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Discrimination and Prediction of Pork Freshness by E-nose

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Abstract. An electronic nose (e-nose) was used to establish a freshness evaluation model for pork. A pre-experiment was performed to acquire optimum parameters (10 g sample mass with 5 min headspace-generation time in 500 mL vial) for later e-nose detection of pork. Responding signals of the e-nose were extracted and analyzed. Linear Discriminant Analysis (LDA) results showed that the e-nose could classify pork with different storage time (ST) well. Back Propagation Neural Network (BPNN) was performed to predict the ST, and the results showed that 97.14% of the predicting set (with 95.71% of the training set) was classified correctly; and Multiple Linear Regression (MLR) was used to predict the sensory scores, with the results showing that the correlation coefficients (R^2 = 0.9848) between the e-nose signals and the sensory scores was high. These results prove that e-nose has the potential of assessing pork freshness.

Keywords: Electronic nose, Pork freshness, Prediction, Back Propagation Neural Network, Multiple Linear Regression

1 Introduction

Due to its high nutritional value and tasty taste, the consumption of pork has been increasing dramatically during the last decades. However, pork is highly susceptible

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to spoilage and contamination by micro-organisms. The main ingredients of pork are water, protein, fat and a small amount of carbohydrates. During the storage, the ingredients will be decomposed by enzymes and bacteria, producing odor: the protein will be decomposed into ammonia, hydrogen sulfide, ethyl mercaptan, etc.; the fat will be decomposed into aldehydes and aldehyde acids odor; the carbohydrates will be decomposed into alcohols, ketones, aldehydes, and carboxylic acid gases [1]. The odor gets more and more intense with the decrease of pork freshness. Consumption of spoilage pork could cause serious health hazards [2]. Thus, it is necessary that a rapid and accurate detection system be developed for microbiologically spoiled or contaminated pork [3-4].

At present, there are mainly three traditional methods in the meat industry to detect pork freshness: sensory evaluation based on the texture, color, organization status, viscidity and odor of the pork [5]; detection of the total volatile basic nitrogen (TVBN) [6]; and aerobic plate counts of the pork samples using standard protocols (FDA-US Food and Drugs Administration, 1998) [7]. The first method provides immediate quality information but suffers from some disadvantages, for example, the subjective nature of the assessment. Furthermore, errors may arise from fatigue of panelists and low threshold concentrations of stale odor compounds may not be perceived [8]; The latter two methods are objective, but destructive, complicated and time consuming. Moreover, they are just used to analyze one or two specialized components instead of giving the whole information of pork quality. Consequently, these traditional methods are unsuitable for fast and on-line application in pork industry.

Electronic nose (e-nose), also known as artificial olfactory, is a simulation of biological functions to identify some simple or complex odor [9-10]. A typical e-nose system contains a selective chemical sensor array, a signal processing subsystem and a pattern recognition subsystem. The sensors in the sensor array are sensitive to different substances. For example, some sensors can discern ammonia and some can discern aldehydes. Thus the whole sensor array can discern complex odor. Instead of detecting one or two components of the substances, the e-nose extracts the whole information for identification. In the last decade, a few researchers have been studying the potential of using e-nose as a non-destructive method for food detection. García et al. [11] used a metal oxide semiconductor thin-film sensors based electronic nose to characterize and classify four types of red wines of the same variety of grapes which came from the same cellar. Two pattern recognition methods: Principal

Component Analysis (PCA) and Probabilistic Neuronal Network (PNN) were performed, and the results showed that electronic nose was able to identify the wine well; Torri, Sinelli, and Limbo [12] used a commercial electronic nose to monitor the freshness of minimally processed fruit (packaged pineapple slices) during storage. The samples were stored at three different temperatures (4-5, 7-8, and 15-16 ^{o}C) for 6-10 days. After a continuous monitoring of the headspace around the fruit, the result showed that the fruit freshness was maintained for about 5 days at 4 ^{o}C, 2 days at 7.6 ^{o}C and 1 day at 16 ^{o}C.

