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Identification of different wheat seeds by electronic nose

Bo Zhou[1], *Jun Wang*[1]*, *and Jinfeng Qi*[2]

[1]Department of Biosystems Engineering, [2]Institute of Applied Entomology,
Zhejiang University, 268 Kaixuan Road, Hangzhou 310029, China

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A b s t r a c t. The potential of electronic nose to distinguish of wheat seeds was studied. The reproducibility and practicability of electronic nose data was evaluated by repeating the analysis of samples with a time difference of two months. The principle components analysis and linear discriminant analysis were applied to the generated patterns to distinguish the varieties of wheat seeds. The results showed that they could distinguish the wheat varieties properly. The stepwise discriminant analysis and a three-layer back-propagation neural network were developed for pattern prediction models. The results showed that both models could identify the wheat varieties, the back-propagation neural network presented the higher percent of correct classifications in comparison to stepwise discriminant analysis. Moreover, gas chromatography mass spectrometry analysis of the headspaces of same samples confirmed that electronic nose as a powerful tool is able to identify the wheat seeds.

K e y w o r d s: electronic nose, wheat seeds, identification, gas chromatography mass spectrometry

INTRODUCTION

The usual methods for identification of wheat seeds are seed protein electrophoresis, DNA molecular markers techniques, morphological identification and field evaluation (Li *et al*., 2006). In most cases, these methods are expensive and time-consuming, have low reproducibility, both in their commercial as well as in their technological implications. Several attempts have been made recently to classify wheat varieties using nondestructive methods such as machine vision (Douik and Abdellaoui, 2008; Li e*t al*., 2007), near infrared spectrometer (Li *et al*., 2008) and thermal imaging (Manickavasagan *et al*., 2010). Most of them used kernel morphological features of a single grain for variety identification. It would be highly desirable to have an alternative method for classification of wheat varieties, which may use some characteristics of the kernels other than morphological features. A simple variety identification system with less complexity for bulk sample testing (not single grain analysis) would be most desirable in the grain-handling facilities.

Electronic nose (E-nose) is instrument which mimics the sense of smell. These devices are typically array of sensors used to detect and distinguish odours precisely in complex samples and at low cost (Peris and Escuder-Gilabert, 2009). In contrast to the well known analytical gas chromatography mass spectrometry (GC-MS) and sensory techniques that have been used for the analysis of flavour compounds, the E-nose does not give any information about the compounds causing the investigated aroma; neither about the identity of the compounds nor their sensorial properties. Using E-nose the aroma is judged by the so-called 'aroma pattern', which should be characteristic to the investigated substrate (O'Sullivan *et al*., 2003). With the use of appropriate mathematical methods, E-nose should be capable of recognizing the aroma pattern or of distinguishing it from aroma patterns of other samples (Peris and Escuder-Gilabert, 2009; Zhang and Wang, 2008). E-nose could gain importance in the food industry.

Several successful applications of E-noses to the monitoring of flavour and/or aroma components along a food production process have been published. In a recent study (Defilippi *et al*., 2009) evaluated the aroma of Castlebrite apricots that were harvested at two different stages of ripening and stored under different refrigeration conditions using GC, sensory panel and E-nose techniques, and concluded that aroma-related volatile compounds were more influential in dictating E-nose response to different apricot samples. In a study on determination of the shelf life of milk, it was found that E-nose could clearly detect both bacteria growth in milk and shelf life (Labreche *et al*., 2005). In

*Corresponding author's e-mail: jwang@zju.edu.cn

another study an E-nose was designed to monitor bakery products, and satisfied results were obtained (Needham *et al*., 2005). Nowadays, E-nose has already been used in various field, such as: pork (O'Sullivan *et al*., 2003), mandarin (Gomez *et al*., 2007), peach (Benedetti *et al*., 2008; Di Natale *et al*., 2001), apple (Li and Heinemann, 2007), pear (Oshita *et al.*, 2000), wine (Garcia *et al*., 2006) and grain (Balasubramanian *et al*., 2007; Evans *et al*., 2000). Moreover, E-nose and GC-MS have also been used for detection of spoilage of grain and mycotoxins, ergosterol, and odour volatiles in durum wheat (Abramson *et al*., 2005; Presicce *et al*., 2006). However, most of them are on qualitative identification of stored grains, no information has been available on the applicability of an electronic nose for the identification different varieties of wheat seeds.

