Chapter 8

Application of Near-infrared Spectroscopy for Assessing Meat Quality and Safety

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Additional information is available at the end of the chapter

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1. Introduction

Meat and meat products are important sources for human to obtain protein, vitamins and minerals, and has become an essential ingredient in the diet greatly prized by the consumer [1]. According to the analysis based on the Food and Agricultural Organization of the United Nations (FAO) Food Balance Sheet data, there has been a significant increase in global meat consumption over time (as is shown in Table 1). Aggregate meat consumption increased by almost 60% between 1990 and 2009, from 175,665 billion to 278,863 billion. It indicates that the meat and meat products occupy a large proportion in people's food items.

	1990	2009	%change
Bovine Meat	54065	63835	18.1
Mutton and Goat Meat	9100	12763	40.2
Pig meat	68692	105503	53.6
Poultry Meat	40173	90664	125.7
Others	3634	6098	67.8
Aggregate	175665	278863	58.7

Table 1. Global Meat Consumption, 1990-2009, 'billion'

With the continuous development of living standards and the relative change of dietary structure, consumers' rising and persistent demand for safe meat and better quality of meat is emphasized. The meat industry is no exception to this expectation. Superior quality of these products is always demanded by consumers and is considered as a key factor for success in today's highly competitive market [2]. The characteristics of raw meat are easily affected by a

great many factors, such as breed, sex, age, pre-slaughter and some post-mortern factors including transporting, storing time, temperature condition, et al. The changes muscles may undergo during these periods can influence many characteristics such as color, tenderness, flavor, and juiciness [3]. Hence, it's of great importance to obtain reliable information about raw meat., In the production of meat products, meat quality is also an important manufacturing requirement, because consumers are susceptible to any forms of contamination that may occur during the manufacturing processes. The great variability in raw meat often leads to highly variable products being marketed without a controlled level of quality, which imposes great pressure on the food manufacturing industry to guarantee the quality of meat [4]. Hence, quality assurance and control are among the main tasks in production and processing of meat and meat products.

On the other hand, meat and meat products are also potential vehicles of hazards to human health. Types of hazards that may be present in meat products include chemicals (causing acute or long-term toxicity), biological agents (pathogenic bacteria, viruses), as well as physical objects (may cause injury) [5]. Biological hazards are of most concern. Their occurrence in meat and meat products is unavoidable because contaminants are present in and on the animals and in their environment. Raw meat and not fully heated (canned) or fermented/dried meat products are highly perishable, which makes them prone to quick spoilage due to microbial presence and growth. In addition, we should also pay attention to the authenticity of meat and meat products as adulteration occurred frequently in recent years and had been the limiting factor which would restrict the healthy and rapid development of meat industry. Some people may replace high value meat with low cost meat or offal or add some improper additive into meat products. The determination and detection of adulteration are indispensable as there are some people who do not accept specific meat for religious reasons. In order to guarantee the legitimate rights and interests of consumers, to eliminate adulteration is necessary...

In conclusion, to realize these needs mentioned above and to fulfil consumers' satisfaction, it is very important to provide meat and meat products that can better meet the customers' needs and market requirements. Therefore, it is a crucial element within the meat industry to accurately assess meat and guarantee the quality and safety. Different techniques such as sensory analysis, chemical procedures and instrumental methods have been employed to provide information about meat quality [6]. Sensory analysis is often implemented by professional staff. This method has been widely used in many food research fields. However, it is subjective, labourious, time-consuming and inconsistent. Chemical procedures and instrumental methods have been used in detecting quality attributes for a long time, which are more convenient, precise and effective than sensory analysis [7]. For chemical methods, the long-time standard for protein analysis is the Kjeldahl method and the method of choice for fat analyses is a solvent-based method for measuring the total fat content in meat. In terms of instrumental methods, pH is traditionally measured by pH meter by inserting it into the muscle directly after incision of the muscle, and colorimeters are commonly utilized for meat color evaluation.

However, most of the above-mentioned techniques are destructive, tedious, time consuming and require lengthy sample preparation. Therefore, these traditional methods have limited

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applicability and are not suitable for fast analysis and early detection of quality attributes in industrial and commercial processing. In contrast to conventional methods, many novel and automatic technologies based on mechanical, optical, dielectrics, X-rays, spectroscopy, and nuclear magnetic resonance have emerged for detecting these quality and safety attributes. The Table 2 compared several methods. Among them, spectroscopy technique included near infrared spectroscopy (NIR), mid-infrared spectroscopy (MIR), far infrared spectroscopy (FIR) and Raman spectroscopy has been considered as one of the most promising technique [8].

Methods	Advantages	Disadvantage
Ultrasound technique	Rapid, Non-destructive, Non-polluting, High sensitivity	Easily affected by operators, measurement sites as well as the ultrasonic frequency, Only detecting chemical compositions for some specific parts
CT scanning	Non-invasive, Providing detailed images	Expensive, Longer evaluation time, Limited range of application
Computer vision	Providing spatial information, Higher accuracy than manual inspection, Able to detect external attributes, Suitable for on-line detection	Limited multi-constituent information, Unable to detect internal attributes
Spectroscopy technique	Simple, Providing spectral information, Able to detect internal attributes	Limited sensitivity to minor components and complicated analysis

Table 2. Non-destructive techniques for meat quality and safety determination

Infrared spectroscopy is the electromagnetic radiation waves between the visible light (Vis) and middle infrared (MIR). In general, the absorptions observed in the near infrared region are overtones or combinations of the fundamental stretching bands which are usually due to C-H, N-H or O-H stretching [9]. Table 3 summarizes the bands commonly observed for organic molecules in the near infrared region. Feature information of organic molecules in a sample could be obtained after scanning the sample.

Wavelength (nm)	Assignment
2200-2450	Combination C-H stretching
2000-2200	Combination N-H stretching, Combination O-H stretching
1650-1800	First overtone C-H stretching
1400-1500	First overtone N-H stretching, First overtone O-H stretching
1300-1420	Combination C-H stretching
1100-1225	Second overtone C-H stretching
950-1100	Second overtone N-H stretching, Second overtone O-H stretching
850-950	Third overtone C-H stretching
775-850	Third overtone N-H stretching

Table 3. Common near infrared bands of organic compounds

NIR spectroscopy has a great potential for the estimation of quality and safety attributes in meat and meat products in recent years, and has been considered as one of the effective and progressive techniques [10].. It's a potential analytical tool for sensitive and fast analysis with simplicity in sample preparation allowing a simultaneous assessment of numerous meat properties [11]. NIR has shown enormous potential to predict quality attributes, such as protein, fat, moisture, ash, myoglobin, pH value, water-holding capacity (WHC), color, marbling, tenderness and safety attributes (freshness, total bacterial count, adulteration) [12]. The existing researches indicated that NIR has been successfully applied to the quantitative determination of such attributes in meat with high accuracy and the coefficients of determination (R) of some indicators were up to 0.90 between predicted and reference values. In general, the whole process of prediction was carried out as what is shown in Fig.1.

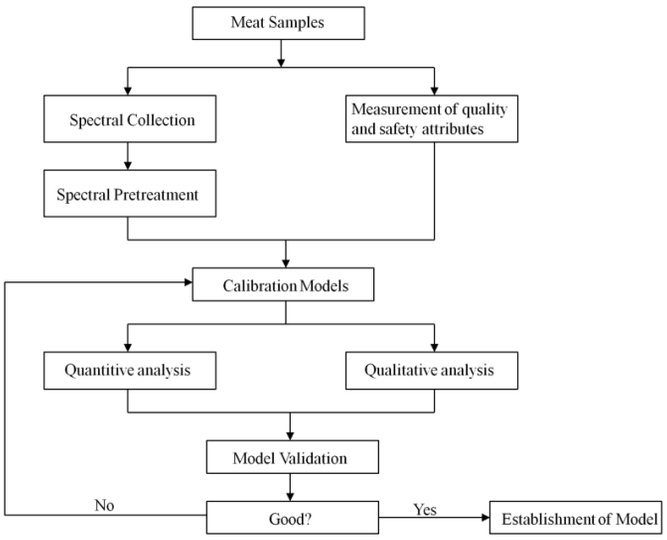

Figure 1. The whole process of prediction

2. Quality Attributes

A number of attributes are related to the quality of meat and meat products, for example, water content, fat [13], protein, ash [14], myohemoglobin, pH, tenderness, WHC, color, and marbling. The constituents are intrinsic reasons that affect meat quality [15]. Among them, water content, pH value, tenderness, WHC and color are especially important [16]. As water content is the reflectance the freshness of meat and is technologically and financially important for food-processing industry. Especially in recent years, the emergence of water injection in meat has made the fresh pork water content test become particularly important [17]. The pH value of fresh meat which may have influence on other meat properties, such as color, water-holding capacity and shelf-life, serves an important function in grading meat [18]. WHC is defined as the ability of muscle to retain water or resist water loss during postmortem storage. It is of great significance for commercial value and consumer acceptance. The quality of fresh meat

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depends to a large extent on WHC. Because it has a great influence on the appearance of fresh meat during retail and might affect the sensory properties of cooked meat. Color influences the acceptability of meat and meat products and plays a major role in the purchase decision. Consumers often use meat color variations as an indicator of freshness and wholesomeness. Tenderness is also an important attribute influencing consumer opinion about the eating quality of fresh meat [19].

