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Nondestructive evaluation of pork freshness using a portable electronic nose (E-nose) based on a colorimetric sensor array

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This paper attempted the feasibility on rapid nondestructive evaluation of pork freshness using a portable electronic nose (E-nose) based on a colorimetric sensor array. A novel and low-cost colorimetric sensors array was fabricated using printing 12 chemically responsive dyes on a silica-gel flat plate. The colorimetric sensors array has a specific colorific fingerprint to volatile compounds released from pork samples and can be successfully used to evaluate the pork freshness with the help of multivariate calibration. Linear discriminant analysis (LDA) and back propagation artificial neural network (BP-ANN) were used for modeling. Experimental results showed that the performance of BP-ANN model was superior to LDA model. The optimum discrimination rates were 100% and 97.5% in the training and prediction sets respectively. The results demonstrated that this technology has a high potential in its real-time use of monitoring pork quality in meat processing industries.

Introduction

Pork meat is a nutrient rich medium that is ideal for many pathogens and spoilage microbes to colonize [1]. The spoilage of meat occurs in a matter of hours or days if left untreated which results unappetizing, poisonous or infectious meat. Spoilage is caused by practically unavoidable infection and subsequent decomposition of meat by bacteria and fungi, which are borne by the animal itself, by the people handling meat and by their implements [2]. During microbial spoilage of pork meat, protein components in pork gradually decompose and produce some toxic small molecular components including histamine, tyramine, putrescine, and tryptamine[3, 4]. Present economic trends lead to increasing distances between consumption and productions zones and ultimately to an extension of the delivery chain. Considering these changes, it is necessary to set out methods for reliable objective safety control to guarantee quality and freshness at all stages of the commodity chain.

Meat freshness is a complex parameter including different microbiological, physicochemical and biochemical attributes. Traditionally, there have been two main methods to evaluate meat freshness; one consists of a sensory evaluation which involves the estimation of organoleptic attributes with the help of skillful experts and the other is the chemical or biochemical measurement of postmortem deteriorative changes associated with sensory quality, microbial growth and chemical changes. Latter one includes determination of pH value, total viable counts (TVC), and total volatile basic nitrogen (TVB-N). In most cases, the former is subjective and costly whereas the latter is time-consuming and destructive. The meat industry needs rapid analytical methods or tools to determine and select suitable processing of their raw material and predict the shelf life of their products. Similarly, the inspection authorities need reliable methods for quality control while the wholesale and retail sectors

also need valid methods to ensure the freshness and safety of their products and to resolve potential disputes between buyers and sellers. Therefore, the necessity to develop a practical analytical tool for the rapid evaluation of pork freshness has increased in recent years.

Electronic nose (E-nose), with the help of multivariate calibration techniques, represents an alternative approach for the evaluation of the freshness of meat or meat products[5]. These techniques are alternative to traditional methods and are quick, easy to handle and do not require sample preparation or the use of chemical reagents. The sensors array in an E-nose system usually consists of numerous non-specific sensors and an odor stimulus generates characteristic fingerprint from the sensors array. Patterns of fingerprints from known odors are employed to construct a database and train a pattern recognition system so that unknown odors can subsequently be classified and identified [6]. Most of E-nose systems consist of the metal oxide semiconductor (MOS) sensors although there are several other types of gas sensors such as conducting organic polymer (COP), quartz crystal microbalance (QCM), surface acoustic wave, carbon nanotubes (CNT), and conductive polymer nanocoposites (CPC) sensors. Most of these sensors are usually conductometric in nature and their resistance changes (decreases/increases) when subjected to the odors vapor molecules. Therefore, this type of E-nose consisting MOS sensors array and CNT sensor (or CPC sensor) is sensitive to the variation of humidity[7, 8].

Herein, a novel and portable colorimetric sensor is being probed which is not sensitive to humidity due to the hydrophobicity of the sensor materials and sensors plate[9]. The basic principle of this technique is that it utilizes the colour change induced by the reaction between the volatile compounds and the array of chemically responsive dyes upon ligand binding for chemical vapour detection and differentiation. Chemically responsive dyes were selected according to their sensitivity

to volatile compounds that need to be detected. Reverse phase plate has been chosen as a non-interacting dispersion medium for the chemically responsive dyes array as well as a suitable surface for diffuse reactance spectral measurements. The colorimetric sensors array can be made by printing selected dyes on a reverse phase plate. A colour change profile for each object can be obtained by differentiating the images of the sensors array before and after exposure to the VOCs of objects. The digital data representing the color change profiles were analyzed using multivariate calibration [10]. Recently, this novel E-nose technique has been used for molecular recognition [11], sugar recognition [12,] detection of H_2S [13] and volatile organic compounds (VOCs) [14], monitoring vinegar acetic fermentation[15] and classification of tea categories[16].

