ii) responses to volatiles using electronic nose and (iii) identification and quantification of volatiles by GC–MS. The water content was measured by weight determination before and after drying 15 g of whole grain at 103°C during 72 h. The moisture content varied between 7.9 and 19.8%, with an average of $13.8 \pm 0.5\%$ (mean$\pm$S.E., $n = 40$; Table 1). The 10 samples with normal odours had lower average water content (10.3%) than the 30 samples with off-odour (15.0%).

2.2. Automatic sampling apparatus and sampling procedure for electronic nose

An automatic sampling apparatus for grain analysis was used. The equipment has previously been described in detail by Börjesson et al. (1996). Three modifications were made. On the outlet from the heating unit a filter fitted with a metal screen (10 μm, 10SR2 Valco Europe, Switzerland) was placed,

Table 1
Colony forming unit (CFU), ergosterol and water content in the used grain samples sorted according to the odour judged by grain inspectors at the reception: the off-odours were graded as weak (A), pronounced (B) or strong (C)

Odour	Log CFU DG18	Ergosterol (mg/kg)	Water content (%)	Sample no.
Normal	2.2	10.5	13.5	590
Normal	4	5.8	10.3	591
Normal	5.5	18.0	7.9	592
Normal	–[a]	8.4	11.4	593
Normal	2.7	4.1	10.4	594
Normal	2.0	3.0	10.1	595
Normal	4.7	2.6	10.1	596
Normal	3.3	4.2	9.9	597
Normal	4.6	3.7	9.6	598
Normal	2.0	4.1	9.6	599
Acid A	6.2	35.0	19.8	471
Acid A	6.6	27.0	17.3	491
Mouldy A	4.4	7.7	15.1	492
Mouldy B	4.4	15.0	13.9	291
Mouldy B	5.6	7.8	13.8	292
Mouldy B	6.6	39.0	18.0	531
Mouldy B	6.0	31.0	16.8	533
Mouldy B	4.5	11.0	14.4	550
Mouldy B	4.7	11.0	11.9	553
Mouldy B	5.6	12.5	12.4	555
Mouldy B	4.0	10.0	13.9	558
Mouldy B	4.2	8.6	14.3	564
Mouldy B	4.7	11.0	14.7	568
Mouldy B	4.0	6.0	13.3	572
Mouldy B	5.1	10.0	16.6	588
Mouldy C	7.1	49.0	16.7	573
Mouldy C	7.4	64.0	9.9	580
Mouldy C	7.3	55.0	9.8	582
Mouldy C	5.3	11.0	16.8	587
Mouldy C	6.0	25.0	17.2	600
Mouldy C	4.4	14.0	16.5	602
Mouldy C	4.4	6.5	14.5	603
Musty B	2.0	11.4	13.8	569
Musty B	4.7	6.0	15.4	574
Musty B	4.6	10.0	17.3	589
Musty C	4.7	13.0	13.0	6
Sour B	5.3	13.5	15.9	552
Sour B	5.1	6.5	14.3	561
Sour B	5.9	25.0	15.9	565
Sour B	3.9	9.1	15.4	601

[a] Not analysed due to low amount of sample.

preventing dust from the grain or fungal spores to reach the sensors. Technical air (80% N_2, 20% O_2) was also flushed into the heating unit of the automatic sampling apparatus, and the samples were collected in a bottom carousel, so that the samples could be used several times.

A 100-g portion of each sample was split into three subsamples (33 g) which were analysed three times each with the electronic nose.

The start tube in the carousel was chosen randomly. The first tube was left empty, while in the second tube clean barley grain (cultivar Golf) of good hygienic quality was used as a reference sample. The third tube was left empty and in the fourth tube a randomly chosen subsample was put. Thereafter every second tube was left empty and every fifth tube contained the reference sample.

2.3. Electronic nose

An electronic nose named VCM 422 (volatile compound mapper) built by S-SENCE, Linköping University, Linköping, Sweden was used. The sensor array was made up of 10 MOSFET sensors, six SnO_2-based Taguchi sensors and one Gascard CO_2 monitor. A more detailed description of the instrument and the sensors can be found in Eklöv et al. (1998). Both the electronic nose, i.e., the sample time, valve switch, temperature control of the sensor and the automatic sampling apparatus were controlled from the computer program Senstool 2.5f (Nordic Sensor Technologies, Linköping, Sweden). The program was run on a PC with Windows 95.

A measurement cycle started by heating the grain to 50°C for 2 min. During this period and an additional 30 s the sensors were exposed to technical air. During the next 90 s the sensors were exposed to the headspace gas in the heating unit of the automatic sampling apparatus. Finally, the sensors were exposed to technical air for 15 min to recover the sensors before the next analysis. The flow rate was kept at 92 ml min^{-1}.