The following are some examples about the application of NIR to predict the water content, pH value, WHC and tenderness, respectively.

2.1. Water Content

Zhang. *et al* [20] collected a total of 57 samples from 31 fresh loins from three different slaughtering houses in 3 days. Each sample was vacuum-packed with serial number. All samples were stored at 4°C in a special cold storage box and transported to the nondestructive detection laboratory immediately. After removing the fat and connective tissue from the sample, all of the fresh pork samples were chopped to approximate dimensions of 8 cm×5 cm×3 cm for further analysis in laboratory. And samples were packaged again into the corresponding original vacuum bag and stored at 4°C in the refrigerator for retaining freshness. Then reflectance [21] spectrum was captured by the VIS/NIR spectra system (as is shown in Fig. 2) which consisted of an AvaSepc-USB double channel VIS/NIR spectrometer (the wavelength range is from 200 nm to 1750 nm), the matched spectrum acquisition software, light source with two halogen lamps and voltage-stabilized source, Y type fiber, optical probe, a PC, platform and white ceramic standard plate. The original spectrogram was shown in Fig.4. Standard values of water content were measured immediately after VIS/NIR spectrum data were collected according to GB-5009.3-2010. The original spectrum and statistics data of pork water content were shown in Fig. 3 and Table 4, respectively.

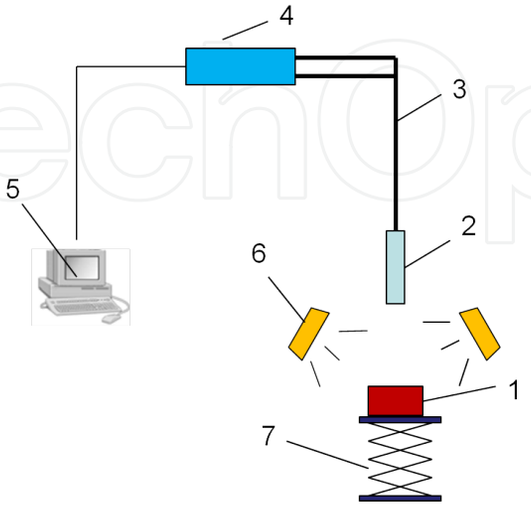

Figure 2. VIS/NIR; spectral system 1. Sample 2. Optical Probe 3. Fiber 4. Spectrometer 5. PC 6. Light Source 7. Platform

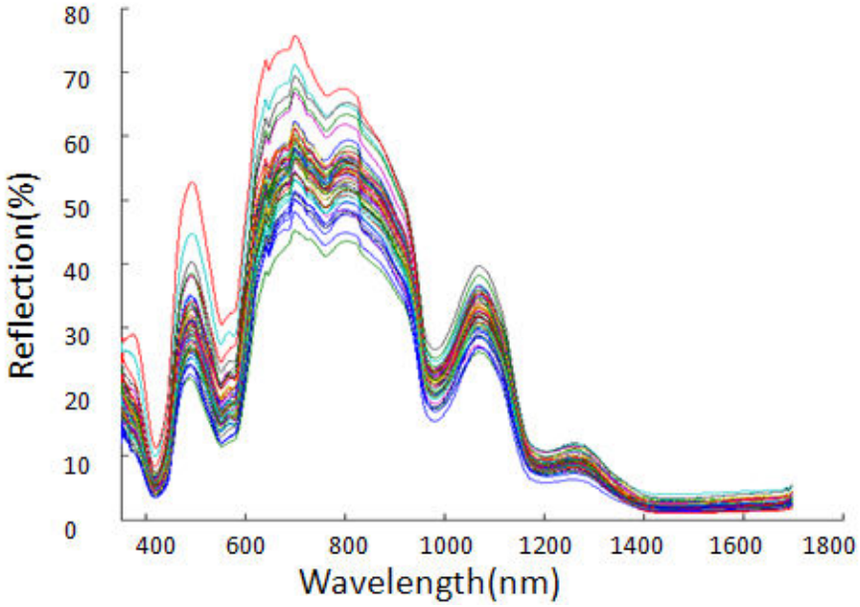

Figure 3. Original spectrum

Samples sets	Water content range	Average value	Variance
Correction set	73.05-78.08	75.52	1.44
Validation set	73.75-77.69	75.54	1.22
Total samples	73.05-78.08	75.53	1.38

Table 4. The Statistics data of pork water content

Then spectral data were analyzed using MATLAB7.6 statistical software package. Firstly, total 57 samples were randomly divided into calibration and validation sets. 43 samples were placed in the calibration set used for building the model, and others were the validation set used for testing model. To overcome the influence of thickness and surface roughness of samples and to improve modeling accuracy, different kinds of pretreatment methods included Median smooth filter (M-Filter), multiple scattering correction (MSC), first order differential (FD), standard normalized variants (SNV) and combination of them were adopted. Then partial least squares regression (PLSR) and multivariate linear regression (MLR) methods were used to establish the testing models. The models were evaluated by correlation coefficient (R) and root mean square error (RMSE) of calibration set and validation set. Good models possessed higher R and lower RMSE, and differences between the calibration set and prediction set were less.

The PLSR model use full band spectrum information and results for two sets were shown in Table 5 by using different pretreatment methods. Results show that the model was better by using of M-Filter, MSC, FD and SNV. Optimal principal components number was 15, Rc and RMSEC of calibration set were 0.90 and 0.50 respectively. Rp and RMSEP were 0.81 and 0.70 for validation set respectively.

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Pretreatment method	Optimal principal components number	Rc	RMSEC	Rp	RMSEP
MSC	10	0.69	1.23	0.58	1.11
SNV	7	0.64	0.80	0.62	0.92
MSC+FD+SNV	10	0.89	0.50	0.78	0.81
FD+MSC+SNV	15	0.90	0.50	0.81	0.70

Table 5. Results of PLSR models based on different pretreatments

In the MLR model, most significant wavebands were selected by using stepwise regression analysis and according to relative coefficient graph, MLR equations and coefficients were obtained and the results of model were shown in Table 6. The Fig. 4 represents the residual of calibration set after using M-Filter, FD and MSC pretreatment. Residuals of No.26 and 34 samples were unusual and the two samples were eliminated. Rc was higher by using M-Filter, FD and MSC compound pretreatment, and much higher R and lower RMSE were obtained after eliminating abnormal samples. Results indicated that there was big distinction of R between calibration and validation sets, which demonstrated that stability of the model was not good.

Pretreatment method	Variables	Rc	RMSEcv	Rp	RMSEP
M-Filter+MSC	6	0.63	1.02	0.58	1.23
M-Filter+FD+MSC	4	0.86	0.66	0.77	1.20
Eliminate the abnormal samples	5	0.91	0.50	0.80	1.07

Table 6. Results of MLR models based on different pretreatments

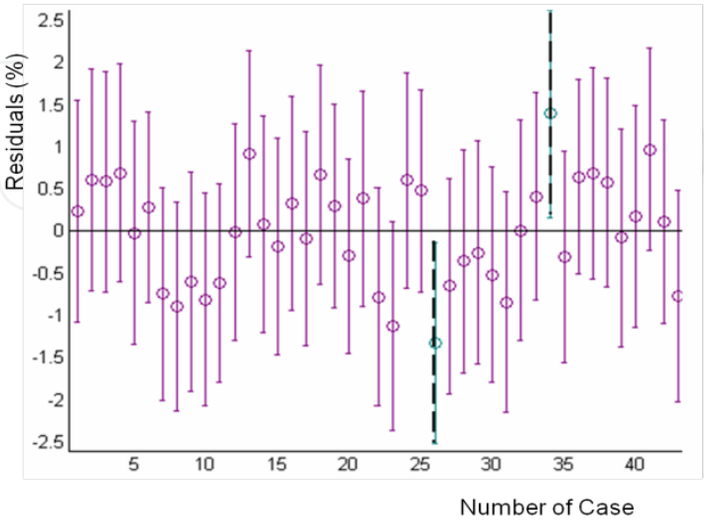

Figure 4. Residual plot

2.2. pH Value

Ma. *et al* [22] had ever applied NIR technology into prediction of pH value of beef. The sample preparation process was similar to the method mentioned above. Total 120 fresh samples were chopped to approximate dimensions of 8 cm×6 cm×2.5 cm. In order to obtain the pH value of entire shelf period, the experiment cycle was designed as 18 days. During the first 6 d, take out one sample to test every 12 h, and then take out two samples to test every 12 h for the next 12 d. The framework of detection system with visible/near infrared spectroscopy was shown in Fig. 5. The enclosed space is designed mainly to form a dark room, and exclude external light interference when testing samples.

