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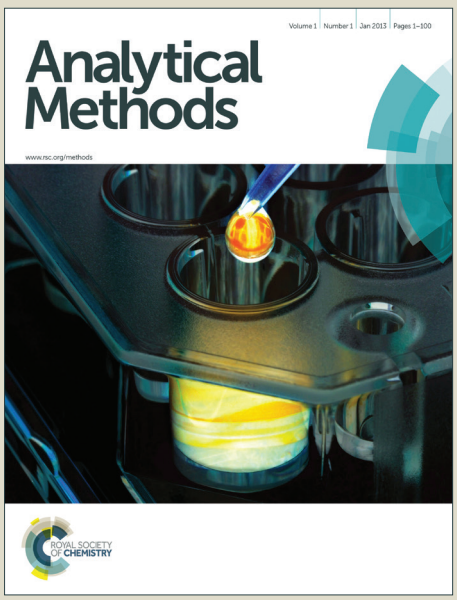

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Quantifying of total volatile basic nitrogen (TVB-N) content in chicken using a colorimetric sensor array and nonlinear regression tool

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Total volatile basic nitrogen (TVB-N) content is an important indicator for evaluating meat's freshness. This study attempted to quantify TVB-N content non-destructively in chicken using a colorimetric sensors array with the help of multivariate calibration. First, we fabricated a colorimetric sensor array by printing 12 chemically responsive dyes on a C2 reverse silica-gel flat plate. A color change profile was obtained by differentiating the image of sensor array before and after exposure to volatile organic compounds (VOCs) released from chicken sample. In addition, we proposed a novel algorithm for modeling, which is back propagation artificial neural network (BP-ANN) and adaptive boosting (AdaBoost) algorithm, namely AdaBoost-BPANN, and we compared it with the commonly used algorithms. Experimental results showed the optimum model was achieved by AdaBoost–BPANN algorithm with *RMSEP* = 7.7124 mg/100 g and R = 0.8915 in the prediction set. This work sufficiently demonstrated that the colorimetric sensors array has a high potential in non-destructive sensing chicken's freshness, and AdaBoost-BPANN algorithm has a strong performance in solution to a complex data calibration.

Introduction

Consumption of chicken has increased in many countries, as it is not only a health conscious diet but also relatively inexpensive protein source, which is ideally suited to the many forms of convenience foods in China [1]. The demand of chicken is increasing every year, which makes its freshness a major concern. Recent incidents about food-borne illnesses have been a global food safety problem. Since there has not been many useful instruments (simple, rapid and handy, low cost and accurate) for quality control concerned with chemical measurement, it seems that the food-borne illnesses might actually be caused due to the poor quality control and quality test measures after all [2]. At present, quality control measurement in the meat industry is mostly done by two methods in order to evaluate meat freshness. One is the chemical and microbial measurement like total viable bacterial counts (TVC) and total Volatile Basic-Nitrogen (TVB-N); the other is by sensory evaluation that involves the estimation of organoleptic attributes with the help of skillful experts [3] . The former is a very objective method, but also destructive method that takes 2–5 days to obtain results. This means that the method cannot simultaneously evaluate correct meat freshness when the meat is sold. The latter is very rapid but costly method. It is also very difficult for this method to evaluate slight differences in the meat freshness before the initial stage of putrefaction. The TVB-N content in chicken, as an important reference index, has been being used to evaluate chicken's freshness[4, 5]. TVB-N compounds in chicken contain mainly ammonia, trimethylamine (TMA) and dimethylamine (DMA) and the levels of TVB-N compounds increase with spoilage by either bacterial or enzymatic degradation. This method is however extremely time consuming, expensive and destructive, it is also not competent with modern industrial processing and production technologies. Therefore, the quality

control in the meat industry demands the development of a freshness sensor, which can measure the meat freshness in situ, rapidly, simply, accurately and most preferentially in a non-destructive manner.

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[6-8]. 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. 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 nanocomposites (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.

