INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
11–058/SAE/2011/13–5–707–712
http://www.fspublishers.org

Full Length Article

Detection of Insect Infestations in Paddy Field using an Electronic Nose

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ABSTRACT

An electronic nose was used to predict the number of infesting insects and the storage time of paddy rice. The multivariate statistical techniques such as principal component analysis (PCA), linear discriminant analysis (LDA), principle component regression (PCR), partial least square (PLS) and back-propagation neural networks (BPNN) were used to evaluate the electronic nose data, respectively. The PCA and LDA results showed that the electronic nose can distinguish paddy rice with different storage time (ST) and different number of infesting insects (NI). After employing PCR, PLS and BPNN, respectively to predict the infestation index (NI & ST), the three methods all had good prediction performances. The correlation coefficient between the NI real and the three predicted values was 0.955, 0.864, and 0.996 for the PCR, the PLS and the BPNN, respectively. The correlation coefficient between the ST real and the three predicted values was 0.992, 0.852 and 0.998. BPNN model had the highest prediction accuracy. The results implied that it is possible to predict the characteristics of insect infestation in stored paddy rice from signal of electronic nose.

Key Words: Electronic nose; Insect infestation; Rice; Storage time; Prediction

INTRODUCTION

Rice is the world's most important food and a primary food source for more than a third of the world's population. However, stored rice can have losses in both quantity and quality. Losses occur when the rice is attacked by stored product insects and microorganisms (Rahman *et al.*, 2009; Gandhi *et al.*, 2010). Thus, it is a top priority to find effective methods to reduce the level of insect infestation in rice during storage. One of the most important aspects of the integrated pest management is the early detection. Traditional detection methods are manual samples, traps, and probes (Neethirajan *et al.*, 2007). These methods for insects' identification request repetitive work and trained personnel, while sometimes observations through naked eyes are subjective and imprecise.

In recent years there have been many techniques studied for insect detection in stored grain, such as acoustic detection, carbon dioxide measurement, uric acid measurement, near-infrared spectroscopy and soft X-ray method etc. These methods have the potential for use at the industry level to detect insects in rice samples (Neethirajan *et al.*, 2007). However, they are not always applicable both for the cost and labor time to analyze one sample. Recently, a new technique for rapid detection of insects in stored rice has been reported, an Electronic nose (E-nose) systems could accurately and rapidly identify the quality of stored rice by analyzing the headspace volatiles in the rice (Balasubramanian *et al.*, 2007; Zhang & Wang 2007; Pang *et al.*, 2008).

An E-nose is an instrument that combines electronic chemical gas sensors with partial specificity and appropriate pattern analysis techniques for the detection, identification or quantification of volatile compounds. Commercial E-noses have been available since the early 1990s, which as compared to the traditional odor analysis methods (sensory panel or headspace GC), are quick, safe, less expensive, non-destructive to samples and can be automated (Peris & Escuder-Gilabert 2009). Because of their convenience and fast inspection characteristic, E-noses have been reported widely, especially in the field of food control, such as beef (Balasubramanian *et al.*, 2004), milk (Labreche *et al.*, 2005), oil (Hai & Wang, 2006) and fruit (Marrazzo *et al.*, 2005).

There are some literatures about the application of E-nose in the detection of fungal contamination of cereal grain samples (Needham *et al.*, 2005; Paolesse *et al.*, 2006). Some recent studies have demonstrated E-nose capability in order to discriminate between non-infected and infected samples with different species through the production of volatile secondary metabolites and to demonstrate the variation of the metabolic pathway due to the contamination of grain (Evans *et al.*, 2000; Presicce *et al.*, 2006). An E-nose was used to detect five different stored duration wheat successfully (Zhang & Wang, 2008). However, there have been few studies on the prediction of insect infestation in stored rice by E-noses.

Appropriate data analysis and pattern recognition techniques are needed to construct reliable algorithms for interpreting the acquired signals or smell patterns for classification or prediction purposes. The smell patterns obtained from the E-nose detectors can be analyzed using various statistical and neural network tools. Pattern recognition techniques like principal component analysis (PCA), linear discriminant analysis (LDA), partial least squares (PLS), discriminant analysis (DA), cluster analysis (CA), artificial neural network (ANN) *et al*., have been used for data analysis in E-nose applications (Pavon *et al.*, 2006; Zhang *et al.*, 2007; Peris & Escuder-Gilabert, 2009). Balasubramanian *et al.* (2007) used linear and quadratic discriminant analysis techniques to evaluate the quality of stored barley based on the resistance generated by the E-nose system. Evans *et al.* (2000) used a radial basis function artificial neural network to correlate sensor array responses with human grading of off-taints in wheat. It also becomes critical to build a reliable and robust classification model that could perform satisfactorily in real world conditions.