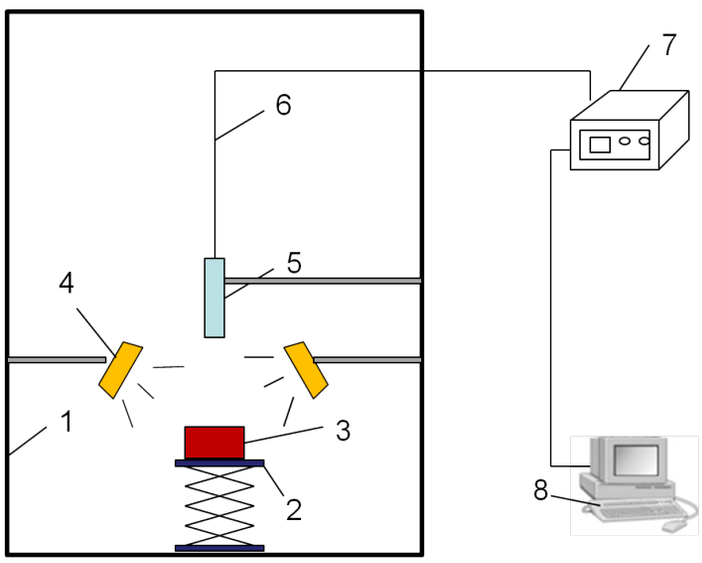

Figure 5. Framework of detection system with visible/near infrared spectroscopy; 1. Enclosed space 2. Platform 3. Sample 4. Light source 5. Fiber Probe 6. Fiber 7. Spectrometer 8. Computer

Once the spectral data of the samples were obtained, the pH values were determined with a pH meter. Six measurements on each sample were taken at different locations across the sample surface and averaged. Spectral data were analyzed using MATLAB7.6 statistical software package. Spectral data and pH of all samples were used for principal component analysis (PCA) and statistical analysis, respectively. Then use spectral leverage value (Leverage) and chemical absolute error (Residual) to test all the spectral anomalies or chemical value anomaly of the sample. Weed out 8 abnormal the remaining 112 samples were used for the following analysis. Total 112 samples were randomly divided into calibration and validation sets. 84 samples were placed in the calibration set used for building the model, and others were the validation set used for testing model. To eliminate the phenomenon of atlas offset or drift and high frequency noise interference, MSC and S-G smoothing were used for processing reflection spectrum of the sample data. The original spectrum and spectrum after MSC pretreatment were shown in Fig. 6 and Fig. 7. Then establish prediction model of MLR, PLSR and least square-support vector machine (LS-SVM) for prediction of pH value in beef based on full-spectrum and effective wavelengths selected by genetic algorithm (GA). GA is a

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computational model based on natural selection and genetic mechanism of biological evolution and is a kind of method to search the optimal solution by simulating natural evolutionary process. It has been successfully applied to select the best feature variables in VIS/NIR spectroscopy to build a stable model, especially for PLSR (GA-PLSR) which combines the advantages of GA and PLSR.. The best model occurred when the RMSECV between the true values and predicted values was the lowest. The results were shown in Table 7.

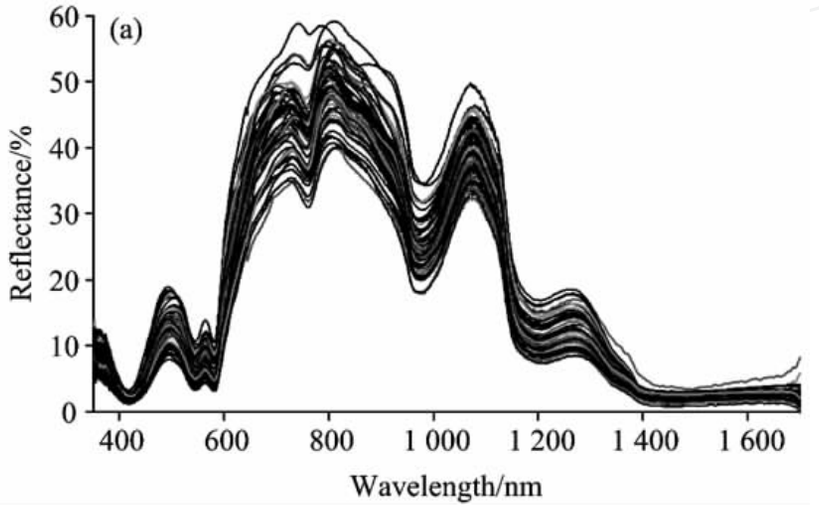

Figure 6. Original spectrum

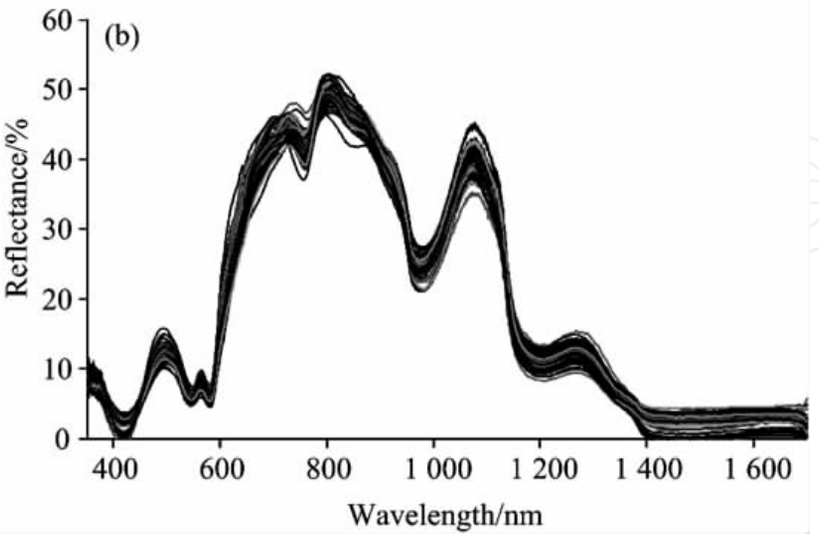

Figure 7. Spectrum after MSC

Pretreatment methods	MLR				PLSR				LS-SVM			
	Rc	SEC	Rv	SEV	Rc	SEC	Rv	SEV	Rc	SEC	Rv	SEV
MSC	0.783	0.144	0.770	0.183	0.864	0.118	0.729	0.189	0.889	0.109	0.756	0.185
MSC+SG	0.806	0.137	0.867	0.150	0.925	0.088	0.831	0.155	0.950	0.075	0.865	0.142

Table 7. Prediction results of beef pH value based on different models and different pretreatment methods

As is shown, three kinds of prediction revealed better results after MSC combined with SG smoothing pretreatment. Hence adopt MSC combined with SG smoothing as the processing method below. Besides, LS-SVM model prediction accuracy was the highest, MLR prediction accuracy was the poorest. However on the whole three kinds of model prediction accuracy was not high, this mainly because useless redundancy information decreased the model prediction ability and prediction accuracy.