At present, a novel low-cost colorimetric sensor array is being probed, which is not sensitive to humidity due to the hydrophobicity of the sensor materials and sensors plate [9] . Recent studies show that the colorimetric sensor array is one of the very low cost, rapid, and non-destructive quantitative measurement methods to predict the chicken freshness. The design of the colorimetric sensor array is based on two fundamental requirements: (1) the chemo-responsive pigment must contain a centre

(functional group) to interact strongly with analytes, and (2) this interaction centre must be strongly coupled to an intense chromophore. Chemo responsive pigments are those pigments that change color in either reflected or absorbed light, upon changes in their chemical environment [3, 10]. The basic principle of this method is the utilization of the color change induced by reaction between volatile compounds and an array of chemical dyes upon ligand binding for chemical vapor detection and differentiation. Chemical dyes are often selected according to their sensitivity to the specific volatile organic compounds (VOCs). For example, metalloporphyrin is a natural choice for the detection of volatile organic vapors [11, 12]. A colorimetric sensor array is usually fabricated by printing the selected chemical responsive dyes on a reverse phase silica gel plate. The array responds to the selective and specific interactions between the VOCs of interest and the chemically responsive dyes. A color change profile for each sample can be obtained by differentiating the images of the sensor array before and after exposure to the VOCs of objects [13]. Thus the colorimetric sensors array has a specific colorific fingerprint to (volatile organic compounds) VOCs released from chicken samples that can be successfully used to evaluate the chicken freshness with the help of multivariate calibration [14]. However, most studies focused on the qualitative classification of meat freshness using the colorimetric sensor, and few studies on the use of colorimetric sensors in quantifying meat freshness indictor using are reported up to date[15, 16].

This study attempted to quantify TVB-N content non-destructively in chicken using a colorimetric sensors array with the help of multivariate calibration. In addition, we attempted to compare different multivariate calibration algorithms to construct model for TVB-N content prediction. Moreover, the performance of the final model was evaluated according to root mean square error of prediction

(*RMSEP*) and correlation coefficient (*R*) in the prediction set.

Materials and Methods

Sample preparation

The chicken breast fillets were purchased from the local Auchan supermarket and brought to our laboratory within 20 min. Then fillet was cut into a piece each weighing approximately 25 gram with the dimension of (4 × 4 ×1) cm. Such 72 samples were put in a sealed plastic bag and stored in a refrigerator at 4°C before further analysis. For following 9 days, samples were randomly taken out from the refrigerator to determine its TVB-N content every another day (i.e. 1^{st}, 3^{rd}, 5^{th}, 7^{th} and 9^{th} day).

Fabrication of colorimetric sensor array and data acquisition

In our previous experiments[15, 17], many materials were tested to choose the optimum chemically responsive dyes. Eventually nine metalloporphyrins materials and three pH indicators were accepted in this work, for details, see supplementary information. As previously implied, the design of a colorimetric sensor array is often based on two fundamental requirements: (1) each chemical responsive dye must contain a center to interact strongly with analytes and (2) the interaction center must be strongly coupled to an intense chromophore. This explains the use of specific 4×3 sized sensor array. The detailed steps of fabricating colorimetric sensor were arranged as follow: (1) each chemically responsive dye (20 mg) was dissolved in 10 mL of chloroform solution. The mixture was preprocessed for 2 hour by ultrasound at room temperature, and eventually obtained 12 kinds of pigments solution. (2) Each pigment solution was spotted on C2 reverse phase silica gel plates (Merck KGaA, Frankfurter, Germany) using 0.1 μL microcapillary tubes. (3) Once printed, the arrays

were stored in a nitrogen-flushed glove bag before the further usage in this experiment. Eventually, we can get a 4×3 colorimetric sensor array consisting of 12 chemically responsive dyes by the above method. The images of sensor array were captured by 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 exposure to the chicken sample that was considered as an 'initial image'. In this experiment, the sensors array chip was mounted in an inert platform inserted into the lid of the closed glass vessel and the chicken sample was placed in the 250 mL glass vessel for its contact with the array. The exposure was made with the help of ventilator support. The sample was stored at 4°C before data acquisition but the ambient temperature was controlled at 25°C while sampling. The typical diagram of the experimental setup of colorimetric sensory system for data acquisition is shown in Fig. 1.

