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Detection and quantification of ochratoxin A and deoxynivalenol in barley grains by GC-MS and electronic nose

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Abstract

Mycotoxin contamination of cereal grains can be detected and quantified using complex extraction procedures and analytical techniques. Normally, the grain odour, i.e. the presence of non-grain volatile metabolites, is used for quality classification of grain. We have investigated the possibility of using fungal volatile metabolites as indicators of mycotoxins in grain. Ten barley samples with normal odour, and 30 with some kind of off-odour were selected from Swedish granaries. The samples were evaluated with regard to moisture content, fungal contamination, ergosterol content, and levels of ochratoxin A (OA) and deoxynivalenol (DON). Volatile compounds were also analysed using both an electronic nose and gas chromatography combined with mass spectrometry (GC-MS). Samples with normal odour had no detectable ochratoxin A and average DON contents of 16 μg kg^{-1} (range 0–80), while samples with off-odour had average OA contents of 76 μg kg^{-1} (range 0–934) and DON contents of 69 μg kg^{-1} (range 0–857). Data were evaluated by multivariate data analysis using projection methods such as principal component analysis (PCA) and partial least squares (PLS). The results show that it was possible to classify the OA level as below or above the maximum limit of 5 μg kg^{-1} cereal grain established by the Swedish National Food Administration, and that the DON level could be estimated using PLS. Samples with OA levels below 5 μg kg^{-1} had higher concentration of aldehydes (nonanal, 2-hexenal) and alcohols (1-penten-3-ol, 1-octanol). Samples with OA levels above 5 μg kg^{-1} had higher concentrations of ketones (2-hexanone, 3-octanone). The GC-MS system predicted OA concentrations with a higher accuracy than the electronic nose, since the GC-MS misclassified only 3 of 37 samples and the electronic nose 7 of 37 samples. No correlation was found between odour and OA level, as samples with pronounced or strong off-odours had OA levels both below and above 5 μg kg^{-1}. We were able to predict DON levels in the naturally contaminated barley samples using the volatile compounds detected and quantified by either GC-MS or the electronic nose. Pentane, methylpyrazine, 3-pentanone, 3-octene-2-ol and isooctylacetate showed a positive correlation with DON, while ethylhexanol, pentadecane, toluene, 1-octanol, 1-nonanol, and 1-heptanol showed a negative correlation with DON. The root mean square error of estimation values for prediction of DON based on GC-MS and electronic nose data were 16 and 25 μg kg^{-1}, respectively.

Keywords: Ochratoxin A/DON prediction; Fungal volatile compounds; Mould; Fungi; PCA; PLS; Asymmetric data

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1. Introduction

Cold and wet weather conditions of temperate climates, such as that of Sweden, can during some years favour growth of the storage mould *Penicillium verrucosum* in inadequately dried cereal grain (Olsen et al., 1993). *P. verrucosum* can produce the mycotoxin ochratoxin A (OA), which has immunotoxic, nephrotoxic, teratogenic, and carcinogenic properties (Krogh, 1987). Seasonal and geographic variations in OA contamination of grain have been observed (Breitholtz et al., 1991; Holmberg et al., 1990). OA has also been detected in the blood of pigs and humans (Breitholtz et al., 1991; Holmberg et al., 1990; Olsen et al., 1993).

Whereas *Penicillium* species mainly cause storage problems, *Fusarium* species infect cereal crops and produce mycotoxins under field conditions (Miedaner and Perkowski, 1996). Deoxynivalenol (DON) belongs to the trichothecenes, a family of mycotoxins that are cytotoxic, phytotoxic, antifungal, and have insecticidal activity (Ciegler, 1979). *F. culmorum* and *F. graminearum* are the most common *Fusarium* species producing DON and the relative dominance of these two species is temperature-dependent (Eriksen and Alexander, 1998).

To reduce mycotoxin contamination, quality controls are performed in Europe at several levels of the grain-handling chain. In Sweden, an evaluation of off-odours by trained inspectors is the most commonly used control method for cereal grain (Smith et al., 1994; Börjesson et al., 1996). The odour classification system is used to determine whether the grain should be used for human or animal consumption or if the granary should reject the grain.

The use of human inspectors for odour determination has obvious drawbacks. The first aspect is the disagreement between different individuals in how they perceive types and intensities of odours. Börjesson et al. (1996) showed that when two trained grain inspectors classified 235 samples as having either normal, acid, sour, mouldy, musty, burnt or foreign odours, they only agreed on 54% of the samples. The second aspect is that smelling of grain for estimation of quality constitutes a potential health hazard through inhalation of mould spores and mycotoxins (Jonsson et al., 1997).

A need therefore exists for alternative methods that can accurately quantify mould and mycotoxin contamination of grain in a few minutes. We have previously shown that volatile compounds desorbed from whole grain and measured with either an electronic nose or GC-MS, can be used to predict ergosterol and CFU in natural grain samples (Olsson et al., 2000).

In the present study, the OA and DON contents, mould counts, the degree of fungal infection and the moisture contents of 40 naturally contaminated barley samples of different odour classes were determined. GC-MS was used to identify and quantify the volatile compounds that were desorbed from these grain samples and the volatile compounds emitted from the samples were analysed using an electronic nose.

The main objectives of this study were to investigate if the electronic nose or the GC-MS could be used to quantify OA and DON in natural grain samples and to identify the volatile compounds that correlate with the OA and DON contents.

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 Sweden. Among these, 10 samples were described as having normal odour while 30 were described as having some kind of off-odour (Table 1). The off-odours were described as mouldy, musty, sour or acid and the odour strength was graded as weak (A), pronounced (B) or strong (C). The samples were stored at +2 °C.

Each sample was split into four 100-g parts for analyses of (i) mycotoxin content, (ii) water content, fungal counts and ergosterol levels, (iii) responses to volatiles using the electronic nose and (iv) 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 (ASAE, 1983).

