PREDICTION OF BEEF FRESHNESS USING A HYPERSPECTRAL SCATTERING IMAGING TECHNIQUE
/
利用高光谱散射图像技术预测牛肉新鲜度

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ABSTRACT

A rapid and non-destructive method based on hyperspectral scattering technique for determination of beef freshness (i.e.,TVB-N) was studied in this study. Hyperspectral images of 60 beef samples stored at 4°C for 1-18 days were acquired using a VIS/NIR hyperspectral imaging system in the wavelength range of 400-1100 nm, and the scattering profiles of every wavelength were fitted to a Lorentzian distribution function to give three parameters a (asymptotic value), b (peak value) and c (full width at b/2). The single parameters (a, b or c) or their combined parameters (a+b/c or (b-a)/c) were used to develop least square-support vector machine (LS-SVM) models for prediction of beef freshness. The performance of a LS-SVM model developed using (b-a)/c was the best among all the tested models, showing R_c=0.91, R_p=0.86, SEC=5.83 mg/100 g and SEP=5.21 mg/100 g. A genetic algorithm was used to optimize the parameter (b-a)/c for developing a GA-LS-SVM model, which performed best showing R_c=0.97, R_p=0.96, SEC=3.38 mg/100 g and SEP=3.85 mg/100 g. This study provided a new non-destructive method based on a hyperspectral imaging technique combined with a genetic algorithm for rapid prediction of beef freshness.

摘要

利用高光谱散射技术预测牛肉挥发性盐基氮（TVB-N），实现了牛肉新鲜度的无损快速检测。用可见近红外高光谱成像系统，获取储藏在4°C下1~18天60个牛肉样品的400~1100nm波长范围的高光谱图像，利用洛伦兹分布函数拟合各个波长处的散射曲线，获取不同波长处散射曲线的洛伦兹参数。利用洛伦兹单参数a、b、c及组合参数a+b/c、(b-a)/c建立TVB-N的最小二乘支持向量机（LS-SVM）预测模型，结果显示组合参数(b-a)/c所建模型的预测精度最佳，校正集和预测集的预测相关系数和标准差分别为0.91、0.86和5.83mg/100g、5.21mg/100g。用遗传算法(GA)对组合参数(b-a)/c进行优选后重新建GA-LS-SVM预测模型，校正集和预测集的预测相关系数分别提高到0.97、0.96，标准差分别降低为3.38mg/100g、3.85mg/100g。研究结果表明，用高光谱散射特征结合遗传算法能够很好地预测牛肉的TVB-N，为实现牛肉新鲜度的无损快速检测提供了一种新方法。

INTRODUCTION

Beef is a kind of high-value nutritional meat. Because it is important in human diet and highly valued by consumers, beef consumption shows a trend of increase in meat product market *(Ortega et al., 2016)*. Freshness is one important quality of beef, which influences beef consumers' purchase choice and the diet's safety. With the improvement in living standards and life quality, beef consumers show more concerns about the evaluation and grading of beef freshness *(Owusu-Sekyere et al., 2014).* During the course of processing, circulation and marketing of fresh beef, many factors such as environment temperature and microbial breeding can influence beef freshness, making beef physico-chemical characters change with time. Under influences from bacteria and enzymes, beef proteins may be decomposed into basic nitrogen substances such as amines. Those basic nitrogen substances, when subjected to organic acids generated in beef decomposition processes, may form salt-ground nitrogen substances that gather in the meat. Those kinds of substances are volatile and referred to as total volatile base nitrogen (TVB-N). With the increase of storage time and corruption degrees of meat, meat TVB-N content increases gradually. Meat TVB-N content can reflect the freshness of meat. TVB-N content in fresh meat and in metamorphic meat differs significantly, and the content difference is in agreement with consumers' sensory evaluation. Therefore, TVB-N content is an objective criterion for the evaluation of meat freshness. China's current food hygiene evaluation criterion considers TVB-N content as the only indicator for the determination of meat freshness. Therefore, TVB-N content is an important indicator for the determination of beef freshness, and now in China beef freshness is graded according to TVB-N content as required by China's national standard GB2707-2005. However, the detection of beef TVB-N is currently conducted with traditional physico-chemical testing methods showing some limitations such as time-consuming processes and sample-destructive processes as well as influences from artificial factors. The traditional testing methods fail to meet the requirements for rapid, non-destructive and automated detection of beef TVB-N.