The objectives in this research are:

– to investigate the capacity of E-nose identifying the different varieties of wheat samples, using a specific E-nose device (PEN 2) based on sensor array and suitable pattern recognition techniques;
– to study whether the results of E-nose correspond with the GC-MS analysis.

MATERIALS AND METHODS

The experimented samples were three different varieties of winter wheat seeds (Varieties: Jiangsu18, 13 and 9023, and labeled W18, W13 and W9023, respectively), which were supplied by the Yangzhou farm, Jiangsu. The three varieties of wheat were harvested in early June 2009. The three groups of samples were stored at room temperature. The volatiles emitted by wheat change slightly during storage, but for long-term storage the wheat quality will be deteriorated and an electronic nose could successfully discriminate the different age of wheat (Pang *et al*., 2008). Thus, to investigate the capacity of E-nose identifying the different varieties of wheat samples at different storage-time. The three varieties of wheat seeds were measured with a time difference of two months (SET I and SET II). 75 samples (25 duplicates for each variety, respectively) were prepared for each set. Thus, there were 150 samples in all for detection by E-nose. For each treatment, 50 g of wheat sample was weighed out. The sample was placed in 500 ml flasks for analyses of response to volatiles using E-nose. The flask was closed tightly after introducing wheat sample and was held at the temperature (50±1°) for 30 min before static headspace sampling.

The volatile collection system has been described by Lou (Lou *et al*., 2005). Temperature was kept at 28°C during the whole process. After the 6 h collection period, compounds were eluted from the adsorbent traps with 200 μl dichloromethane. Collections were replicated six times for each wheat variety. Volatiles analyses were done with an HP 6890 series gas chromatograph equipped with a flame ionization detector and coupled to a HP 5973 mass selective detector. An HP-5 (30 m, 0.25 mm in dia, 0.25 μm film thickness; Alltech, Deerfield, IL, USA) capillary column was equipped for separation. Helium (24 ml min^{-1}) was used as the carrier gas. A splitless injection (injection injector temperature 250°C and the injection amount is 3 μl) was used, Followed injection, the column temperature was performed as followed: from 40°C (2 min hold) to 250°C at 6°C min^{-1}, and held at 250°C for 2 min. All compounds were analyzed by the HP 5973 mass spectrometer. Compounds were identified by comparison of retention times and mass spectra with those of authentic standards. The authentic standard chemicals were obtained from Fluka, Sigma, Aldrich.

A PEN2 E-nose (Win Muster Airsense, Schwerin, Germany) was used to obtain the odour signal patterns from headspace of wheat samples. This E-nose contains an array of 10 different metal oxide sensors positioned into a small chamber. Each sensor has a certain degree of affinity towards specific chemical or volatile compounds. Table 1 lists all the sensors used and their main applications. This table contains current known or specified reaction.

During the measurement process, the headspace gas of a sample was pumped into the sensor chamber at a constant rate of 100 ml min^{-1} via a Teflon-tubing connected to a needle. When the gas accumulated in the headspace of vials was pumped into the sensor chamber, the ratio of conductance of each sensor changed. The sensor response was expressed as ratio of conductance (G/G0) (G and G0, conductivity of the sensors when the sample gas or zero gas blows over). The measurement procedure was controlled by a computer program. The flush time was set to 40 s. The measurement time was 65 s, which was enough for the sensors to reach stable values. The interval for data collection was 1 s. A computer recorded the response of E-nose every second, thus 65 data were recorded for each sensor. When the measurement was completed, the acquired data was properly stored for later use.