2.2. Automatic sampling apparatus and sampling procedure for electronic nose

An automatic sampling apparatus previously described by Börjesson et al. (1996) was used with three modifications. On the outlet from the heating unit, a

Table 1
Fungal counts, internal infection, mycotoxin, ergosterol, and moisture contents of barley of different odour classes

Sample	Odour	Moisture (%)	OA (μg kg^{-1})	DON (μg kg^{-1})	Ergosterol (μg kg^{-1})	CFU (log g^{-1})					Fusarium	Int. Inf. (%)[b]	
						Total	Pen	Asp	Eur	Fus	#[a] on CZID	DG18	CZID
590	normal	13.5	0	0	10.5	2.2	<3	<3	<3	4.0	4	90	50
591	normal	10.3	0	60	5.8	4.0	<3	<3	3.6	n.d.[c]	n.d.	n.d.	n.d.
592	normal	7.9	0	5	18.0	5.5	<3	4.0	4.9	3.0	0	70	64
593	normal	11.4	0	0	8.4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
594	normal	10.4	0	80	4.1	2.7	<2	<2	<2	<2	0	20	10
595	normal	10.1	0	5	3.0	2.0	<2	<2	<2	<2	0	22	4
596	normal	10.1	0	0	2.6	4.7	4.7	4.4	n.d.	<2	1	80	20
597	normal	9.9	0	0	4.2	3.3	2.6	2.0	2.9	<2	0	92	6
598	normal	9.6	0	0	3.7	4.6	4.5	<3	<3	<2	0	80	20
599	normal	9.6	0	5	4.1	2.0	<2	<2	2.0	<2	0	50	12
471	acid A	19.8	346	97	35.0	6.2	6.0	n.d.	5.0	<2	n.d.	n.d.	n.d.
491	acid A	17.3	9	58	27.0	6.6	n.d.	n.d.	6.6	<2	0	100	54
492	mouldy A	15.1	3	48	7.7	4.4	3.8	n.d.	3.8	<2	5	100	20
291	mouldy B	13.9	274	42	15.0	4.4	3.7	n.d.	4.0	<2	1	95	96
292	mouldy B	13.8	11	48	7.8	5.6	n.d.	4.5	n.d.	<2	0	50	8
531	mouldy B	18.0	289	37	39.0	6.6	5.9	n.d.	6.5	<2	5	84	50
533	mouldy B	16.8	11	50	31.0	6.0	3.8	4.0	4.8	<2	0	100	40
550	mouldy B	14.4	0	0	11.0	4.5	3.3	4.5	<3	<2	0	100	96
553	mouldy B	11.9	0	0	11.0	4.7	3.3	3.5	3.4	<2	0	100	2
555	mouldy B	12.4	3	25	12.5	5.6	4.7	<3	<3	3.6	6	64	18
558	mouldy B	13.9	0	0	10.0	4.0	3.8	4.0	<3	<2	0	92	20
564	mouldy B	14.3	6	0	8.6	4.2	4.0	3.0	<3	<2	0	100	50
568	mouldy B	14.7	0	12	11.0	4.7	4.0	4.0	4.1	<2	2	100	6
572	mouldy B	13.3	4	14	6.0	4.0	3.5	<3	3.3	<2	4	80	40
588	mouldy B	16.6	3	26	10.0	5.1	4.3	<2	<2	3.3	1	86	70
573	mouldy C	16.7	178	37	49.0	7.1	4.7	5.8	4.5	<2	0	100	60
580	mouldy C	9.9	934	35	64.0	7.4	6.2	<3	<3	4.0	9	100	0
582	mouldy C	9.8	4	857	55.0	7.3	6.3	<3	<3	6.2	20	100	96
587	mouldy C	16.8	81	14	11.0	5.3	4.0	<3	<3	<2	1	100	60
600	mouldy C	17.2	26	110	25.0	6.0	5.3	5.4	<4	<2	7	98	86
602	mouldy C	16.5	43	0	14.0	4.4	6.0	<4	<4	<2	1	100	78
603	mouldy C	14.5	0	0	6.5	4.4	3.0	<3	4.4	<2	0	100	80
569	musty B	13.8	0	281	11.4	2.0	<2	<2	2.3	<2	1	56	30
574	musty B	15.4	5	16	6.0	4.7	4.0	4.0	3.8	3.3	0	980	30
589	musty B	17.3	13	60	10.0	4.6	4.6	3.7	<3	<2	0	96	2
6	musty C	13.0	10	89	13.0	4.7	3.0	n.d.	3.5	<2	0	98	93
552	sour B	15.9	0	80	13.5	5.3	4.4	4.3	4.5	<2	0	100	30
561	sour B	14.3	0	18	6.5	5.1	4.0	3.6	4.2	<2	0	100	52
565	sour B	15.9	14	10	25.0	5.9	4.6	4.3	4.7	<2	0	100	92
601	sour B	15.4	0	0	9.1	3.9	3.2	<2	3.1	<2	0	100	60

A = weak; B = pronounced; C = strong; Pen = *Penicillium* species; Asp = *Aspergillus* species; Eur = *Eurotium* species; Fus = *Fusarium* species.
[a] # = number of *Fusarium* colonies on CZID.
[b] Int. Inf. = % internal infected kernels.
[c] n.d. = not determined.

filter fitted with a metal screen (10 μm, 10SR2 Valco Europe, Switzerland) was placed to prevent dust from the grain or fungal spores from reaching the sensors. Technical air (80% N_2, 20% O_2, Air Liquide, Malmö, Sweden) was flushed into the heating unit, and the samples were collected in a bottom carousel for samples to be reanalysed.