From the response curve Senstool calculated the maximum signal amplitude (response), increasing and decreasing derivatives (on/off derivatives) during 6 s and increasing and decreasing integrals (on/off integrals) during 20 s. An example of response curves for a gas sensor with a schematic description of the extracted sensor signals that were described above is given in Fig. 1. Further information about parameter extraction can be found in Eklöv et al. (1997).

In total more than 850 measurements were done

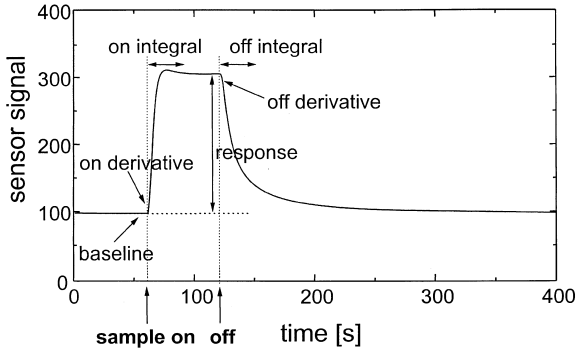

Fig. 1. Typical response curve for a gas sensor with a schematic description of the extracted sensor signals.

with the electronic nose. A PCA of every measured sample and the sensor signals was performed. The scores of the first principal component (t_1) were plotted against measurement order. The results showed that the scores for t_1 decreased for each measurement of a sample (data not shown), proving that the first measurement was most useful for further modelling. As a consequence of this, the average of the first (of three) measurement of each subsample was calculated and used for modelling.

2.4. Adsorption of volatiles from grain samples for GC–MS analysis

An 85-g sample was placed in a glass tube with a total volume of 190 ml, with glass wool covering both ends of the tube (Fig. 2). The ends were capped with teflon plugs with a $1/8'$ Swagelok connection to which teflon tubes ($1/8' \times 1.6$ mm) were connected. To collect volatile compounds a porous polymer adsorbent (Chromosorb 102, 80–100 mesh, 300 mg) was connected after the desorption unit. The glass tube with the sample was placed in a water bath set at 50°C. Technical air with a flow of 40 ml min^{-1} was passed through the tube until 5 l of air had passed through. The flow rate was measured with a flowmeter (ADM 1000, J&W Scientific) every 10 min. After the adsorption, excess water was removed by flushing the adsorbent with 20 ml min^{-1} nitrogen during 15 min. The adsorbents were stored at −20°C until GC–MS analyses were performed.

Between every sample the glass tubes, teflon plugs, tubes and Swagelok connections were first washed in water with a detergent, then washed three times with distilled water and kept at 115°C during 2 h. Finally, the adsorbents were kept at 200°C during 15 min while nitrogen gas (100 ml min^{-1}) was passed through them.

2.5. GC–MS analysis

The volatiles were thermally desorbed from the Chromosorb adsorbent using a Perkin-Elmer ATD 400 (Perkin-Elmer, Stockholm, Sweden) set at 170°C and with a helium stream of 100 ml min^{-1} for 5 min.

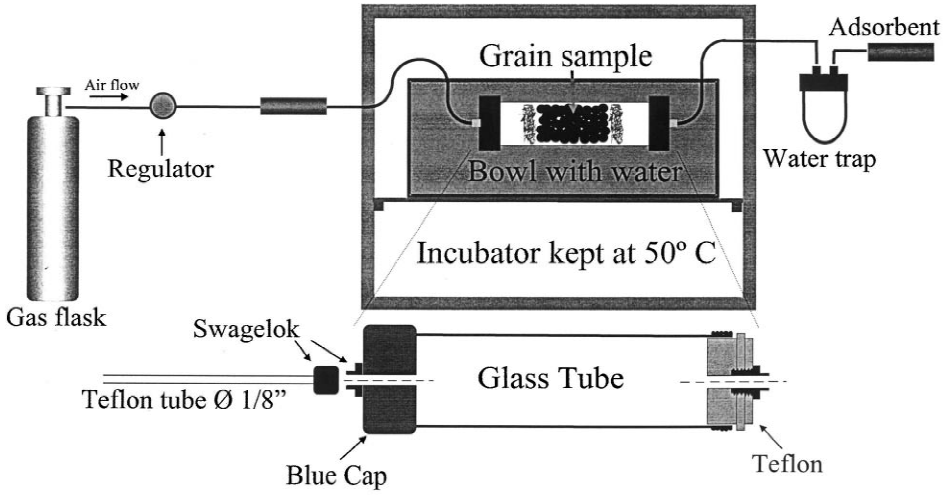

Fig. 2. Overview of the equipment design used to collect volatiles from grain onto the adsorbent for subsequent GC–MS analysis.