The objectives of this work were: (1) to study the feasibility of the E-nose technique to evaluate the insect infestation in stored rice at different storage time; (2) to develop models for predicting the number of adult insects (NI) and storage time (ST) using E-nose signals.

MATERIALS AND METHODS

Experimental materials: Paddy rice samples were supplied by the experimental farm of Zhejiang University (variety: Zhou 903, grown in Hangzhou, China at 120:07E & 30:10N). The paddy rice samples were harvested in August 2009 and dried in ambient conditions to l2.0±1.0% moisture content. Test insects were Rice weevils, *Sitophilus oryzae* (L.). The female adult *S. oryzaes* 5 d-old were taken from a culture that was kept at the laboratory for two generations at 27±1°C and 70±5% relative humidity (r.h.) on the rusty grain.

The paddy rice samples were divided into 4 groups (5 kg each group). Each group was placed in a 10 L cylindrical glass container, which was closed, except for a hole 3 cm in diameter (at the top of the container), which was covered with muslin for aeration. Then, different numbers of 5 d-old female adults of *S. oryzae* were introduced into each container. Based on the initial numbers of adult insects introduced, three groups of rice were P5 (introduced into 5 insects male & females), P10 (introduced into 10 pests) and P40 (introduced into 40 pests), respectively. The corresponding uninfested normal rice served as control group, which labeled P0. The four groups were placed in an artificial climate chamber at 30±1 with 70±5% (r.h.) for up to 4 weeks. At each week, rice samples were taken for examination and measurement. For each group, 20 samples (50 g per sample) were prepared, and the adult insects were picked out before the measurements.

E-nose and data acquisition: An E-nose device PEN2, provided by (WMA Airsense Analysentechnik GmbH) Schwerin, Germany, was used. PEN2 system consisted of a sampling apparatus, a detector unit containing the array of sensors and pattern recognition software (WinMuster v.1.6) for data recording. The sensor array was composed 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 I lists all the sensors used and their main applications. This table contains current known or specified reaction.

Each rice sample was placed into an airtight glass jar with a volume of 500 mL. The glass jar was then closed and kept at room temperature (25±1) for 30 min before static headspace sampling. During the measurement process, the headspace gas of each sample was pumped into the sensor chamber at a constant rate of 200 mL/min 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 the ratio of conductance (G/G_0) (G & G_0, the 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 the E-nose every second. When the measurement was completed, the acquired data was properly stored for later use.

Pattern recognition: The NI and ST are the two most important indices to describe the rice damage level after infestation. In this paper, PCA and LDA were used to classify four groups of rice samples with different initial numbers of adult insects at different storage time. Multivariate calibration methods PCR, PLS and BPNN were employed to build the modes for predicting NI and ST values using E-nose signals. In order to evaluate the goodness of fit of these three models, multiple correlation coefficients (R^2), standard error calibration (SEC) and standard error of prediction (SEP) were used. A low SEC and high R^2 are evidences of a good regression model.

PCA is a statistical technique for determining the key variables in a multidimensional data set that explain the differences in the observations and can be used to simplify the analysis and visualization of multidimensional data sets (Peris & Escuder-Gilabert, 2009). Scores, called principal components (PCs), are extracted, which explain decreasing amounts of variation within a group, with the proviso that it is orthogonal to the first. In general, the vast majority of the variation is contained in a few PCs without significantly reducing the value of the information stored within it.

LDA is one of the most used classification procedure. The method tries to maximize the variance between categories and minimize the variance within categories. This means that in theory, it should be superior to PCA in classifying subjects into groups.

PCR and PLS Regression are two of the most widely employed multivariate calibration methods, which compress all the statistically significant information down into low dimensional spaces characterized by a small number of orthogonal latent vectors. Generally, the analytical applications are based on the assessment of a linear model; the model has the following general form:

$$Yi = \beta_0 + \beta_i \times X_i \qquad (i=1,2,3,\ldots\ldots, N).$$

Where *Yi* is the dependent variable; β_0 is the intercept and β_i are the regression coefficients of the independent variable *Xi*; N is the number of the independent variable. These methods form linear combinations of the predictive variables *X* and use them as regressors for the dependent variable *Y*. The rationale behind this is to determine a maximum compromise between having a small least-squares error on the calibration set and having a stable model, which can be used safely for routine analysis.