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 automatic sampling apparatus contained 30 sample tubes mounted on a carousel. The start tube in the carousel was chosen randomly. The first tube was left empty, while in the second tube, barley grain, cultivar Golf, of good hygienic quality was used as a reference sample. The third tube was left empty and the fourth tube contained a randomly chosen sample. Subsequently, 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 metal oxide semiconductor field effect transistor (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). The sample time, valve switch and 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 sampler. Finally, the sensors were exposed to technical air for 15 min to permit the sensors to recover 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 can be found in Olsson et al. (2000). For further information about parameter extraction refer to Eklöv et al. (1997).

More than 850 measurements were carried out with the electronic nose. An initial evaluation of the data were performed with a principal component analysis (PCA) of the sensor signals for every measured sample. 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. Consequently, for three subsamples, the average of the first of three measurements was calculated and used for further modelling. For further explanation of the decrease in the sensor signals refer to Olsson et al. (2000).

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

An 85-g sample was placed in a 190-ml glass tube, with glass wool covering both ends of the tube. The ends were capped with Teflon plugs with a 1/8″ Swagelok connection to which Teflon tubes (1/8″ × 1.6 mm) were attached. To collect volatile compounds, a porous polymer adsorbent (Chromosorb 102, 80–100 mesh 300 mg) was connected after a desorption unit. The glass tube with the sample was placed in a 50 °C water bath. Technical air at a flow rate 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 for 15 min with 20 ml min^{-1} nitrogen. 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 for 2 h. Finally, the adsorbents were kept at 200 °C for 15 min while nitrogen gas (100 ml min^{-1}) was passed through them. A schematic figure of the apparatus can be found in Olsson et al. (2000).

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 flow of 100 ml min^{-1} for 5 min. The compounds were injected directly into the gas chromatograph, a Varian 3400 GC with FID detector, a fused silica capillary column with chemi-

cally bound methyl-polysiloxan, OV1CB, 1 μm, 60 m × 0.32 mm i.d. (Lars Johansson, Mölndal, Sweden), temperature program rising from 35 °C to 220 °C at 4 °C per min, the carrier gas was helium at 23.1 psi at a rate of 3–4 ml min^{-1}. The data collection system was a Hewlett-Packard HP 3350. The mass spectrometer was a Finnigan Incos-50. The library of mass spectra came from both the National Institute of Standards and Technology and National Bureau of Standards (Finnigan, San Jose, USA). The analyses were performed by Institutet för Livsmedel och Bioteknik (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, extracted and analysed for ergosterol using a HPLC technique according to procedures described in Olsson et al. (2000).

2.7. Fungal counts

Fungal counts were determined according to Methods 154 (Thrane, 1996) and 98 (Åkerstrand, 1995) of the Nordic Committee on Food Analysis (NMKL). Samples (40 g) were soaked for 30 min in 0.1% peptone water (360 ml) before being pummelled in a Stomacher (Laboratory Blender 400, Seward Medical, London, UK) for 2 min. Ten-fold dilutions (0.1 ml) were plated in duplicate on Dichloran 18% Glycerol agar (DG18; Hocking and Pitt, 1980) and Czapek Dox Iprodione Dichloran agar (CZID; Samson et al., 1996). The DG18 plates were incubated upright in darkness while CZID plates were incubated under alternating illumination (12 h light + 12 h dark) at 25 °C for 5 days. Total counts and counts of *Aspergillus*, *Penicillium*, *Eurotium* and *Fusarium* species were obtained.

2.8. Direct plating

Internal infection of the kernels was determined by direct plating (Åkerstrand, 1995; Thrane, 1996). Kernels (200) were surface-disinfected in NaOCl (0.4% active chlorine; 10 volumes), dried with sterile filter papers, and plated (10 kernels per plate) on DG18 and CZID. The plates were incubated at 25 °C for 5–7 days in darkness (DG18) and alternating illumination (CZID). Percentages of internal infection and *Fusarium* species were calculated.

2.9. Ochratoxin A

The method used was described by Rhone-Poulenc (1996). Finely ground samples (50 g) were added to 60% (v/v) acetonitrile/water (200 ml), blended at high speed for 2 min, and filtered through Whatman No. 4 filter. The filtrate (4 ml) was diluted with 33 ml phosphate buffered saline pH 7.4 (33 ml). The whole extract was applied to an immunoaffinity column containing monoclonal antibodies against OA (Ochraprep, Rhone-Poulenc, Glasgow, Scotland). The column was washed with distilled water (20 ml; 5 ml min^{-1}) and dried by passing air through the column. The bound ochratoxin was eluted with acetic acid/methanol (2:98 v/v; 1.5 ml; one drop per min) followed by distilled water (1.5 ml) to give a total volume of 3 ml. From this, 100 μl was injected onto a HPLC system. The mobile phase was acetonitrile/water/acetic acid (51:47:2) at a flow rate of 1 ml min^{-1}. The column was a Spherisorb S5 ODS2 (25 × 0.46 cm) and detection was by a fluorescence detector measuring emission at 443 nm. The analyses of OA were made by the Swedish National Veterinary Institute, Uppsala, Sweden.

2.10. Deoxynivalenol (DON)

The method used was described by Möller and Gustavsson (1992). Ground samples (50 g), water (10 ml), and ethyl acetate/acetonitrile (4:1; 250 ml) were shaken for 30 min on a flask shaker, then filtered extract (10 ml) was evaporated to dryness under a stream of nitrogen on a 45 °C water bath. To each tube, hexane (4 ml), acetonitrile (3 ml) and water (2 ml) were added and mixed on a Vortex. After a brief centrifugation, the upper layer was discarded, and the last step repeated with another 4 ml hexane. Combined acetonitrile fractions were evaporated to dryness, dissolved in 2.5 ml chloroform/methanol (90:10), and applied to an active Sep-Pak Florisil cartridge. The Florisil cartridge was eluted with chloroform/methanol (90:10). The collected eluate was evaporated to dryness, dissolved in chloroform, and again evaporated to

dryness under a stream of nitrogen. The sample was then derivatized by adding 50 μl Tri-Sil/TBT and injected into a capillary gas chromatograph in splitless mode. The analyses of DON were made by the Swedish National Veterinary Institute.