Each sample data of E-nose was a matrix with 65 rows and 10 columns (65 measurement times and 10 sensors). The analysis was carried out using the signal stability at 58 s in wheat. The pattern-recognition techniques used were principal component analysis (PCA), linear discriminant analysis (LDA), stepwise discriminant analysis (SDA) and back-propagation neural network (BPNN).

RESULTS AND DISCUSSION

In order to obtain sufficient volatile compounds information about the samples, GC-MS measurements were carried out. Volatile compounds were identified by comparison of GC data with reference compounds. The typical chromatograms for three varieties of wheat seeds are shown in Fig. 1. The peaks in chromatogram at times shorter than 5 min were due to solvent-solvent interactions and extracted impurities, thus each chromatogram started at 5 min. A wide variety of compounds belonging to various functional groups including alcohols, aldehydes, esters, ketones, acetates, and furans

T a b l e 1. Sensors used and their main applications in PEN 2

Number in array	Sensor name	General description	Reference
S1	W1C	Aromatic compounds	Toluene, 10 mg kg^{-1}
S2	W5S	Very sensitive, broad range sensitivity, react on nitrogene oxides, very sensitive with negative signal	NO_2, 1 mg kg^{-1}
S3	W3C	Ammonia, used as sensor for aromatic compounds	Propane, 1 mg kg^{-1}
S4	W6S	Mainly hydrogen, selectively, (breath gases)	H_2, 100 μg kg^{-1}
S5	W5C	Alkanes, aromatic compounds, less polar compounds	Propane, 1 mg kg^{-1}
S6	W1S	Sensitive to methane (environment) *ca*. 10 mg kg^{-1}. Broad range, similar to No. 8	CH_3, 100 mg kg^{-1}
S7	W1W	Reacts on sulfur compounds, H_2S 0.1 mg kg^{-1}. Otherwise sensitive to many terpenes and sulfur organic compounds, which are important for smell, limonene, pyrazine	H_2S, 1 mg kg^{-1}
S8	W2S	Detects alcohol, partially aromatic compounds, broad range	CO, 100 mg kg^{-1}
S9	W2W	Aromatics compounds, sulfur organic compounds	H_2S, 1 mg kg^{-1}
S10	W3S	Reacts on high concentrations >100 mg kg^{-1}, sometime very selective (methane)	CH_3, 10 CH_3, 100 mg kg^{-1}