In the exemplary study of this article, we reported a novel and portable E-nose based on a colorimetric sensor array to evaluate pork freshness. The major contribution of this research is to explore a low-cost and portable E-nose system based on colorimetric sensor array for nondestructive evaluation of pork freshness. Compared with other colorimetric sensors, the selected metalloporphyrins in this sensor array are nearly ideal for the detection of VOCs released from pork because of their open coordination sites for axial ligation, their large spectral shifts upon ligand binding and their intense coloration. Meanwhile, the metalloporphyrins is not sensitive to the variation of humidity. The specific work was arranged as follows: (1) fabricating a colorimetric sensors array using dyes printing on a plate; (2) developing a low cost and portable E-nose system based on a colorimetric sensors array; (3) linear discriminant analysis (LDA) and back propagation artificial neural network (BP-ANN) were comparatively used for data analysis.

Materials and methods

Sample preparation

In this study, all samples were from the pork's logissimus dorsi (LD) muscles and 10g of the longissimus muscle of size 3 cm ×2 cm×2 cm as a sample was cut from the 4th lumbar vertebra of the right carcass side. Herein, all 80 samples were collected from 80 pigs' carcasses (approx. 100 kg live weight) on the same day in a local pork farm. Pigs were slaughtered under commercial conditions (stunned electrically, exsanguinated, scalded, de-haired, eviscerated and split into sides). No different treatments at slaughter were carried out and pig slaughter was operated according to the National Standard of PR China (GB/T17236-2008). Before analysis, all samples were labeled one by one and stored in a refrigerator at 4 °C.

Reference measurement of pork freshness

In this study, the reference measurement of pork freshness was carried out using measuring TVB-N content in pork. TVB-N content in pork was measured by a steam distillation method, according to Chinese standard GB/T 5009.44[17]. For details of methods, see the supplementary information.

Measurements using the electronic nose

E-nose system and data acquisition

Before data acquisition, we fabricated the sensor array. For details of methods, see supplementary information. A functional prototype of E-nose for pork freshness detection was conducted and the schematic diagram is shown in Fig. 1. Images of the sensor array were captured by a HP Scanjet 4890 flatbed scanner (Hewlett Packard Inc., Shanghai, China). The scanner's resolution was set at

600 dpi. First, the sensors array was captured by the flatbed scanner before exposed to the pork sample and an original image of the sensors array was achieved. Next, the array was exposed to the pork sample with the help of ventilatory support. In this experiment, the sensors array chip was mounted in an inert platform inserted into the lid of the closed glass vessel and the pork sample was placed in the 250 mL glass vessel. The sample was stored at 4 ^{o}C before data acquisition but the ambient temperature was controlled at 25 ^{o}C when sampling. This experiment was achieved with the uniform arrangements including the ambient temperature, the volume of sample, the size of Petri dish, and the headspace time. Once reaching nearly complete equilibration, we took out the sensors array from the glass vessel to scan it again and got a "final" image. We could get a colorful difference image by subtracting the "initial" image from the "final" image, the difference image provided a color change profile that is a characteristic fingerprint to volatile compounds in pork sample. In this work, the equilibration time of sensor reaction was determined by the preliminary experiments. According to the results of preliminary experiments, we found that the reaction between the dyes and VOCs reached nearly complete equilibration after 5min. Eventually, the reaction time was set as 5min in this work. To avoid factitious non-uniformity, the center of each dye spot (a round area consisting of 800 pixels) was averaged. The difference image is a RGB color image consisting of 3 color components images (i.e. R image, G image, and B image). Thus each dye can provide 3 variables (R, G, and B gray value) and 12 dyes in the sensors array can provide 36 variables (12 dyes×3 color components). Herein, the color RGB image is an 8-bit image and the range of color values is [0 255]. In other words, the original data of colorimetric sensors array include 36 variables.