The PCR is simply a PCA analysis followed by a regression step. PCA is firstly applied to the matrix of independent variables *X*. Principal components obtained from PCA are then used as regressors instead of the original variables. When the variables are noticeably collinear, only a small number of components need to be introduced in the model. In PCR, the scores, which are used as regressors, are assessed by using no information from *Y*. Thus, if another dependent variable is to be predicted from *X*, the PC scores remain equal.

PLS is related to PCR in that the spectral decomposition is also performed, but this decomposition step is performed differently. In PLS, the linear combinations used in the prediction equation are obtained by taking both *X* and *Y* into account. The stages of determination of the regressors and of the calibration regression can be considered as linked together. The PLS components primarily describe the variations of the independent variables, which are relevant for modelling the variations of *Y*.

BPNN is a type of neural networks that most widely used to solve problems in modeling and classification (Zhang & Wang, 2008). The model has the ability to simulate a nonlinear system. The typical back-propagation network consists of an input layer, an output layer and at least one hidden layer. Each layer contains neurons and each neuron is a simple micro-processing unit, which receives and combines signals from other neurons. Each neuron has weighted inputs, summation function, transfer function and output. The behavior of a back-propagation network is mainly determined by the transfer functions of its neurons.

RESULTS AND DISCUSSION

E-nose response to rice volatile: In order to compare the response signals of e-nose between infested rice and normal rice, P 5 and P 0 were used. Fig. 1 showed the typical response signals of ten sensors for normal rice and infested rice by 5 adult insects at the 4th week. Each curve represented the variation in conductivity of each sensor against time when the rice volatiles reached the measurement chamber. The sensor response signal represented by the ordinate was the gas response G/G_0, where G and G_0 express the resistance of a sensor in clean air and in a detecting gas, respectively. In the initial period, the ratio of conductance (G/G_0) 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 58th s was used in analysis.

The value of sensor response signals of the normal rice and the infested rice differed with the 4th week (Fig. 1). Fig. 1a showed that the value G/G_0 (0.8-1.5) of normal rice was lower than G/G_0 (0.6-2.6) (in Fig. 1b) of the rice infested by 5 adult insects, indicating that the sensor responses of the E-nose differed between normal rice and infested rice. Previous study has shown that the volatile compound profiles differed between contaminated wheat samples and no fungi inoculated samples using gas chromatography-mass spectrometer (GC-MS). Comparison with un-contaminated wheats, the contaminated samples had higher concentration of aromatic compounds (Presicce *et al.*, 2006). E-nose could successfully classify wheat at different stroage age (Pang *et al.*, 2008). Those implied that the E-nose technique might be used to distinguish with rice by different level of insect infestations.

PCA and LDA Analysis

NI analysis: To investigate whether the E-nose was able to distinguish the four groups of rice at different storage times (week 1, week 2, week 3 & week 4), PCA and LDA were employed. The results are shown in Fig. 2. Principal component 1 (PC1) vs principal component 2 (PC2) were shown in (Figs. 2 a1-a4), together explaining more than 85% of the variance. First linear discriminant (LD1) vs second linear discriminant (LD2) were shown in (Figs. 2 b1-b4), together explaining more than 91% of the variance. All these implied that the system has enough resolution to explain the classification.

The PCA discrimination result of the four groups (P 0, P 5, P 10 & P 40) was shown in the (Fig. 2a1) at the week 1. Each group was distinguishable from the others, except P 5 or P 10 overlapped each other lightly. And the distinction between P 0 group and P 40 group was greater. While in week 2 and week 3, groups P 0 were clearly distinguishable from the other three groups; groups P 5, groups P 10 and groups P 40 overlapped each other. The distinction between P 0 and other three groups in week 2, week 3 and week 4 were better than in week 1. These results demonstrated that the volatiles of normal rice samples become more and more significantly different from those infested by different numbers of insects with storage time, which indicated that the quality of rice declines faster under insect infestation.