The compounds were injected directly into a column of the gas chromatograph. The instrumental parameters were as follows. Gas chromatograph: Varian 3400 GC with FID detector; column, fused-silica capillary column with chemically bound methylpolysiloxan, OV1CB, 1 μm, 60 m × 0.32 mm i.d. (Lars Johansson, Mölndal, Sweden); temperature program, 35–220°C, 4°C min^{-1}; carrier gas, helium 23.1 psi (3–4 ml min^{-1}). Data collection system, Hewlett-Packard HP 3350; mass spectrometer, Finnigan Incos-50; library of mass spectra, both National Institute of Standards and Technology (NIST) and National Bureau of Standards (NB) mass spectral search programs were used (Finnigan, San Jose, USA). The analyses were performed by laboratory engineer Karin Wedenström at SIK, Gothenburg, Sweden.

From the GC–MS analysis of all samples a total of 103 compounds were detected, identified and used for data evaluation.

2.6. Ergosterol

Samples were dried at 65°C for 14 h, ground to pass a 0.5-mm sieve and 25 g of each sample were weighed into a flask with ground joint. To the flask 150 ml methanol, 50 ml ethanol (95%), 20 g potassium hydroxide and 0.2 ml pyrogallol in methanol (10%, w/v) were added. The flasks were weighed, fitted on to a reflux condenser and refluxed at 100°C for 30 min whilst stirring. The flasks were cooled and the weights were checked and if necessary corrected to initial flask weight with methanol. The samples were allowed to sediment before the supernatants were filtered, and 20 ml of the filtrate were placed on an extraction column (Chem Elut, 1219-8008 Varian, Harbour City, CA, USA). After 20 min the ergosterol was eluted with 90 ml *n*-hexane and collected in a round-bottom flask, evaporated to dryness and dissolved in 5 ml heptane/2-propanol (98 + 2).

Ten μl were injected into a HPLC with a LC-mobile phase of *n*-heptane + 2-propanol (98 + 2) with Nukleosil 50-5 (Macherey-Nagel, Düren, Germany) as LC-analytical column. Ergosterol was detected at 282 nm with a variable UV-detector. The analyses were performed by AnalyCen (Lidköping, Sweden).

2.7. Colony-forming units (CFU)

Fungal CFU were determined according to method 98 proposed by the Nordic Committee on Food Analysis (NMKL; Åkerstrand, 1995). Samples (40 g) were soaked for 30 min in 360 ml 0.1% peptone water before being homogenised in a Stomacher (Laboratory blender 400, Seward Medical, London, UK.) for 2 min. The homogenate was diluted and 0.1 ml from each dilution was plated in duplicate on dichloran 18% glycerol agar (DG18; Hocking and Pitt, 1980). The plates were incubated upright in darkness at 25°C for 5 days.

2.8. Data evaluation

For data evaluation, principal component analysis (PCA), partial least squares (PLS), PLS discriminant analysis (PLS-DA) and soft independent modelling of class analogy (SIMCA) classification were used. These techniques extract information from data with many variables (*m* variables) using all the variables simultaneously. In PCA, *m* axes will define an *m*-dimensional space in which each sample (object) can be described by a point (Wold et al., 1984, 1987). The whole set of samples will be defined as a swarm of points in the *m*-dimensional space. The first principal component (PC) describes the direction through the swarm which explains the largest variation in the data. The second principal component is orthogonal to the first one and explains the second largest variation, etc. The main results from PCA are the score and loading plots. The score plot shows how the samples are related to each other, while the loading plot shows how the variables relate too each other.

The program SIMCA-P 7.01 (Umetrics AB, Umeå, Sweden) calculates R^2- and Q^2 values for every PC. R^2 shows how much of the total variation that is explained by a PC, ranging from 0 to 1, while Q^2 shows the predicted variation after cross-validation for the PC. R^2 and Q^2 values above 0.8, and not separated by more than 0.2 are required for a satisfactory model. The sum of R^2 for all used PCs is described by the R^2_{Cum} value.

PLS have many similarities to PCA, but with the main difference that PLS contains both *X* data (e.g., sensor signals) and an *Y* data (e.g., colony forming units) (Höskuldsson, 1988; Sjöström et al., 1983). In

PLS the principal components are calculated to maximise the covariance between X and Y space. In PLS R^2 is calculated both for the X and the Y variables. The R^2 for X variables is denoted R^2_X, while R^2_Y is used for the Y variable. The geometry of PLS has been described by Phatak and DeJong (1997).

PLS can also be used to distinguish classes. A dummy variable can be constructed, representing the sample odour (e.g., normal $= -1$, off-odour $= 1$) and then used as Y (Sjöström et al., 1986). This type of PLS is called PLS discriminant analysis and is very useful for interpreting which variables in X that best describes each class (here normal and off-odour).

When the data contain classes as in this study (normal or off-odour), SIMCA classification (Soft Independent Modelling of Class Analogy) can be used to visualise the distance between the classes (Albano et al., 1981; Coomans et al., 1983; Sjöström and Wold, 1980). In SIMCA classification a PCA model is made for every class. For each sample, the distances to the two models is computed and plotted along with the class membership limit (critical distances). The result can be visualised in a Cooman's plot (see Fig. 4a–b). Samples that are occurring in the lower right rectangle belong to normal samples, and samples occurring in the upper left rectangle belong to off-odour samples. The samples that appear in the lower left corner belong to both classes, while samples located in the upper right rectangle do not belong to any of the classes.