[Here for Fig. 1]

Multivariate calibrations

The colorimetric sensors array in this E-nose system can generate a characteristic ngerprint to an odor stimulus. Patterns or fingerprints from known odors are often employed to construct a database and train a pattern recognition system so that unknown odors can subsequently be classified and identified. Hence, E-nose was used for classification of pork freshness by developing a classification model ~~to evaluate pork freshness~~. In this work, the data of E-nose was used for modeling with the help of the multivariate calibration. A classification model was calibrated on the training samples with reference category. The data of TVB-N contents of samples were just used to determine the reference category of these training samples. Eventually, we can use the developed classification model to predict the category of an unknown sample. Linear discriminant analysis (LDA) and back propagation artificial neural network (BP-ANN), as two commonly used multivariate calibration algorithms, were used for modeling. Principal component analysis (PCA) was conducted on the sensors data to extract some principal components (PCs) as the inputs of the model. All data analysis was carried out in Matlab Version 7.10.0 (Mathworks, Inc. Natick, USA) in Windows 7.

Results and discussion

Reference measurements results of pork freshness

Fig. 2 shows the reference measurement results of TVB-N contents for all 80 samples. When the TVB-N contents were between 0 and 15mg/100g level, sample was defined as “fresh sample” and otherwise the “unfresh sample”. Herein, the TVB-N content with 15 mg/100g was defined as the pork freshness baseline. These classification results of 80 samples were used for the references of the further work. In this work, all 80 samples were divided into 40 unfresh samples and 40 fresh samples according to their TVB-N contents. The 80 samples were divided into 2 subsets. One was called the

calibration set used for calibrating model and the other was called the prediction set which was used to test the performance of the model. To achieve a robust model, the selection of samples were done as follows: first, all samples were sorted according to their respective TVB-N contents; then one sample of every two samples were selected into the prediction set. Eventually, the calibration set contained 40 samples and the prediction set contained 40 samples. Table 1 shows the relevant information of all samples in the calibration and prediction sets.

Table 1 Relevant information for all samples in the calibration and prediction sets

Subsets	Fresh sample		Unfresh sample	
	Sample number	Range of TVB-N content (mg/100g)	Sample number	Range of TVB-N content (mg/100g)
Calibration set	20	9.0362~14.9872	20	15.4633~32.0738
Prediction set	20	9.0876~14.9756	20	15.6687~30.1397

[Here for Fig. 2]

Sensor responses

Fig. 3 shows the difference images for the fresh pork sample (a) and the unfresh pork samples (b). And the difference image was obtained by subtracting the original image from the final image. Each difference image has its particular colorific fingerprint. During microbial spoilage of pork meat, proteins components in pork gradually decomposed by microorganisms giving off the spoilage metabolites especially volatile bases, hypoxanthine, organic acids and biogenic amines. The selected metalloporphyrins dyes in the sensors array have sensitive responses to hypoxanthine and biogenic amines due to their open coordination sites for axial ligation, large spectral shifts upon ligand binding, and intense coloration. The additional dyes consisting of 3 pH indictors have sensitive responses to volatile bases and organic acids. All these mentioned above show that the behavior of

the spoilage metabolites is related to colorific change of chemical responsive dyes. Microbial metabolites increased gradually along with the process of pork spoilage, thus the sensors array showed unique colorific fingerprint to each pork sample corresponding to its freshness as shown in Fig. 3. However, the selected dyes in the sensors array have non-specific sensitivity and wide cross-sensitivity toward volatile compounds (i.e. spoilage metabolites). That is to say, one of dyes in the sensors array could be simultaneously sensitive to numerous volatile compounds and also different dyes could be simultaneously sensitive to one of volatile compounds. So this sensors technique is not like the conventional component-by-component analyses (e.g., gas chromatography (GC) and gas chromatography – mass spectrometry (GC-MS)) and is difficult to assign specific colorific profile to a specific volatile compound. Thus, the digital data representing the color change profiles were analyzed with the help of an appropriate multivariate calibration.

[Here for Fig. 3]

Results of classification

Principal component analysis (PCA) is a linear, unsupervised and pattern recognition technique used for analyzing, classifying and reducing the dimensionality of numerical datasets in a multivariate problem [18]. It can transform original variables into a few new variables known as principal components (PCs). Each principal component is a linear combination of the original variables. These PCs account as much as possible for the variability contained in the original data. The first principal component (PC1) accounts for the maximum of the total variables, the second (PC2) is not correlated with the first and accounts for the maximum of the residual variance and so on. The top two or three PCs constructed a two or three dimensional coordinates. The two or three

dimensional corresponding score plot shows the relation between the observations. To visualize the cluster trends of these samples, a scatter plot (also called a score plot) was obtained using the top three principal components (i.e. PC1, PC2, PC3) issued from PCA.