2.11. Multivariate data evaluation

To find patterns in the data matrix related to different classes, a technique called pattern recognition (PARC) was used (Wold et al., 1984). Soft independent modelling of class analogy (SIMCA) is a special case of PARC which also works on asymmetric data structures. Asymmetric data are commonly found in medical diagnosis (one disease vs. all others), chemical structure determinations (one type of structure vs. all others), biomedical applications (biological active compounds vs. inactive compounds), and quality control (good vs. all types of inferior) (Albano et al., 1981; Thelin et al., 1995). Asymmetric data are analysed by describing the well-defined class by a principal component (PC) model. Unknown samples are then evaluated with regard to whether the samples are located inside the well-defined class, i.e. belonging to that class or not. This is done by projecting, for example GC-MS data from a new sample down to the PC model, describing the well-defined class, which will result in the scores describing the place of the new sample with respect to the ones used in the construction of the PC model.

For the PC, PCA, and PLS models, the variables were scaled to unit variance. Each variable was first divided with the standard deviation for that variable and then mean-centred. This gave all variables equal influence on data analysis.

3. Results

3.1. The mycotoxins and fungal content of the grain samples

The OA, DON, and fungal content (ergosterol and counts and percentages of infection) of the 40 barley samples are shown together with moisture contents in Table 1. The OA level varied between 0 and 934 μg kg^{-1}, with an average of 57 μg kg^{-1} (SE = 26; $n = 40$). The DON content varied between 0 and 857 μg kg^{-1}, with an average of 55 μg kg^{-1} (SE = 22; $n = 40$). Samples with normal odour had no detectable OA and an average DON content of 16 μg kg^{-1} (range 0–80), while samples with off-odour had average OA contents of 76 μg kg^{-1} (range 0–934) and DON contents averaging 69 μg kg^{-1} (range 0–857). The moisture content varied between 7.9% and 19.8% with an average of 13.8% (SE = 0.5; $n = 40$). The 10 samples with normal odour had a lower average water content (10.3%) than the 30 samples with off-odour (15.0%).

A PCA model was constructed using the data from Table 1 as X variables. The OA and DON levels were log transformed before the PCA analysis was performed. For counts of genera below the limit of reading, one log CFU unit below that was used in the PCA analysis. Sample 593 was excluded from the analysis due to missing data, while samples 580 and 582 were excluded since they were found to be outliers.

The PCA using two PC gave an R^2_{Cum} of 0.57 with a Q^2_{Cum} of 0.33. Fig. 1(a) shows the score plot t_2 vs. t_1, with the normal samples (590–599) located in the left part of the plot, with some overlapping with off-odour samples. The corresponding loading plot shows how the measured variables were described by the two first PC (Fig. 1(b)). The variable's total counts on DG18, counts of *Penicillium*, *Aspergillus* and *Eurotium* species, ergosterol, OA, and water content were highly correlated, as indicated by their close proximity on the plot. The variables internal infection on CZID, and to a lesser extent, internal infection on DG18 are also quite closely correlated to the variables just mentioned. The variable counts of *Fusarium* species on CZID was negatively correlated to the variables mentioned above since this variable has a negative value for PC 1. Fig. 1(b) also shows that the number of *Fusarium* colonies growing from the surface-disinfected kernels on CZID were correlated to the detected DON levels.

A new PC model was performed using mycological data (Table 1) from the samples with normal odour ($n = 9$) as X variables. The four significant PC explained almost all variations in X ($R^2_{Cum} = 0.95$), while the Q^2_{Cum} reached 0.38. To study if samples with normal odour were different compared to samples with off-odour, the class memberships were predicted and are displayed in Fig. 2. Among the 28 samples with off-odour, three were classified as normal since the predicted distance for these samples is within the displayed critical distance (0.05 level) for the normal

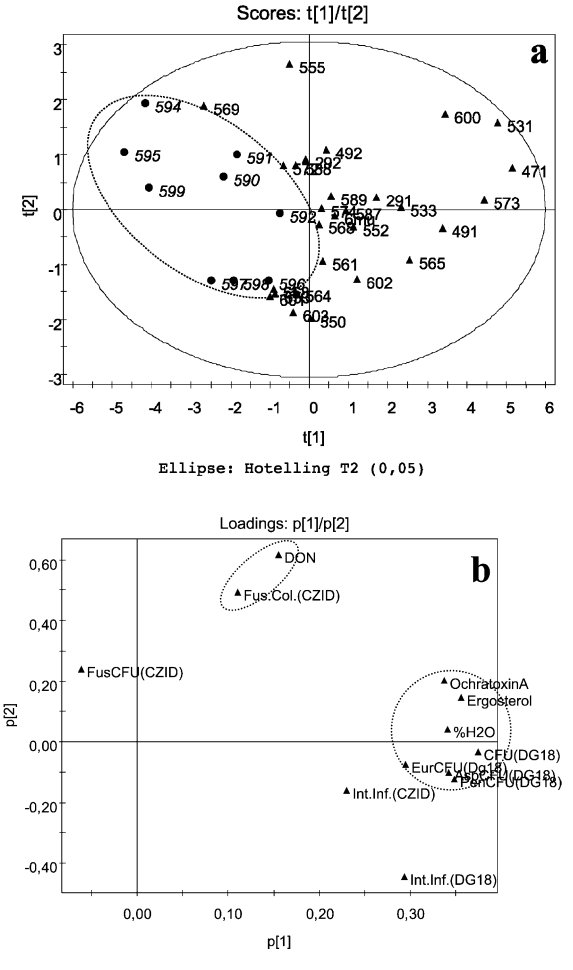

Fig. 1. (a) PCA score plot with t_2 vs. t_1 and indicated sample numbers and (b) the corresponding loading plot based on the mycological data shown in Table 1. The two first PC account for 62% ($R^2_{Cum}=0.62$) of the total variation. Samples described as having normal odour (●) are located to the left (dotted ellipse) in the score plot (1a). Variables located close to each other inside the dotted circles of the loading plot are correlated (1b).

samples. This shows that these three samples had similar fungal, mycotoxin, and moisture contents as the normal samples. Of these three samples, two had been described as mouldy B and one as musty B (Fig. 2).