Spectrum-based detection methods are convenient, rapid and non-destructive with good measurement reproducibility, and have been widely used in the detection of fresh meat quality and safety *(Peng and Zhang, 2013; Wang et al., 2013; Liu et al., 2016; Lohumi et al., 2015)*. Some studies reported the detection of meat freshness based on near-infrared (NIR) spectrum *(Hou et al., 2006; Xu et al., 2009)*, where the investigators carried out preliminary studies on the detection of meat freshness by using NIR. *Cai et al. (2009; 2011)* predicted pork TVB-N content by using NIR spectrum in two studies, where the prediction correlation coefficients (R_p) were 0.823 and 0.808, respectively. *Ma et al. (2012)* developed a prediction model based on partial least squares-support vector machine (LS-SVM) and predicted beef pH with non-destructive visible and near-infrared spectrum, achieving a good prediction result with a R_p of 0.935 and a *SEP* (standard error of prediction) of 0.111. *Morsy et al. (2013)* developed robust linear and non-linear models of NIR spectroscopy for detection and quantification of adulterants in fresh and frozen-thawed minced beef, which were mixed with pork, beef offal and beef fat trimming, but there are some limitations in the NIR application. Hyperspectral imaging is a new detection technology that can provide many spatially resolved spectral data in a sample, and can comprehensively reflect the surface and internal characteristics of the sample. The hyperspectral imaging technology has been used with high precision and accuracy for the determination of chemical constituents or quality attributes in food and agricultural products *(Gowen et al., 2007; Wu and Sun, 2013; Xie et al., 2015; Kamruzzaman et al., 2016)* and has been used to detect meat quality attributes in recent studies *(Wu et al., 2009; El Masry et al., 2012; Li et al., 2015; Yang et al., 2017). Zhang et al. (2012)* predicted pork TVB-N with a correlation coefficient (R_v) of 0.90 and a *SEP* of 7.80 by using reflectance spectra of hyperspectral images. Spatially resolved hyperspectral scattering profiles were used to predict pork tenderness, *Escherichia coli* contamination in pork and microbial spoilage of beef *(Tao et al., 2012; Peng et al., 2011)*. The hyperspectral scattering technique were used to predict fresh beef tenderness (expressed in terms of the values of Warner-Bratzler Shear Force or WBSF) and color parameters (L*, a*, b*), showing R_{cv} of 0.91 for beef WBSF, and R_{cv} of 0.96, 0.96 and 0.97, respectively, for colour parameters (*Wu et al., 2012*). The aforementioned research examples show that hyperspectral scattering techniques can predict meat quality attributes. However, studies about prediction of beef freshness based on hyperspectral scattering imaging have not yet been reported. This study aimed to build a hyperspectral detection system for beef freshness measurement. In this system, hyperspectral images of fresh beef would be collected and analyzed for extraction of hyperspectral images scattering characteristics that would be used to establish a prediction model for determination of fresh beef TVB-N and beef freshness.

MATERIAL AND METHOD

Hyperspectral imaging system

A hyperspectral imaging system in the wavelength range of 400-1000 nm was established and used to acquire the images of beef meat samples in this study, and is sketched in Fig. 1. The hyperspectral imaging system mainly consisted of a high-performance back-illuminated 12-bit charge-coupled device (CCD) camera, an imaging spectrograph, a light source unit equipped with optical fibers, a computer installed with a data acquisition and control software, a motor, a screw, a sample holder and a shield case. The imaging system was enclosed in the shield case in order to minimize effect of ambient light. The optical fiber functioned as a 150-W point light source in the imaging system, and the diameter of the point light beam was 5 mm. The system worked in a line scanning mode, and all scans were achieved by scanning sample positions at a 4 mm distance from the incident light centre to avoid signal saturation on the CCD detector. The spectral resolution of the imaging system was 2.8 nm. Any image obtained with this system comprised 1376×1040 (spatial×spectral) pixels.