Fig. 4 shows a 3-Dimension plot constructed by PC1, PC2, and PC3 and all samples appear clustered along the three principal components axes confirming the presence of four groups. PC1 can explain 30.22% of the variance, PC2 can explain 12.92% of the variance and PC3 can explain 9.36% of the variance. The total accumulative contribution rate of variance from PC1, PC2, and PC3 was 52.6%. This is also to say, the 3-D plot can only explain 52.6% variance of raw data and therefore, was no neat separation between fresh samples and unfresh samples. Seen from Fig. 4, the distribution of fresh samples is relatively centralized; whereas it was a spatial distribution for unfresh samples. As we all known fresh pork meat has no peculiar smell but for unfresh samples, protein components in pork gradually decompose into some spoilage metabolites, especially volatile bases, hypoxanthine, organic acids and biogenic amines. Most of spoilage metabolites are the volatile components and the colorimetric sensors array has its specific response to them therefore the sensor array can bring a corresponding colorific fingerprint. Compared with fresh samples, the quantity of volatile component varies significantly among the unfresh samples in this work which is due to their different spoilage degrees. The TVB-N contents of pork samples can also account for the results which are shown in Fig. 4.

[Here for Fig. 4]

Geometrical exploration based on PCA score plots can give the clusters trends in this study but PCA is an unsupervised technique. Usually application of sensors data in solutions to classification problem must depend on a classification model that is often developed by the supervised pattern

recognition. Supervised pattern recognition refers to the technique that finds the relationships between a set of descriptive variables (i.e. sensor data variables) and a qualitative variable (i.e. samples categories). The classification model is developed on a training set of samples with categories. The model performance is evaluated by means of some independent samples from a prediction set then the final results are computed by comparing their predicted categories with their own true categories. Supervised pattern recognition has numerous classification algorithms and how to choose the most appropriate one is of great significance. In this study, two different classification algorithms which were linear discriminant analysis (LDA) and back propagation artificial neural network (BP-ANN) were attempted to develop the classification models, respectively. We systematically studied and discussed the effects of two classification algorithms on qualitative analysis of this sensors data. Besides, the number of PCs and some other model parameters were optimized.

[Here for Fig. 5]

Among many possible techniques for data classification, linear discriminant analysis (LDA) is a commonly used one. LDA is used to find the linear combination of features which best separates two or more classes of object or event. The resulting combinations may be used as a linear classifier. This method maximizes the ratio of between-class variance to the within-class variance in any particular data set thereby guaranteeing maximal reparability [16, 18]. The number of principal component (PCs) is crucial to the performance of the LDA discrimination model. The number of PCs was optimized in developing the LDA model in this study. The optimum number of PCs was achieved according to the highest discrimination rates in the calibration and prediction sets. Fig. 5(a) shows the discrimination rates of LDA model with different PCs in the calibration and prediction sets. As seen from Fig. 5 (a),

the optimal LDA model was achieved when 6 PCs were included and the best discrimination rates were 80% and 75% in the calibration and prediction sets, respectively.

Back propagation artificial neural network (BP-ANN) is the most classical feed-forward multi-layer networks consisting of neurons arranged in layers (an input layer, one or more hidden layers and an output layer) being the connections (weights) unidirectional from input to output[19]. And it is a strong tool to capture and reveal complex relevance between inputs and outputs. As an important supervised pattern recognition method, many parameters exert to some extent certain influence on the performance of ANN models. These parameters include the number of neurons in the middle layer, scale functions, learning rate factor, momentum factors, and initial weights. In this work, the most classical back propagation artificial neural network (BP-ANN) with 3 layers construction was used to construct the discrimination model. These parameters of the BP-ANN model were optimized by the minimal mean square error (MSE) value as follows: the number of neurons in the hidden layer was set to 5, the learning rate factor and momentum factor were set to 0.1, the initial weight was set to 0.3 and the scale function was set as 'tanh' function. It is crucial to select the appropriate number of PCs in constructing a BP-ANN model. Fig. 5 (b) shows the discrimination rates of BP-ANN model with different PCs in the calibration and prediction sets. Seen from Fig. 5 (b), the optimum of BP-ANN was obtained when the 5 PCs were included. Its identification rate of BP-ANN model was 100% in the training set, 97.5% in the prediction set. It shows that only few sample has been misclassified compared with other PCs discrimination rates.

Discussion

To get good performance in discrimination of pork freshness using this E-nose, we systematically studied the multivariate calibrations and parameter optimization that had to be done through

developing the classification model. Table 2 shows the discrimination results from LDA and BP-ANN approaches used in this study. Investigating these results, we can conclude that (1) most of the misclassified samples were unfresh samples; (2) their TVB-N contents were close to the freshness baseline (i.e. 15mg/100g); (3) BP-ANN model is superior to LDA model in the discrimination results. Such results can be accounted for from the following aspects.