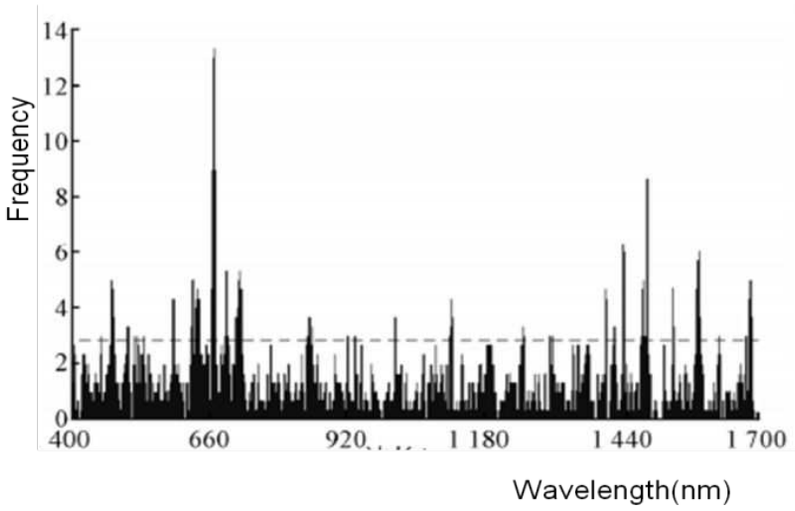

Figure 8. Frequencies of variables selected by GA

GA was employed for effective spectral variables selection after all band spectral variables were pretreated by MSC combined with SG smoothing. GA group size was set to 30 individuals and each individual genes variable was set to 17, mutation probability and crossover probability was set to 1% and 50% respectively, genetic algebra was 100 generations. As the GA initial population was generated randomly, 5 times screening processes were repeated for establishment of models. The Fig. 8 showed frequencies of variables selected by GA. Effective wavelength variables whose selected frequency was higher than the horizontal dotted line in the figure was used for modeling. Table 8 showed the MLR, PLSR and LS-SVM prediction results based on the variables selected by GA. Compared with the Table7, the accuracies of three kinds of modeling had been improved, MLR model predicted results was a bit poor, PLSR and LS-SVM modeling prediction were better. The LS-SVM modeling based on effective spectrum selected by the fourth iteration selected have the best prediction results, with the correlation coefficient of calibration set and validation set of 0.950 and 0.935 respectively,

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predict standard deviation was 0.074 and 0.111. The corresponding selected effective spectral variables were shown in Fig. 9. Vertical lines in the graph represent the GA selection by the wavelength of the effective variables. This study demonstrated that the LS-SVM model built by using VIS/NIR spectroscopy with GA could nondestructively and rapidly determine pH value in beef during its whole shelf-life. This research provided a basis of further developing device for nondestructive and rapid determine pH value in beef.

Times of GA	MLR				PLSR				LS-SVM			
	Rc	SEC	Rv	SEV	Rc	SEC	Rv	SEV	Rc	SEC	Rv	SEV
1	0.931	0.080	0.854	0.152	0.968	0.056	0.835	0.162	0.980	0.046	0.851	0.154
2	0.948	0.073	0.826	0.175	0.972	0.052	0.906	0.112	0.982	0.040	0.876	0.135
3	0.927	0.086	0.862	0.151	0.965	0.063	0.918	0.108	0.986	0.037	0.903	0.123
4	0.965	0.064	0.889	0.142	0.960	0.072	0.931	0.101	0.950	0.074	0.935	0.111
5	0.912	0.102	0.768	0.181	0.962	0.068	0.924	0.103	0.954	0.070	0.902	0.123

Table 8. Prediction results of GA-MLR, GA-PLSR, GA-LS-SVM

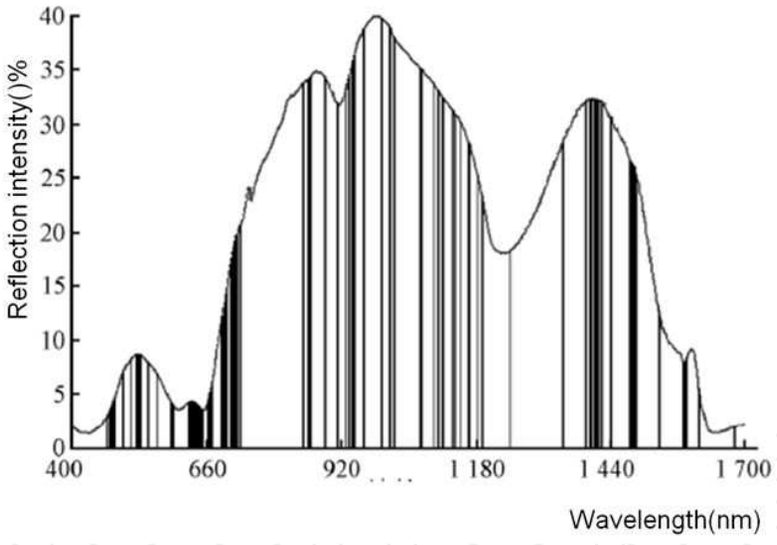

Figure 9. Spectra effective variables selected by GA

2.3. Tenderness

VIS/NIR Hyperspectral Imaging was employed by Tian *et al* [23] to prediction of beef tenderness. The hyperspectral imaging system can capture both spectral and spatial data simultaneously and process immediately in the VIS/NIR regions of the spectrum [24]. A total of 42 beef samples were collected at various retail stores and each steak was ensure to come from different animals by selecting from various retail stores. All the samples were taken from the

part of longissimus dorsi (LD) between 12 th rib and 13 th rib and then all the samples were cut into 4 cm×6 cm×2.5 cm chops. Each beef steak was first scanned by the hyperspectral imaging system. The system used in this study was a line-scan spectrograph which records a whole line of an image rather than a single pixel at a time. As shown in Fig. 10, the system consists of CCD camera along with focusing lens (12 mm), a spectrograph, a electric translation stage operated by a stepper motor and controller and a computer supported with Spectral Cube data acquisition software. In order to illuminate the sample and keep better distribution of optical intensity, two 100W halogen lamps was fixed above the sample from both sides at the height of 50 cm and at an angle of 45°.

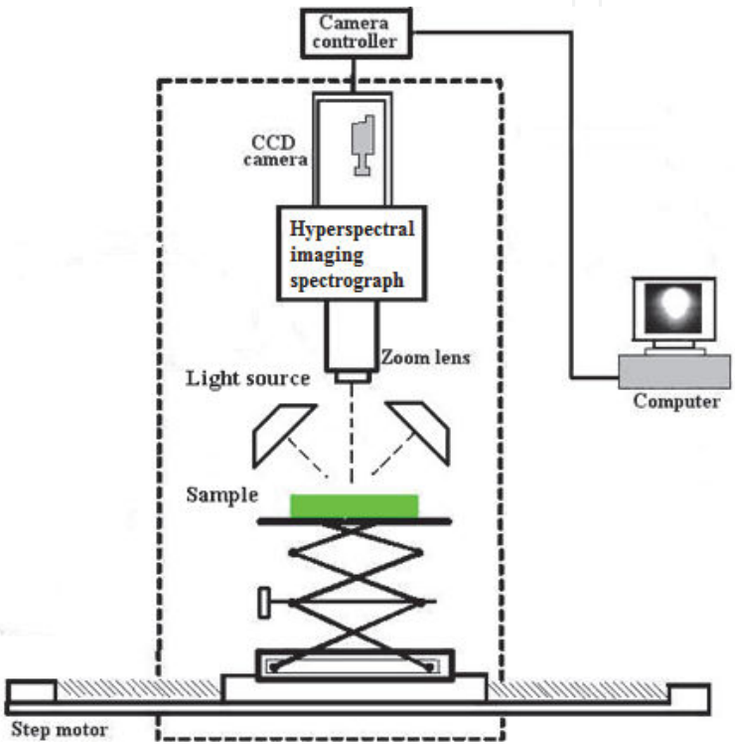

Figure 10. Hyperspectral imaging system

Beef sample was placed on the electric translation stage to be scanned line by line using 50 ms exposure time to build a hyperspectral line image which is then stored to composite 3D hyperspectral image. Several congruent sub-images which represent intensities with 520 wavelength bands from 380 to 1100 nm formed 3D image. The acquired hyperspectral image include both spatial and spectral information and we can get physical, geometric features and chemical information from it [25]. After that reference values of tenderness were determined. Steaks were cooked in 80°C water bath to an internal temperature of 70°C, which was monitored by thermoelectric couple inserting into center of samples, avoiding fat and connective tissue. Then the samples were vacuum packaged and put in the refrigerator to store at 4°C for 12 h before removing 6-8 cores parallel to the longitudinal orientation of muscle fibers [26]. Each core was sheared perpendicular to the longitudinal orientation of the muscle fibers by a Warner-Bratzlar Shear Force (WBSF) apparatus [27]. For each sample, six cores were taken and

