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Comprehensive Identification of Beef Freshness with Multi-index Using Visible/Near-Infrared Spectroscopy

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Abstract

Freshness is an important quality attribute that affects the quality and safety of meat. Spectral technology has been widely used to detect the attributes of agricultural products. However, the selected freshness indexes and the accuracy of prediction models are the main factors that affect their practical application. Visible (Vis)-near-infrared (NIR) (Vis–NIR) spectroscopy with multi-index was used to realize the rapid and nondestructive detection of beef freshness and improve the accuracy of prediction. The laboratory Vis–NIR spectroscopy system was constructed to collect the reflectance spectrum range of 400–1700 nm of 56 beef samples stored 4 °C for 1–17 days. The changes in the physical and chemical indexes of beef color, total volatile basic nitrogen (TVB-N), pH value, and total bacterial count with time were studied, and which could be taken as the indexes for the comprehensive assessment of beef freshness was studied. The different spectrum pretreatment methods and prediction models of indexes were investigated. Results reveal that the five indexes of meat color (L^* for bright index, a^* for green and red), TVB-N, pH value, and total bacterial count are taken as indexes to evaluate beef freshness. The linear discriminant analysis (LDA) model refers to the comprehensive identification model of beef freshness. The best spectrum pretreatment methods and spectral prediction models of the five indexes are determined. The correlation coefficients of the five aforementioned indexes' prediction model in the verification set are 0.900, 0.890, 0.862, 0.902, and 0.875, and their standard deviations are 2.025, 3.027, 0.183, 7.502 $mg\cdot(100g)^{-1}$, and 0.447 $logCFU\cdot g^{-1}$, respectively. The multi-index comprehensive identification LDA model of beef freshness is verified, and its discriminating rate is verified for 100%. The proposed algorithm provides evidences for the further development of the device for the rapid and nondestructive determination of beef freshness.

Keywords: Beef freshness, Multi-index comprehensive identification, Visible/near-infrared spectroscopy, Nondestructive determination

1. Introduction

Beef is a high-value nutritional meat. Beef consumption shows an increasing trend in meat product market because it is important in human diet and is highly valued by consumers [1]. Due to the nutrient-rich and own environment characteristics of fresh meat, it is prone to corruption and produces toxic and harmful chemicals that seriously affect the quality of meat products and the diet's safety. Therefore, the freshness of fresh meat is an important index in evaluating the quality grade of meat products. With the improvement in living standards and life quality, consumers show more concerns about the evaluation and grading of meat freshness [2]. During the course of processing, circulation, and marketing of fresh beef, many factors, such as environment temperature and microbial breeding, can influence beef freshness and change the beef's physico-chemical characteristics with time. Under the influences of bacteria and enzymes, beef proteins may be decomposed into basic nitrogen substances, such as amines. When subjected to organic acids generated in beef decomposition processes, these basic nitrogen substances may form salt-ground nitrogen substances that gather in the meat. These kinds of substances are volatile and referred to as total volatile base nitrogen (TVB-N). With the increase in the storage time and corruption degrees of meat, meat TVB-N content increases gradually [3]. Meat TVB-N content can reflect the freshness of meat. At the same time, during the course of the process, the physical and chemical attributes of meat, such as the color, pH value, and total bacterial count, which also reflect the meat freshness, are changed correspondingly. At present, consumers judge meat freshness mainly according to their personal experience of sensory evaluation, such as sight, smell, and touch, and the error of evaluation is larger. China's current food hygiene evaluation criterion considers TVB-N content as the only indicator for determining meat freshness. In China, meat freshness is currently graded according to TVB-N content, as required by China's national standard. To evaluate the freshness of meat comprehensively, the physical and chemical indexes, such as TVB-N, pH value, meat color, and total bacterial count, which reflect meat freshness, must be detected. However, the detection of TVB-N and total bacterial count is currently conducted using traditional physico-chemical testing methods that show some limitations, such as time-consuming processes and sample-destructive processes, as well as influences from artificial factors. Traditional testing methods fail to meet the requirements for the rapid, nondestructive, and automated detection of TVB-N and total bacterial count, as well as the highly increasing fast-paced industrial meat sector. Many methods and techniques have been studied to detect meat freshness and attributes nondestructively and rapidly [4]. Scholars evaluated meat

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freshness by estimating the ability of e-nose based on a metal oxide sensor microarray and linear discriminant analysis (LDA) pattern recognition. However, the differences of various kinds of meats supplied by slaughterhouses are large, hence resulting in the need of a large number of meat samples for training the e-nose sensors; moreover, establishing the prediction model is heavy workload [5,6]. A kind of edible sensor was developed, and when the edible sensor was exposed to the headspace above meat samples, clear colorimetric changes were observed as the meat samples degraded and realized the detection of meat freshness. However, the manufacturing cost of this kind of sensor is high, and its wide application is difficult [7]. Spectrum-based detection methods are convenient, rapid, and nondestructive with good measurement reproducibility and have been widely used in the detection of fresh meat quality and safety [8-12]. The dominant information present in the spectra arises from vibrations from overtone and combination bonds in molecular groupings, such as O-H, N-H, C-H, and S-H, which are typically very broad, thereby making it a complex task to identify detailed structure and assign individual features from specific chemical components [13]. The essence of meat quality detection using spectral technique is to establish a spectral prediction model that is established by obtaining meat samples' spectral information and the indexes that reflect the meat quality features. Thus, how to choose the physical and chemical indexes that reflect the meat quality and how to establish a reliable prediction model are important problems to be solved.

Therefore, this study aimed to explore the physical and chemical indexes that reflect the meat quality and the spectral prediction model of these indexes by establishing Vis-NIR detection system and using physical and chemical experiments. This main objective is to determine the indexes that can reflect the freshness of fresh beef and the prediction model of beef freshness comprehensively. The findings of this study can serve as a reference for the rapid and nondestructive assessment of beef freshness and application by using spectroscopic technology.

