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Evaluation of rapeseed quality by the electronic nose

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The quality of rapeseed, which is supposed to be free of any detectable foreign aromas, was evaluated by an electronic nose equipped with an array of 8 quartz microbalance sensors and 4 metal oxide semiconductor sensors. Signals generated by the sensors were analysed by Principal Component Analysis (PCA) and Discriminant Functions Analysis (DFA). The aim of the analysis was to distinguish between good quality rapeseed and rapeseed that contained small percentages of mouldy and/or burnt rapeseed. All sound rapeseed samples (7 % moisture content) could be distinguished from samples with high moisture content (14.4 % weight by weight (w/w)) that were incubated for different numbers of days, and which consequently became mouldy. Another set of sound rapeseed samples was mixed with different percentages (1 % to 10 % w/w) of mouldy rapeseed which was obtained by incubating rapeseed with high moisture content for six days. All in all 95.6 % of the samples were correctly classified by DFA of the sensor signals. Also 96.1 % of samples which contained different amounts of burnt rapeseed were correctly classified.
However the natural variability of rapeseed, which might influence the correctness of the classification of quality parameters, was not considered in this paper. The study was conducted to evaluate the applicability of the particular set of sensors for the intended purpose.

1. Introduction

Rapeseed oil received a lot of attention in recent years due to the increasing demand of biodiesel, which is produced in large quantities by transesterification of edible oils, mostly rapeseed oil. This trend will continue, especially with regard to the goal set by the European Commission in 2007 to reduce the dependence on fossil fuels by supplying ten percent of the fuels consumed in EU by 2020 from renewable sources (Communication from the Commission, 2007). However, the quality criteria for rapeseed used for fuel production might be less stringent then the criteria for rapeseed intended for the production of edible oil. The two major quality deficiencies of rapeseeds are linked to high moisture content. A moisture content above 7 % w/w, which is often caused by improper storage conditions, makes rapeseed prone to growth of mould. The presence of even small percentages of mouldy rapeseed in a particular production batch deteriorates the quality of edible oil obtained from it (Matthaus et al. 2005). Harvested rapeseed has in some European countries, mostly due to climatic conditions, high moisture content which demands artificial drying, during which part of the rapeseed might be burnt. The presence of burnt rapeseed in the production batch

could lead to an increased contamination of the crude oil with polycyclic aromatic hydrocarbons (PAHs) (Cejpek et al., 1998). Since some PAHs are classified by the International Agency for Research on Cancer (IARC) as carcinogenic to humans (class 1), probably carcinogenic to humans (class 2a), or possibly carcinogenic to humans (class 2b) (Straif et al. 2005) the detection of even small amounts of burnt rapeseed in a batch of sound rapeseed is important. Rapid and efficient analytical methods for the evaluation of rapeseed quality would be of interest not only for edible oil producers, but also for farmers who could immediately and independently judge the quality of their product.

Quality assessment of rapeseed samples is commonly done olfactometrically by a panel of trained experts. However, this procedure is not always effective, particularly if the smell of a mixture of sound and mouldy rapeseed, or if sound and burnt rapeseed has to be assessed. In addition it is expensive due to the required manpower and limited sample throughput caused by limitations of the human scent. Hence a simple, cheap, and automated instrumental analysis method that could replace the panel of experts would be very much appreciated.

This paper focuses on the detection of rapeseed contaminated with small amounts (ranging from 1% to 10%) of mouldy or burnt rapeseed by the electronic nose.

2. Materials and instrument setup

2.1 Materials

Rapeseed samples of the same variety used for this study were obtained directly from Belgian rapeseed producers. All samples were stored in the dark at ambient temperature. Samples of five grams of rapeseed were placed in 22 ml vials and sealed with PTFE/butyl septa and aluminium crimp caps. The samples were analysed with the electronic nose without further treatment. The water content of rapeseed was determined by coulometric Karl Fischer titration (Bernreuther and Klein, 2005). The average water content of sound rapeseed samples was 6.7±0.1 g/100 g. Mouldy rapeseed was prepared by adding 20 ml of water to 200 g of sound rapeseed and storing at ambient temperature in polypropylene boxes, intended for storage of food products, for different periods of time. The average water content of mouldy rapeseed evaluated after 5 days of storage was 14.4±0.4 g/100 g. It should be noted that the rapeseed samples were not inoculated with mould spores and that the mould infestation was not quantified. However the assumption was made that the level of mould infestation is directly linked to the incubation time. The authors are aware of the fact that potentially different numbers of naturally present mould spores would lead to different levels of mould growth after a certain period, as well as this technique has limitation as a predictive quantitative tool which decrease the precision of the elaborated model. In order to compensate for potential extreme values at a certain level the number of replicate analyses was set to 36 per level (sound rapeseed; one day, three days, and five days of incubation).