In the previous research, most sensor arrays used in e-nose are Metal-Oxide Semiconductors (MOS). However, seldom information about the quality assurance of the e-nose performance has been given regarding sensor drift and humidity (MOS sensors used in e-nose are water sensitive), so it is impossible to rule out the fact that their research results may be significantly affected by either a day to day sensor drift of the sensor system, or the fact that the temporal changes observed in the sensor reading is due to the fact that the sensors perceive increasingly proportions of humidity due to increase in water vapor during storage. Moreover, few papers have mentioned the study of optimum experimental parameters, and the recognition models used are just focused on discrimination rather than prediction. In most cases, only e-nose was used, with no other experiments carried out. So even if we could predict the storage time of the food, we still can't precisely identify its freshness degree, since we don't have other indexes for cross-reference.

In this research, two experiments were conducted: e-nose detection and sensory evaluation. The main objective of this research is to evaluate the capacity of an electronic nose to classify pork samples stored for different time, as well as to predict their storage time and sensory scores. As for the e-nose detection, the sensor drift and humidity problem were taken care of by controlling the environment parameters (temperature and humidity) and by choosing calibrated data as the initial data. A pre-experiment was conducted to study the effects of headspace-generation time and pork sample mass on the response of e-nose performance. The optimum experimental parameters of headspace-generation time and sample mass were determined after employing Multivariate Analysis of Variance (MANOVA) and One-Way Analysis of Variance (ANOVA), and the later e-nose experiment was taken under the optimum experimental parameters. Linear Discriminant Analysis (LDA) and Back Propagation Neural Network (BPNN) were employed to observe if the e-nose could classify the pork samples stored for 0-6 d well, as well as to predict the storage time; and Multiple

Linear Regression (MLR) was employed to build the prediction model between e-nose data and sensory scores.

2 Materials and Method

2.1 Sample Preparation

Fresh lean pork samples were purchased twice: the first purchase was for the pre-experiment, and the second purchase was for the discrimination of pork samples stored for 0-6 d. For both of the time, all the samples were obtained from the same parts of pigs and the same supplier in the local farmers' market (30.26 N, 120.19 E, Zhejiang province, China) 4 h after been killed, and were minced immediately on the spot. All the samples were packaged immediately using polystyrene base trays and were covered with commercial food grade polymer wraps before being transported to the lab. The samples were stored at 10 ^{o}C in the fridge before detection, except the ones detected on the first day (marked as day 0).

2.2 Electronic Nose System

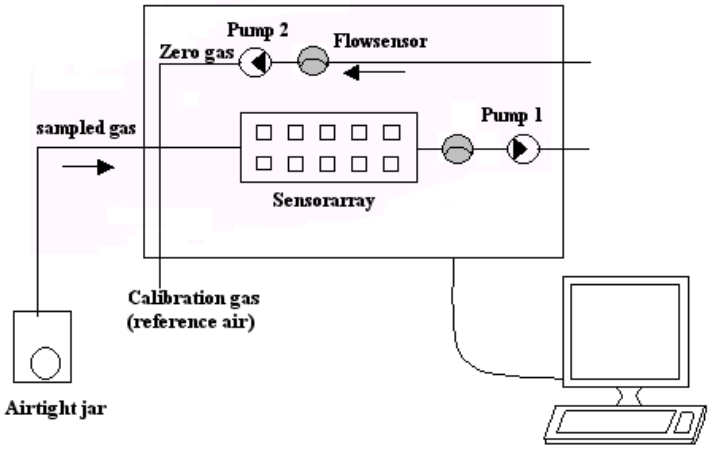

Fig. 1. Schematic diagram of the electronic nose (e-nose) measurements

The experiment was performed with a portable electronic nose (PEN2, Airsense Analytics, GmBH, Schwerin, Germany) (Fig. 1), which is consisted of an auto-sampling apparatus that is exposed to the volatiles, a sensor array, and pattern recognition software that is ran on a computer. The sensor array is composed of ten MOS. A description of the ten MOS is given in Table 1.