[Here insert Fig. 1]

The uniform arrangements were made for parameters: the ambient temperature, the volume of sample, the size of Petri dish and the headspace time. On complete equilibration, the sensors array from the glass vessel was taken out to rescan and achieve a "final" image. Finally, a colorful difference image was obtained by simply subtracting the "initial" image from the "final" image; the difference image provided a color change profile and that is a characteristic fingerprint to volatile oxidative compounds (VOCs) in chicken 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 were at complete equilibration after 5 min. Therefore, the reaction time was set to 5 min in this work. To avoid factitious non-

uniformity, the center of each dye spot (a round area consisting of 800 pixels) was averaged.

Reference measurement

TVB-N content in chicken was measured by a steam distillation method, as per to the Chinese standard GB/T 5009.44 [18]. After scanning initial and final images of the chicken meat samples, fat was removed from the tissue samples and passed three times through a meat grinder with 4 mm holes. Ten grams of the ground chicken was taken into a beaker, and blended with 100 mL distilled water, and impregnated still for 30 min and shook the beaker every 10 min. Next, the solution was centrifuged at 3000 rpm for 10 min, and the homogenate was filtered through the filter paper. Five milliliter of filtrate was made alkaline by adding 5 mL of 10 g L^{-1} magnesia (MgO). Steam distillation was performed using Kjeldahl distillation unit (Shanghai jianqiang glass Co., China) for 5 min. The distillate was absorbed by 10 mL of 20 g L^{-1} boric acid, and then titrated with 0.1 mol L^{-1} HCl. TVB-N content was calculated and expressed with a unit of mg/100 g.

Multivariate calibrations

Due to a dynamic process for chicken meat spoilage, the relationship between the freshness of chicken meat and these characteristic variables from the sensors array is very complicated. Therefore, we proposed a novel algorithm for modeling in this work, which is back propagation artificial neural network (BP-ANN) and adaptive boosting (AdaBoost) algorithm, namely AdaBoost-BPANN.

The AdaBoost algorithm, short for Adaptive Boosting, introduced by Freund & Schapire (1995) [19, 20], solved a lot of practical problems related to the earlier boosting algorithms. The advantages of AdaBoost include less memory and computational requirements [21]. Boosting is a method of combining performances of weak learners to build a strong classifier that performs better than any of

the individual weak classifiers does. A weak learner is a simple rule whose classification accuracy may be just slightly better than a random guess. Enhanced performance of the resulting combined classifier is due to added weights given to training examples, which are difficult to classify. AdaBoost is a machine-learning algorithm that is often used in conjunction with many other weak learning algorithms to improve their performance forming a strong classifier, which is sensitive to noisy data and outliers. In general, more improvements can be accepted when the classifiers are diverse and yet accurate [15]. In this study, we use the BP-ANN (i.e. an input layer, a hidden layer and an output layer) as the weak learning algorithm for AdaBoost and name it AdaBoost-BPANN prediction algorithm. The detailed steps of AdaBoost-BPANN algorithm are arranged as follows:

1) Initialization: m indicate the training dataset, the weight of this initial distribution on training example X is denoted as $D_1(i) = 1/m$, determine the prediction error threshold(Φ), and then configure the initial BP neural network parameters based on the input and output dimension.