3.2. Prediction of ochratoxin A

3.2.1. GC-MS

The data were first studied using PCA, before a PLS model using GC-MS data as X and OA as Y was constructed. However, it was not possible to obtain a PLS model (volatile compound = X variables) that could predict OA in the grain samples with satisfactory accuracy. The SIMCA classification technique based on pattern recognition was used instead. The Swedish National Food Administration has established 5 μg kg^{-1} as the maximum limit for OA in cereal grain in Sweden. Hence, data on volatiles from samples with an OA level below 5 μg kg^{-1} were used to build a PC model, while the rest of the samples were used to predict if these samples fitted the PC model. The water content was added and used as an X variable together with the 103 volatile compounds identified. Sample numbers 291, 531, and 555 had not been analysed with the GC-MS and were therefore excluded. This model gave a R^2_{Cum} value of 0.5, while Q^2_{Cum} was 0.08 using four PC. The distances to the PC model were calculated for samples with OA higher than 5 μg kg^{-1}. The result is visualised in the DmodX plot (Fig. 3), showing that 3 samples (564, 292, 587) of 37 were wrongly classified into the PC model (marked with arrows). The samples with OA below 5 μg kg^{-1} were included in the plot to see if these samples were predicted as belonging to the model.

To the data matrix containing water content and GC-MS data, two binary variables were added according to whether the sample had an OA below (addressed: 1 0) or above (addressed: 0 1) the limit 5 μg kg^{-1}. A PCA was performed with the new class marker variables, water content, and GC-MS data. PC 1 explained 19%

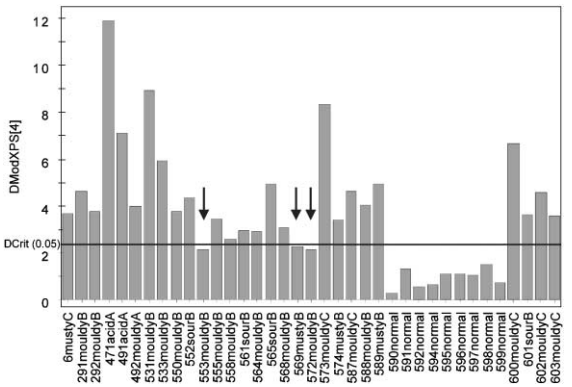

Fig. 2. Prediction of odour class (normal or off-odour) using mycological data from samples with normal odour (Table 1) as the X variables. The misclassified samples are indicated with arrows.

($R^2 = 0.19$) of the total variation ($Q^2 = 0.1$), while PC 2 explained an additional 12% ($R^2 = 0.12$) of the total variation ($Q^2 = 0.05$). The result showed that samples with OA levels below 5 μg kg^{-1} had higher concentrations of aldehydes, e.g. 2-heptenal, nonanal, octanal, decanal, 2-hexenal, and heptanal and alcohols such as 1-penten-3-ol, 1-hexanol, 1-nonanol, 1-butanol, and 1-octanol. Samples with OA levels above 5 μg kg^{-1}, on the other hand, had higher concentration of ketones, e.g. 2-pentanone, 3-pentanone, 2-hexanone, 3-octanone, 2-heptanone, and methylheptanone. The highest concentration of compounds such as dimethylpyrazine, methylisooctanoate, geosmin and ethanol were found in samples with OA levels above 5 μg kg^{-1} (data not shown). No correlation was found between odour and OA level, as samples with pronounced or strong off-odours had OA levels both below and above 5 μg kg^{-1} (Table 1 and Fig. 3). However, all samples with an OA content above 5 μg kg^{-1} had been classified as having some kind of off-odour.

3.2.2. Electronic nose

The data were first studied using PCA before the classification technique based on pattern recognition was used. A PC model was performed with data from the 24 samples with an OA level below 5 μg kg^{-1}, using the 85 sensor signals from the electronic nose and water content as X variables. Two samples (552 and 573) were found to be outliers and the PC model was recalculated without them. The R^2_{Cum} was 0.72 while the Q^2_{Cum} was 0.61 using three PC. The PC model was then used to predict the distance in X space for the samples with OA above 5 μg kg^{-1} as illustrated in the DmodX plot (Fig. 4). The samples with OA below 5 μg kg^{-1} were included in the plot to see if these samples were predicted to belong to the model. One of the samples (492, arrow marked) was wrongly predicted as not belonging to the model, while six samples (564, 6, 292, 533, 471, and 580), all marked with arrows were wrongly classified as belonging to it (Fig. 4).

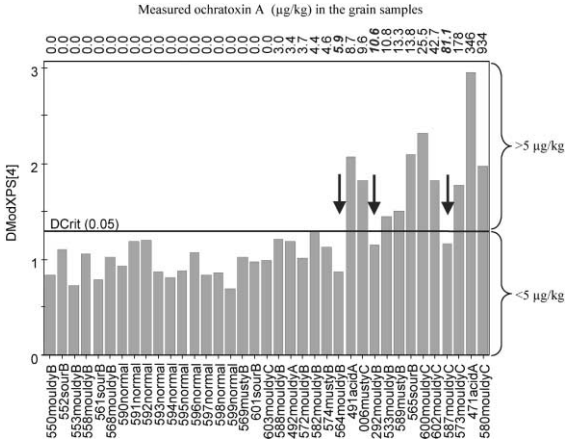

Fig. 3. Prediction of OA class (< or >5 μg kg^{-1}) from GC-MS volatile compounds data. The samples are marked with numbers in combination with odour classes and the ochratoxin A content for each sample is shown at the top of the figure. The misclassified samples are indicated with arrows.