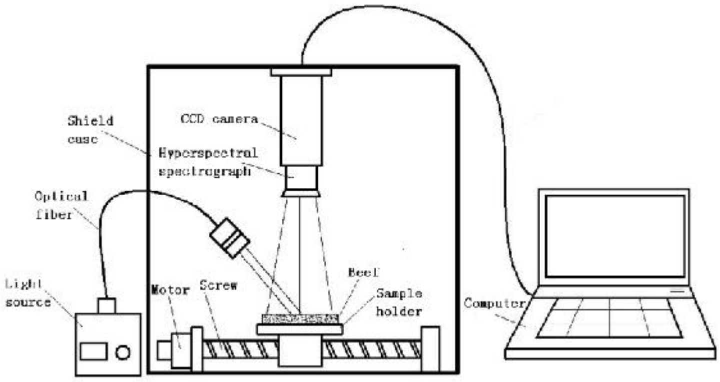


Fig. 1 - The sketch of the hyperspectral imaging system

Fig. 2 - The view of the test system

Experimental procedures

Sample preparation

A fresh beef meat product was purchased from a local supermarket on the day of the experiment and immediately transported to a lab under refrigeration. Sixty beef samples were aseptically prepared by trimming the meat into 60 pieces each in a uniform size of 8 cm×5 cm×2.5 cm (length×width×thickness). The 60 samples were packed separately in commercial food-grade polyethylene bags and placed orderly in a refrigerator at 4°C for 1-18 days. According to characteristics of beef corruption, the time period of experiment was set to be 18 days. During the early 6 days, one sample was randomly withdrawn for the hyperspectral imaging and reference TVB-N analysis with 12-h time intervals. During the late 12 days, two samples were randomly withdrawn for the hyperspectral imaging and reference TVB-N analysis with 12-h time intervals.

Acquisition of hyperspectral images

Before acquiring the hyperspectral imaging and reference TVB-N analysis in each experiment, the beef samples were removed from the polyethylene bags and placed in the air for 20 minutes, which could allow the beef surface's moisture to volatilize minimizing moisture effect on the measurements. In order to eliminate dark current effect of the imaging system, dark images were first collected by covering the camera lens before the imaging of each beef sample. Before imaging, the object distance was measured by a vernier caliper and maintained at a preset value by fine tuning of the vertical stage. For any sample, six positions were selected for imaging, and the mean of the six images was used to denote the hyperspectral image for that sample. In order to improve the signal-to-noise ratios, 2×2 binning was performed on the original images for each sample. Therefore, the resulting image size of each sample was 688×520 (spatial×spectral) pixels. All the acquired sample images were saved in TIFF format for further analysis.

Total volatile base nitrogen (TVB-N) test

Fig. 2 provides a view of the test system. The TVB-N content in the beef samples was measured according to the semi-micro Kjeldahl method as required by China's national standard GB/T 5009.44 for hygienic assessment of fresh and frozen meat of livestock (*Hygiene and Committee, 2003, 2005; Huang et al., 2014*). All beef samples for testing were individually ground using a meat grinder (JYL-C022, Joyong Company Ltd., China). Each ground beef meat sample (10±0.1 g) was placed in a beaker and impregnated with 100 mL of distilled water for 30 min under sporadic beaker shaking every 2 min. The mixture was filtered through a filter paper. Five millilitres of the filtrate was made alkaline by adding 5 mL of 10 g L^{-1} Magnesia (MgO). Steam distillation was conducted for 5 min using a Kjeldahl distillation apparatus (KDY-9820, Jinan Hanon Instrument Co. Ltd., China). The distillate was absorbed by 10 mL of 20 g L^{-1} boric acid and then titrated in triplicate with 0.01 mol L^{-1} HCL. Before the titration of the beef samples' distillate, a blank samples' distillate was titrated in triplicate and the data was subtracted from the above data of the beef samples. The amount of TVB-N in each beef sample was calculated according to eqn. 1.

$$TVB-N(mg/100g)=\frac{(V_1-V_2)\times c\times 14}{m\times 5/100}\times 100 \tag{1}$$

where V_1 is the mean titration volume (in mL) for the test sample measured in triplicate, V_2 is the mean titration volume (in mL) of the blank sample measured in triplicate, c is the concentration (in mol L^{-1}) of HCL, and m is the weight (in g) of the ground beef sample.