A sample quantity of five grams of rapeseed was common to all analyses.

A semi-quantitative approach for the determination of low percentages of mouldy rapeseed in sound rapeseed consisted of breeding of mouldy rapeseed and adding defined amounts of mouldy rapeseed to sound rapeseed. The quantities representing 1%, 3 %, 5 % and 10 % (all w/w) of the final sample amount were added to 200g of sound

rapeseed. The mixtures were homogenised and 30 replicate samples of each were analysed by electronic nose and compared to the results of 60 replicate analyses of sound rapeseed samples. The higher number of replicate analyses was performed to get a good picture of the variability of analysis data for sound rapeseed, which was then used as a reference for the measurements of off-flavour samples.
Samples of burnt rapeseed were prepared in the laboratory by baking 800 g of rapeseed in a laboratory drying cabinet for 20 minutes at 180°C. After this time the burnt aroma was well developed and the colour of the inner part of the rapeseed seeds changed from bright yellow into a dark brown. Semi-quantitative experiments were performed in analogy to the experiments described before.

2.2 Instruments setup
The instrument setup consisted of the electronic nose VOCmeter (AppliedSensor GmbH, Reutlingen, Germany) connected to the headspace sampler G1883 (Agilent Technologies, Cernusco, Italy) (Fig.1).

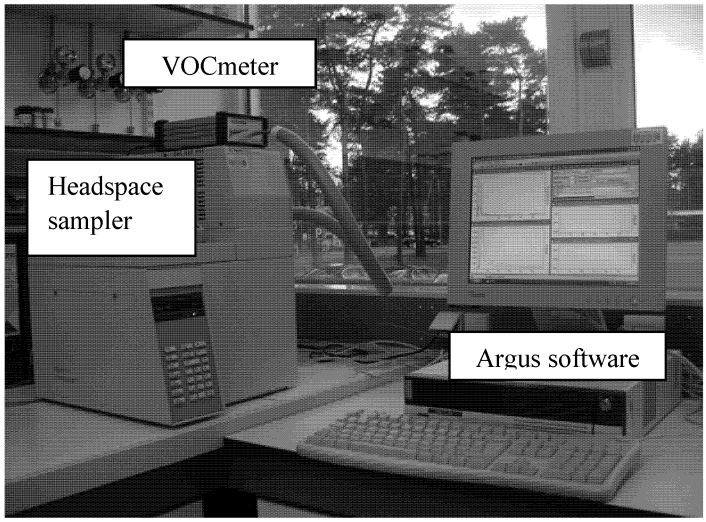

Figure 1: Instrument setup of the electronic nose experiments

The VOCmeter contained two types of sensors: eight quartz microbalance (QMB) sensors and four metal oxide semiconductor (MOS) sensors. Different polymers airbrushed on both sides of the quartz plates of QMB sensors could adsorb different substances depending on their physicochemical properties. The MOS sensors (Figaro, USA) were TGS 2610 (with high sensitivity to propane and butane), TGS 2611 (with high sensitivity to methane and alcohol vapours), TGS 2620 (with high sensitivity to vapours of organic solvent), and TGS 2600 (with high sensitivity to hydrogen and carbon monoxide). In order to minimize the influence of the moisture content of rapeseed samples on the response of the sensors the temperature of the oven of the headspace sampler was set to 45°C. The loop temperature was set to 50°C and the transfer line temperature was set to 55°C. The other parameters were as follows:

equilibration time 30 min, vial pressurization time 30 s, loop fill time 18 s, loop equilibration time 6 s. The volume of the injection loop in the headspace sampler was 3.0 mL. The carrier gas flow (dry synthetic air of high purity) was set to 25.0 ml/min. Signals of 8 QMB and 4 MOS sensors were recorded and the maximum value of signal changes was calculated and used in further analyses. Data were normalized in order to give equal weight to each sensor. Principal Component Analysis (Argus Software1.51, AppliedSensors GmbH, Germany) was applied first for visualization of the multivariate output data and next the Discriminant Functions Analysis (Statgraphics Plus 5.1, StatPoint, USA) classification was performed. For cross-validation of the DFA model the leave-one-out method was applied.