2) Train weak learner: t=1 …T, T is the size of the weak learner. when trained the t weak learner, first, using the training dataset to train the BP neural network and obtain the hypothesis $h_t(x)$, then to calculate the error of h_t:

$$er(i) = \left| h_t(x_i) - y_i \right|, \quad (1)$$

3) Calculate the weight of weak learner w_t:

$$\varepsilon_t = \sum_{i:er(i)>\Phi} D_t(i), i = 1,2,\cdots,m. \quad (2)$$

$$w_t = 0.5 \times \ln(\frac{1-\varepsilon_t}{\varepsilon_t}) \quad (3)$$

4) Setting the D_{t+1} :

$$D_{t+1}(i)=\begin{cases}\dfrac{\exp(w_t)\times D_t(i)}{B_t}, er(i)>\Phi \\ \dfrac{D_t(i)}{B_t} i=1,2,\cdots,m.\end{cases} \quad (4)$$

$$B_t=\frac{1}{\sum_{i=1}^{m} D_{t+1}(i)} \quad (5)$$

Where B_t is the normalization factor and Φ is a threshold.

5) Output the strong learner $F(x)$:

$$F(x)=\sum_{t=1}^{T} w_t \times f(h_t(x), w_t). \quad (6)$$

In addition, to highlight the performance of AdaBoost-BPANN in the solution to complicated data regression, we compared it with the three commonly used regression algorithms, which are partial least square (PLS), genetic algorithm-partial least squares (GA-PLS) and back propagation artificial neural network (BP-ANN).

All data algorithms were implemented in Matlab R2009b (Matworks Inc., Natick, MA, USA) on Windows 7.

Results

Reference measurement results

TVB-N content of the 72 chicken samples was determined by Steam distillation method according to Chinese Standard GB/T 5009.44 (2003). The reference measurement result of TVB-N content for all samples is as shown in Fig. 2. The graph shows the change in TVB-N content of chicken samples in various storage day; the TVB-N values were ≤ 12 mg/ 100 gm after a day of storage, ≤ 32 mg/ 100 gm for 3rd day. These results approve the meat freshness and suggest that the chicken meat can still

be fresh until 3 days when stored at 4 degree Celsius. By the 5th day of storage, the values increased up to 55.4 mg/ 100 gm and up to 63 mg/ 100 gm after 7^{th} day, which points to the spoilage of chicken in a severe way.

[Here insert Fig. 2]

All 72 samples were divided into 2 subsets. One was called the calibration set in which all samples were used for calibrating model, and the other was called the prediction set in which all samples were used to test the model. To achieve a robust model, the selection of samples was done by first sorting all samples according to their respective TVB-N contents. Selection of samples into the prediction set was done by leaving one sample out of every three samples. Ultimately, the calibration set contained 48 samples and the prediction set contained 24 samples. As shown in Table 1, the range of Y-value in prediction set can be covered by the range in the calibration set. Thus, the samples in the prediction can be used to test the robustness of the final model.

[Here insert Table 1]

Colorimetric sensor responses

Fig. 3 shows the difference images of sample obtained after subtracting its initial image from the final image. Each difference image has its specific colorific fingerprint. The difference image is a RGB color image consisting of three color components images (i.e. R image, G image, and B image). 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). The RGB image is an 8-bit image and the range of color values is [0 255]. A difference map is easily generated by digital subtraction, pixel by pixel, of the image of the array before and after exposure.

[Here insert Fig. 3]

Colorimetric sensor detects the odor changes of chicken samples during spoilage, which was produced by decomposition of the main internal chemical ingredients like protein, fat and carbohydrates. As day passes by, microbial spoilage of chicken sample occurs during which a wide variety of volatile organic compounds (VOCs): hydrogen sulfide, dimethyl disulfide, indole, lactic acid, acetic acid, other fatty acids (propionic, isobutyric, isovaleric, n-butyric), C2-C5 alcohols, C6-C8 hydrocarbons, C3-C4 ketones, diacetyl-acetoin, putrescine, cadaverine, tyramine and other biogenic amines are produced [22]. The metalloporphyrins dyes in the sensors array respond to most of the VOCs during chicken spoilage because of their open coordination sites for axial ligation and easy modification of their molecular structure. The additional dyes of three pH indictors also respond to hydrogen sulfide and the organic acids. Microbial metabolites increased gradually along with the process of sample spoilage, and thus the sensors array has its unique colorific fingerprint to each sample corresponding to its freshness as in Fig. 3.