Compared with fresh samples, the quantity of volatile component has a bigger varying among the unfresh samples in this work which is due to their different spoilage degrees. The colorimetric sensors array has its specific response to the volatile components, and has a corresponding colorific fingerprint. Accordingly, the relative dispersion degree of unfresh samples is bigger than that of fresh samples, thus more unfresh samples were misclassified. All these mentioned above can account for the first conclusion that most of the misclassified samples were unfresh samples. This agrees with the ~~the~~ standard deviation of unfresh samples (2.8509) is bigger than that of fresh samples (1.6904). Because of the geometric growth of microorganisms during pork spoilage, the increasing of microorganism metabolites is very subtle at the beginning but faster and faster over time as pork meat decay[20]. In general, the pork sample is at the beginning of spoilage if its TVB-N content is less than 20 mg/100g. The microorganism metabolites, most of which are volatile compounds, produced from the pork sample are similar to those from fresh sample[21]. Accordingly, the differences of volatile compounds among the samples whose TVB-N contents are close to the freshness baseline (i.e. 15mg/100g) are so subtle that cannot be easily differentiated by the colorimetric sensors array and also cannot be actually differentiated by human panel test. All these account for why the TVB-N contents of misclassified samples are close to 15mg/100g.

Table 2 Comparison of identification results from LDA and BP-ANN models

Models	PCs	Subsets	Sample type	Sample number	Discrimination results		
					Fresh	Unfresh	Discrimination Rate
LDA	6	Calibration set	Fresh	20	17	3	80% [a]
			Unfresh	20	5	15	
		Prediction set	Fresh	20	16	4	75% [b]
			Unfresh	20	14	6	
BP-ANN	5	Calibration set	Fresh	20	20	0	100%
			Unfresh	20	0	20	
		Prediction set	Fresh	20	20	0	97.5% [c]
			Unfresh	20	1	19	

[a] LDA model in the calibration set: 3 fresh samples and 5 unfresh samples were misclassified; TVB-N contents of the misclassified samples are 13.6353 mg/100g, 14.4275 mg/100g, and 14.9756 mg/100g, 15.4633 mg/100g, 15.7654 mg/100g, 15.9667mg/100g, 16.0798 mg/100g, and 16.9959 mg/100g, respectively.

[b] LDA model in the prediction set: 4 fresh sample and 6 unfresh samples were misclassified; TVB-N contents of the misclassified samples are 13.5762 mg/100g, 14.1090 mg/100g, 14.9678 mg/100g, 14.9872 mg/100g, 15.6987 mg/100g, 15.8713 mg/100g, 16.0489 mg/100g, 16.2624 mg/100g, 17.3761 mg/100g, and 19.6915 mg/100g, respectively.

[c] BP-ANN model in the prediction set: one unfresh sample was misclassified with 15.4633 mg/100g TVB-N content. The discrimination rate (%) = (N1/N2) × 100%, where N1 is the number of correctly classified sample, and N2 is the number of all samples in the calibration set.

From the principles of statistical learning theory, the classification algorithm of BP-ANN has its own unique advantages in contrast to LDA classification algorithms. BP-ANN is a nonlinear classification algorithm; while, LDA is a linear one. Considering that microbial meat spoilage is a complex process which involves nonlinear growth of microorganisms, the increasing of microorganism metabolites as pork spoilage is also nonlinear[4]. Accordingly, colorimetric sensors array provide its particular colorific fingerprint to microorganism metabolites but the colorific fingerprint has a very complex relation to pork freshness. Thus, the linear tools would not provide a

complete solution to complicated classification problem: (1) nonlinear method is stronger than linear method in the level of self-learning and self-adjust; (2) the topological network architecture of BP-ANN might be more suitable for the solution to this classification problem in this work. All these account for why BP-ANN model is superior to LDA model in the discrimination results.

Conclusion

A novel colorimetric sensor array in this work was fabricated using printing 12 chemically responsive dyes on a C2 reverse silica-gel flat plate. A portable E-nose based on the colorimetric sensors array was successfully developed and used to evaluate the freshness of pork meat with the help of multivariate calibration. It can be concluded that the E-nose based on colorimetric sensors array with multivariate calibration has a high potential in evaluating pork freshness.