Table 1. Sensors used and their object substances in PEN2

Array number	Sensor	Substances for Sensing
MOS 1	W1C	Aromatics
MOS 2	W5S	Nitrogen oxides
MOS 3	W3C	Ammonia, aromatic molecules
MOS 4	W6S	Hydrogen
MOS 5	W5C	Methane, propane, and aliphatic non-polar molecules
MOS 6	W1S	Methane
MOS 7	W1W	Sulfur-containing organics
MOS 8	W2S	Broad alcohols
MOS 9	W2W	Aromatics, sulfur- and chlorine-containing organics
MOS 10	W3S	Methane and aliphatics

The operating process is based on Win Muster V1.6 software. There are two kinds of data obtained from the e-nose, one is R (the resistance value of the sensors when the sample gas flow through them), the other is G/G_0, where G and G_0 are the conductivities of the sensor when exposed to the sample gas and the zero gas, respectively. The G/G_0 value is more reliability cause it could avoid sensor drift in some degree, so in this study, the G/G_0 value is chosen as the initial data.

2.3 Experimental Procedure

Sensory Evaluation. A sensory evaluation of pork is a method for description of the quality in an objective way. A descriptive method in accordance with GB/T 5009.44─2003 [13] was carried out at the Department of Biosystem Engineering and Food Science by a selected and trained sensory panel consisting of 8 assessors. All

assessors were familiar with pork characters and descriptive analysis procedure. Scoring standard is given in Table 2.

Table 2. Sensory evaluation standards

Parameters	Scores		
	5	3	1
Color	Muscle: shiny, red, uniform Fat：white	Muscle: a little dark red Fat：lack of luster	Dark color with sign of depravity
Viscosity	Slightly dry or moist	Sticky or dry moist new cut section	High viscosity
Elasticity	Instantly and completely recover from acupressure	Slowly and incompletely recover from acupressure	Low elastic
Flavor	Normal	Ammonia or sour odor	Strong odor

E-nose Sampling Procedure. The concentration of the volatile gas is affected by the mass and headspace-generation time of the samples [14], so a set of pre-experiments were performed to determine the optimum experimental parameters. Three main factors were considered: mass of the pork samples (M: 10 and 25 g), storage time (ST: 0 and 1day) and headspace-generation time (HGT: 5, 15 and 25 min). The samples stored for 0 and 1 day were divided into six groups respectively, marked as 10-5, 10-15, 10-25, 25-5, 25-15, 25-25. The number format was mass – headspace-generation time, for example, the 10-5 group means 10 g sample mass with 5 min headspace-generation time. The multifactor pre-experiment was conducted with seven replicates of each group (Table 3). After acquiring the optimum experimental parameters, the pork samples stored for 0-6 d (15 replicates each day) were detected by e-nose under such parameters.

Each pork sample was placed in a 500 mL airtight glass vial that was sealed with plastic wrap. The glass vial was closed for a certain time (headspace-generation time) to collect the volatiles from the pork sample. During the measurement process, the headspace gaseous compounds were pumped into the sensor array at a constant flow rate of 50 mL min^{-1} through Teflon tubing connected to a needle in the plastic wrap, making the ratio of conductance of each sensor change. The measurement phase lasted for 65 s, which was long enough for the sensors to reach stable signal values. The signal data from the sensors were collected by the computer once per second

during the measurements. When the measurement process was complete, the acquired data were stored for later mathematic analysis. After each measurement, zero gas (air filtered by active carbon) was pumped into the sample gas path from the other port of the instrument for 50s (flush time). In case of sensor pollution which could cause sensor drift, after all the measurements were done every day, nitrogen gas was pumped into the sample gas path to clear the sensor array. All the measurements were carried out at a temperature of 20 ^{o}C ± 1 ^{o}C and 50% to 60% relative humidity (controlled by air-conditioning).