2. State of the art

Many studies have investigated the spectrum technique for detecting meat quality attributes. Douglas F. B et al. [14] studied the potential applications for the quality control of turkey cuts and processed turkey meat products by using NIR spectroscopy and revealed the effect of chemical composition and quality feature on the spectra. Matthew I. K. et al. [15] classified the pH, tenderness, and intramuscular fat content of Australian lamb by using Vis-NIR spectroscopy with predictive regression model. These models classified the predicted pH, tenderness, and intramuscular fat content at above or below a threshold value with 94%, 98%, and 88% accuracy, respectively. However, the standard error of cross validation (SECV) of intramuscular fat content was higher, and further development is required to improve the SECV for intramuscular fat content. Fazal M. et al. [16] developed a fast analytical method that combined near infrared reflectance spectroscopy and multivariate analysis for the detection and quantification of pork meat in other meat samples. A partial least-squares regression (PLSR) model was built to predict the pork meat contents in other meats, which provided the correlation coefficient R-square (R^2) value of 0.9774 and root mean square error of interactive validation (RMSECV) value of 1.08%. Marina N. B. et al. [17] used Vis-NIR spectroscopy to test intramuscular fat (IMF) and tenderness of Nellore cattle with determination coefficients of calibration models for tenderness ranging from 0.17 to 0.53 and for IMF ranging from 0.12 to 0.14. However, that model wrongly classified all tender samples as tough, and more robust models for the prediction of tenderness must be evaluated. Lorena C. R. S et al. [18] used a portable NIR spectrometer to detect adulteration in ground meat, which were adulterated in the range of 0–100 wt% in binary blends (chicken/beef; beef/pork; pork/chicken) and ternary blends (beef/chicken/pork). The values of R^2 ranged from 0.78 to 0.99 for the binary blends, but only the prediction of beef content was considered good with R^2 =0.98 for the ternary blends. Olga M. M. et al [19] determined the collagen content in ground beef by using near-infrared spectroscopy with $R^2 = 0.82$ and RMSECV = 0.11%, but the prediction error was high. Raquel C. M. et al. [20] used Raman spectroscopy with PLS regression models to predict the Warner–Bratzler shear force (WBSF), IMF, pH, drip-loss, and cook-loss of beef, with R^2 ranging from 0.5 to 0.9. The predictive ability of models of WBSF, pH and cook-loss were low. Gardis J. E. G. et al. [21] developed the prediction models of moisture content and color to monitor the quality with hyperspectral imaging (400–1000 nm) during the drying of beef slices. This model provided non-invasive product monitoring systems for beef drying processes. Eva M. A. et al. [22] developed a prediction model of the bacterial growth on beef Longissimus dorsi (LD) muscle under simulated normal (4 °C) and abuse (10 °C) storage conditions by using Vis-NIR hyperspectral imaging (HSI) technique. This study demonstrated the potential of HSI for real-time monitoring to predict microbial growth on LD along the meat supply chain. Shin S. H et al. [23] classified beef freshness using a deep spectral network fused with myoglobin information. The accuracy of the proposed model improved to 91.9%, and it provides a basis for future studies on the investigation of myoglobin information associated with meat freshness. Ahmed R. et al. [24] investigated the use of Vis-NIR spectroscopy system in the range of 400–1000 nm to assess and estimate plant and animal proteins as potential adulterants in minced beef. Their optimal models for predicting adulterant levels yielded correlation coefficient (R) of 0.78–0.86, and the results illustrate the potential application of spectroscopic technology to detect adulterants in minced beef rapidly and accurately. However, they did not study the prediction models of beef freshness indexes. Liu J. X. et al. [25] established a potential of multispectral imaging analysis in the visible and near-infrared (405–970 nm) regions to identify water-injected beef. The partial least squares regression (PLSR) algorithm was employed to establish prediction models and acquire quantitative estimations of actual water increase with a correlation coefficient (R) of 0.923. Their results demonstrate the capability of multispectral imaging technology as a rapid and nondestructive tool for the identification of water-injected beef. However, they also did not study the prediction models of beef freshness indexes. Maduro D. et al. [26] attempted to predict the moisture, total fat, and crude protein of beef by using NIR spectroscopy. The best calibration for the chemical properties under evaluation showed coefficient of determination and standard error of cross validation (SECV) values of 0.93 and 1.25%, respectively, for fat content, 0.89 and 0.99% for crude protein, and 0.72 and 2.18% for

moisture. The use of NIR spectroscopy provides a good estimate of the fat and protein contents of raw bovine meat. Urmila K. et al. [27] established hyperspectral imaging system and assessed the chicken quality by detecting the TVB-N content of chicken nondestructively. The prediction model of TVB-N content was established by using the classic back propagation artificial neural network algorithm combined with principal component analysis and ant colony optimization (ACO) algorithms. The dimension and number of input variables for predicting model were reduced, and the calculation speed was improved. However, its prediction accuracy is lower, with a prediction correlation coefficients $R = 0.754$ in the prediction set. Stuart O. J. et al. [28] explored the nondestructive classification of different statuses of meat and realized to judge the meat freshness by using Vis-NIR hyperspectral imaging. They illustrated the threshold detection for pH level in beef with different freshness levels. The support vector machine (SVM) classification model of pH was built with an accuracy of 91% for the classification of beef samples with a pH above 5.9. However, it only implemented a qualitative classification of beef freshness. Jiang et al. [29] used and adopted visible and near-infrared hyperspectral imaging system in the near infrared region (400–1000 nm) to assess the final color and pH of broiler breast fillet nondestructively. The PLSR prediction models of color (L^*, a^*) and pH were established. The prediction accuracies of color parameters (L^*, a^*) were achieved with prediction correlation coefficients R of 0.75, 0.87. This study added the prediction of color parameters of meat, but the prediction accuracy color parameters was still relatively low, and the prediction of pH was unsatisfactory. Oto N. et al. [30] investigated the nondestructive evaluation of total bacterial count on pork meat surface stored aerobically at 15 °C for three days by using NIR spectroscopy technique. The PLSR prediction model of total bacterial count was established, and the total bacterial count was predicted with good determination coefficient (0.94–0.97 in calibration and 0.84–0.88 in validation). However, it also only achieved one single prediction model of beef freshness indexes. The author has studied the non-destructive prediction of TVB-N and pH of fresh beef using Vis-NIR spectroscopy. The results show that detecting the index parameters of beef freshness using Vis-NIR spectroscopy is feasible [31, 32].