Figs. 2b1-b4 are the LDA results of the same data analyzed by PCA. Similar to the PCA analysis of Figs. 2a1,

Figs. 2b1 also showed a partial overlapping between P 5 group and P 10 group. In Figs. 2b2 and Figs. 2b3, all the four groups were clearly distinguished from each other, but in Figs. 2b4, the P 5 group and P 10 group were overlapped again. The results could be explained by the deterioration process of the infested rice. The deterioration of rice quality and the variation of volatiles were limited at the first week, thus the difference in volatiles between P 5 group and P 10 group were not obvious, which caused the overlapped phenomena on PCA and LDA results. The rice quality deteriorated faster after 2 weeks.

The performance results for LDA were much better than PCA. This is because LDA tries to summarize the separation of samples among groups into a reduced space, while PCA is a projection method of the original variables onto new ones, orthogonal and arranged according to their eigenvalue.

ST analysis: In order to study the deterioration process of the insects infested rice samples during storage, PCA and LDA were applied and the results are shown in Fig. 3.

After employing PCA, Figs. 3a1 showed that there is no clear discrimination among the four storage times (week 1, week 2, week 3 & week 4) of the normal rice samples (P 0 group), this caused by the high level of similarity among the rice samples in the pattern classification space. However, in (Fig. 3a2-a4), the four storage times of the samples from group P5, P 10, P40 could be clearly discriminated, respectively and in each figure, there was greater distinction between week1's data and other weeks' data. In general, expect group P 0, different storage times of the other three groups (P5, P10, P40) could be all distinguishable by PCA.

The same datasets were analyzed by LDA. The results were shown in (Fig. 3b1-b4). Compared to PCA, the four groups (P0, P5, P10, P40) all had perfect classification of their different storage times, even the P0 group was clearly divided into four regions representing its four storage times. In general, LDA had better results than PCA.

Prediction of insect infestations: Early detection and accurate prediction are required in insect prevention process, so the week 1 data of the four groups were chosen for predicting the NI; the data of P5 group were chosen for the prediction of ST (from first week to the fourth week).

Prediction of NI: The four rice groups (P0, P5, P10 & P40) at week 1 were chosen to construct the prediction models for predicting the NI. 60 rice samples (15 samples of each group) were selected randomly to establish calibration model; the rest of 20 rice samples (5 samples of each group) were chosen as the prediction sample.

The PCR calibration model for NI prediction was given as follows:

$$NI = -269.073 + 14.808\times S1 + 16.127\times S2 + 12.342\times S3 - 14.153\times S4 + 281.607\times S5 - 10.613\times S6 + 11.361\times S7 - 2.599\times S8 - 32.626\times S9 - 27.919\times S10 \quad (1)$$

Fig. 1: Response curves of ten sensors for rice volatiles at 4th week: (a) normal rice; (b) rice infested by 5 adult insects

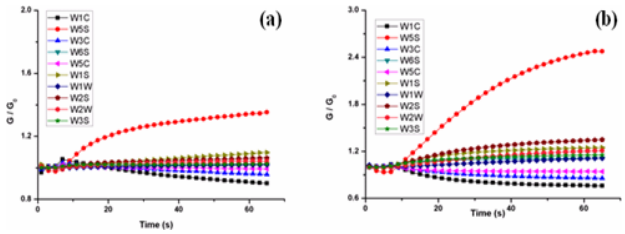


Fig. 2: Scores plot of four groups of rice at different storage times (week 1, week 2, week 3 & week 4): (a1-a4) PCA and (b1-b4) LDA