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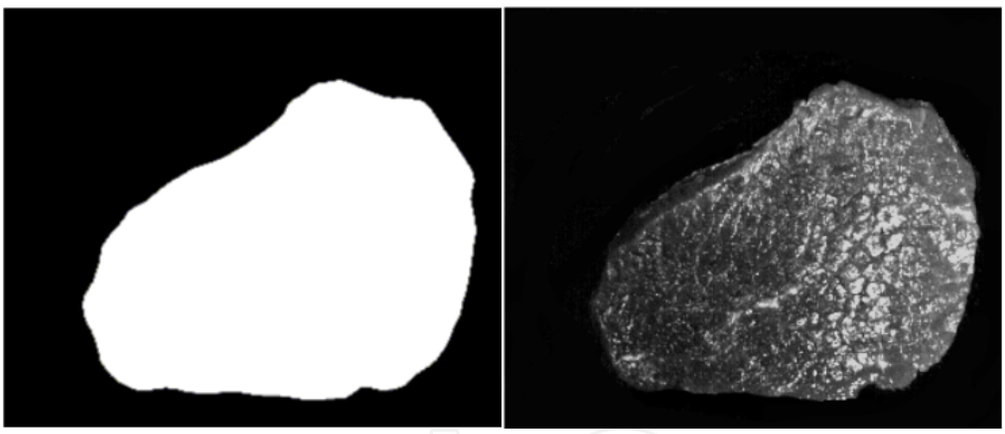
the mean peak WBSF value was used for data analysis. In Table 9 the relevant statistics of tenderness of beef steaks were presented.

Quality attributes	n	Mean	Minimum	Maximum	SD	CV
WBSF (N)	42	50.41	30.66	75.01	18.79	0.37

Note: n = number of samples; SD = standard deviation; CV = coefficient of variation.

Table 9. The reference values of WBSF values of beef steaks

Images were processed for analysis utilizing processing algorithms and analysis for identification of background from lean and fat texture parameters. The muscle lean areas were extracted from background with initial values for textural threshold which were selected from the plot of pixel intensities as shown in Fig. 11. After image segmentation, the lean muscle area was used for future texture feature and spectral information extraction. Before extraction of textural features, median filtering was used to reduce noise [28].



(a) Full mask of muscle lean (b) Muscle lean areas of sample

Figure 11. Muscle lean area of beef steak extracted from hyperspectral image

After processing, the hyperspectral image of beef samples contained only the lean part of the sample without fat, which was then used as the main region of interest (ROI) for further analysis. The average spectrum of each sample was extracted by locating the lean parts of the beef sample as the main region of interest (ROI). The extracted spectral data from all steaks were then used to construct a data matrix in which the row represents the number of samples and the column represents the number of variables. Furthermore, the reflection intensity hyperspectral data was converted to absorbance spectra by taking the base-10 logarithm of the reciprocal reflectance spectrum (log10 (1/R)) [29]. All processes of image segmentation, denoising and spectral data extraction were programmed in ENVI 4.3 and Matlab 7.6. An obvious absorbance peak within 530-580 nm and a small peak at 650 nm were presented. The

former related with oxyhaemoglobin absorption bands, and the latter with O-H third overtone or an absorption band produced by myoglobin oxidation. The samples of different tenderness presented the same spectral patterns all over the wavelength region (430-1000 nm), but there were still some differences in the magnitudes of absorbance intensities as shown in Fig. 12. The reason was because different samples from carcass were different in their major chemical composition such as fat, protein and dry matter. The tough samples had the higher absorbance intensities throughout the whole spectral region, meanwhile the tender ones had the lower absorbance (highest reflectance) compared to the mean overall average value.

For each absorbance spectrum, the first derivative was calculated to correct multiplicative scatter, baseline drifts, and to avoid the overlapping peaks. Spectra smoothing was performed on the absorbance spectra using the S-G method and moving average filter method with a span of 20 points. Fig. 13 showed the first derivative spectra of all samples. The main obvious variations bands can be identified at 450, 535 nm and 575nm. Obvious changes at 450 nm was related with Soret absorption band, attributed to traces of erythrocytes of hemoglobin, and the changes at 575 nm related with oxyhaemoglobin absorption.

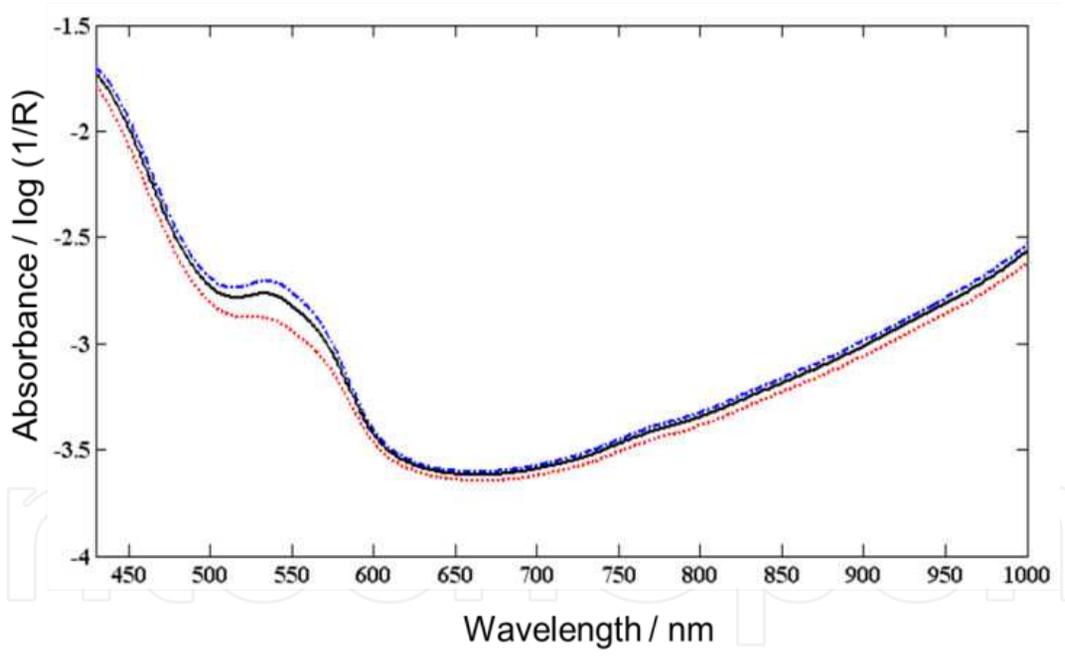


Figure 12. Difference in absorbance spectral profiles of beef steaks

The stepwise was performed with first derivative spectra to determine the optimal wavelengths. As shown in Table10, for tenderness, 8 wavelengths were determined as optimal wavelength to construct MLR prediction model, with coefficient of determination (Rv) of 0.88 and a root mean square error (RMSEV) of 6.49 and RPD value as 2.94 which indicated the model of meglio performance.

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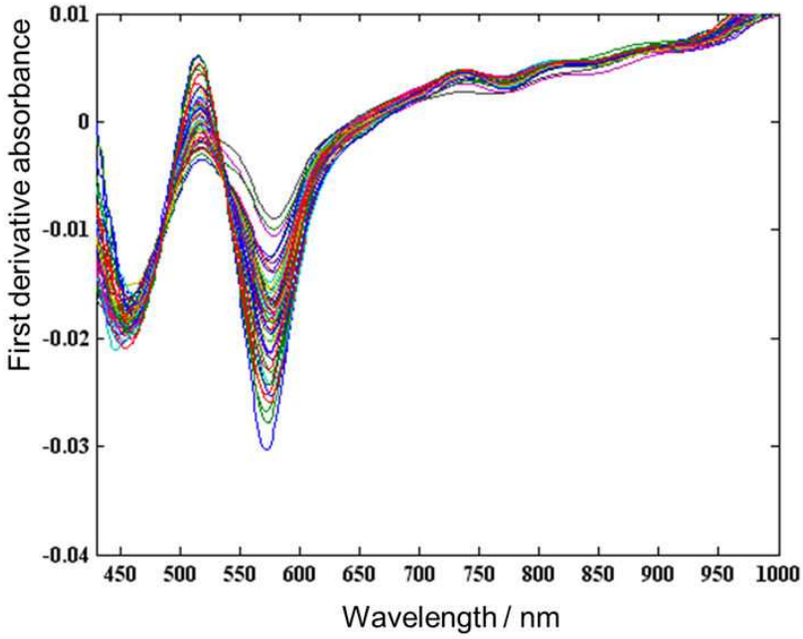

Figure 13. First derivative of absorbance spectral

Quality attributes	Optimal Wavelengths	Rc	RMSEC	Rv	RMSEV	RPD
Tenderness	437, 534, 549, 646, 700, 718, 776, 849	0.90	5.27N	0.88	6.49N	2.94

Table 10. MLR models based optimal wavelength of longissimus dorsi (LD)

3. Safety Attributes

The action of tissue enzymes, microorganisms and the degeneration of meat are the main factors which lead to meat spoilage. Meat spoilage is a dynamic and complex process which often manifest in internal changes (chemical components) and external changes (colour, texture, smell, et al). During storage, total volatile basic nitrogen (TVB-N) formed along with the changes of many substances and other basic nitrogenous compounds. TVB-N content in meat was considered as an important reference index to evaluate meat freshness. Total viable bacterial count (TVC) is one of the most important parameters during pork spoiling, by which we can have a knowledge of the degree of spoilage of meat [30]. Meats with excessive bacteria cause harm to human health, thus it is critical to guarantee the safety of meats. In addition, because of high commercial value meat industry, it has attracted the attention of adulterators for centuries. Therefore the determination and detection of adulteration are indispensable as there are some people who do not accept specific meat for religious reasons. In order to guarantee the legitimate rights and interests of consumers, to eliminate adulteration is necessary. The Fig. 14 revealed the potential authenticity problems in daily life [31].