Data analysis

Lorentzian function fitting to scattering profiles

When acquiring the hyperspectral images of the beef samples, we illuminated the samples with a small continuous-wave light beam. The hyperspectral scattering image of any beef sample is a diffusely reflected image generated at the sample's surface around the incident light point, and the image was a result of light propagation and light backscattering inside the sample *(Mendoza et al., 2011*). When applying the hyperspectral scattering imaging to the determination of the TVB concentrations, we need to use appropriate mathematical equations to describe the spectral scattering profiles and to reflect the information in the profiles. A Lorentzian function was proposed to fit hyperspectral scattering images and a good fitting result was obtained (*Lu and Peng, 2006*). Following *Lu and Peng* (2006), this study proposed a three-parameter Lorentzian function as shown in eqn. 2 to fit the scattering profiles of the beef samples.

$$I_{wi} = a_{wi} + \frac{b_{wi}}{1+(x/c_{wi})^2} \tag{2}$$

In eqn. 2, I is the light intensity in the CCD count; x (in mm) is the scattering distance measured from the beam incident centre; a is the asymptotic value of light intensity; b is the peak value of the scattering profile at the light incident centre; c is the full scattering width of the scattering profile at one half of the peak value; the subscript w_i represents the i^{th} specific wavelength in the wavelength range of 400-1100 nm where i=1,2,3,…,n and n represents the total number of wavelengths.

Development of Prediction models

In this study, except using individual Lorentzian parameters to develop prediction models for beef TVB-N, we used a combination of the parameters to fully utilize the information associated with the three individual parameters. An LS-SVM model was taken as the prediction model in this study; this kind of model has been widely used as a prediction model and has shown good prediction results in many studies. The details of the LS-SVM model principle can be referred to the study *by Xu et al. (2015)*. Spectra with optimal parameters were defined as best-parameter spectra for developing prediction models. A genetic algorithm method was employed to optimize the spectra variables. In order to reduce effect of noise and light scattering, the best-parameter spectra were subjected to pre-treatment methods such as Multiplication Scatter Correction (MSC) and Savitzky-Golay smoothing (SG). All the data analysis was performed in a Matlab 7.11 software package (Mathworks, Ltd., USA).

Genetic Algorithm

The method of genetic algorithm (GA) is a kind of numerical optimization method based on random search and is particularly suitable for dealing with complex non-linear optimization problems and combination optimization problems. GA begins with a population initialized randomly over the search space of the optimization problem. By simulating the Darwinian evolution principle of "survival of the fittest", GA generates improved approximate solutions or optimal solutions through an iterative procedure. A detailed description of the algorithm can be referred to the literature *(Leardi and Gonzalez, 1998, 2000)*. GA has been successfully used as a feature selection technique, improving predictive ability of prediction models and making it very easy to perform model predictions. In this study, GA-selected feature variables of the best-parameter spectra were taken as input variables for developing prediction models.

Evaluation of model performance

The stability, reliability and dynamic adaptability of prediction models were taken as the evaluation criteria of model performance. As shown in eqns. 3-6, four statistical indices, namely the correlation coefficient of calibration set (R_c), the standard error of calibration (*SEC*), the correlation coefficient of prediction set (R_p) and the standard error of validation were calculated to evaluate the performance of the established models.