3. Results and discussion

3.1 Identification of the most suitable sensors

The aim of these experiments was the identification of the most suitable type of sensor for the evaluation of the target parameters. For this purpose the matrix of measured signals was evaluated by DFA for each sensor type (MOS and QMB) separately and was compared to the DFA analysis results of the merged data set. Samples representing different dilution levels of a mouldy as well as a burnt rapeseed sample (1 %, 3 %, 5 %, and 10 % w/w) with sound rapeseed were used. The results of these experiments are presented in Table 1.

Table 1: Percentage of samples correctly classified by DFA

Rapeseed type:	Sensor Type	Percentage: 0 %	Percentage: 1 %	Percentage: 3 %	Percentage: 5 %	Percentage: 10 %
mouldy	MOS	100.0	66.7	80.0	76.7	77.3
	QMB	100.0	100.0	76.7	73.3	96.7
	QMB+MOS	**100.0**	**100.0**	**86.0**	**80.0**	**100.0**
burnt	MOS	88.3	100.0	56.7	63.3	100.0
	QMB	96.7	83.3	73.3	96.7	86.7
	QMB+MOS	**100.0**	**100.0**	**76.7**	**100.0**	**100.0**

As seen in Table 1, mouldy rapeseed samples were more accurately classified by DFA analysis of single sensor type signals than burnt rapeseed samples. The separate analysis of both MOS and QMB sensors signals of sound rapeseed samples did not give any false positive results. However the mixtures of sound and mouldy rapeseed were less correctly classified. The percentages of samples of one class ranged for the combination mouldy rapeseed and MOS sensors from 66.7 % for the 1 % class to 80.0 % for the 3 % class. In that respect the classification using the QMB signals was better for these samples. Superior to the separate evaluation of sensor signals of the individual sensor types was the evaluation of the combined data set. All samples of three out of five sample classes were classified correctly. False classifications were found only for samples of the classes 3 % and 5 %, which were partially recognised as belonging to the respective other class. In case of burnt rapeseed, only the combined signals of MOS and QMB sensors led to 100 % correct classifications. Only samples of the class 3 % were misclassified as belonging to the adjacent classes. More details on the classification of different samples are given in Table 3 and Table 4. However it should be highlighted

that false negative classifications, which means in that respect the classification of off-flavour samples as sound rapeseed, were not found. The misclassifications might be the consequence of residual sample inhomogeneity. Consequently the application of larger sample vials that could take up higher sample quantities might be advantageous.
Based on these results the merged signals of MOS and QMB sensors were applied for the evaluation of further experiments.

3.2 Analysis of samples developing mould during storage

Samples of rapeseed with high moisture content incubated for different period of time: 1, 3 and 5 days were used in this experiment. PCA as well as DFA analyses were performed on the gained sensor signals. Figure 2 shows the PCA analysis data of the four sample classes. Based on this analysis, a clear distinction between sound rapeseed samples (0 day) and mouldy (after 1, 3, 5 days) rapeseed samples is possible. However, the clusters of the latter overlap strongly, which indicates that more detailed information on e.g. the level of contamination cannot be obtained by PCA.