Prediction results of model

Application of sensors data in solutions to the quantitative problem often depends on a prediction model developed by a multivariate calibration algorithm. The prediction model is often developed using the samples with reference results in the calibration set. The model performance is tested by means of some independent samples from a prediction set. There are numerous regression algorithms for modeling, and how to choose the most appropriate algorithm is of great significance. In this study, we proposed a novel AdaBoost-BPANN algorithm for modeling.

AdaBoost is an ensemble method, which is possible to increase the accuracy of BP-ANN by

averaging the decisions of ensemble of BP-ANN. Adaboost helps us get the best results as it works in a sequential order as; first, we attempt to choose the parameters of weights associated with a given family of functions called weak hypotheses in the boosting literature . It is usually described as a procedure that works together with a subroutine called the weak learner i.e. BP-ANN in our work. On each series of rounds, the weak learner picks a weak hypothesis. It always chooses the weak hypothesis with smallest error rate i.e. with the smallest weighted number of mistakes relative to a distribution over training examples selected by AdaBoost [23]. Then, AdaBoost sequentially updates these parameters one by one and on each of a series of iterations, a single feature is adjusted. Therefore, in this study, we proposed a novel algorithm Adaboost-BPANN to construct the model for TVB-N content prediction. The number of PCs and the prediction error threshold (Φ) have a significant effect on the Adaboost-BPANN model, thus, they were optimized by cross-validation, and determined by the lowest *RMSEP*. Firstly, the threshold (Φ) was optimized in a lager scope, and we find that when the parameter (Φ) was selected within 0.01-0.19, the model is ideal. Fig. 4 (a) shows the RMSEP of Adaboost-BPANN model with different PCs and thresholds. From Fig. 4 (a), the lowest RMSEP could be obtained when the 3PCs and Φ=0.13 were included. Eventually, the optimum Adaboost-BPANN model was achieved with *Rp* = 0.8915, and *RMSEP* = 7.7124g/100 mL in the prediction set. The scatter plot between reference measurement of TVB-N content and Adaboost-BPANN predicted results is shown in Fig. 4 (b).

[Here insert Fig. 4]

Discussion

To highlight the good performance of the Adaboost-BPANN algorithm, we attempted to compare it

with three commonly used algorithms- partial least square (PLS), genetic algorithm-partial least squares (GA-PLS) and back propagation artificial neural network (BP-ANN) in this work. Details about PLS, GA-PLS and BP-ANN can refer to some literatures[24-28]. Table 2 shows the results from PLS, GA-PLS, and BP-ANN approaches used in this study.

[Here insert Table 2]

The main reason of the above-mentioned results is that spoilage of meat is rather complex processes, where the nonlinear growth of microbiology generates the nonlinear accumulation of metabolites. The selected dyes in the sensors array have non-specific sensitivity and wide cross-sensitivity toward volatile compounds. Each dye in the sensors array could be simultaneously sensitive to numerous volatile compounds, and 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., GC and GC-MS), and is difficult to assign specific colorific profile to a specific volatile compound. Moreover, the chemical reactions between the colorimetric dyes and the VOCs of metabolism are also extraordinarily complicated. Based on the reasons mentioned above, it is difficult for the simple linear algorithm to quantify the chicken freshness.

The other significant reason of the results can be illustrated from the theory of algorithms. First, BP-ANN and Adaboost-BPANN are nonlinear statistical learning algorithms, PLS and GA-PLS are linear approaches. Nonlinear models, with a stronger capability of self-learning and self-adjust, can handle the complex problem as this work, so it can give better results than linear models[29, 30]. The results by BP-ANN and Adaboost-BPANN algorithms are, therefore, better than results by PLS and GA-PLS algorithms. Secondly, in contrast to BP-ANN and Adaboost-BPANN, the algorithm of