Although the aforementioned studies have mainly focused on the nondestructive prediction of fresh meat freshness by using spectroscopic technology, these studies mainly focused on the nondestructive prediction of the single index of freshness. Moreover, the prediction accuracy needs to be improved further. The process of fresh meat change, which is influenced by many factors, such as animal carcass, slaughtering process, and transportation condition, is complex. Evaluating meat freshness accurately and comprehensively with only a single index is difficult. The study of meat freshness with multi-indexes is lacking. In the present work, we attempted to develop the classification model of beef freshness with the multi-indexes, such as TVB-N, pH, color, total bacterial count, combined with sensory evaluation, by using Vis-NIR spectroscopy. The spectral prediction models of TVB-N, pH, color, and total bacterial count were established. The multi-index comprehensive, rapid, and nondestructive identification of beef freshness was realized using classification and prediction models.

The remainder of this study is organized as follows. Section 3 describes the methodology. Section 4 makes a discussion on the results. Section 5 summarizes the conclusions.

3. Methodology

3.1 Vis-NIR spectroscopy detection system

In this study, a Vis-NIR spectroscopy detection system in the wavelength range of 350–1800 nm was established and used to acquire the spectrum of beef samples (Fig. 1). The Vis-NIR spectroscopy detection system mainly consisted of a Vis–NIR spectrometer, a light source unit equipped with optical fibers, a computer installed with a data acquisition software, a sample holder, and a shield case. The spectroscopy detection system was enclosed in the shield case to minimize the effect of ambient light. The spectral resolution of the Vis–NIR detection system was 0.8 nm in the visible band and 10 nm in the NIR band.

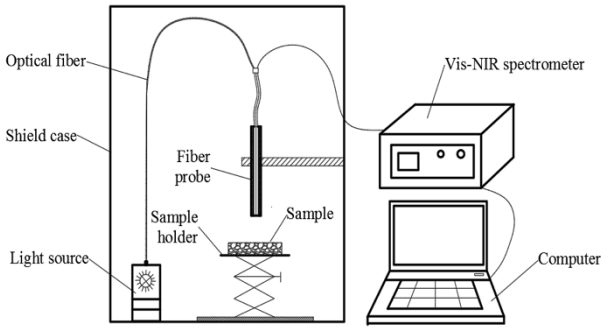


Fig. 1. Sketch of the Vis-NIR detection system

3.2 Sample preparation

Slaughtered fresh beef products were purchased from a local supermarket on the day of the experiment and immediately transported to the laboratory under refrigeration. A total of 56 beef samples were aseptically prepared by trimming the meat into 56 pieces each in a uniform size of 7 cm× 6 cm× 3 cm (length× width× thickness). The 56 samples were packed separately in commercial food-grade polyethylene bags and placed orderly in a refrigerator at 4 ℃ for 1–17 days. According to the characteristics of beef corruption, the time period of the experiment was set to 17 days. During the early 6 days, one sample was randomly withdrawn for the spectrum collection and reference analysis of TVB-N, pH, color, total bacterial count, and sensory evaluation with 12-h time intervals. During the late 12 days, two samples were randomly withdrawn for the spectrum collection and reference analysis of TVB-N, pH, color, total bacterial count, and sensory evaluation with 12-h time intervals.

3.3 Vis-NIR reflectance spectral data collection

The spectral data of the beef samples were collected in reflectance mode with the wavelength range of 350–1800 nm by using the Vis-NIR spectroscopy detection system. Before acquiring the spectral data and reference value analysis in each experiment, the beef samples were removed from the polyethylene bags and placed in air for 20 minutes to allow the beef surface's moisture to volatilize, hence minimizing the effect of moisture on the measurements. Before acquiring data, the sample's distance from the optic probe was measured by a vernier caliper and maintained at a

preset value of 20 mm. For any sample, four positions were selected to collect spectral data, and the mean of the four spectral data was used to denote the spectral data for that sample. The process of acquiring the spectral data of beef samples is shown in Fig. 2.