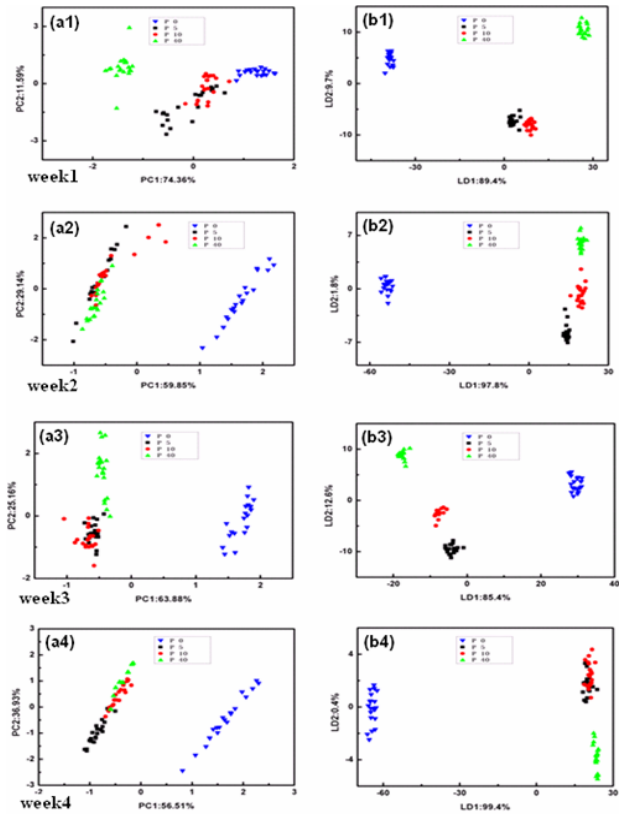
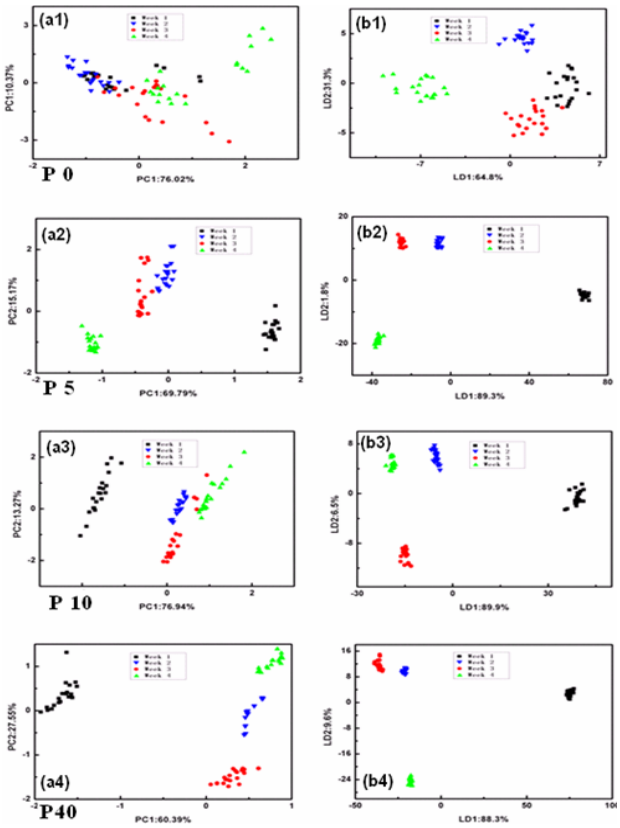


Table II shows the R^2, SEC and SEP for infestation indices predicted for rice. The prediction ability of the E-nose was shown in (Fig. 4a), where each rhombus represents the predicted values versus the real values. The R^2 of calibration model of NI was high as 0.955, with the SEC of 0.566. When the model was used to predict the rest 20 samples, prediction results were also high (R^2 = 0.923, SEP = 1.277).

The PLS calibration model for NI prediction was given as follows:

$$NI = 8.549 + 9.461\times S1 + 3.065\times S2 + 9.833\times S3 - 20.016\times S4 + 101.809\times S5 - 14.496\times S6 - 8.158\times S7 - 10.837\times S8 - 36.003\times S9 - 42.585\times S10 \quad (2)$$

Fig. 3: Scores plot of different storage times for four groups (P 0, P 5, P 10 & P40): (a1-a4) PCA and (b1-b4) LDA



The PLS prediction results for NI were presented in (Fig. 4b). The R^2 was 0.864, with the SEC of 1.308. When the model was used for prediction, the results (R^2 = 0.855, SEP =1.687) were not as good as the PCR model.

While applying the BPNN, the response values of the E-nose at 58th s were optimum to be used as the input vector of ANN. The architecture of the artificial neural network chosen was 10×18×1 three-layer back-propagation according to Kolmogorov's theorem, hereinto, ten was the num of input neurons, the value of NI as target output, respectively. The training algorithm was the variable learning rate back-propagation (traingdx) algorithm available in MATLAB's Neural Network Toolbox. After several attempts, training parameters were chosen with maximum epoch of 1000 and goal of 0.01, respectively. The R^2 was 0.996, with the SEC of 0.159. When the model was used to predict the rest 20 samples, the prediction results were great (R^2 = 0.995, SEP = 0.319).

Prediction of ST: Four weeks' datasets of P 5 group were chosen for prediction of ST. 60 rice samples (15 samples of each week) were selected randomly to establish calibration models; the rest of 20 rice samples (5 samples of each week) were predicted using the models.