Adaboost-BPANN shows its advantages in solution to complex data. BP-ANN as a nonlinear regression tool, has solved the complex problem effectively, nevertheless, although BP-ANN has proved its powerful capability in quantitative analysis, it also has its own deficiencies, which may lead to the following problems: (1) local minimum problem, (2) decreased rate problems, and (3) relatively low stability. Detailed demonstration of these three problems can be referred to[31, 32]. In terms of the above problems, an Adaboost-frame based on BP-ANN algorithm, namely Adaboost-BPANN, was attempted to enhance the performance of the BP-ANN model. It constructs a more powerful prediction system by developing a sequence composed by original forecasting algorithm. In this case, BP-ANN is used as weak predictor, aiming at developing a new predictor based on Adaboost algorithm, which takes the influence of weights into consideration, and increases the iteration time apparently. AdaBoost includes less memory and computational requirements. The enhanced performance of the resulting BP-AdaBoost is also due to added weights given to training examples by BP-ANN, which are difficult to classify. AdaBoost has been used in conjunction with weak learning BP-ANN algorithm to improve its overall performance by forming a strong joint classifier, which is sensitive to noisy data and outliers. Generally, more improvements can be accepted when the classifiers are diverse and yet accurate. Hence, Adaboost-BPANN model has achieved much better performance and reliability in contrast to BP-ANN regression tool as can be seen in Table 2.

Conclusion

We fabricated a novel colorimetric sensor array using printing 12 chemically responsive dyes on a 4 ×3 C2 reverse silica-gel flat plate in this work. A novel colorimetric sensors array was developed and

successfully used for quantifying the TVB-N content in chicken with the help of multivariate calibration. Besides, the AdaBoost-BPANN algorithm, in contrast to commonly used multivariate algorithms, showed its excellent performance and reliability. It can be concluded that the colorimetric sensors array has a high potential in quantifying of TVB-N content in chicken with the help of a suitable multivariate calibration.

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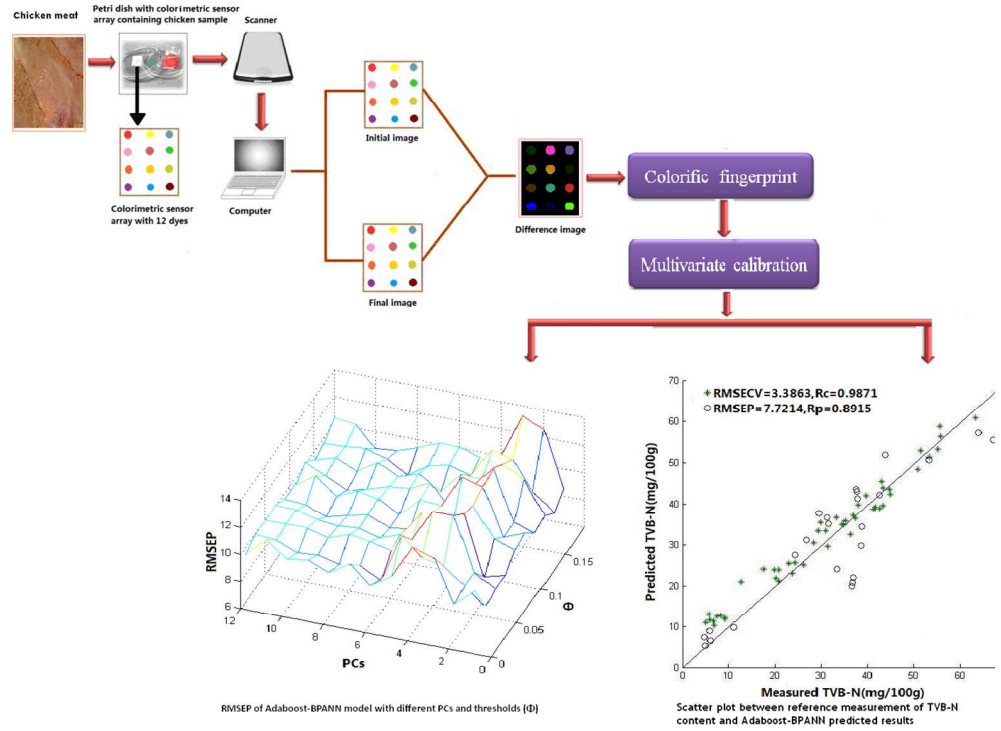
Figure Captions