Table 3. Pre-experiment method

Storage time/ d	Mass/ g	Headspace-generation time/ min	Number of replications
0	15	5	7
0	15	15	7
0	15	25	7
0	25	5	7
0	25	15	7
0	25	25	7
1	15	5	7
1	15	15	7
1	15	25	7
1	25	5	7
1	25	15	7
1	25	25	7

2.4 Statistical Analysis Methods

LDA is a widely used statistic method. Similar to PCA, it is also a linear combination of the original variable to construct a discriminant function [15]. Compared with PCA, the LDA method can notice the distribution of points in the same category and the distance between them. It maximizes the variance between categories and minimizes the variance within categories to improve the resolution of classes [16]. The graphical view of LDA analysis is similar to a PCA display.

BPNN has been a widely-used method for e-nose [17-18]. BPNN can be described as a non-linear projection between the input vectors and output vectors. A typical BPNN model includes the input layer, the hidden layer (one layer or more) and the output layer. The dimensions of the input and output layer are usually decided by the dimensions of the input and output vectors, respectively, while the dimensions of the hidden layer is usually decided by try and error methodology. While determining the suitable network topology, the network processed the inputs and compared the resulting outputs against the desired outputs. Errors were then propagated back through the system, causing the system to adjust the weights that control the network [19]. This process continued over and over until the error matched the training goal error.

MLR analysis is a common method used in quantitative analysis. Equations relating the dependent variable behavior to the descriptors are developed with the following form: contribution, with numbers enclosed in parentheses and set on the right margin.

$$Y_i = \beta_0 + \beta_i X_i , \quad (i = 1, 2, 3, \ldots, \mathrm{N}). \tag{1}$$

Where Y_i is an independent variable; β_0 is the intercept and β_i are the regression coefficients of the independent variables X_i; and N is the number of independent variables.

ANOVA is a method of portioning variability into identifiable sources of variation and the associated degree of freedom in an experiment. The frequency test (F-test) is utilized in statistics to analyze the significant effects of the parameters, which form the quality characteristics [20]. MANOVA is a generalized form of univariate ANOVA. It is used when there are two or more dependent variables. It helps to answer : 1. do changes in the independent variable(s) have significant effects on the dependent variables; 2. what are the interactions among the dependent variables and 3. among the independent variables [21].

The data processing method LDA was performed in WinMuster, which is combined in the e-nose software; (M)ANOVA and MLR were performed in SAS software, and BPNN was proceeded using the network toolbox in MATLAB R2008a.

3 Results and Discussions

3.1 Discussions of Sensory Evaluation Results

Sensory evaluation result is shown in Fig. 2. The sensory scores of color, elasticity, viscosity and odor attributes decreased as the storage time increased. It is also noticeable that both of the curves had declined quickly since the third day, while in the first 3 days, the total score curve only declined slightly, and the odor score nearly kept the same. This manifested that the pork sample didn't corrupt until the third day. So in the first 3 days, it remained fresh, with its appearance or odor change little.

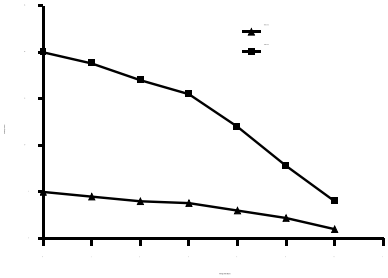

Fig. 2. Sensory evaluation result

3.2 Discussions of Pre-experiment Results

Responding Curves of E-nose. Fig. 3 shows two typical responding curves of the ten sensors during measurement of a pork sample in day 0 (Fig. 3a) and day 1 (Fig. 3b), respectively. Each curve represents a sensor's ratio of conductance (G/G_0), which increased in the first few seconds and finally stabilized at about the 60^{th} s. Compare Fig. 3a and Fig. 3b, the ratio of conductance values of all the sensors increased with the storage time of pork. It is also noticeable that the sensor MOS 2, which is sensitive to nitrogen oxides (one of the main odor in pork putrefaction), increased most observably with storage time, with its G/G_0 value differing significantly by the storage time. This indicates that it is potential to monitor pork freshness by e-nose.