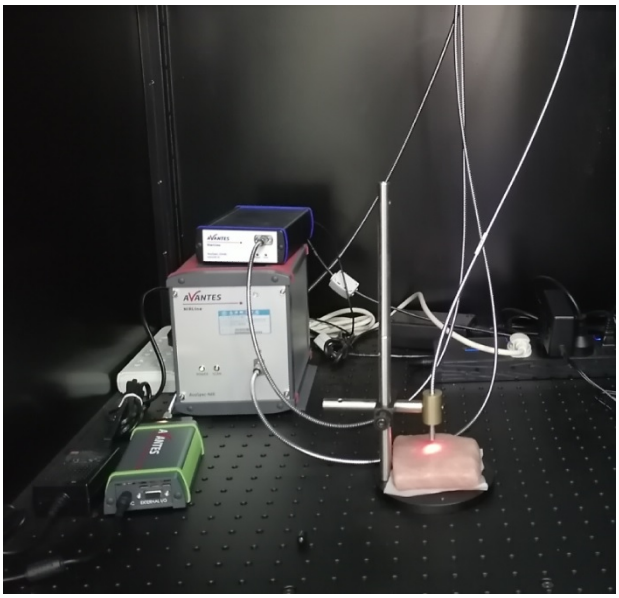


Fig. 2. View of the detection system

3.4 Measurement of reference value and sensory evaluation

3.4.1 Measurement of TVB-N

The TVB-N content in beef meat was measured by the semi-micro Kjeldahl method based on the procedure mandated by the Chinese national standard GB/T 5009.44 (Hygiene and Committee, 2003), which was adopted for the hygienic assessment of fresh and frozen meat of livestock by the Chinese national standard (Hygiene and Committee, 2005). All beef samples for testing were ground individually using a meat grinder (JYL-C022, Joyong Company Ltd., China). A total of 10±0.1g of the ground beef meat was taken into a beaker, blended with 100 mL distilled water, impregnated still for 30 min, and the beaker was shaken every 2 min. Next, the solution was filtered through the filter paper. A total of 5 milliliters of filtrate were made alkaline by adding 5 mL of 10 $g \cdot L^{-1}$ magnesia (MgO). The steam distillation was distilled 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 $g \cdot L^{-1}$ boric acid and then titrated with approximately 0.01 $mol \cdot L^{-1}$ H_2SO_4. Before titrating the distillate of beef samples, three titrations of distillate of blank samples and three titrations of distillate of beef samples were conducted. The amount of TVB-N was calculated using Eq. (1). The result stated for each sample corresponds to the mean value of three measurements:

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

where V_1 is the titration volume for the test sample (mL), V_2 is the titration volume of blank (mL), c is the actual concentration of H_2SO_4 ($mol \cdot L^{-1}$), and m is the weight of the ground beef sample (g).

3.4.2 Measurement of pH

The pH value of the sample was measured with a pH meter, which was calibrated with pH 4.0 and pH 7.0 standard buffers, and the probe was rinsed with de-ionized water between measurements. The result of pH was recorded as the average of four readings, which were measured at four different locations of a beef sample.

3.4.3 Measurement of color

Color (CIE L^*, for bright index, a^* for green and red) measurements were measured on beef surfaces using a colorimeter NR200 (3NH Technology Co., LTD). The colorimeter was calibrated with black and white plates before measurements. Four measurements were taken at four different locations on the beef surface, and each measurement was recorded as the average of four readings, which was taken as the reference value of the beef sample flesh color.

3.4.4 Measurement of total bacterial count

The detailed process of detecting the total bacterial count was performed by referring to Literature [33]. Total bacterial count refers to the total number of colonies formed per gram of sample in colony-forming units ($CFU \cdot g^{-1}$), which is determined by plate counting, as defined in GB / T 4789.2-2010. To facilitate data processing, the total number of colony is taken as the reference value of the total number of bacteria samples because when the total number of colonies counted is larger, the unit is expressed as $\log CFU \cdot g^{-1}$.

3.4.5 Sensor evaluation

The sensory evaluation of beef freshness was performed by a sensory evaluation score of 10 assessors who had undergone professional training. The sensory score was evaluated on the basis of the color, odor, elasticity, and viscosity of meat by using the 10-point system. The total sensory scores of beef freshness in terms of color, smell, elasticity, and viscosity were 20%, 30%, 20%, and 30%, respectively. The average sensory evaluation score of 10 persons was taken as the sensory evaluation score of the freshness of each sample. Higher sensory evaluation score means better beef freshness.

3.5 Data analysis and model establishment

3.5.1 Data preprocessing and model establishment methods

The spectra of the samples were pretreated by multiple scattering correction (MSC) and Savitzky–Golay smoothing (SG) to eliminate noise interference and reduce analysis error, respectively. The detailed description of the MSC algorithm can be referred to in Literature [34]. A detailed description of the SG algorithm can be referred to in Literature [35]. The prediction models of different freshness indexes were developed by multiple linear regression (MLR), partial least-squares regression (PLSR), and least squares support vector machine (LS-SVM). The comprehensive discriminant model of beef freshness was developed by LDA. Finally, the best pretreatment and predictive models were determined.

MLR is a statistical method [36] that attempts to model the relationship between two or more interpretive variables and a response variable by fitting a linear equation into the observed data. The MLR model is expressed as:

$$y_i = a_0 + a_1 x_{i,1} + a_2 x_{i,2} + \ldots + a_j x_{i,j} + e_i \quad (2)$$

where y_i is the dependent variable, a_0 is a constant, $x_{i,j}$ is an independent variable, a_j is the vector of regression coefficient, and e_i is a random measured error.

PLSR is a widely used multivariate linear regression method in the present spectroscopic analysis [37]. PLSR considers the target chemical property matrix Y (the properties of interest) and the variable matrix X (the spectrum) simultaneously and finds the fundamental relations between them. In this study, PLSR analysis was performed to develop a regression model for the prediction of kiwifruit SSC. PLSR was also applied as a regression method to extract latent variables (LVs). LVs were considered new eigenvectors of the original spectra to reduce the dimensionality and compress the original spectral data. Certain selected LVs were used as the inputs of PLSR. The number of LVs is a critical parameter in establishing a PLSR model. The optimal number of LVs is helpful in avoiding model overfitting or underfitting. Full cross-validation was used in the development of the PLSR model to determine the optimal number of LVs by the root-mean-square error of cross-validation to prevent overfitting problems.