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 The difference images of sample obtained after subtracting its initial image from the final image

Fig.4 The RMSEP of Adaboost-BPANN model with different PCs and thresholds (Φ) (a) Scatter plot between reference measurement of TVB-N content and Adaboost-BPANN predicted results (b)



RMSEP of Adaboost-BPANN model with different PCs and thresholds (Φ)

Scatter plot between reference measurement of TVB-N content and Adaboost-BPANN predicted results

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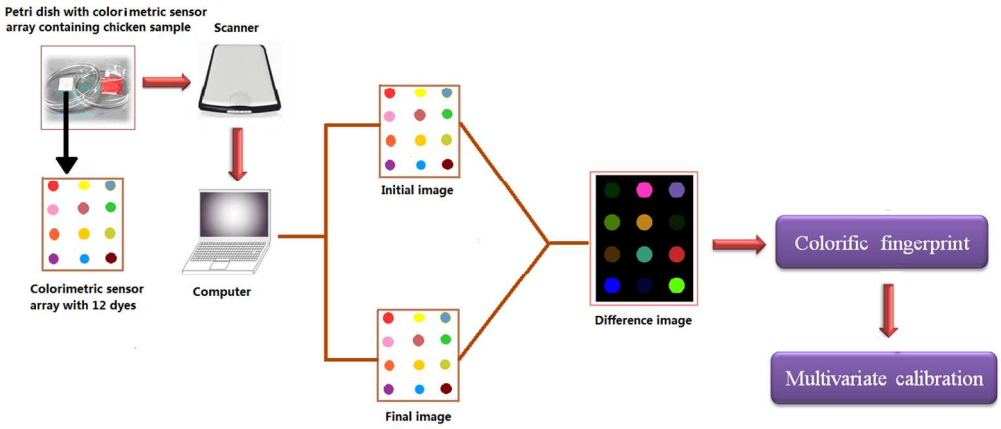