$$Rc = \sqrt{\sum_{i=1}^{n_c}(\hat{y}_i - y_i)^2} \Bigg/ \sqrt{\sum_{i=1}^{n_c}(\hat{y}_i - y_m)^2} \tag{3}$$

$$Rp = \sqrt{\sum_{i=1}^{n_p}(\hat{y}_i - y_i)^2} \Big/ \sqrt{\sum_{i=1}^{n_p}(\hat{y}_i - y_m)^2} \quad (4)$$

$$SEC = \sqrt{\frac{1}{n_c - 1}\sum_{i=1}^{n_c}(\hat{y}_i - y_m)^2} \quad (5)$$

$$SEP = \sqrt{\frac{1}{n_p - 1}\sum_{i=1}^{n_p}(\hat{y}_i - y_m)^2} \quad (6)$$

Here, $\hat{y}_i$ is the i^{th} predicted value; y_i is the i^{th} measured value; y_m is the mean of the calibration or prediction set; n_c is the number of samples in the calibration set; n_p is the number of samples in the prediction set. Generally, models with higher R_c and R_p or with lower *SEC* and *SEP* are more satisfactory than models with lower R_c and R_p or with higher *SEC* and *SEP.*

RESULTS

Results of the TVB-N analyses

Sixty beef samples were measured during an 18-day period of storage. Fig. 3 depicts the reference TVB-Ns of all the beef samples tested on each day of the experiment, and shows the change trends of TVB-Ns with time. In Fig. 3, the horizontal axis represents the storage time (day) and the ordinate axis represents the TVB-N content (mg/100 g). The TVB-N trend curve showed the daily mean (with error bars) of TVB-N content tested on each day of the experiment. As shown in Fig. 3, the TVB-Ns content of the reference beef sample stored at 4°C increased with the storage time during 1-18 days of storage, thereby indicating a decrease of the beef freshness with the storage time. After the 7th day, the TVB-N content exceeded 15 mg/100 g, a threshold value for edible beef as mandated in the aforementioned China's national standard, and therefore the beef after the 7th day was no longer edible.

The statistics of the 60 sample reference TVB-N contents are given in Table 1. As shown in Table 1, the maximum TVB-N content was 62.40 mg/100 g and the minimum TVB-N content was 8.64 mg/100 g. The mean value and the standard deviation (SD) were 28.96 and 13.41 mg/100 g, respectively. These data showed a wide range of data coverage for beef meat TVB-N contents, implying that the TVB-N contents measured in this study may represent a complete data set during the course of freshness change in stored beef. Therefore, the freshness prediction model established based on this study's data was likely to be representative.

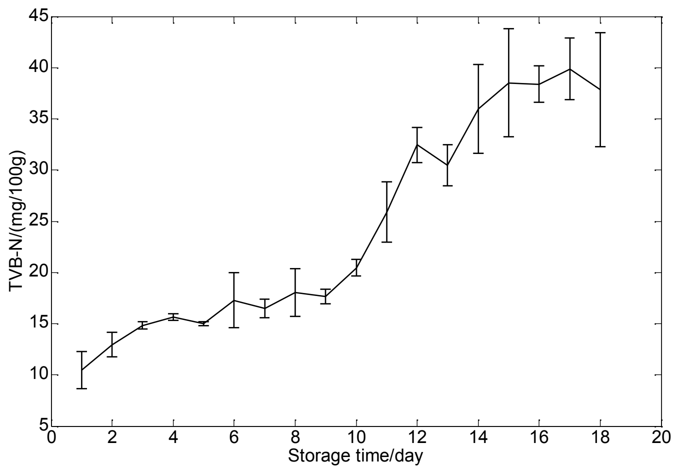


Fig. 3 - Reference beef TVB-N tested on each day of the experiment

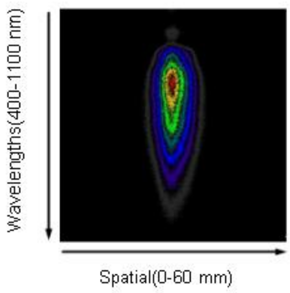
Table 1

A summary of measured beef TVB-N contents (in mg/100g)

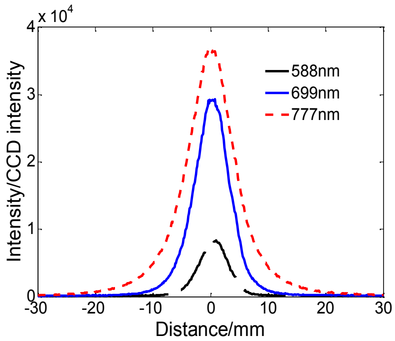
Number of samples	Max	Min	Mean	Standard deviation
60	62.40	8.64	28.96	13.41