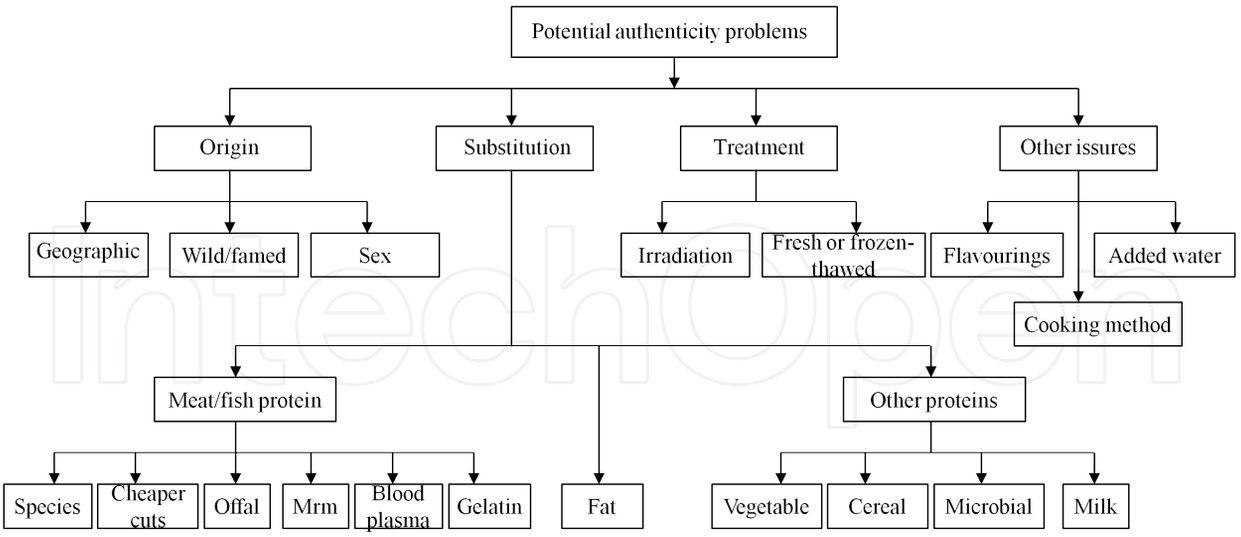

Figure 14. Potential authenticity problem

3.1. Total Volatile Basic Nitrogen (TVB-N)

Real-time and on-line detection of freshness is current industrial desire. An on-line multi-channel visible and near infrared spectral system (300-2500nm) has been developed by Zhao *et al* [32] which was progress on prediction of pork freshness. The whole system mainly included three sections: the hardware system, the software system and the model.

Among them, the hardware system mainly consisted of the visible and near infrared spectral system, signals transmission system and motion system (as is shown in Fig.15). The visible and near infrared spectral system was used for collecting spectral data. It was mainly composed of two fiber optic spectrometers (AvaSpec-2048×14 and AvaSpec-NIR256-2.5, Avantes Netherlands), fiber optic multiplexer (FOM-IR400-2×8, Avantes Netherlands), a light unit with a tungsten lamp as the light source (AvaLight-HAL, Avantes Netherlands), 7 reflection probes (FCR-7IR400-2-ME, Avantes Netherlands) and an electronic computer. The wavelengths of first fiber optic spectrometer ranged from 300 nm to 1100 nm with a spectral resolution of 0.04-20 nm, the second ranged from 1000 nm to 250 0nm with a spectral resolution of 6.0-90 nm. Various spectral data operation was completed by the spectrometers software package (Avantes AvaSoft). By using multiplexer control software (Avantes AvaFom), the switching sequence of many channels was achieved. In order to implement on-line visible and near infrared spectroscopy system [33], a secondary development was performed on the SDK platform of spectrometers software and multiplexer software.

During the actual experiment, more test points in the sample were required. Due to the need for multi-point measurement, firstly the quantity of the reflectance probe has to be determined. In multiple measurements, with increase of the number of measurements, the standard deviation of arithmetic mean decreases. To improve the accuracy of the measurement results, the number of measurements cannot be increased indefinitely. The general number of measurements is 5-10 times. There are six fibers in the outer ring of the probe

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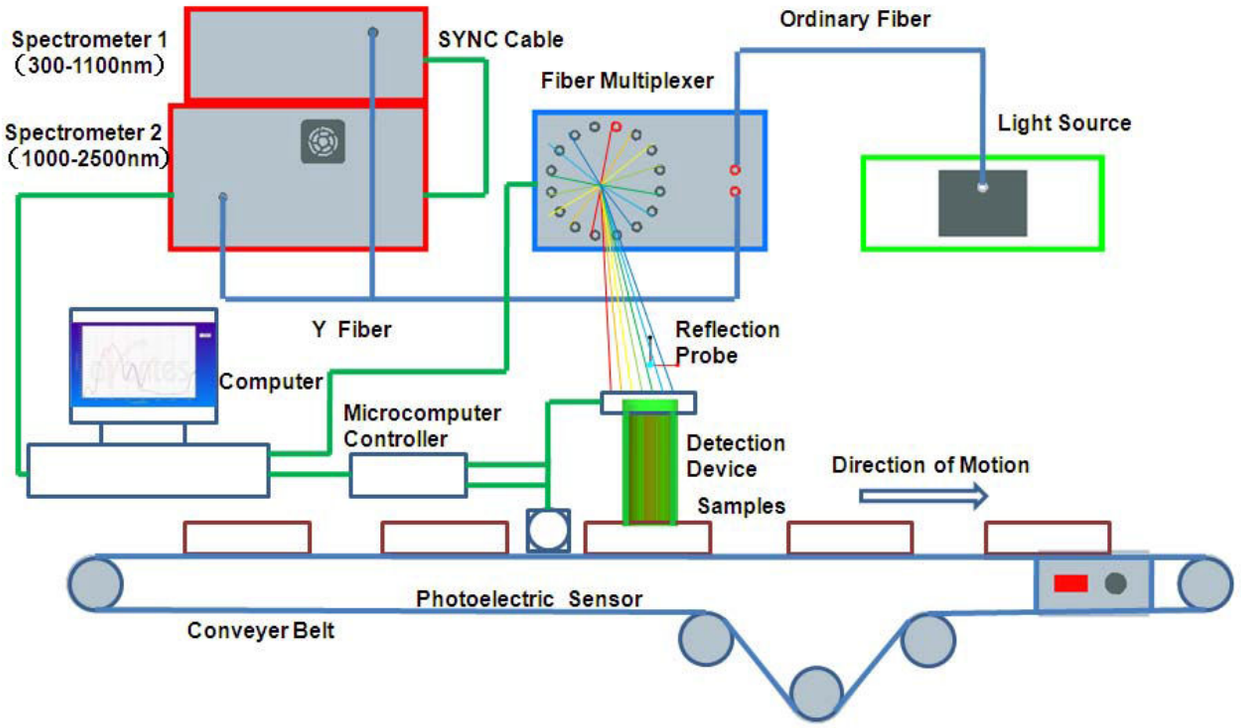

Figure 15. Real-time visible and near infrared multi-channel spectral system

and once fiber in the inner ring. From the arrangement of probe, seven fiber probes was chosen to design the detection device, and when the number is seven, the detection device could make the best of the information of the detection region. The arrangement of reflectance probe is shown in Fig. 16.