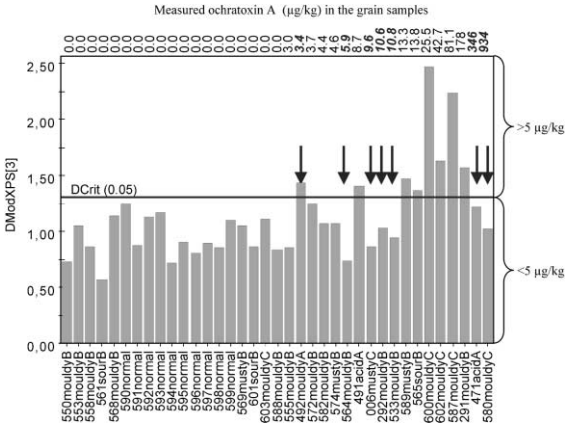

Fig. 4. Prediction of OA class (< or >5 μg kg^{-1}) from electronic nose data. The samples are marked with numbers in combination with odour classes and the ochratoxin A content for each sample is shown at the top of the figure. The misclassified samples are indicated with arrows.

3.3. Prediction of deoxynivalenol (DON)

3.3.1. GC-MS

A PLS model was calculated using the 103 volatile compounds detected by GC-MS as X variables and the measured DON levels as Y variables. In the PLS model samples 569 and 582 were outliers, probably because of higher DON levels than the rest of the samples (Table 1). A new PLS model without the outliers was calculated. The recalculated PLS model using two PC obtained a $R^2_{X\mathrm{Cum}}$ of 0.24, while the $R^2_{Y\mathrm{Cum}}$ was 0.77 and the $Q^2_{Y\mathrm{Cum}}$ 0.23. A few samples were recognised

as weak outliers in the distance to model in X space (DModX plot), Y space (DmodY plot), and the score plot (t_1/t_2), but were kept in the model. The root mean square error of estimation (RMSEE) was 16 μg kg^{-1} DON in the grain. Pentane, methylpyrazine, 3-pentanone, 3-octene-2-ol, and isooctylacetate showed a positive correlation with DON, while ethylhexanol, pentadecane, toluene, 1-octanol, 1-nonanol, and 1-heptanol showed negative correlations with DON (data not shown).

3.3.2. Electronic nose

A PLS model was also constructed using the sensor signals from the electronic nose as X variables and the DON levels as Y variables. A nonlinear relationship between the t_1 and u_1 scores was obtained for the samples with DON levels of 0 μg/kg. These 12 samples were therefore excluded from the PLS model. In addition, samples 531 and 573 were excluded since they were found to be outliers. In the recalculated PLS model with two PC, $R^2_{X\mathrm{Cum}}$ became 0.58 while the $R^2_{Y\mathrm{Cum}}$ was 0.42 and the $Q^2_{Y\mathrm{Cum}}$ 0.10. The RMSEE was 25 μg DON per kilogram of grain. The MOSFET sensors and the CO_2 sensor showed a positive correlation to DON, while the Taguchi sensors showed a negative correlation to DON (data not shown).

4. Discussion

Odour classification of grain is widely used as a fast method for determining the grain quality. The granaries in Sweden accept grain with pronounced odour (grade B) but with a price reduction, while samples with strong odour (grade C) are not accepted. Schnürer and Jonsson (1992) found no correlation between grain odour and ergosterol content in a large number of naturally contaminated oat and barley samples. Results presented in this work show that odour at an intensity described as pronounced (grade B) could contain OA both below and above 5 μg kg^{-1} grain (Table 1). Sample 471, with an OA content of 346 μg kg^{-1}, was described as having a weak acid odour, indicating a low predictive ability of the odour classification system for OA.

Initially, PLS was used for quantification of OA using GC-MS or electronic nose data as X variables and OA concentrations as Y variables. However, as the relation between the volatile compounds detected by GC-MS or electronic nose and the OA levels was found to be asymmetric, PLS modelling could not be used. Instead, PARC was used by performing PC models, based on GC-MS or electronic nose data using the samples with OA levels below 5 μg kg^{-1} (Figs. 3 and 4). This only allowed a qualitative (± 5 μg kg^{-1}) prediction of OA. Nevertheless, a qualitative prediction would be sufficient, as samples exceeding the Swedish legal limit of 5 μg kg^{-1} OA could be detected.

When predicting the OA class from GC-MS data (Fig. 3), 3 samples out of 37 were misclassified (564, 292, 587). Sample number 564 contained 5.9 μg kg^{-1} OA, which is only marginally above 5 μg kg^{-1}, while sample numbers 292 and 587 contained 10.6 and 81 μg kg^{-1} OA, respectively (Table 1). A PCA model was also constructed based on the GC-MS data. The misclassified samples (564, 292, 587) were clustered near the samples with OA < 5 μg kg^{-1} in the score plot (t_2 vs. t_1), showing that the patterns of volatile compounds for these samples are more similar to this group (data not shown). The GC-MS chromatogram for sample number 587 was reevaluated without finding any deviation. As it was not possible to measure the volatile compounds from sample 587 by GC-MS a second time, we cannot determine the reason for this discrepancy. However, the sample was correctly predicted by the electronic nose, indicating an inaccurate GC-MS analysis.

Pasanen et al. (1996) reported that a toxic strain of *P. verrucosum* produced more ketones but lower levels of alcohols than a nontoxic strain. These result agrees with our findings, but unlike Pasanen et al. (1996), we found 1-octen-3-ol in all samples in about equal amounts, suggesting that this compound could not be used as an indicator of OA production in naturally contaminated grain.