Hyperspectral scattering images

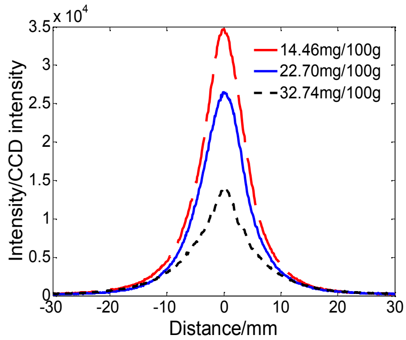
A typical averaged hyperspectral scattering image is shown in Fig.4a.The horizontal axis is a spatial axis and the vertical axis is a wavelength axis. Different colours in the figure represented different reflectance intensities. A vertical line taken from the image represented a spectral profile collected from a particular point of the scanning line at the surface of beef. In essence, each scattering image was composed of hundreds of spectra and each spectrum came from a different point at the beef surface. Therefore, each scattering image reflected the character of beef quality. Fig.4b shows three spectral scattering profiles at 588, 699 and 777 nm wavelength for a beef sample. All the spectral scattering profiles were symmetric about the center point of scanning line, but showed large wavelength-dependence of the scattering intensities. Fig.4c depicts the spatial scattering profiles of different beef samples with different TVB-N contents (14.46, 22.70 and 32.74 mg / 100 g) at the wavelength of 777 nm. The peak intensities of the scattering profiles decreased with the increase of TVB-N, but the half wave bandwidths changed in the opposite direction. To describe the scattering characteristics reflecting the freshness of beef samples, a Lorentz function was used to fit each of the scattering profiles, thereby generating a series of parameters (*a, b* and *c*) that were all wavelength-dependent. The parameters were plotted versus wavelengths to give the best-parameter spectra that were subjected to further analysis, as elaborated below.



a) An averaged scattering image



b) Spectral scattering profiles at 588, 699 and 777nm



c) Scattering profiles of different samples at 777 nm

Fig. 4 - Hyperspectral scattering images of beef

Curve fitting of scattering profiles

The scattering profiles of all the 60 beef samples in the wavelength range of 400-1100 nm were fitted to a Lorentzian function (eqn. 2). Fig. 5 represents the correlation coefficients of the fitting at different wavelengths. As shown in Fig. 5, most of the correlation coefficients for the samples within the wavelength range of 500-1000 nm were greater than 0.95, while the correlation coefficients were lower at wavelengths outside that wavelength range. Therefore, only the data between 500 and 1000 nm were used in the development of beef freshness prediction models.

Fig. 6 showed the extracted Lorentzian function parameters of all the beef samples; different beef samples showed similar patterns of the parameter spectra for the same Lorentzian parameter (*a, b* or *c*) despite the spectra intensity differences among different samples. The three types of parameters spectra contained all scattering profile information in the hyperspectral images of all the beef samples. As shown in Fig. 6, the parameter spectra contained much noise and drifted at the both ends of the wavelength range of 400-1100 nm, thereby indicating that the spectra should be subjected to pre-treatment to improve the data quality prior to the development of prediction models.