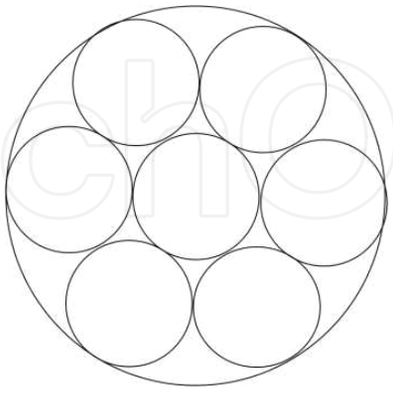

Figure 16. The arrangement of reflectance probe

The reflection probes were fixed in a device made up of a detection probe. The probe can acquire the spectral data from the pork cuts surface. Multi-point testing of the samples in different locations can be achieved through fiber multiplexer. Detection device was connected

with electric translation stage through connection frame. With the control of stepper motor, detection device could go up and down following the electric translation stage. The construct of connecting detection device is shown in Fig. 17.

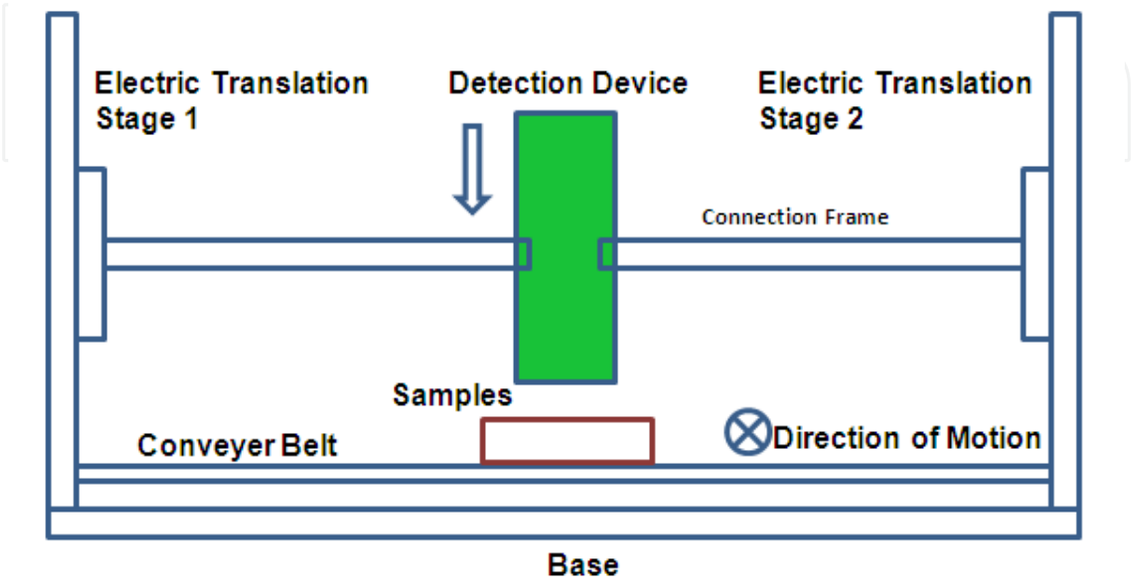

Figure 17. The construct of connecting detection device

Signal was transmitted and processed between the various parts of hardware by signal transmission system. The two spectrometers were connected with synchronization cable to ensure that the two spectrometers were synchronized. The second spectrometer was made the master spectrometer. The connected pattern between spectrometers makes them collect spectral data and display prediction results on the same software interface. Fiber multiplexer was connected with computer through the serial cable, so that computer could control the switching sequence of the fiber multiplexer. When sample approaches the required position, the photoelectric sensor produces an output signal to single chip microcontroller. The model of photoelectric sensor was E3Z-T61. Programmable System on Chip was used to receive signals from computer parallel port to control the stepper motor. With the control of stepper motor, the position of sample to be detected was controlled. Sample on arrival to the detection zone, signal is generated to control spectrometer and collect optical data with certain delay time. After collection of spectral data, control system retuned fiber multiplexer and detection device.

Motion system was used to deliver detection probe and samples and maintain constant distance between the detection probe and the surface of the sample to be detected. Motion system included motion parts of the conveyor belt and motion parts of detection device. Conveyor belt was designed to convey samples. Conveyor speed was adjustable. When testing the same batch of samples, the conveyor speed should remain constant. The detection device

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moved up and down which was controlled by motor, and when the detection device was in contact with the sample surface, the spectral data collection procedure was started.

The initial software interface is shown as Fig. 18. Before collection of spectral data, the spectrometer was calibrated with black reference and white reference. The measurement of black reference which is dark current spectral data was measured by turning off the light source. And the white reference was measured by the use of the white reference tile. After connection of hardware device, the software was operated. After receiving signal from the serial port, with proper delay time, the software triggered spectrometer to collect the spectral data from the sample. The delay time was determined according to the hardware structure. The spectral data acquisition was achieved through switching sequence of multi-channel fiber multiplexer, and then the multi-point measurement results were averaged, and finally the diffuse reflectance of sample were calculated. After the pre-treatment of spectral data, the corresponding freshness detection prediction model was called in the software to assess every indicator of freshness, finally the prediction results were displayed in the software interface.

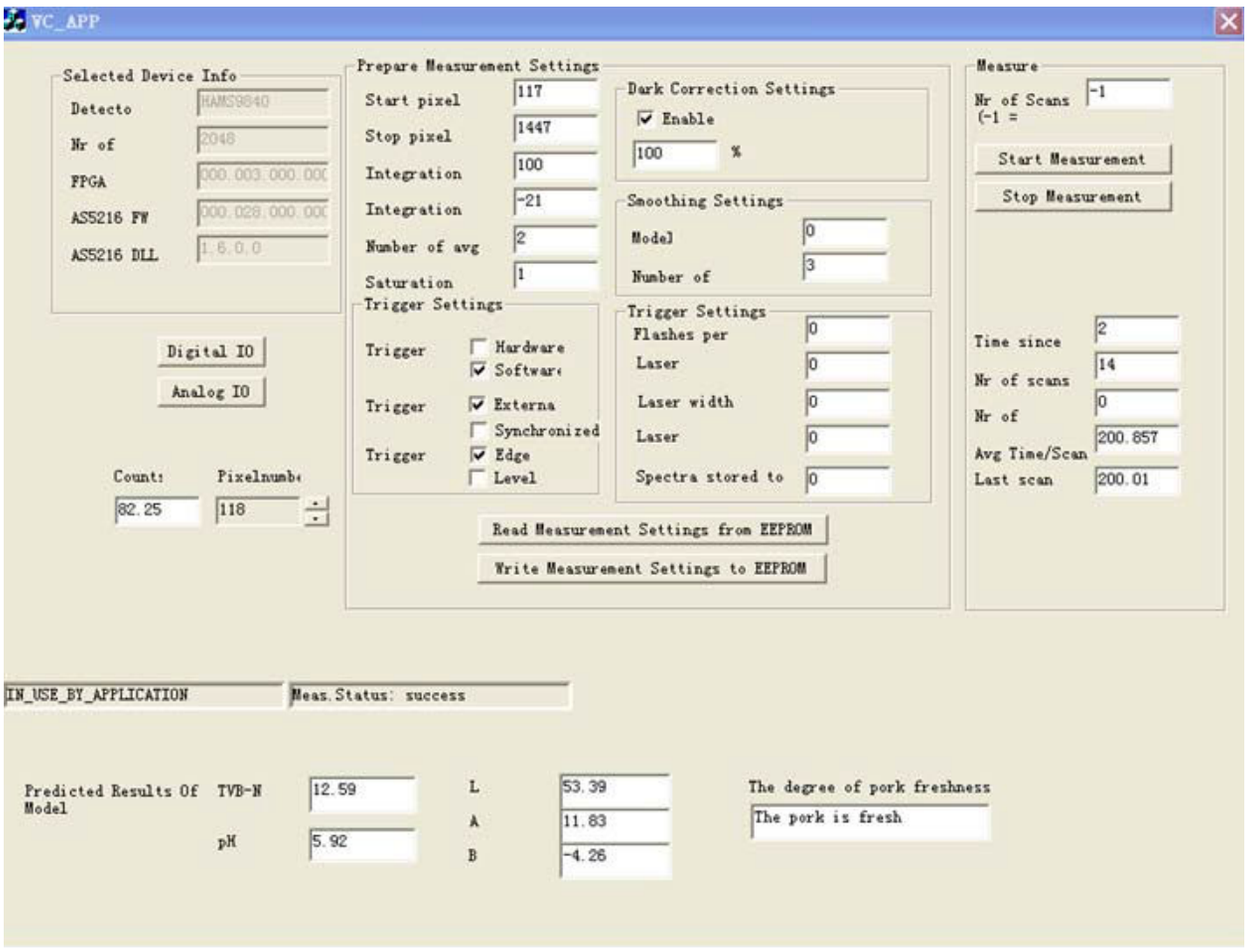

Figure 18. Start interface of software system

The prediction model was based on an experiment carried out under static environment. Diffuse reflectance spectroscopy data from the pork surface of 380-1080 nm was obtained by use of multi-channel visible near-infrared spectroscopy system. The pre-treatments of the spectral data were multiplicative scattering correction (MSC) and standard normal variables (SNV), then using partial least squares regression to establish pork freshness of prediction model, and then evaluated the freshness of pork. The results show that the partial least squares regression model after standard normal variables (SNV) treatment is relatively stable, and the performance is better. The correlation coefficient of TVB-N is 0.9. Finally, the accuracy rate of pork freshness assessment is 92.9% by this model.