Acknowledgements

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Figure captions

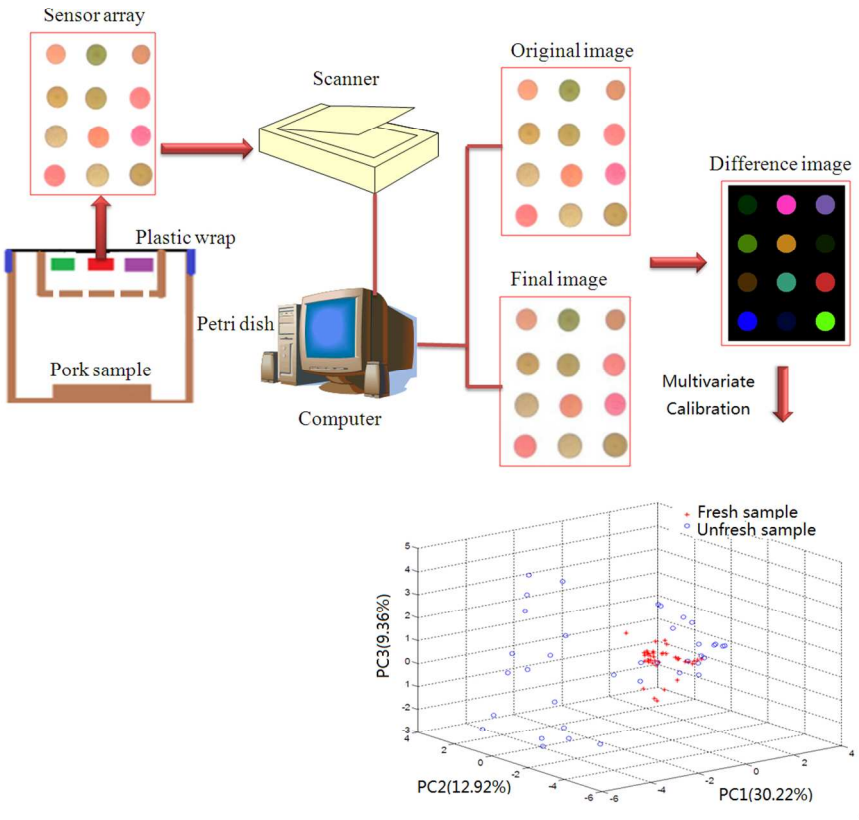
Fig. 1 Schematic diagram of E-nose system based on a colorimetric sensors array.

Fig. 2 Reference measurement results of TVB-N content for all samples.

Fig. 3 Difference images for fresh pork samples (a) and unfresh samples (b).

Fig. 4 Cluster plot constructed by PC1, PC2 and PC3 for fresh samples and unfresh sample.

Fig. 5 Discrimination rates of the model with different PCs: LDA model (a) and BP-ANN model (b).



Graphical Abstract
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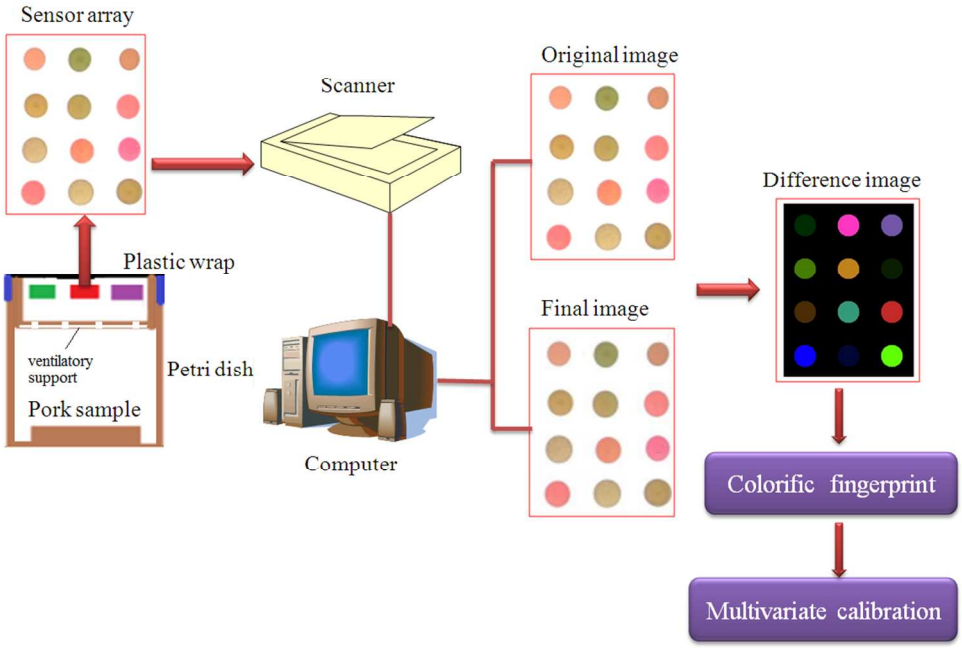