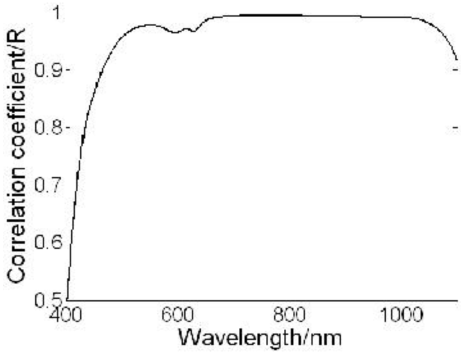
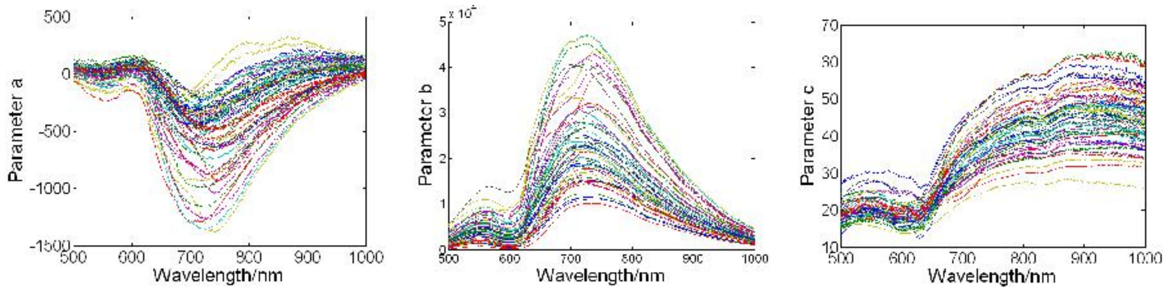


Fig. 5 - Correlation coefficients of fitting at different wavelengths



a) Asymptotic parameter *a* **b) Peak-value parameter *b*** **c) Full-width parameter *c* at *b/2***

Fig. 6 - Lorentzian-fitting parameters for 60 beef samples

Modeling results with full-spectra data

The 60 samples were randomly divided into a calibration set of 45 samples and a prediction set of 15 samples. The 45 calibration samples were used to establish the prediction model, and the 15 prediction samples were used to verify the accuracy and reliability of the prediction model. As mentioned earlier in this study, LS-SVM was used to establish two separate quantitative prediction models, one based on the individual parameters of *a, b, c* and the other based on parameter combinations, namely *a+b/c* and *(b-a)/c*. The prediction results were compared among models established by LS-SVM after three different types of data pre-treatment, namely , the MSC pre-treatment, the SG pre-treatment and the MSC+SG pre-treatment , and it was found that the MSC+SG pre-treatment generated the best results. Therefore, the parameter spectra were all subjected to the MSC+SG pre-treatment prior to the development of the aforementioned two quantitative prediction models. Table 2 shows all the TVB-N prediction results of the LS-SVM models that were established either with the individual parameters or with the parameter combinations. As shown in Table 2, the modeling results obtained with the individual parameter *a* and with the parameter combination *a+b/c* were very close. The models using the individual parameter *b* or *c* over-fitted the data. The modeling result obtained with the parameter combination *(b-a)/c* was the best, reporting R_c=0.91, *SEC*=5.83 mg/100 g, R_p=0.86 and *SEP*=5.21 mg/100 g. This showed that the parameter combination *(b-a)/c* contained more information of the scattering profiles of the samples. Therefore, the parameter combination *(b-a)/c* was taken as the best input variable for establishing the prediction model of beef TVB-N.

Table 2

TVB-N prediction results of LS-SVM models using Lorentzian-fitting parameters

Parameter	R_c	SEC	R_p	SEP
a	0.87	7.13	0.88	5.01
b	0.94	4.83	0.75	6.97
c	0.93	5.24	0.83	6.10
a+*b*/*c*	0.86	7.36	0.86	5.21
(*b*-*a*)/*c*	0.91	5.83	0.86	5.21
(*b*-*a*)/*c(GA)*	0.97	3.38	0.96	3.85

Modeling results with optimized-spectra data by GA

In order to improve the prediction precision, the GA method was used to optimize the parameter combination and select the effective variables for developing the LS-SVM model. In the GA method, the population size of chromosome was set to be 30, the probability of mutation and the probability of cross-over were set as 1% and 50%, respectively, and the number of run times was set to be 100. Because the initial population of GA was generated randomly every time, the GA was randomly run 5 times to select effective variables. Fig. 7 illustrates one among five variable-selection processes, where the selected parameter-combination spectra variables were above the dotted line. On the whole, the GA-selected variables were similar in each of the five selection processes, and were mainly centered at the wavelengths of approximately 630, 710, 930 and 980 nm, greatly reducing the number of variables required for developing the prediction model as well as reducing information redundancy. The optimized variables used to develop the prediction model of TBN-N (referred to as GA-LS-SVM) and the modeling results are shown in Table 2. The prediction results of GA-LS-SVM were relatively satisfactory, as indicated by R_c=0.97, *SEC*=3.38 mg/100 g (Fig.8a) and R_p=0.96, *SEP*=3.85 mg/100 g (Fig.8b). These results also showed that GA was able to extract effective wavelength variables, thereby greatly improving the model's prediction accuracy.