Sample 492 was wrongly predicted as having >5 μg kg^{-1} OA, while samples 564, 6, 292, 533, 471, and 580 were mispredicted as having OA levels <5 μg kg^{-1} (Fig. 4). The PCA model based on GC-MS data was again used to check the patterns of volatile compounds for these samples. Four of these samples (6, 292, 564, 580) were found to be clustered close to the group of samples with <5 μg kg^{-1} OA, indicating that these samples contained more alcohols and aldehydes than ketones. Even so, the GC-MS man-

aged to predict the correct class for samples 6 and 580 but not for 292 and 564 (Fig. 3). The explanation as to why the electronic nose was less successful in a qualitative estimation of OA levels compared to GC-MS could partly be due to the fact that the volatile compounds were concentrated on an adsorbent before injecting into the GC-MS. The detection principle also differs between the two systems.

A PLS regression model was performed using GC-MS data as *X* variables and DON concentrations as *Y* variables. The compounds pentane, methylpyrazine, 3-pentanone, 3-octene-2-ol, isooctylacetate, ethylhexanol, pentadecane, toluene, 1-octanol, 1-nonanol, and 1-heptanol were the most important for predicting the presence of DON (data not shown). None of these compounds have previously been reported as being important for prediction of this compound. A correlation has been reported between the production of the terpene trichodiene, a volatile trichothecene precursor, and the synthesis of trichothecenes, both from wheat kernels (Jelen et al., 1997a) and incubated grain spikes with natural *Fusarium* head blight infestation (Jelen et al., 1997b). Pasanen et al. (1996) reported that terpenes and ketones were produced at approximately equal amounts, and alcohols to only a minor extent, when a trichothecene producing strain of *F. sporotrichioides* was grown on sterile oat and wheat grains. In our study, no terpene and only one ketone were among the most important compounds positively correlated with DON contents. Alcohols dominated among the six volatiles compounds negatively correlated with DON (4/6). The scarcity of terpenes detected in this study is likely to be a reflection of the GC-MS technique used.

The mycological data showed a strong correlation between OA level and ergosterol, total counts, the counts of *Aspergillus*, *Penicillium* and *Eurotium* species as well as the water content (Fig. 2(b)). The ratios between *Aspergillus*, *Penicillium* and *Eurotium* species varied among the samples. This gave rise to different patterns of fungal volatiles, which in turn gave an asymmetric data distribution.

The ergosterol and OA contents were correlated, while the ergosterol and DON contents were not (Fig. 2(b)). In earlier studies, ergosterol had been found to correlate to both OA (Boley and Müller, 1986) and DON (Perkowski et al., 1995). However, both these studies dealt with inoculated grain kernels as opposed to our work where naturally contaminated grain was used. Miller and Young (1983) showed that ergosterol is useful for estimating *Fusarium* biomass within grain kernels from artificial *Fusarium* head blight infections since these contain few other fungi. It is possible that the correlation we found between ergosterol and OA level and the lack of correlation between DON level and ergosterol (Fig. 2(b)) is due to the fact that the OA producing fungi contribute more to the ergosterol contents than the DON-producing *Fusarium* spp.

Our results show that it is possible to use volatile compounds to detect OA and to predict whether the OA level is below or above 5 $\mu g\ kg^{-1}$ grain. The volatile compounds can be detected using either a GC-MS system or an electronic nose. The GC-MS system predicted the OA with a higher accuracy than the electronic nose used in this report, since the GC-MS misclassified only 3 of 37 samples, while the electronic nose misclassified 7 of 37. We were also able to predict the DON level in the naturally contaminated barley samples using the volatile compounds detected and quantified by either GC-MS or electronic nose. However, it is important to mention that neither the GC-MS system nor the electronic nose has been optimised to classify OA or DON levels in grain. The RMSEE value for prediction of DON based on GC-MS data and electronic nose were both quite low, 16 and 25 $\mu g\ kg^{-1}$, respectively. For comparison, Dowell et al. (1999) obtained a standard error of around 40 $\mu g\ kg^{-1}$ when they predicted DON in single wheat kernels using near-infrared spectroscopy. This system is faster than either the GC-MS or the electronic nose as it does not require any sample preparation. The second fastest is the electronic nose system, which in this paper has proved to be useful for prediction of mycotoxins in grain. The electronic nose technology is being rapidly developed, both with regard to sensor and data processing capabilities. Future research on the relationship between fungal volatiles and mycotoxin contents of grain should analyse a large number of samples of naturally infected grains from all the major cereal crops.

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References

Åkerstrand, K., 1995. Mould and yeast. Determination in foods. NMKL Method No. 98, 3rd edn. Nordic Committee on Food Analysis, Esbo, Sweden.

Albano, C., Blomqvist, G., Coomans, D., Dunn, W.J., Edlund, U., Eliasson, B., Hellberg, S., Johansson, E., Nordén, B., Sjöström, M., Söderström, B., Wold, H., Wold, S., 1981. Pattern recognition by means of disjoint principal components models (SIMCA). Philosophy and methods. In: Höskuldsson, A.E.A. (Ed.), Proceedings Symposium i Anvent Statistik. NEUCC, RECAU and RECKU, Copenhagen, Denmark, pp. 183–218.

ASAE (American Society of Agricultural Engineers), 1983. Moisture measurement—grain and seeds. Agricultural Engineers Yearbook of Standards. American Society of Agricultural Engineers, St. Joseph, MI, USA, p. 328.

Boley, A., Müller, H.M., 1986. Prediction of ochratoxin A, citrinin and ergosterol by *Penicillium viridicatum* in autoclaved and non-autoclaved wheat at low temperature. Mycotoxin Res. 2, 99–103.

Börjesson, T., Eklöv, T., Jonsson, A., Sundgren, H., Schnürer, J., 1996. Electronic nose for odor classification of grains. Cereal Chem. 73, 457–461.