In order to enhance the predictive power of the PLS models, it could be necessary to remove systematic noise that occur in data measured with the electronic nose and GC–MS by using orthogonal signal correction (OSC). In this method, variables not correlated to the Y responses are weighed down and, thereby have less influence in the generated model (Wold et al., 1998).

For the PCA and PLS models, the variables were scaled to unit variance. This is the default scale for the program, where the variables are first mean-centred and then scaled to the same variance. This will give all variables equal opportunities of influencing the data analysis.

3. Results

3.1. Classification of grain odour using data from electronic nose or GC–MS

A PCA was used to investigate how well electronic nose analysis and GC–MS could separate grain with normal and off-odour (Fig. 3). For the PCA model based on sensor signals from the electronic nose, 13 significant principal components (PC) were calculated using cross-validation. These 13 PCs explained 0.97 of the total variation in the data

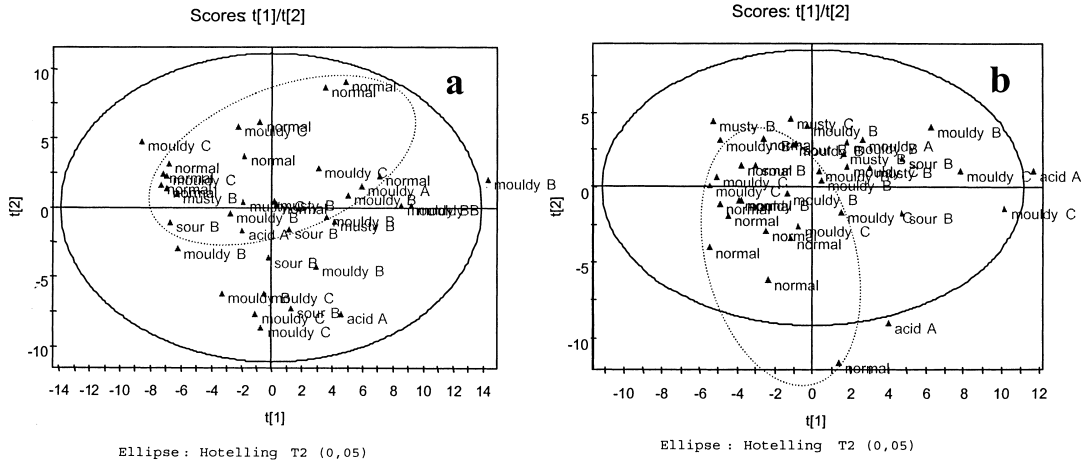

Fig. 3. Score plot of 85 sensor signals from the electronic nose (a) and 103 compounds detected and identified with GC–MS (b) from the 40 grain samples. The samples are labelled with odour described by grain inspector (see Section 2.1).

(Q^2_{Cum} 0.77). Fig. 3a shows the score plot for sensor signals registered by the electronic nose where t_1 ($R^2_X = 0.35$, $Q^2 = 0.29$) is plotted against t_2 ($R^2_X = 0.22$, $Q^2 = 0.28$). For the 103 volatile compounds identified and quantified from the GC–MS measurement a PCA model with 13 significant PCs was obtained. Here the 13 PCs explained 0.78 of the data, but with a Q^2_{Cum} value of only 0.14. The score plot t_1 ($R^2_X = 0.19$, $Q^2 = 0.10$) against t_2 ($R^2_X = 0.13$, $Q^2 = 0.04$) based on data from the 103 volatile compounds is shown in Fig. 3b.

To study the discrimination between the two odour classes (normal and any type of off-odour) SIMCA classification and Coomans's plot were used. For both electronic nose and GC–MS data one PCA model for normal odour and one for off-odour were performed. Fig. 4a shows the Cooman's plot where the PCA models for normal and off-odour based on electronic nose data are compared to each other, while Fig. 4b is based on GC–MS data. Data measured with the electronic nose classified the samples into normal or off-odour with higher certainty than GC–MS. Only three samples of the 40 samples were classified as belonging to both classes with the electronic nose data, compared to six samples using GC–MS data (Fig. 4a–b).

3.2. Difference in the profile of volatile compounds from grain with normal or off-odour

The differences in the profile of volatile compounds from grain with normal odour and any type of off-odour were evaluated by creating a discriminant odour variable (normal odour = −1 and off-odour = 1) that was used as *Y*-variable in PLS modelling. As *X*-variables either the 85 sensor signals from the measurement with the electronic nose or the 103 compounds identified by the GC–MS measurements were used. The regression coefficients plot and the loading plot from both models were studied and the most important variables were identified and used to recalculate the models. Score plots and the regression coefficients plot from one of the recalculated PLS discriminant analysis models are shown in Fig. 5a–c.