The PCR calibration model for ST prediction was given as follows:

Fig. 4: Comparison of the NI real values with the predicted ones from the PCR, PLS and BPNN models: (a) PCR; (b) PLS; (c) BPNN

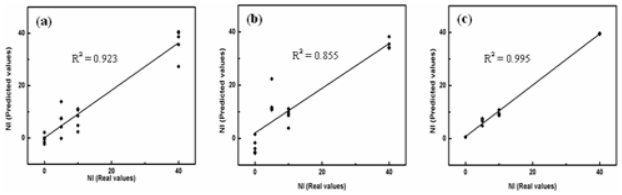
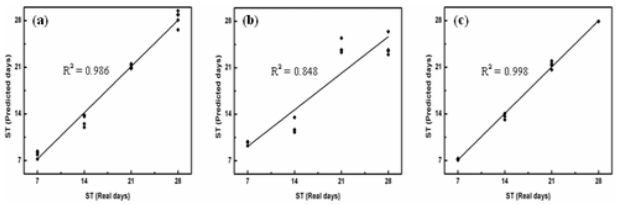


Fig. 5: Comparison of the ST real values with the predicted ones from the PCR, PLS and BPNN models: (a) PCR; (b) PLS; (c) BPNN



ST=130.189 - 9.643×S1 + 5.394×S2 - 8.547×S3 - 19.579×S4 - 49.733×S5 - 7.591×S6 - 13.108×S7 - 23.268×S8 + 11.662×S9 - 5.844×S10 (3).

The prediction ability of the E-nose was shown in (Figs. 5a), where each rhombus represented the predicted values versus the real values. The R^2 of calibration model of ST was high as 0.992, the SEC of 0.308. When the model was used to predict the 20 samples, the prediction results were also high (R^2 = 0.986 SEP =0.534).

The PLS calibration model for ST prediction was given as follows:

ST=83.718 - 5.238×S1 - 1.825×S2 - 6.321×S3 - 4.099×S4 - 27.509×S5 + 7.089×S6 - 3.986×S7 + 5.982×S8 + 3.260×S9 - 29.484×S10 (4).

The correlation coefficient of calibration model of ST was 0.852, the SEC of 0.815. When the model was used to predict the 20 samples, the results (R^2 = 0.848, SEP=1.506) were not as good as the PCR model.

BPNN was also applied to predict the ST. The network topology was designed 10×18×1. The correlation coefficient of calibration model of ST was 0.998, with the SEC of 0.062. When the model was used to predict the rest 20 samples, the result was also high (R^2 = 0.998, SEP = 0.209).

Comparison of PCR, PLS and BPNN models: The predictive ability of PCR, PLS and BPNN model are shown in (Figs. 4-5). The corresponding SEP and R^2 values obtained for all models were listed in Table II. Comparing R^2 values of PCR, PLS and BPNN, in all cases better results were obtained by the BPNN method (0.995 for NI, 0.998 for ST). Comparing corresponding SEP values of PCR, PLS and BPNN, better results were also obtained by the BPNN method (0.319 for NI, 0.209 for ST). The models built by

Table I: Sensors used and their main applications in PEN 2

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

Table II: Results of calibration and prediction for rice infestation indices based on the E-nose signals

Insect infestation indices	Model	Calibration		Prediction	
		R^2	SEC	R^2	SEP
NI	PCR	0.955	0.566	0.923	1.277
	PLS	0.864	1.038	0.855	1.687
	BPNN	0.996	0.159	0.995	0.319
ST	PCR	0.992	0.308	0.986	0.534
	PLS	0.852	0.815	0.848	1.506
	BPNN	0.998	0.062	0.998	0.209

the BPNN appeared to be of high ability of prediction.

CONCLUSION

Both PCA and LDA had good distinction for paddy samples with different storage time and different numbers of infesting insects, but the LDA analysis had better result than PCA. PCR, PLS and BPNN methods all had the ability of predicting the NI and ST of the rice samples. The BPNN model had the best prediction results. The results indicated that it is possible to use e-nose technique for predicting the characteristics of insect infested rice.

Acknowledgement: We acknowledge the financial support of the Chinese National Foundation of Nature and Science through Project 30771246, the National High Technology Research and Development Program of China through Project 2006AA10Z212.

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(Received January 2011; Accepted 23 May 2011)