LS-SVM, which was proposed by Suykens and Vandewalle, is a simplified and improved SVM model. LS-SVM is used in classification and regression problems. LS-SVM uses a set of linear equations for training and improves the convergence speed of the algorithm. Therefore, LS-SVM has been widely used in classification and regression [38]. The prediction belongs to the regression problem. For regression problems, the training set is supposed to be (x_i, y_j), where $i = 1,2,3,...n$; x_i is the input variable, and y_i is the output variable. The optimization problem and the constraint condition of LS-SVM can be described as follows:

$$\begin{cases} \min J(w,b,\xi)=\frac{1}{2}w^T w+\frac{1}{2}\gamma\sum_{i=1}^{n}\xi_i^2 \\ s.t\, y_i[w^T\varphi(x_i)+b]=1-\xi_i, i=1,...,n \end{cases} \tag{3}$$

where w is the weight vector; ξ_i is the slack variable for x_i ; γ is the regularization factor, which is used to adjust the confidence interval of LS-SVM and the proportion of empirical risk; and b is a partial vector.

Proper kernel function and optimal LS-SVM parameters should be solved before the application of LS-SVM. Linear kernel, polynomial kernel, and radial basis function (RBF) are frequently obtained as the kernel function of LS-SVM. RBF could handle the nonlinear relationships between the spectra and target attributes, reduce the computational complexity of the training procedure, and perform well. Therefore, RBF was used as the kernel function of LS-SVM in this study. The regularization γ and RBF kernel function parameter σ^2 are two important parameters that determine the learning, prediction, and generalization abilities of LS-SVM. In this study, K-fold cross-validation was applied to determine the optimal values of γ and σ^2.

The LDA is the most popular technique that has been successfully applied to many classified fields. A key feature of LDA is that it allows the creation of a statistical classification model based on a training dataset and then allows a test dataset to be independently classified without user input [39]. This study constructed the LDA model of beef freshness.

3.5.2 Model evaluation standard

The stability, reliability, and dynamic adaptability of the prediction models were used as the evaluation criteria of model performance. Four statistical indexes, 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 prediction (SEP), the correlation coefficient of verification set(R_v), and the standard error of verification (SEV), were calculated in Equations (4)–(9) to evaluate the performance of the established models.

$$Rc = \sqrt{\sum_{i=1}^{n_c}(\hat{y}_i - y_i)^2} \Big/ \sqrt{\sum_{i=1}^{n_c}(\hat{y}_i - y_m)^2} \tag{4}$$

$$Rp = \sqrt{\sum_{i=1}^{n_p}(\hat{y}_i - y_i)^2} \Big/ \sqrt{\sum_{i=1}^{n_p}(\hat{y}_i - y_m)^2} \tag{5}$$

$$Rv = \sqrt{\sum_{i=1}^{n_v}(\hat{y}_i - y_i)^2} \Big/ \sqrt{\sum_{i=1}^{n_v}(\hat{y}_i - y_m)^2} \tag{6}$$

$$SEC = \sqrt{\frac{1}{n_c - 1}\sum_{i=1}^{n_c}(\hat{y}_i - y_m)^2} \tag{7}$$

$$SEP = \sqrt{\frac{1}{n_p - 1}\sum_{i=1}^{n_p}(\hat{y}_i - y_m)^2} \tag{8}$$

$$SEV = \sqrt{\frac{1}{n_v - 1}\sum_{i=1}^{n_v}(\hat{y}_i - y_m)^2} \tag{9}$$

where $\hat{y}_i$ is the *ith* predicted value, y_i is the *ith* measured value, y_m is the mean of the calibration, verification 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, and n_v is the number of samples in the verification set. Generally, models with higher R_c, R_p, and R_v or lower SEC, SEP, and SEV are more satisfactory than models with lower R_c, R_p, and R_v or higher SEC, SEP, and SEV.

In this study, data analysis was performed in the MATLAB R2010 software (The Mathworks, Inc., Natick, MA, USA).

4. Results and discussion

4.1 Spectral analysis of sample and indexes value of beef freshness

The spectral curves of the beef samples over the 350–1800 nm range were obtained. Nevertheless, the spectral data in the Vis-NIR spectral range of 400–1700 nm were selected for future research because of their larger noise at both ends of the spectral range. Mahalanobis distance outlier detection approach was used before establishing the models to detect the samples' spectral data. The index value of beef freshness

was used to identify the potential abnormal samples and eliminate the influence of unavoidable outliers in the training sample on the predictive ability and accuracy of the predictive models [40]. Three outlier samples were detected and removed from the 56 samples. The original reflectance spectra of the 53 remaining beef samples at the 400 nm to 1700 nm wavelengths are shown in Fig. 3. Obviously, a similar tendency was presented throughout the examined wavelength region at different beef samples, and some numerical differences were observed in the variation amplitude of the spectral reflectance, which was possibly attributed to the changes in the main chemical components when the freshness of beef samples was lost. The spectra in the Vis-NIR region were generally sensitive to organic compounds, which were composed of the molecular bonds of C–H, O–H, and N–H, and the absorption peaks were connected with overtones and combinations of fundamental vibrations of these functional group overtones of O–H stretching. Four obvious absorption peaks were found around 430, 665, 780, and 1530 nm. In accordance with the typical wavebands indicated in the literature, the absorption peak at approximately 430 nm was meat deoxy-myoglobin, the absorption peak near 665 nm was mainly the third time frequency of NH_3 group, and the absorption peak near 780 nm and 1530 nm was mainly the third and first time frequencies of the N-H group, respectively. This finding indicated that the spectral information reflected the change information of beef color, total bacterial count, TVB-N, and pH. The original spectra exhibited evident scattering, noise, and baseline shift. Therefore, preprocessing methods (e.g., SG, MSC and SG + MSC) were applied to the original spectra.