Two PCs were proved to be significant determined by cross-validation when the PLS discriminant analysis model was based on 24 volatile compounds (Fig. 5a–b). The model explained a large percentage of the variation of the *Y*-matrix ($R^2_{Ycum} = 0.82$, $Q^2_{Cum} = 0.70$), but much less of the variation in *X*-matrix ($R^2_{XCum} = 0.41$). Fig. 5a shows the score plot (t_1/t_2) for the final PLS model. The PLS discriminant analysis model based on the subset of volatile compounds was more efficient in separating grain samples with normal odour and off-odour compared to the PCA model (Fig. 3b). The separation was, however, not complete since one sample described as having normal odour (indicated with an arrow) was located within the off-odour class (Fig. 5a). Fig. 5b shows the regression coefficients plot indicating the volatile compounds that characterise normal odour and off-odour. The main volatile compounds of grain with normal odour were 2-hexenal, benzaldehyde, nonanal, unknown ester (MW 286), butylacetate,

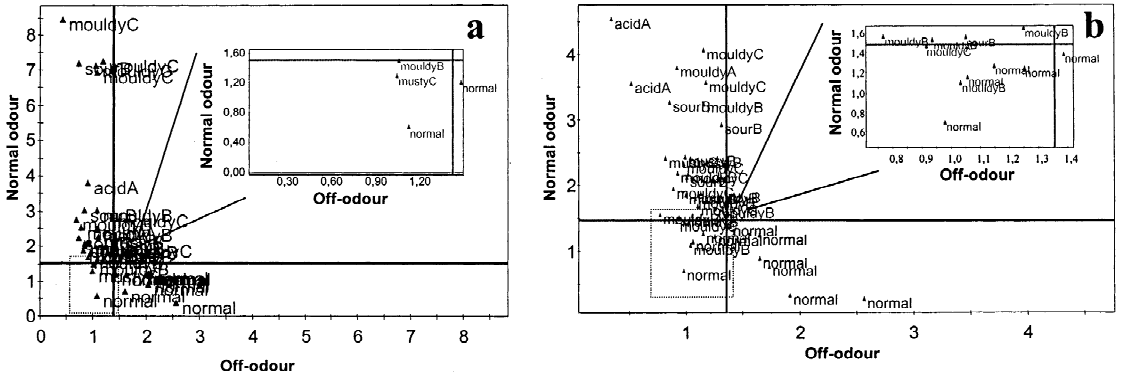

Fig. 4. Cooman's plot of sensor signals from the electronic nose (a) and GC–MS data (b) based on PCA models for normal odour and off-odour.