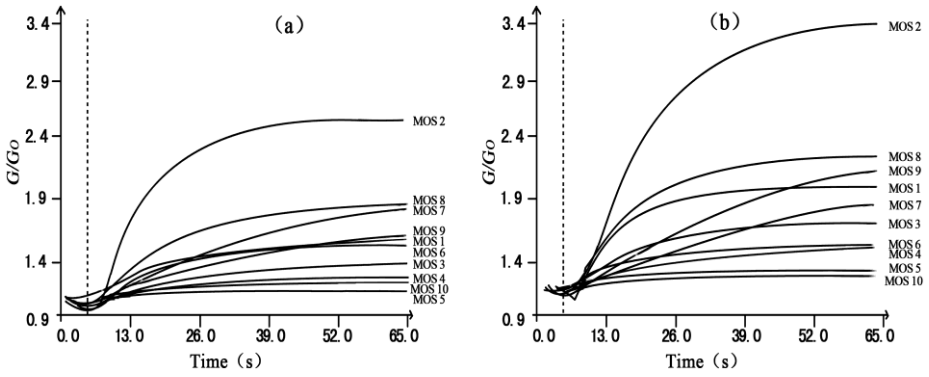

Fig. 3. Responding curves of sensors for fresh pork samples: (a): stored for 0 day; (b): stored for 1 day

Optimum Experimental Parameters.

Multivariate Analysis of Variance Result. A 3-factors analysis of variance was performed to acquire optimum experimental parameters. The three factors are sample mass (M), headspace-generation time (T) and storage time (ST).

Table 4. MANOVA (factors are storage time, mass, and headspace-generation time)

Different Source	Sum of Squares	Degree of Freedom	Mean Squares	*F*	Sig.
M	17.880	1	17.880	172.231	0.000
T	8.043	2	4.021	38.736	0.000
ST	22.875	1	22.875	220.338	0.000
M × T	0.890	2	0.445	4.288	0.017
M × ST	0.145	1	0.145	1.393	0.241
T × ST	0.217	2	0.108	1.044	0.357
M × T × ST	0.678	2	0.339	3.267	0.043

MANOVA was performed to see how these factors affected the response of e-nose. The MANOVA results are summarized in Table 4, where the magnitudes of the *F*-values indicate the relative importance of the factors to some extent. As is shown in Table 4, M, T, and ST all have very significant effect on the response of the e-nose respectively (Sig. < 0.001). The interaction of M × T, and M × T × ST also have a

significant effect on the response of the e-nose (Sig. < 0.05). It can also be observed that the ST has the highest F-value, which means the e-nose signals are differed between samples with different storage time. This proves the feasibility of using the e-nose to distinguish pork freshness. The mass factor has the second highest F-value, next is the headspace-generation time, suggesting that it is very important to determine the sample mass and the headspace-generation time.

One-Way Analysis of Variance Result. ANOVA (the factor is storage time) was applied for group 10-5, 10-15, 10-25, 25-5, 25-15, 25-25 and got each group a *F*-value, respectively. The results of six groups are summarized in Table 5, which shows that all the groups have very significant effects on the response of e-nose, and the combination 10-5 has the highest *F*-value. This means that when the mass is 10 g and the headspace-generation time is 5 min, the e-nose has the most obvious difference in its responding values towards samples with different storage time.

Table 5. *F*-values of six combinations

Combinations	*F*-value	Sig.
10-5	209.883	0.000
10-15	44.131	0.000
10-25	66.676	0.000
25-5	89.451	0.000
25-15	159.226	0.000
25-25	55.049	0.000

Therefore, in this research, the optimum parameters are 10 g pork sample with 5 min headspace-generation time.