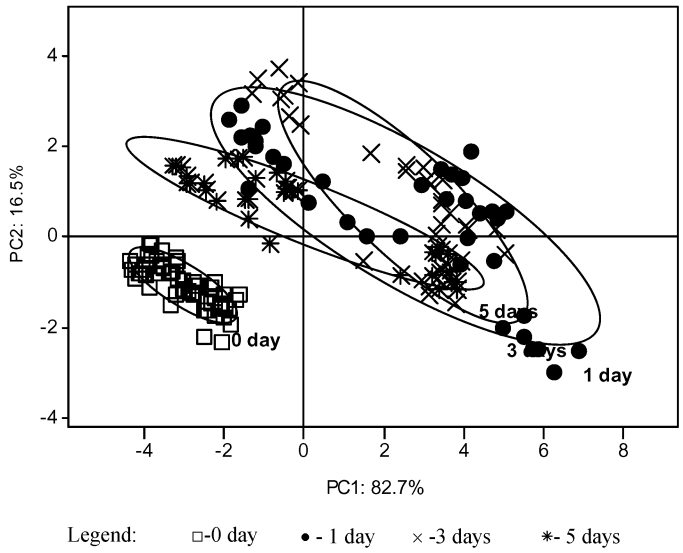

Figure 2: PCA measurements of sound and mouldy rapeseed samples after 1, 3 and 5 days of storage

The DFA results of the classification of rapeseed samples stored for 1, 3 and 5 days are presented in Table 2. All samples of sound rapeseed were correctly classified. The percentage of correctly classified samples after 1, 3, and 5 days of storage, was 86.1 %, 69.4 % and 83.2 % respectively. The reason for the partially wrong classification of mouldy rapeseed samples might be residual sample inhomogeneity and different levels of mouldiness since the number of spores was not controlled at the beginning of the study.

Table 2: DFA classification results of sound and mould rapeseed samples after 1, 3 and 5 days of storage.

Days of storage	Group Size	Predicted values			
		0 day	1 day	3 days	5 days
0 day	60	**100.0 %**	0.0 %	0.0 %	0.00 %
1 day	36	0.0 %	**86.1 %**	**13.9 %**	0.00 %
3 days	36	0.0 %	**22.2 %**	**69.4 %**	**8.3 %**
5 days	36	0.0 %	**5.6 %**	**11.2 %**	**83.2 %**

3.3 Analysis of samples with different concentration of mouldy rapeseed

The PCA analysis of samples of sound rapeseed and samples with different concentration of mouldy rapeseed is presented in Figure 3. The first Principal Component explained approximately 70 % of the variability of the analysed data and the second 27 %. Most of the mouldy rapeseed clusters are overlapping with each other so the full separation based on the first two principal components only was not possible. The results of the classification of rapeseed samples with different concentration of mouldy seeds done by DFA are presented in Table 3. All samples of sound rapeseed were correctly classified. Samples of 1% and 10% concentration of mouldy rapeseed were also correctly classified.

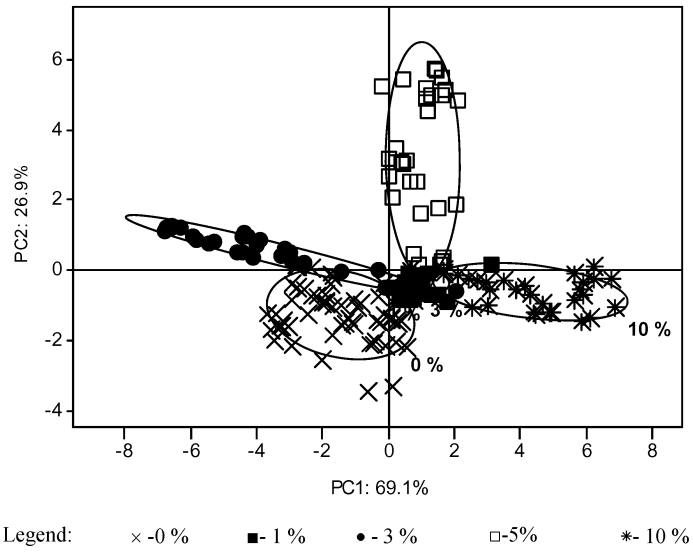

Figure 3: PCA measurements of sound (0%) and mixtures with different contents of mouldy samples in sound rapeseed (1 %, 3 %, 5 %, and 10 %)

Four out of 30 rapeseed samples (13.3 %) of class 3 % were misclassified and recognised as member of class 1 %. Five out of 30 samples (20 %) of rapeseed class 5 % were wrongly recognised as belonging to class 10 %. This could be reasoned - as mentioned before - by a potential lack of sample homogeneity.