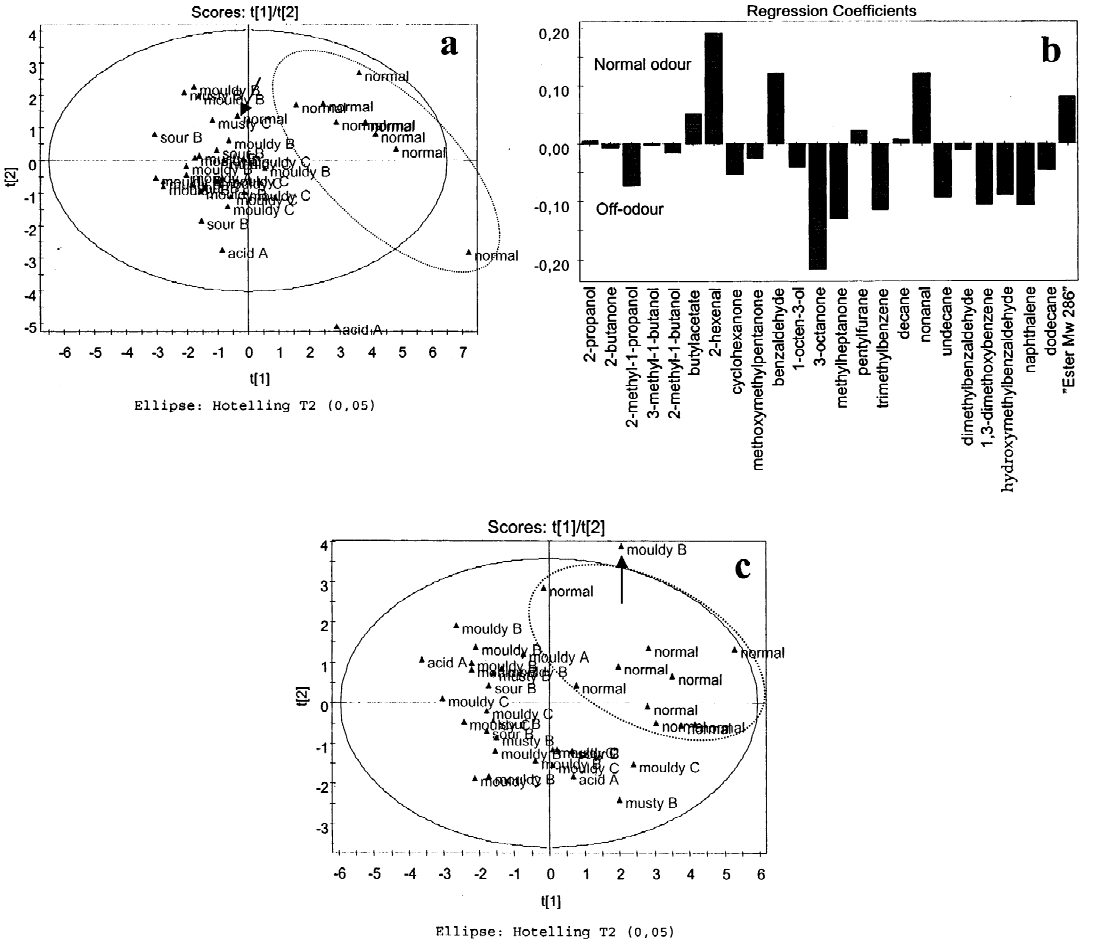

Fig. 5. The score plot (a) and the regression coefficients plot (b) from the PLS discriminant analysis based on 24 volatile compounds. The samples are labelled with odour described by grain inspectors. (c) The score plot from the PLS discriminant analysis based on 18 sensor signals from the electronic nose showing the highest discrimination power for samples with normal and off-odour.

pentylfuran, decane and 2-propanol (Fig. 5b). For grain with off-odour, 3-octanone, methylheptanone, trimethylbenzene, 1,3-dimethoxybenzene, naphthalene, undecane, hydroxymethylbenzaldehyde, 2-methyl-1-propanol, cyclohexanone, dodecane, 1-octen-3-ol, methoxymethylpentanone, 2-methyl-1-butanol, dimethylbenzaldehyde, 2-butanone and 3-methyl-1-butanol were the main volatile compounds (Fig. 5b).

A PLS discriminant analysis model based on the sensor responses only resulted in a R^2_{Ycum} of 0.43 ($Q^2_{Cum}=0.21$) and no separation between samples with normal and off-odour could be seen (data not shown). Instead a new PLS discriminant analysis model based on all types of sensor signals (Fig. 1) was performed. The most important sensor signals were identified and used to recalculate the PLS discriminant analysis model. Fig. 5c shows the score plot from the PLS discriminant analysis model based on data from 18 sensor signals. Also for this model two significant PCs were calculated. The model explained rather large percentage of the variation of both X- and Y-matrix ($R^2_{Ycum}=0.62$; $R^2_{XCum}=0.63$), while the Q^2_{Cum} was 0.41. These results showed that also other signals than only the response were important for the separation of grain samples with

normal and off-odour. Only the MOSFET sensors proved to be useful to classify grain samples into normal or off-odorous (data not shown).

3.3. Prediction of ergosterol content from GC–MS and electronic nose

The ergosterol levels of the analysed samples are listed in Table 1. Some of the grain samples had very high levels of ergosterol and due to this skewed distribution, samples with ergosterol levels above 30 mg/kg were excluded from further evaluation. Data from the remaining 34 samples were log transformed and divided into a calibration set with 28 samples and a validation set with six samples.

The lowest root mean square error of prediction (RMSEP) in combination with the highest R^2_Y and Q^2 were obtained when 3-octanone, trimethylbenzene, undecanal, 1-octanol, 1,3-dimethylbenzene, nonane and decane were used for prediction of ergosterol (data not shown). Samples 592 and 565 were identified as outliers and excluded from the calibration set. An overview of the PLS models, the root mean square error of estimation (RMSEE) and prediction (RMSEP) is given in Table 2.

Among the 85 sensor signals from the electronic nose, 36 proved to be useful to predict ergosterol. Water content was added as an *X*-variable since it improved the PLS-model. Five of the samples in the calibration set (568, 572, 574, 587 and 599) were identified as strong outliers and were therefore kept out of the calibration set. In general, the 10 MOSFET sensors and the Taguchi sensors 1, 4 and 6 proved to be useful to predict ergosterol (data not shown). The PLS models based on data from the electronic nose and GC–MS analyses showed large similarities since $R^2_{Y(\mathrm{cum})}$, $Q^2_{(\mathrm{cum})}$ and RMSEE were almost identical, while only small differences were found in $R^2_{X(\mathrm{cum})}$ and RMSEP value (Table 2).The predictive ability of the two PLS models described above were tested on six samples that had not been used for calibration. The results are shown in Table 3. The PLS model based on GC–MS data had a higher degree of correspondence with measured data than the electronic nose based model.