Breitholtz, A., Olsen, M., Dahlbäck, Å., Hult, K., 1991. Plasma ochratoxin A levels in three Swedish populations surveyed using an ion-pair HPLC technique. Food Addit. Contam. 8, 183–192.

Ciegler, A., 1979. Mycotoxins—their biosynthesis in fungal: biosynthesis of the trichothecenes. J. Food Prot. 42, 825–828.

Dowell, F.E., Ram, M.S., Seitz, L.M., 1999. Predicting scab, vomitoxin, and ergosterol in single wheat kernels using near-infrared spectroscopy. Cereal Chem. 76, 573–576.

Eklöv, T., Mårtensson, P., Lundström, I., 1997. Enhanced selectivity of MOSFET gas sensors by systematical analysis of transient parameters. Anal. Chim. Acta 353, 291–300.

Eklöv, T., Johansson, G., Winquist, F., Lundström, I., 1998. Monitoring sausage fermentation using an electronic nose. J. Sci. Food Agric. 76, 525–532.

Eriksen, G.S., Alexander, J. (Eds.), 1998. *Fusarium* toxins in cereals—a risk assessment. Nordic Council of Ministers, Copenhagen, Denmark, pp. 746–748.

Hocking, A.D., Pitt, J.I., 1980. Dichloran–glycerol medium for enumeration of xerophilic fungi from low moisture foods. Appl. Environ. Microbiol. 39, 488–492.

Holmberg, T., Hagelberg, S., Lundheim, N., Thafvelin, B., Hult, K., 1990. Ochratoxin A in swine blood used for evaluation of cereal handling procedures. J. Vet. Med., Ser. B 37, 97–105.

Jelen, H., Latus-Zietkiewicz, D., Wasowicz, E., Kaminski, E., 1997a. Trichodiene as a volatile marker for trichothecenes biosynthesis. J. Microbiol. Methods 31, 45–49.

Jelen, H., Kaminski, E., Wiewiorowska, M., Kuta, A., Rudzinska, M., 1997b. Assessing the toxigenicity of *Fusaria* contaminating grain spikes on the basis of headspace analysis of trichodiene. Cereal Res. Commun. 25, 331–335.

Jonsson, A., Winquist, F., Schnürer, J., Sundgren, H., Lundström, I., 1997. Electronic nose for microbial quality classification of grains. Int. J. Food Microbiol. 35, 187–193.

Krogh, P., 1987. Ochratoxins in food. In: Krogh, P. (Ed.), Mycotoxins in Food. Academic Press, London, pp. 97–121.

Miedaner, T., Perkowski, J., 1996. Correlations among *Fusarium culmorum* head blight resistance, fungal colonization and mycotoxin contents in winter rye. Plant Breed. 115, 347–351.

Miller, J.D., Young, J.C., 1983. *Fusarium* toxins in field corn: I. Time course of fungal growth and production of deoxynivalenol and other mycotoxins. Can. J. Bot. 61, 3081–3087.

Möller, T., Gustavsson, H., 1992. Determination of type A and B trichothecenes in cereals by gas chromatography with electron capture detection. J. AOAC Int. 75, 1049–1053.

Olsen, M., Möller, T., Åkerstrand, K., 1993. Ochratoxin A: occurrence and intake by the Swedish population. In: Scudamore, K.A. (Ed.), United Kingdom Workshop on Occurrence and Significance of Mycotoxins. Brunel University, London, pp. 96–100.

Olsson, J., Börjesson, T., Lundstedt, T., Schnürer, J., 2000. Volatiles for mycological quality grading of barley grains—determinations using gas chromatography-mass spectrometry and electronic nose. Int. J. Food Microbiol. 59, 167–178.

Pasanen, A.L., Lappalainen, S., Pasanen, P., 1996. Volatile organic metabolites associated with some toxic fungi and their mycotoxins. Analyst 121, 1949–1953.

Perkowski, J., Miedaner, T., Geiger, H.H., Muller, H.M., Chelkowski, J., 1995. Occurrence of doxynivalenol (don), 3-acetyl-don, zearalenone, and ergosterol in winter rye inoculated with *Fusarium culmorum*. Cereal Chem. 72, 205–209.

Rhone-Poulenc, 1996. Instructions for use: Ochraprep, ochratoxin columns. Rhone-Poulenc, Glasgow, Scotland.

Samson, R., Hoekstra, E., Frisvad, J., Filtenborg, O. (Eds.), 1996. Introduction to Food-Borne Fungi, 5th edn. Centraalbureau voor Schimmelcultures, Baarn, Netherlands, pp. 795–797.

Schnürer, J., Jonsson, A., 1992. Ergosterol levels and mould colony forming units in Swedish grains of food and feed grade. Acta Agric. Scand., Sect. B 42, 240–245.

Smith, E.A., Chambers, E., Colley, S., 1994. Development of vocabulary and references for describing off-odors in raw grains. Cereal Foods World 39, 495–499.

Thelin, B., Lundstedt, T., Lundgren, R., Olsson, A., Batra, S., 1995. Classification of estradurin (R) batches—correlation between P-

31 NMR and a biological duration test for batch approval. Chemom. Intell. Lab. Syst. 27, 135–145.

Thrane, U., 1996. *Fusarium*. Determination in foods and feedstuffs. NMKL Method No. 154. Nordic Committee on Food Analysis, Esbo, Sweden.

Wold, S., Albano, C., Dunn, W.J., Edlund, U., Esbensen, K., Geladi, P., Hellberg, S., Johansson, E., Lindberg, W., Sjöström, M., 1984. Multivariate data analysis in chemistry. In: Kowalski, B.R. (Ed.), Chemometrics: Mathematics and Statistics in Chemistry. Reidel, Dordrecht, pp. 1–79.