Table 3: DFA classification results of sound rapeseed and different concentrations of mouldy rapeseed

Actual value	Predicted values				
	0 %	1 %	3 %	5 %	10 %
0%	**100.0 %**	0.0 %	0.0 %	0.0 %	0.0 %
1%	0.0 %	**100.0 %**	0.0 %	0.0 %	0.0 %
3%	0.0 %	13.3 %	**86.7 %**	0.0 %	0.0 %
5%	0.0 %	0.0 %	0.0 %	**80.0 %**	20.0 %
10%	0.0 %	0.0 %	0.0 %	0.0 %	**100.0 %**

3.4 Analysis of samples with different concentration of burnt rapeseed

The PCA of electronic nose measurements of sound rapeseed samples (0 %) and mixture of sound and burnt rapeseed samples are presented in Figure 4. The first Principal Component explained 60.9 % variability of the data and the second PC explained 33.7 %. Most of the clusters are overlapping with each other, so the distinction between different qualities of rapeseed was not possible with the first two PCs.

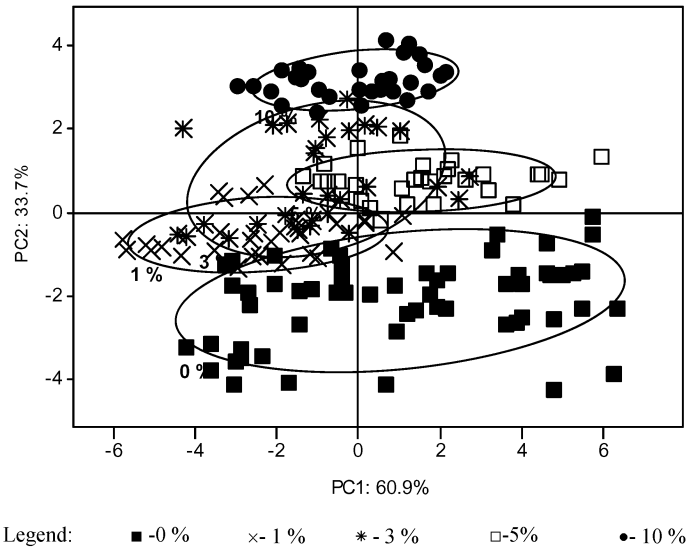

Figure 4: PCA of electronic nose measurements of sound (0%) and mixture of sound and burnt rapeseed samples

Based on the DFA classification of electronic nose data presented in Table 4, all samples of sound rapeseed were correctly classified. Also, samples of rapeseed belonging to the class: 1 %, 5 %, and 10 % of burnt rapeseed were correctly classified. Seven out of 30 samples of rapeseed with concentration 3% were misclassified and recognised as 1 % (one sample) or 5 % (six samples) of burnt rapeseed. The reason for the misclassification of class 3 % samples has not been found yet. However the correct classification of most samples is promising for future studies.

Table 4: DFA classification results of sound rapeseed and different concentrations of burnt rapeseed

Actual value	Predicted values				
	0 %	1 %	3 %	5 %	10 %
0 %	**100.0 %**	0.0 %	0.0 %	0.0 %	0.0 %
1 %	0.0 %	**100.0 %**	0.0 %	0.0 %	0.0 %
3 %	0.0 %	**3.3%**	**76.7 %**	**20,0 %**	0.0 %
5 %	0.0 %	0.0 %	0.0 %	**100.0 %**	0.0 %
10 %	0.0 %	0.0 %	0.0 %	0.0 %	**100.0 %**

4 Conclusion

By applying an array of eight quartz microbalance sensors and four metal oxide semiconductor sensors sound rapeseed could be distinguished from rapeseed contaminated with low amounts of mouldy or burnt rapeseed, both representing severe quality deficiencies. Experiments revealed that the growth of mould can be detected already at the early stage. Misclassifications of samples occurred exclusively within the different classes of contaminated samples, which could be explained by the combined effect of small sample size and sample inhomogeneity. In that respect the headspace extraction of a higher amount of rapeseed per analysis would be favourable. However it should be pointed out that neither false positive nor false negative classifications of samples occurred. Experiments were performed only with one variety of rapeseed so the potential effects on the sensor signals coming from the natural variability of rapeseed were not yet considered and need to be evaluated in future studies.

The presented results encourage further research on a sampling system with online evaluation of rapeseed quality parameters. Future studies should also deal with evaluating the influence of the natural variability of rapeseed on the established model and on the impartial quantitation of mould and burnt rapeseed levels of test materials used for the preparation of test samples.

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