3.4. Prediction of CFU

Data from both GC–MS and the electronic nose measurements had systematic noise, making it much harder than in the case of ergosterol to create a PLS model to predict CFU, even if water content was added as an *X*-variable.

Using OSC on sensor signals from the electronic nose data with subtraction of one latent variable orthogonal to *Y*, gave an improved PLS model where $R^2_{Y\mathrm{Cum}}$ increased from 0.44 to 0.77 and Q^2_{Cum} from 0 to 0.55 while RMSEE decreased from 1.07 to 0.70 log CFU. Subtraction of one OSC variable from

Table 3
Validation of PLS models with ergosterol as *Y*-variable and GC–MS data or sensor signals from the electronic nose as *X* matrix

Sample no.	Measured ergosterol (mg/kg)	Predicted ergosterol (mg/kg)	
		GC–MS	Electr. nose
594	4.1	3.8±1.1	7.2±1.1
561	6.5	5.9±1.1	9.6±1.1
564	8.6	7.3±1.0	16.8±1.1
569	11.4	10.7±1.1	10.1±1.1
589	10.0	12.2±1.1	16.1±1.1
600	25.0	25.7±1.2	15.6±1.1

Table 2
Overview of the PLS models that were used for ergosterol prediction in the interval 0–30 mg/kg and the OSC-PLS models that were used for CFU prediction in the interval log 2–7.4: RMSEE and RMSEP values for these models are also given

Method	Instrument	PC	$R^2_{X(\mathrm{cum})}$	$R^2_{Y(\mathrm{cum})}$	$Q^2_{(\mathrm{cum})}$	RMSEE	RMSEP
Ergosterol	GC–MS	2	0.43	0.88	0.61	1.22	1.13
Ergosterol	Electr. nose	3	0.81	0.90	0.70	1.24	1.62
CFU	GC–MS[a]	2	0.22	0.89	0.50	0.51	1.21
CFU	Electr. nose[a]	3	0.73	0.77	0.55	0.70	1.45

[a] One OSC component was subtracted.

Table 4
Validation of OSC-PLS models with CFU as *Y*-variable and GC–MS data or sensor signals from the electronic nose as *X* matrix

Sample no.	Counted log CFU	Predicted log CFU	
		GC–MS	Electr. nose
599	2.0	3.7±0.1	4.6±0.2
550	4.5	3.3±0.1	2.8±0.5
568	4.7	5.0±0.1	5.0±0.3
587	5.3	4.4±0.1	6.0±0.2
533	6.0	5.4±0.1	6.4±0.2
582	7.3	5.5±0.1	5.9±0.1

GC–MS data also increased the model Q^2_{Cum} from 0.16 to 0.50, while RMSEE decreased from 0.61 to 0.51 log CFU. The compounds 1,3-dimethoxybenzene, phenol, dodecane, dimethylpyrazine, ethylbenzene and nonane all had a positive correlation with CFU, while 1-butanol. 1-pentene-3-ol, 1-heptanol, octadieneone, nonanal, nonanol showed a negative predictive correlation (data not shown). Also pentanal, 1-hexanol, 2-hexenal, hexanal, 2-pentanone and geosmin were found to be important for the prediction of CFU (data not shown). For the prediction of CFU, all five types of sensor signals (Fig. 1) from both MOSFET and Taguchi sensors were needed (data not shown).

As was found for the ergosterol prediction, the PLS-models for prediction of CFU based on data from electronic nose and GC–MS showed large similarities. The $Q^2_{(\mathrm{cum})}$ obtained a similar value while the explained *Y*-variation ($R^2_{Y(\mathrm{cum})}$) was higher for the PLS-model based on GC–MS data (Table 2). However, the explained *X*-variation ($R^2_{X(\mathrm{cum})}$) was higher for the PLS-model based on electronic nose data. Both the RMSEE and the RMSEP were slightly lower for the GC–MS model compared to the PLS-model based on electronic nose data (Table 2). As for the ergosterol models, the predicting ability of the CFU models described above were tested on six samples that had not been used for calibration. The results indicate a slightly higher predictive ability for the model based on GC–MS data (Table 4).

4. Discussion

Börjesson et al. (1996) have previously shown that an electronic nose is able to classify naturally infected grain samples into the odour classes bad and good with high accuracy. Schnürer et al. (1999) showed that the technique also can be used to predict the fungal content measured as CFU and ergosterol in artificially infected grain samples. In this study naturally contaminated barley samples with different moisture contents were used.

Many of the sensors that are used in electronic noses are sensitive to changes in the relative humidity of the air flowing over the sensors. An increase in the relative humidity will often result in an increased sensor signal. By including the water content as an extra *X*-variable, the PLS model provided more accurate predictions of ergosterol, while the model for prediction of CFU was not improved.

The sensor signals from 13 of the 17 sensors of the electronic nose gave lower signals between the first and the third measurement. Seitz (1995) also found, using the same type of dynamic headspace sampling, that low molecular weight compounds were detected in higher amounts in the first purge.