Fig. 1 Schematic diagram of E-nose system based on a colorimetric sensors array.
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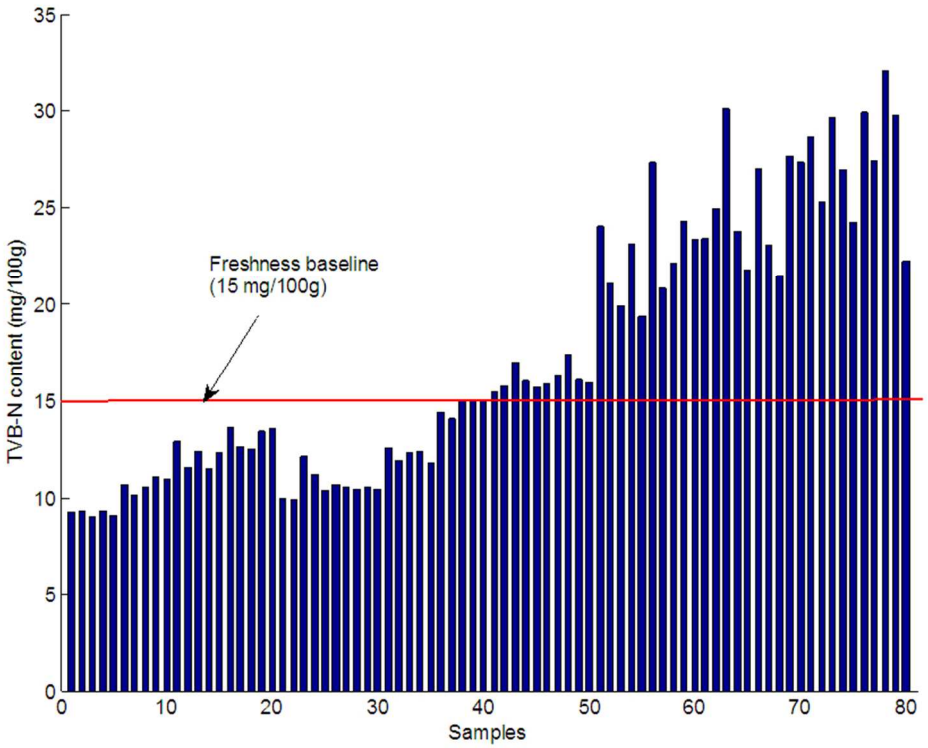

Fig. 2 Reference measurement results of TVB-N content for all samples.
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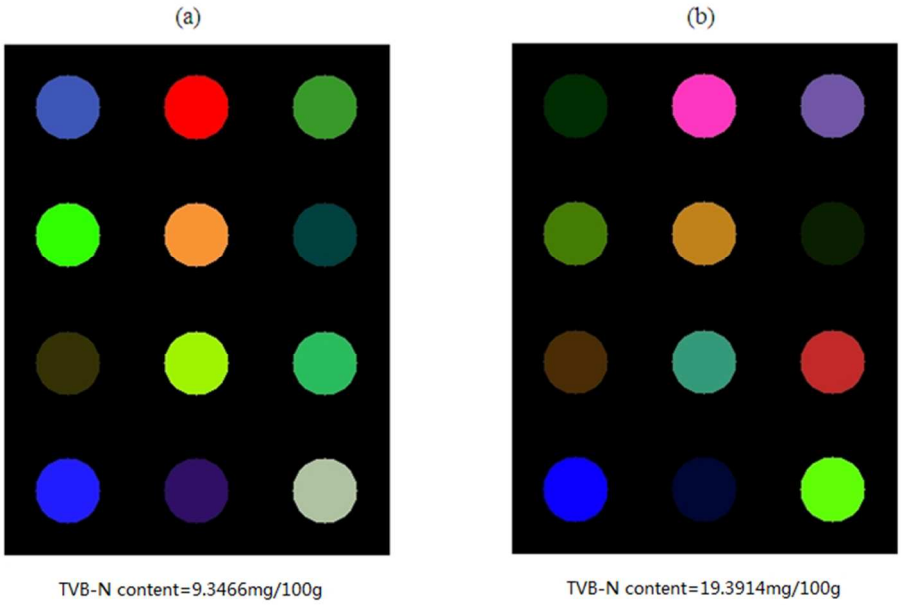