Schematic diagram of E-Nose System based on colorimetric sensor array
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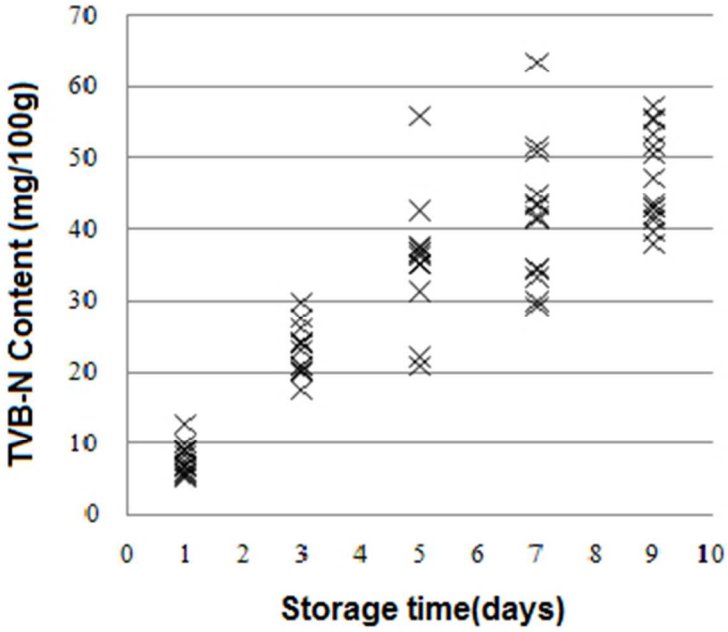
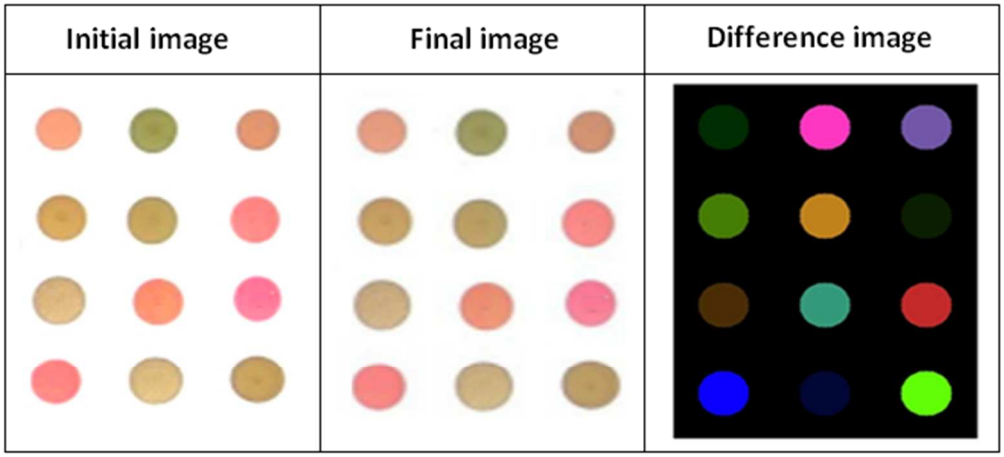
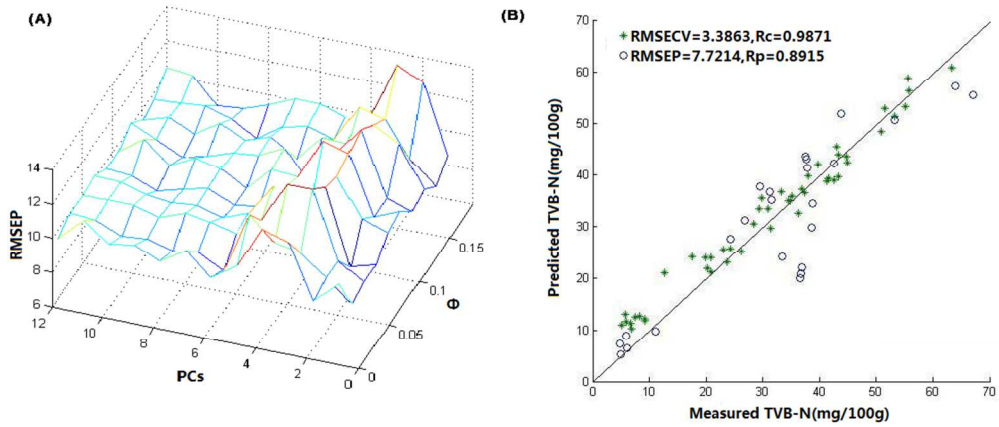


Reference measurement results of TVB-N Content for all samples
112x97mm (96 x 96 DPI)



The difference images of sample obtained after subtracting its initial image from the final image
174x80mm (96 x 96 DPI)



The RMSEP of AdaBoost BPANN with different PCs and thresholds (Φ) (a) Scatter Plot between reference measurement of TVB-N content and AdaBoost-BPANN predicted results (b)
297x128mm (96 x 96 DPI)

Table 1 Reference measurement of TVB-N content of pork samples in calibration and prediction set

Subsets	Sample number	Range (mg/100g)	Mean (mg/100g)	Standard deviation (mg/100g)
Calibration	48	5.04~63.27	30.83	15.92
Prediction	24	5.29~57.12	30.89	15.96

Table 2 Comparison of the results from four multivariate calibration models

Models	Calibration Set		Prediction Set	
	R_c	RMSECV	R_p	RMSEP
GA-PLS	0.8543	8.47	0.8454	8.89
PLS	0.7805	10.2	0.8093	9.75
BP-ANN	0.8936	6.5645	0.8324	7.7045
BP-AdaBoost	**0.9870**	3.3863	**0.8915**	7.7124