The changing trend of beef color (L^*, a^*), total bacterial count (CFU), TVB-N, and pH during the whole 1–17 days of storage period was shown in Fig. 4. The solid line represented the average value of the corresponding physical and chemical indexes of all samples every day, and the short vertical line represented the error between the detected value and the average of the physical and chemical indexes of the beef samples. In Figs. 4 (a)–(e), L^* and a^* showed a downward trend, whereas the trend of CFU, TVB-N, and pH increased with time, indicating that the beef color changed from light to dark and from red to green. Moreover, the values of CFU, TVB-N, and pH increased with storage time, indicating that the indexes of L^*, a^*, CFU, TVB-N, and pH value reflected the beef's freshness.

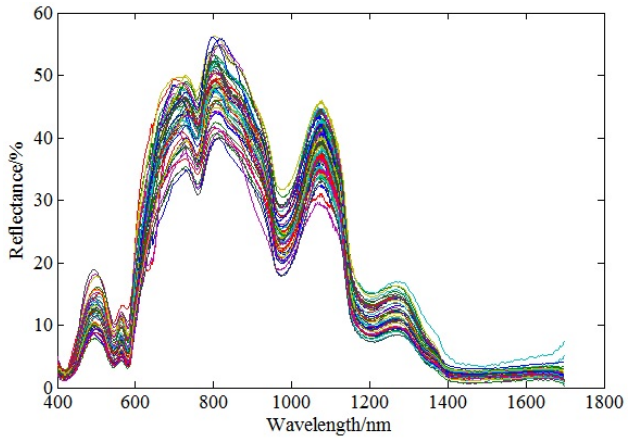
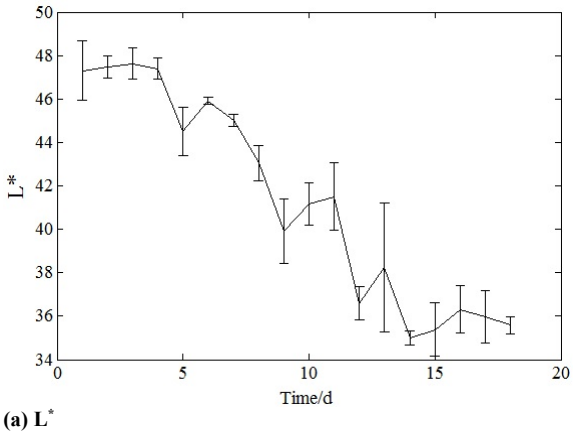
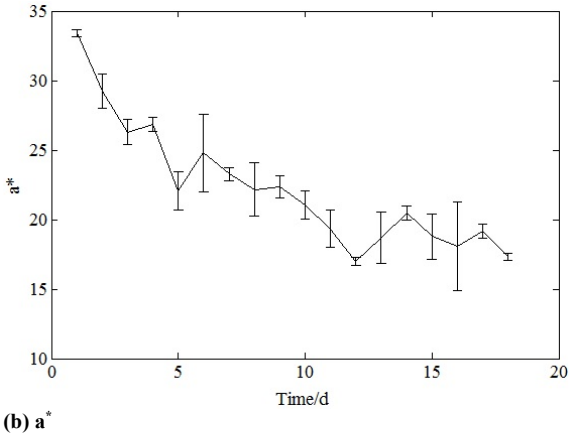


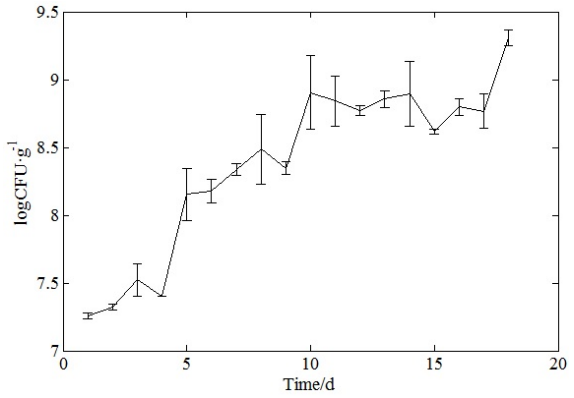
Fig. 3. Raw Vis/NIR spectra of beef samples