Fig.3 Difference images for fresh pork samples (a) and unfresh samples (b).
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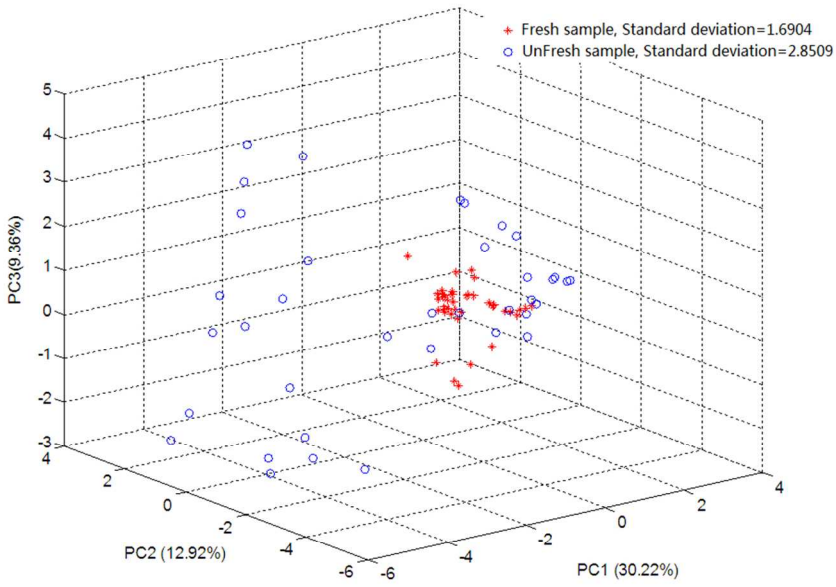

Fig. 4 Cluster plot constructed by PC1, PC2 and PC3 for fresh samples and unfresh sample.
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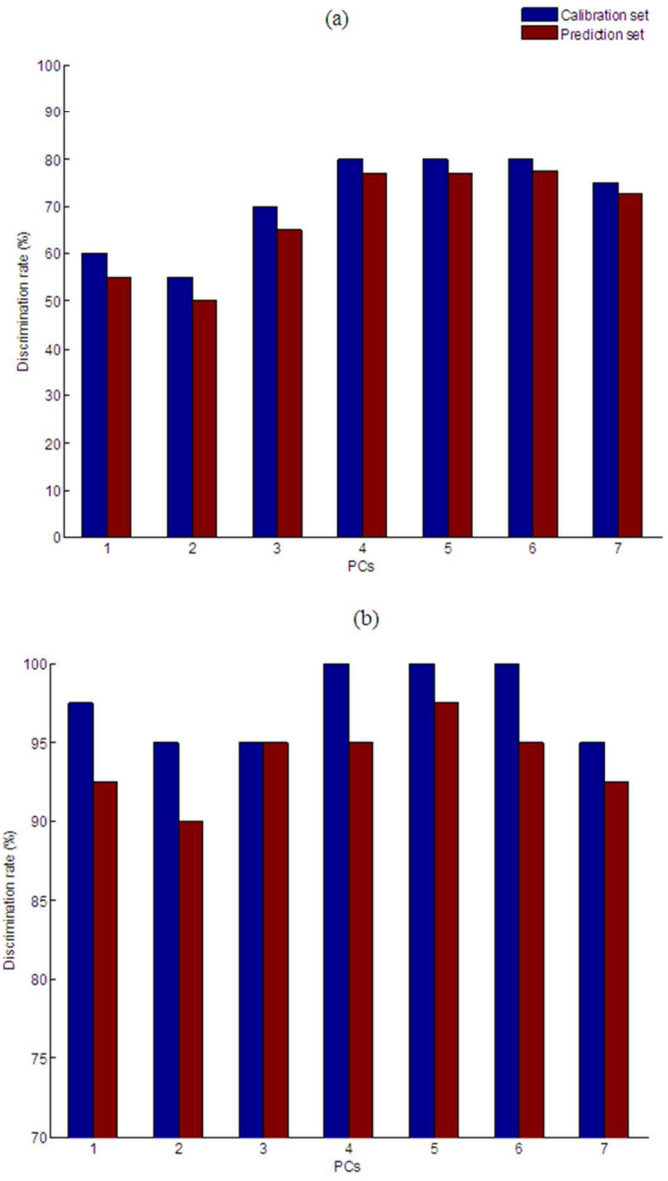

Fig. 5 Discrimination rates of the model with different PCs: LDA model (a) and BP-ANN model (b).
199x323mm (96 x 96 DPI)