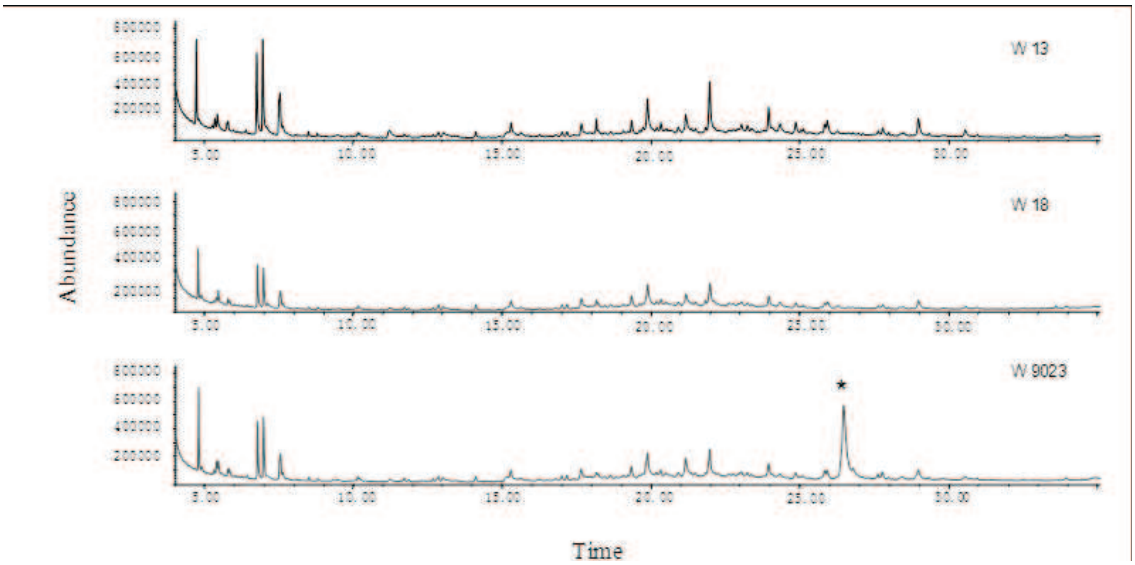

Fig. 1. Typical chromatograms for three varieties of wheat seeds (W13, W18 and W9023).

were identified from the headspace of the wheat samples. The results are consistent with those of other authors (Presicce *et al*., 2006). Based on visual observations of chromatograms, the volatile compounds of three wheat varieties were quite similar, but the content of each compound was different according to peak profiles and the W9023 had a unique peak which labeled with '*' (compound: 2, 6, 10-dodecatrien-1-ol, 3, 7, 11-trimethyl). These indicated that each wheat variety had its unique character.

A typical response by 10 sensors during measuring a wheat sample is shown in Fig. 2. Each curve represents a different sensor transient. The curves represent conductivity of each sensor against time due to the electro-valve action when volatiles reached the measurement chamber. In

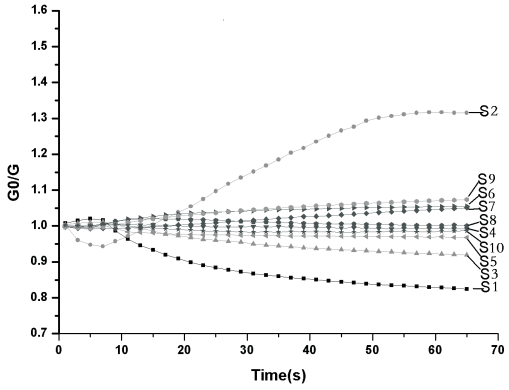

Fig. 2. Ten sensors typical responses to wheat seeds aroma (W9023).

initial period, the ratio of conductance (G/G0) of each sensor was close to 1.0, then increased or decreased continuously, and finally stabilized after about 50 s. In this research, the signal of each sensor at response 58 s was used in analysis.

In order to investigate whether E-nose was able to distinguish among different varieties, PCA and LDA analysis were applied. The analysis was carried out using the signal stability at 58 s in wheat seeds. PCA and LDA analysis results of SET I are shown in Fig. 3. PCA in Fig. 3a shows the score plot inside ellipses and represents the standard variation around different varieties for wheat. The processed data show an erratic shift of different varieties along the first principal component, PC1, which explains 83.38% of the total variance with value 95.58%. The second principal component (PC2) explains 12.1% of the variation and shows no particular trend with varieties. The three varieties of wheat samples were distinguishable from each group, except W13 and W18, had a little overlapped. When using LDA analysis (Fig. 3b), the three varieties of wheat were clearly distinguished from each group. In this plot about 90.26% of total variance of data is displayed. LDA function 1 (LD1) and function 2 (LD2) accounted for 77.07 and 13.19% of variance respectively. PCA and LDA analysis results of SET II are shown in Fig. 3cd. The results were similar to the SET I. PC1 explained 98.65% of total variance, PC2 explained 0.99% of variation in Fig. 3c. There were similar successful classifications by LDA (Fig. 3d). LD1 and LD2 accounted for 89.86 and 7.32% of variance respectively. The three wheat samples were, again, distinguished completely. These results demonstrate that different wheat varieties had different volatiles. Wheat samples could be distinguished completely by LDA. Compared to PCA plot, the response values in every cluster in LDA plot are more concentrated and the intervals of clusters are larger, which implies that LDA method is better than PCA for distinguishing different variety of wheat samples.