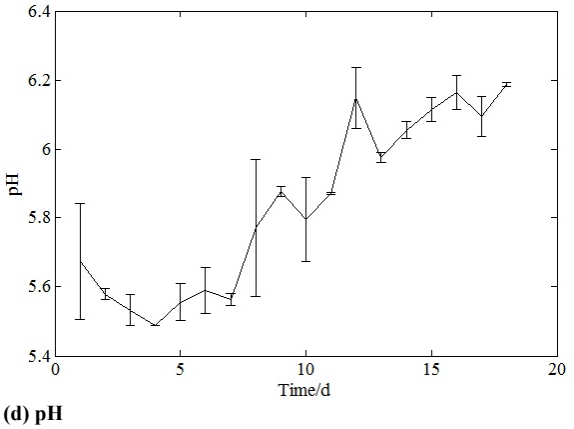
(a) L*



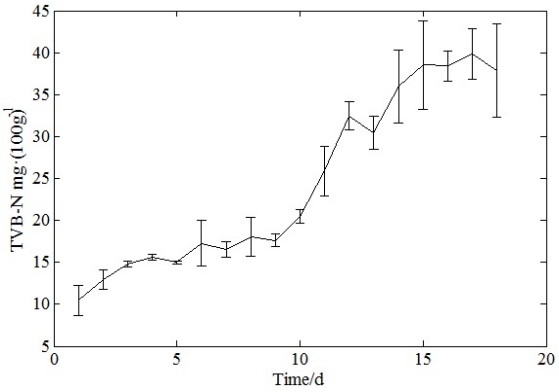
(b) a*



(c) CFU



(d) pH



(e) TVB-N

Fig. 4. Physical and chemical index change tendency chart

4.2 Freshness LDA model

The beef freshness discrimination model has been established by using the LDA method. The 53 beef samples were divided into 2 levels, namely, fresh and stale, by sensory evaluation. A total of 15 beef samples were fresh, and 38 beef samples were stale. The fresh samples were labeled as "0," and stale samples were labeled as "1." A total of 53 samples were randomly divided into training set and test sets at 3:1. The training set samples were used to establish the LDA model. The test set samples were used to test the prediction accuracy and stability of LDA built by the training set. The beef freshness indexes of L^*, a^* CFU, TVB-N, and pH were taken as the input parameters of the LDA model, and the identification label "0" or "1" of the corresponding sample was taken as the output parameter of the LDA model. The prediction results of LDA freshness discrimination model are shown in Table 1. As shown in Table 1, the discriminating rate of the LDA model in the training set is 100%, and the discriminating rate in the test set is 92.86%.

Table 1. Results of LDA model

Model	Training set	Test set
	Discriminating rate/%	Discriminating rate /%
LDA	100.00 (39/39)	92.86 (13/14)

4.3 Prediction model of freshness indexes

After dividing the samples into calibration and prediction sets at 3:1, the full spectrum was pretreated by MSC, SG, or MSC+SG and was then used as the input variable to build the prediction models of L^*, a^*, pH, TVB-N, and CFU. The MLR, PLSR, and LS-SVM models for L^*, a^*, pH, TVB-N, and CFU were built on the basis of the full spectrum. The prediction results are shown in Table 2. The performance of the PLSR and LS-SVM models of the five freshness indexes preprocessed with MSC+SG were better than those preprocessed with MSC or SG only. However, the best pretreatment methods for the MLR model of the five indexes varied. Among them, the best pretreatment method for MLR models of L^*, a^*, and pH indexes was MSC, and that for TVB-N and CFU was SG. This finding also shows that different pretreatment methods have a great impact on the MLR model. After comparative and comprehensive analyses, PLSR, LS-SVM, LS-SVM, PLSR, and MLR models in the prediction sets were the best prediction models for L^*, a^*, pH, TVB-N, and CFU with $R_p = 0.992$, $SEP = 1.995$; $R_p = 0.921$, $SEP = 1.262$; $R_p = 0.934$, $SEP = 0.068$; $R_p = 0.916$, $SEP = 4.635$ $mg \cdot (100g)^{-1}$; and $R_p = 0.911$, $SEP = 0.297$ $\log CFU \cdot g^{-1}$, respectively.

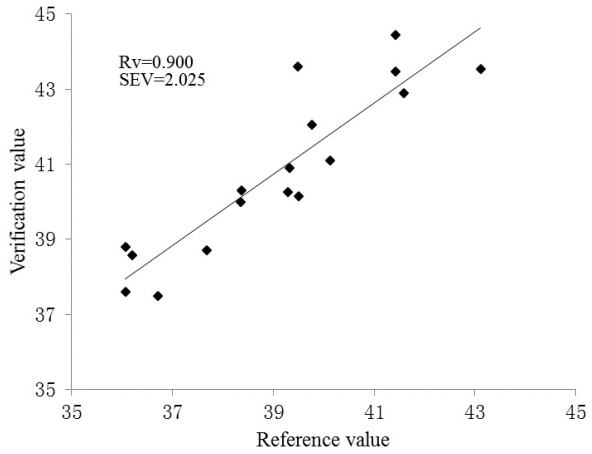
Table 2. Comparison of the prediction results of three different models

Model	Index	The best pretreatment method	Calibration set		Prediction set	
			Rc	SEC	Rp	SEP
MLR	L^*	MSC	0.944	1.650	0.905	1.978
	a^*	MSC	0.907	1.702	0.867	2.014
	pH	MSC	0.918	0.103	0.820	0.110
	TVB-N	SG	0.842	5.767	0.831	5.878
	CFU	SG	0.912	0.228	0.911	0.297
PLSR	L^*	MSC+SG	0.941	1.657	0.922	1.995
	a^*	MSC+SG	0.915	1.626	0.850	1.966
	pH	MSC+SG	0.884	0.121	0.800	0.120
	TVB-N	MSC+SG	0.924	4.858	9.16	4.635
	CFU	MSC+SG	0.832	0.309	0.799	0.383
LS-SVM	L^*	MSC+SG	0.999	0.095	0.931	2.032
	a^*	MSC+SG	0.932	1.284	0.921	1.262
	pH	MSC+SG	0.961	0.072	0.934	0.068
	TVB-N	MSC+SG	0.968	3.230	0.796	9.846
	CFU	MSC+SG	0.897	0.260	0.738	0.453

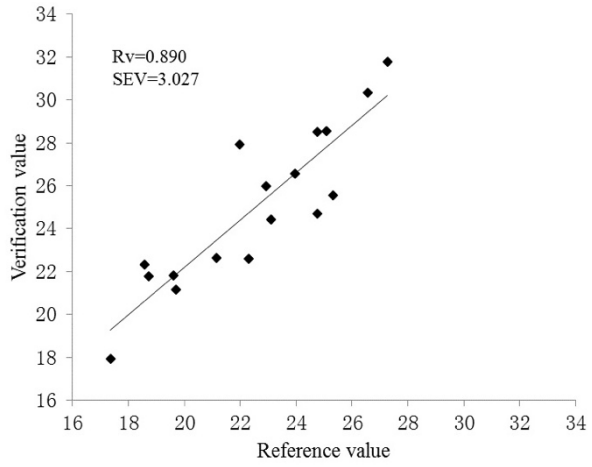
4.4 Validation of freshness index predictions models and freshness discrimination model