Discrimination Power (DP). DP is another index used to observe the magnitude of difference among samples [22]. All combinations with different mass and headspace-generation time (as was described before) were applied the DP analysis and got a value respectively (listed in Table 6). The number format is storage time—mass—headspace-generation time. For example, 0-10-5 means stored 0 day and 10 g sample mass with 5 min headspace-generation time. As is shown in Table 6, the combination 10-5 has the highest DP value, which means the e-nose response of this

combination has the most obvious difference between day 0 and day 1. This result is the same with the ANOVA result.

Table 6. Results of DP test of six combinations

	1-10-5	1-10-15	1-10-25	1-25-5	1-25-15	1-25-25
0-10-5	0.967					
0-10-15		0.555				
0-10-25			0.753			
0-25-5				0.582		
0-25-15					0.852	
0-25-25						0.448

3.3 Discussion of E-nose Detection Results

The pork samples stored for 0-6 days (15 replications of each day) were detected by e-nose under the optimum experimental parameters, and the signals of e-nose were analyzed by multiple data analysis methods.

Linear Discriminant Analysis (LDA). For all 105 pork samples (7 storage time × 15 duplicates), the response signal values of the e-nose at the 60^{th} s were extracted and analyzed by LDA. The results are shown in Fig. 4, which is a two-dimensional spatial plot defined by two discriminant functions. The first discriminant function (LD 1) explains 64.70% of the total variance, and the second discriminant function (LD 2) explains 23.72% of the total variance. The total contribution rate is 88.42%, which means these two reflect 88.42% of the original information.

As is shown in Fig. 4, the samples stored for 1-3 days are very close to each other. This may be explained as follows: in the first 0-3 days, fresh pork stored at 10 ^{o}C in the fridge still remained fresh, and the change in their volatile gases was subtle so the e-nose could not notice the difference well. Thus, the data that e-nose extracted was similar to each other and concentrated. However, freeze & unfreeze could cause cell damage and affect the quality of pork, so the samples stored 0 day, which were taken for experiment directly without being stored in the fridge, are discriminated from those stored in fridge for 1-3 days. It is also noticeable that the samples stored 6 days

are obviously discriminated. In general, except a little overlapped among the samples stored 1-3 days, all the samples can be clearly divided into seven regions according to their storage time.

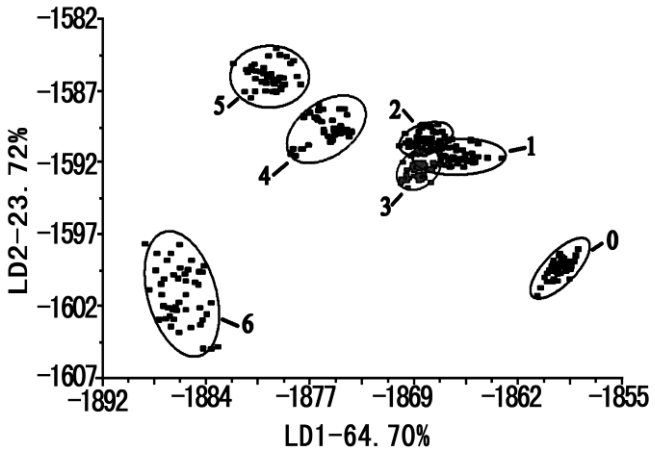

Fig. 4. LDA analysis of pork stored for 0-6 days

Back Propagation Neural Network (BPNN). In this study, a 30-7-7-7 BPNN model was applied for the storage time prediction of the pork samples stored 0-6 days. Three eigenvalues (the 20th s, 40th s and 60th s value) of every sensor signal were adopted, and then all 30 (3 × 10) signal values were used as the input vector of the BPNN. The output vector was designed seven-dimensional in accordance with the seven storage days, and the training goal error was set as 0.001. After many trials, the hidden layer was decided and the network topology was designed as 30-7-7-7.

All the 105 samples were divided into two groups: 70 samples for the training set (10 samples of each group were randomly chosen), and 35 samples for the predicting set. So the input layer for the training set and the predicting set was a 70 × 30 matrix (3 eigenvalues of each sensor) and a 35 × 30 matrix, respectively. The results were shown in Table 7 and Table 8.