In order to investigate whether E-nose was able to predict the wheat varieties, SDA and BPNN analysis were applied. First, the samples of SET I and SET II were used for the direct prediction severally. For each set, 60 wheat samples (20 samples of each variety) were selected randomly for

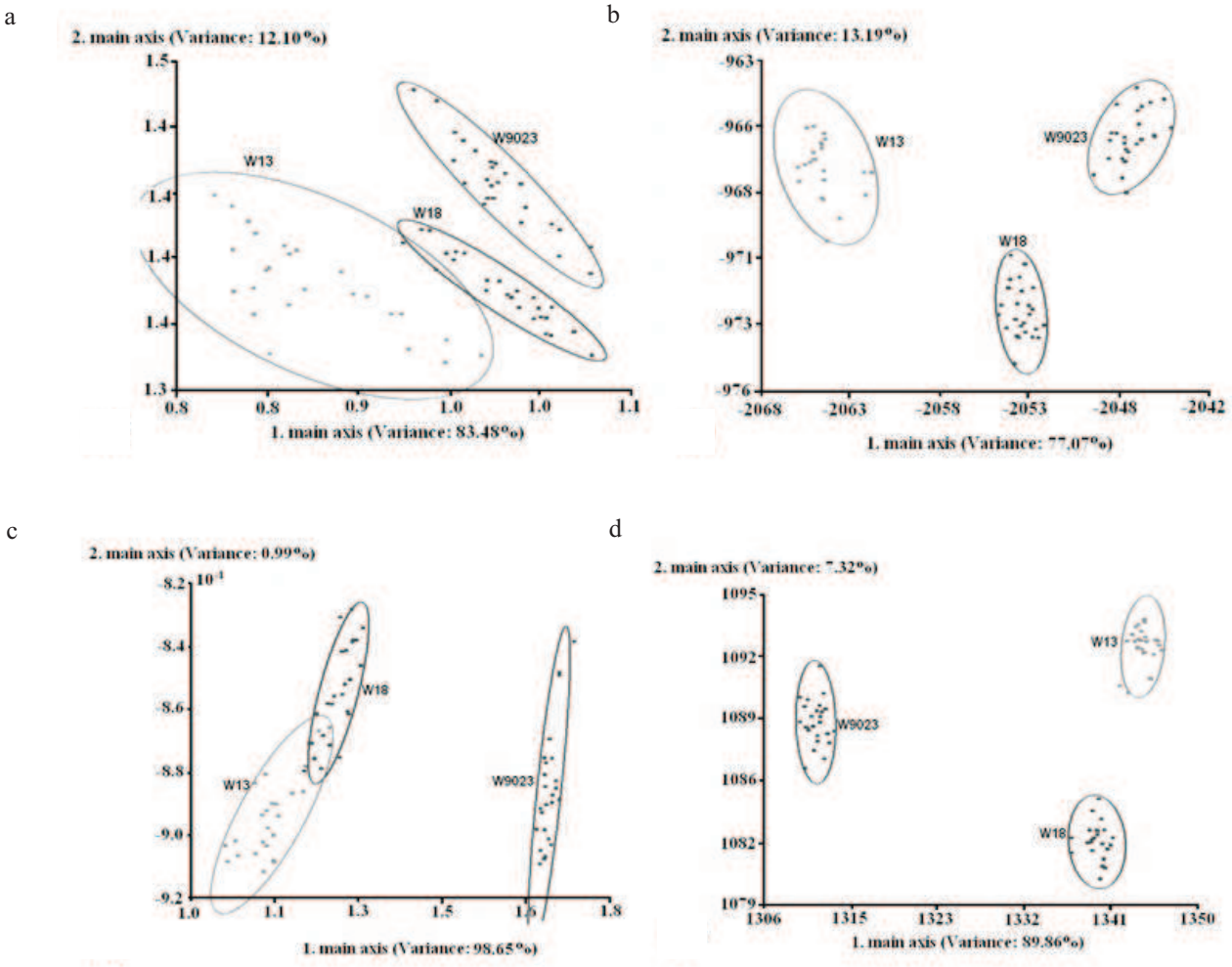

Fig. 3. Scores plot of three different varieties wheat seeds for the: SET I: a – PCA analysis, b – LDA analysis; SET II: c – PCA analysis, d – LDA analysis.