To verify the reliability and accuracy of freshness index prediction and freshness discrimination models, 17 beef samples were used as verification sets to collect the spectral data and test reference values of 5 freshness indexes according to the experimental process during the 17 days. In the experimental process, the sensory evaluation team evaluated the freshness of all samples (i.e., 8 samples were fresh, and 9 samples were stale). The 5 beef freshness indexes were predicted according to the above-mentioned conclusions of the best pretreatment methods and best prediction modes of L^*, a^*, pH, TVB-N, and CFU. The results are shown in Fig. 5. The validation correlation coefficient (R_v) of L^*, a^*, pH, TVB-N, and CFU were 0.900, 0.890, 0.862, 0.902, and 0.875, respectively. The standard deviation (SEV) of L^*, a^*, pH, TVB-N, and CFU were 2.025, 3.027, 0.183, 7.502 $mg \cdot (100g)^{-1}$, and 0.447 $\log CFU \cdot g^{-1}$, respectively. These values show that the accuracy and stability of the prediction model of the five indexes of freshness are relatively good.

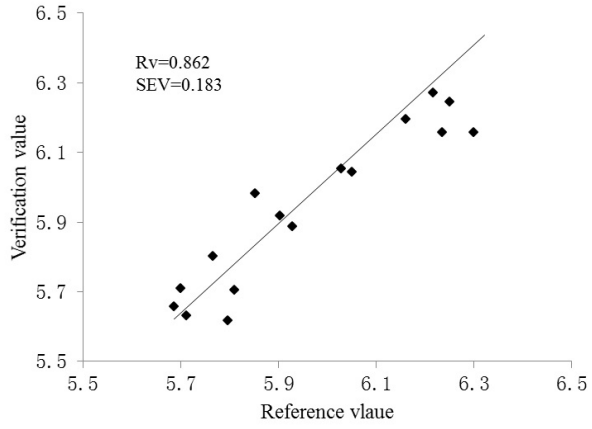
The aforementioned prediction values of L^*, a^*, pH, TFB-N, and CFU of the 17 samples in the verification set were used as the input variables of beef freshness LDA model to predict the freshness of the samples. The discriminating rate was 100%, which indicates that the established multi-index freshness discrimination model was stable and reliable and can be used for grading and discriminating beef freshness.



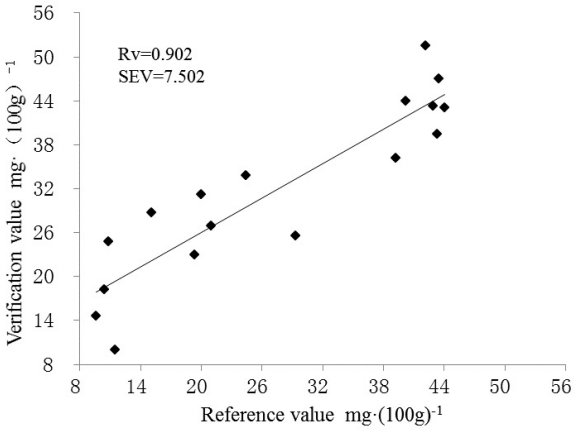
(a) L*



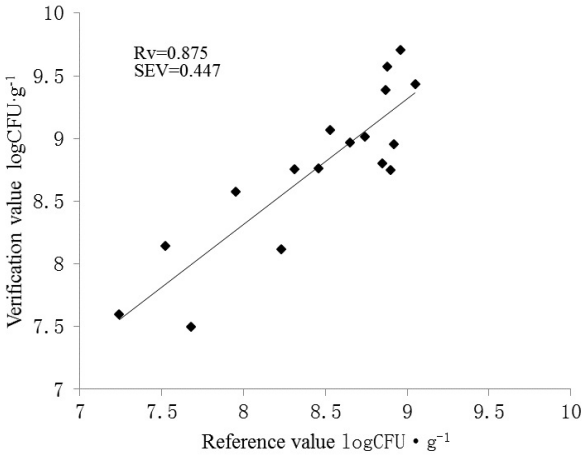
(b) a*



(c) pH



(d) TVB-N



(e) CFU

Fig. 5. Raw verification vs reference values of five indexes in the verification set by prediction models

5. Conclusions

Different beef freshness indexes, pretreatment methods, prediction models of indexes, and LDA model of freshness were analyzed comprehensively to study the performance of Vis-NIR spectroscopy with multi-index in identifying beef freshness. The notable conclusions of this study are presented as follows:

(1) Vis–NIR spectroscopy with multi-index can identify beef freshness comprehensively, rapidly, and nondestructively.

(2) The LDA model of beef freshness was established with multi-index, and its discriminating rate in the verification set was 100%.

(3) The MSC + SG pretreatment method was best for L^*, a^*, pH, and TVB-N, whereas the SG pretreatment method was best for CFU. The best prediction models for beef freshness L^* and TVB-N were PLSR with $R_v = 0.900$ and $SEV = 2.025$ and $R_v = 0.902$ and $SEV = 7.502$ $mg \cdot (100g)^{-1}$; the best prediction models for beef freshness a^* and pH were LS-SVM with $R_v = 0.890$ and $SEV = 3.027$ and $R_v = 0.862$ and $SEV = 0.183$; and the best prediction models for beef freshness CFU was MLR with $R_v = 0.875$ and $SEV = 0.447$ $\log CFU \cdot g^{-1}$.

This study indicates that Vis–NIR spectroscopy combined

with multi-index identify beef freshness rapidly, accurately, and comprehensively without destroying the beef. This method is very likely to be applied to the real-time detection of meat attributes and freshness in the future and provides a basis for the further development of practical, rapid, and nondestructive detection equipment for meat. However, novel methods should be studied in the future to improve the accuracy and robustness of the prediction model further.

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