The total identification rate of the simulated results for the training set was 95.71%, and the total identification rate for the predicting set was 97.14%. It should be noticed that neither the predicting set nor the training set could correctly discriminate the samples stored between 2 and 3days.

Table 7. BP results of the original data in the training set

ST	NS	Recognition results							Identification rate of each day	Identification rate of all days
		0	1	2	3	4	5	6		
0	10	10							100%	
1	10		10						100%	
2	10			8	2				80%	
3	10			1	9				90%	95.71%
4	10					10			100%	
5	10						10		100%	
6	10							10	100%	

Table 8. BP results of the original data in the predicting set

ST	NS	Recognition results							Identification rate of each day	Identification rate of all days
		0	1	2	3	4	5	6		
0	5	5							100%	
1	5		5						100%	
2	5			4	1				80%	
3	5				5				100%	97.14%
4	5					5			100%	
5	5						5		100%	
6	5							5	100%	

Multiple Linear Regression (MLR). The MLR algorithm establishes the model that describes the relationship between sensor signals and odor scores. All variables used in the models are significant at the 0.01 level. The sample data (105 pork samples from 7 storage time) were separated randomly into two groups: one group for the calibration set was used to develop the calibration models (70 pork samples, 10 samples each day) and the other group containing the remaining samples was used for the prediction set (35 pork samples, 5 samples each day).

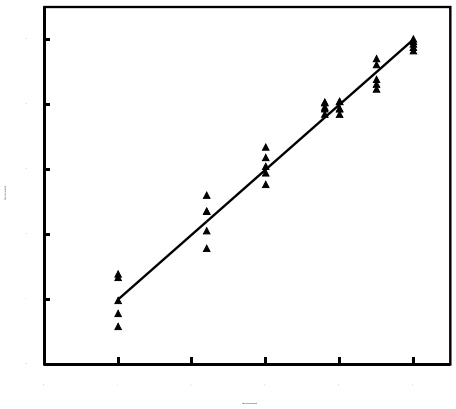

Fig. 5. Prediction of odor scores by MLR model

The predictive model for the odor score is given below.

$$Y_i = 6.695285 + 1.314430X_1 - 0.552935X_2 + 2.401326X_3 - 4.553837X_4 + 3.023900X_5 - 2.250302X_6 - 6.150372X_7 - 1.827269X_8 - 1.609080X_9 + 8.197955X_{10}, \quad (2)$$

where S is the odor score, while X_1、X_2、X_3、X_4、X_5、X_6、X_7、X_8、X_9 and X_{10} represent the 60^{th} s signal data of the ten sensors MOS1 - MOS10, respectively. The R^2 of this model is 0.9848.

Fig. 5 shows the prediction ability of the e-nose, where each triangle represents the predicted values versus the real value of each measurement. The figures illustrate a

linear correlation between the response of sensors and odor scores, indicating that the responses of sensors linearly correlated with the odor scores.

4 Conclusions

A PEN2 e-nose detection combined with sensory evaluation were conducted for discrimination and prediction of pork freshness. A pre-experiment was conducted to observe if the sample mass and headspace-generation time would affect the performance of e-nose, and the optimum experimental parameters (10 g sample mass with 5 min headspace-generation time in 500 mL vial volume) were determined by MANOVA and ANOVA. The later e-nose experiment was taken under such parameters. LDA, BPNN and MLR were employed to observe if the e-nose can classify the pork samples stored during 0-6 d well, as well as to predict the storage time and sensory scores. The results were good: The pork samples could be well discriminated by LDA; 97.14%% of the predicting set (with 95.71% of the training set) was classified correctly using BPNN model; and the MLR model also provided an accurate quality index model between e-nose signals and sensory scores with high correlation coefficients (R^2 = 0.9848). These results prove that the e-nose has the potential of being a reliable instrument for the assessment and prediction of pork freshness.

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