the training set, the rest of 15 wheat samples (5 samples of each variety) composed the testing set. Then, in order to verify reproducibility and practicability, 60 wheat samples of SET I were selected randomly for the training set, 15 wheat samples of SET II composed the testing set for cross prediction. SDA was applied in order to select the most discriminant wheat samples. The correct identification rate of training set was 100%. Table 2 showed the results for testing set of SET I and SET II. The correction ratio of results for testing samples of SET I was 100%. The correction ratio of the results for the testing samples of the SET II was 96.67%; one sample of W13 was classified into W18. The results for testing set of cross prediction are shown in Table 2. The correction ratio of results for testing samples was 83.33%; four samples of W13 were classified into W18 and one sample of W18 was classified into W13. A three-layer back-propagation neural network was used. The architecture of artificial neural network was chosen: 10×18×1 three-layer back-propagation according to Kolmogorov theorem, hereinto, ten is the num of input neurons, the num of wheat variety indices as target output, respectively. The training algorithm was variable learning rate back-propagation (Traingdx) algorithm available in MATLAB Neural Network Toolbox. After several attempts, training parameters were chosen with maximum epoch of 1 000 and goal of 0.01, respectively. The threshold of prediction error was set as 0.2 that means if the actual value differs by more than 0.2 from its prediction value, the prediction result is considered to be failed. The correct identification rate of training set was 100%. The results of BPNN for testing set of SET I and SET II were shown in Table 3. The correction ratio of results for testing samples of SET I was 100%. The correction ratio of results for testing samples of SET II was 96.67%; one sample of W13 was misclassified. The results for testing set of cross prediction are shown in Table 3. The correction ratio of results for testing samples was 90%; two samples of W13 were classified into W18 and one sample of W18 was misclassified. These results indicate that E-nose may be successfully applied as rapid method for classifying different variety of wheat samples. In addition, classification techniques such as neural networks (that are trained with known samples in order to classify unknowns) may provide better results. From GC-MS results we can conclude that volatile compounds of W13 had much more similar to

T a b l e 2. Discrimination of testing set by SDA

Simulated results		W13	W18	W9023	Correct rate (%)
SET I	W13	10			100
	W18		10		100
	W9023			10	100
SET II	W13	9	1a		90
	W18		10		100
	W9023			10	100
SET I and II	W13	6	4[a]		60
	W18	1[a]	9		90
	W9023			10	100

[a]Samples of the incorrectly classified.

T a b l e 3. Discrimination of testing set by BPNN

Samples		Desired outputs	Predict resulting outputs		Correct rate (%)
			Correct	Error	
SET I	W13	1	10	0	100
	W18	2	10	0	100
	W9023	3	10	0	100
SET II	W13	1	9	1	90
	W18	2	10	0	100
	W9023	3	10	0	100
SET I and II	W13	1	8	2	80
	W18	2	9	1	90
	W9023	3	10	0	100

W18 than to W9023. From Fig. 3ac, PCA results showed that W13 and W18 had a little overlapped. SDA and BPNN results indicated that misclassified samples predominantly involved confusion between W13 and W18. The agreement between these results obtained by GC-MS with those obtained by E-nose implies that E-nose combined with GC-MS analysis of the headspace of samples as a powerful tool is able to identify the wheat seeds.

CONCLUSIONS

1. The analysis results showed that the principal component analysis and linear discriminant analysis could properly distinguish the three varieties of wheat seeds.

2. The stepwise discriminant analysis (SDA) and a three-layer back-propagation neural network (BPNN) were developed for pattern recognition models.

3. The results showed that both models for training data sets the discrimination rate of three wheat seed varieties were 100%, for different testing data sets the SDA discrimination rate was over 83.33% and the BPNN was over 90%, the BPNN presented the higher percent of correct classifications in comparison to SDA.

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