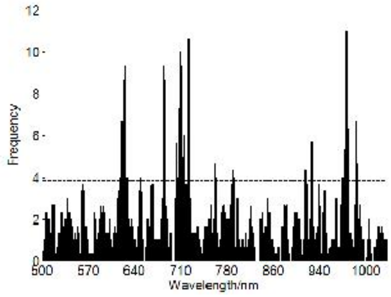
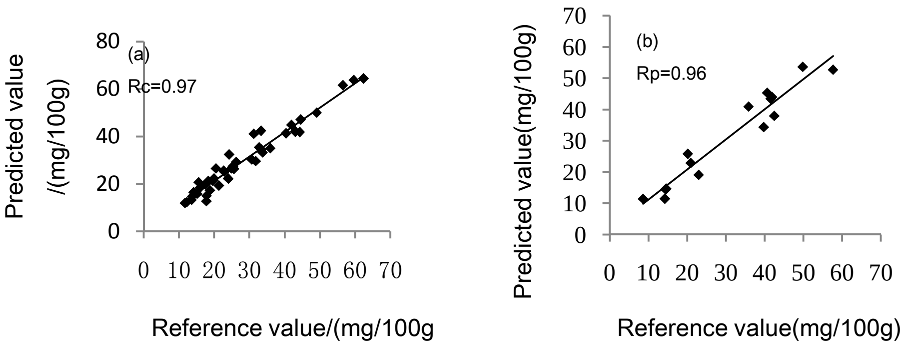


Fig.7 - Occurrence frequency of GA-selected variables



a) calibration set **b) prediction set**

Fig. 8 - Predicted vs. reference values of a GA-LS-SVM model using the optimized *(b-a)/c* parameter

CONCLUSIONS

The study demonstrated that a hyperspectral imaging technique combined with a Lorentzian distribution function could be a rapid and non-destructive tool for prediction of beef meat freshness. A hyperspectral imaging system was used to capture hyperspectral scattering images of fresh beef samples that were stored at 4^{o}C for 1-18 days. Spatial scattering curves were extracted from the hyperspectral images and fitted to a three-parameter Lorentzian distribution function. The fitting-parameters of the Lorentzian distribution function were presented as parameter spectra and used to develop a LS-SVM model for predicting beef TVB-N, an indicator of beef freshness. The primary findings are as follows.

(1) In the wavelengths range of 500-1000 nm, the Lorentzian distribution function could give a better fitting to the scattering curves of the beef hyperspectral images. The beef parameter spectra were obtained and used as input variables for developing prediction models of beef TVB-N.

(2) The MSC+SG pre-treatment method was found to be the best one among the three pre-treatment methods (MSC, SG, and MSC+SG) for developing prediction models. A LS-SVM prediction model of beef freshness was developed. Among the prediction models established with the individual parameters (*a*, *b* or *c*) or with the parameter combinations (*a+b/c* or *(b-a)/c*), the prediction model developed with the parameter combination *(b-a)/c* was the best, showing a correlation coefficient of 0.86 and a standard error of prediction of 5.21 mg/100 g. The variable *(b-a)/c* was optimized by GA and used to develop a prediction model GA-LS-SVM, which showed improved prediction accuracy with R_p=0.96 and *SEP*=3.85 mg/100 g.

This study indicated that hyperspectral scattering imaging combined with Lorentzian distribution functions and genetic algorithms may accurately predict beef TVB-N, providing a promising method for rapid and non-destructive determination of beef freshness. This method is very likely to be applied to real-time detection of meat quality and safety attributes in the future.

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