By using PLS discriminant analysis on the 103 identified volatile compounds, the compounds that best described the two classes normal and off-odour were identified. Only four of these compounds, cyclohexanone, methoxymethylpentanone, methylheptanone and dimethylbenzaldehyde, could not be found in the literature, while all the others have previously been reported as volatile compounds emitted from grain samples or fungal cultures (Börjesson, 1993; Jelen and Wasowicz, 1998; Larsen and Frisvad, 1995; Sunesson et al., 1995). The ketone 3-octanone was the most important compound associated with off-odorous samples in this study. This metabolite is one of the most commonly produced volatiles by *Penicillium* species, and often produced in the largest amounts (Larsen and Frisvad, 1995). The concentrations of benzaldehyde, 2-hexenal, butylacetate, nonanal, pentylfuran, unknown ester (MW 286), decane and 2-propanol were higher in samples with normal odour. These samples also had lower concentration of the compounds that were found in samples with off-odour. Analysis of volatile compounds from sound wheat grain has shown that some alcohols, hydrocarbons, aldehydes, ketones and terpenes are responsible for the odour of sound wheat (Jelen and Wasowicz, 1998). It has also been reported that the profile of volatiles from maize, wheat, rye and triticale is qualitatively similar and only differs in compound concentrations. Com-

pounds such as 3-methyl-1-butanol, 1-octen-3-ol and 3-octanone that have been reported as off-odours produced by fungi can be found even in grain with normal odour, but in concentrations below the odour threshold (Jelen and Wasowicz, 1998). We also found benzaldehyde, 2-hexenal, nonanal, and pentylfurane in higher concentrations in samples with normal odour and that some off-odorous compounds were detected in low concentrations in samples with normal odour. It is therefore important to analyse the total volatile compounds profile by using multivariate analyse techniques, instead of looking at one compound at a time.

The possibilities of quantification of ergosterol and CFU by electronic nose or GC–MS measurements were also investigated. This report confirms that it is possible to quantify ergosterol and CFU using volatile compounds extracted from naturally contaminated grain samples. The volatile compounds that were the most important for quantification of ergosterol and CFU were identified. Of these compounds, trimethylbenzene, 1,3-dimethylbenzene, nonane, decane, 1-octanol and 3-octanone has been reported to be present in sound wheat, but trimethylbenzene, 1,3-dimethylbenzene, 1-octanol and 3-octanone has also been reported as fungal volatiles e.g., produced by isolates of *Penicillium*, *Aspergillus* and *Fusarium* (Jelen and Wasowicz, 1998). The compounds 1-pentene-3-ol, 1-heptanol, and geosmin have been reported as fungal metabolites, while phenol, dodecane, nonane, 2-hexenal and hexanal have been connected to sound wheat odour. Some metabolites like hexanol, ethylbenzene and nonanal have been reported as being important for both sound wheat odour and as fungal metabolites (Jelen and Wasowicz, 1998).

Compounds like 3-octanone, decane and trimethylbenzene were identified as important compounds both during the prediction of ergosterol and for discrimination between normal and any off-odour, while 1,3-dimethoxybenzene, 2-hexenal, dodecane and nonanal were important compounds both for the prediction of CFU and the discrimination between normal and off-odour. Only one compound, nonane, was identified as an important compound both for the prediction of ergosterol and CFU.

This investigation has shown that both GC–MS and electronic nose analyses can be used to discriminate between normal and off-odour grains. The volatile compounds ‘responsible’ for differentiating between these two classes have been identified using GC–MS. Volatiles can also be used to predict ergosterol levels in cereal grains. Prediction of CFU levels was less successful, perhaps reflecting inherent difficulties of the plate count technique. Our results stress the importance of using multivariate data analysis when investigating the relation between volatile metabolite patterns and other indicators of fungal growth in complex environments. The electronic noses that are commercially available are fast and do not require the sample preparations that are common practice in GC–MS systems. Also the investment costs, and thereby the cost per analysis is lower for an electronic nose. On the other hand, with a GC–MS system it is possible to identify and quantify volatile compounds of interest and with lower detection limit than for electronic noses. We are presently investigating the possibility of using both GC–MS and electronic nose detection of volatiles to indicate also ochratoxin A and deoxynivalenol contamination of cereal grain.

Acknowledgements

This work was performed within the Swedish Sensor Centre (S-SENCE), supported by Swedish industry, the Swedish National Board for Technical and Industrial Development (NUTEK), and Linköping University, Sweden. This work has been financially supported by the Cerealia Foundation R&D, Järna, Sweden, Swedish Farmers Supply & Marketing Association, Stockholm, Sweden and Swedish University of Agricultural Sciences, Sweden. We also wish to thank Per Mårtensson for his valuable assistance during the measurements with the electronic nose. Tomas Eklöv, Per M. Andersson and Erik Johansson are thanked for critically reviewing the manuscript.

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