3.2. Bacteria Contamination

Tao *et al* [34] made use of NIR hyperspectral imaging technique to realize determination of *Escherichia coli* contamination of pork. 31 fresh pork meat samples were purchased from a local market earlier on the day of the experiment and transported to the lab under refrigeration in 30 min. In order to avoid the effect from initial contamination, the surface pieces in 1-cm thickness were removed from all pork meat chops under sterile procedures. Then, all samples were trimmed to the size of 9 cm×5 cm×2.5 cm uniformly. The generic E. coli was provided by College of Food Science and Nutritional Engineering, China Agricultural University. The bacteria used were first activated by duplicate transfers and incubation in Nutrient Broth for 24 h at 37 °C respectively, and then delivered into 0.85% sterilized saline solutions to achieve series of bacterial suspensions. Pork meat samples with different E. coli loads were artificially contaminated by submerging into different concentrations of E. coli suspensions accordingly. Additionally, in order to ensure the adhesion of E. coli cells on pork meat samples, the samples were placed in the super clean bench for a certain time uniformly [35].

The cell counts of different E. coli suspensions were determined immediately after the sample preparation. Three original or diluted concentrations for each suspension were selected to be incubated on Nutrient Agar and two repetitions were performed on each concentration. All plates were incubated at 37°C for 48 h and the results were expressed in Log CFU/mL. The data of two repetitions was averaged and then used for further analysis. Then the images of pork samples were acquired by a hyperspectral imaging system in the spectral range of 400-1100 nm. This system was similar to that mentioned above. For each sample, an average image from four images was acquired for further analysis. In addition, 2×2 binning was performed when acquiring images to improve the signal-to-noise ratio. The final hyperspectral image was of 520×688 pixels. The hyperspectral image of pork meat was shown in Fig. 19.

Then Lorentzian distribution function and the modified Gompertz function were performed to fit the scattering profiles of pork in the spectral range of 400-1100 nm. Lorentzian distribution function can be mathematically expressed by Eq. (1).

$$R_{w_i} = a_{w_i} + \frac{b_{w_i}}{1 + \left(z / C_{w_i}\right)^2} \tag{1}$$

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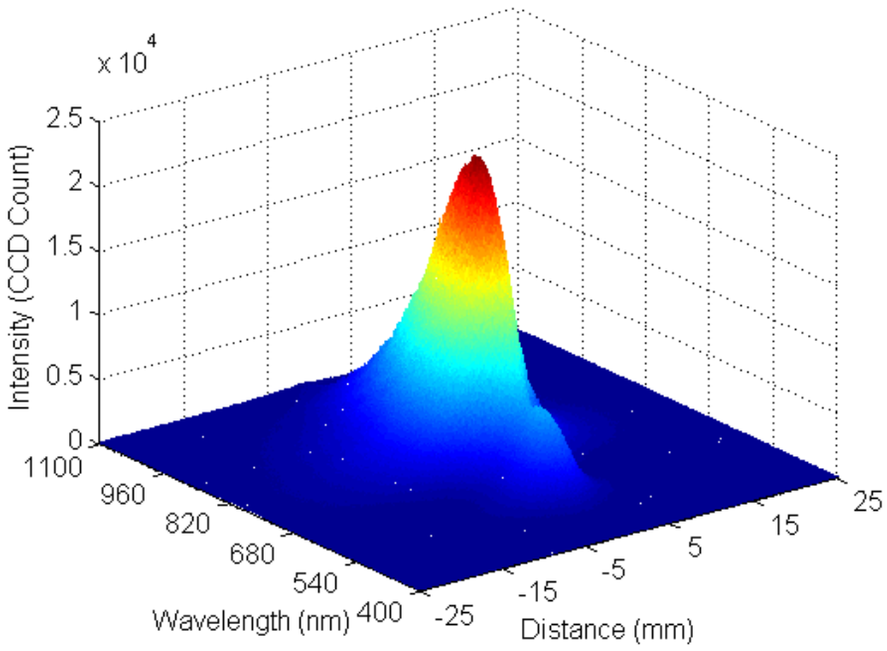

Figure 19. The hyperspectral image of pork meat in 3-D format

where R represents the light intensity, in CCD count; z means the scattering distance from the center of beam incident, in mm; a is the asymptotic value of the light intensity; b is the peak value of the scattering profile at z=0, in CCD count; c is the full width of the scattering profile at $b/2$ (FWHM), in mm. The subscript w_i represents one individual wavelength of the whole spectral range with i=1, 2, 3... N; and N is the total number of wavelengths. The modified Gompertz function with four parameters was shown in Eq. (2) [36].

$$R_{w_i} = \alpha_{w_i} + \beta_{w_i}\left(1 - e^{-\exp\left(\varepsilon_{w_i} - \delta_{w_i} z\right)}\right) \tag{2}$$

where R represents the light intensity, in CCD Count; z means the distance from the detected position to the light incident center, in mm; α is the asymptotic value of light intensity; β is related to the upper value of estimated light intensity at the light incident center; ε is the full scattering width at the inflection point; δ is the slope around the inflection point; w_i represents the designated wavelength in the spectral range of 400-1100 nm in accordance with i=1, 2, 3... N; and N is the number of wavelengths [37].

Then extracted three individual Lorentzian parameters a, b, c, combined parameters of (b-a), (b-a)×c, (b-a)/c, a&b&c and four Gompertz parameters α, β, ε, δ were used to establish MLR models in the study. The extracted parameters a and b for pork samples were shown in Fig. 20. Gompertz parametersα, β, ε, and δ from 31 pork meat samples were shown in Fig. 21.

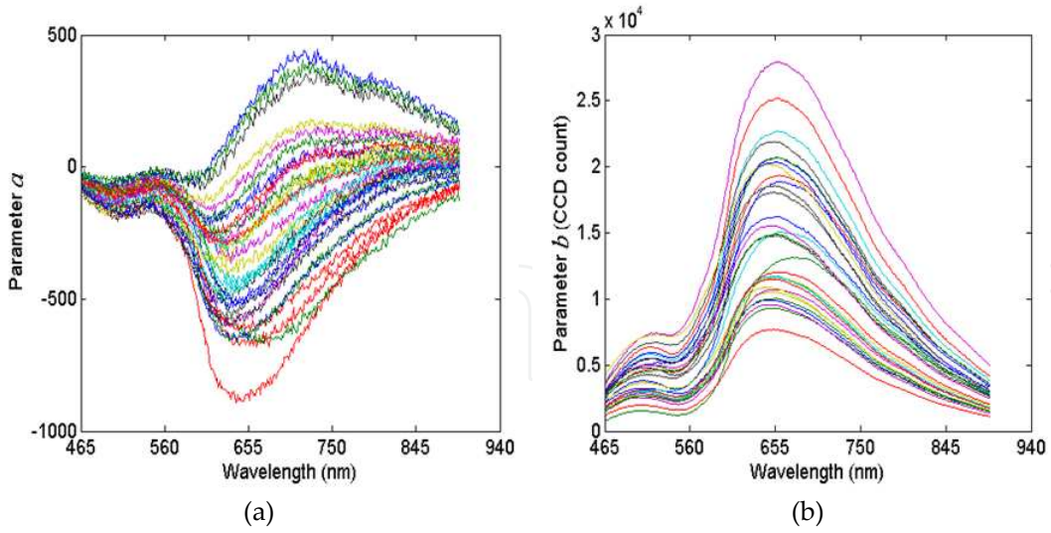

Figure 20. Lorentzian parameters extracted for 31 pork samples: (a) Parameter a (asymptotic value), (b) Parameter b (peak value)

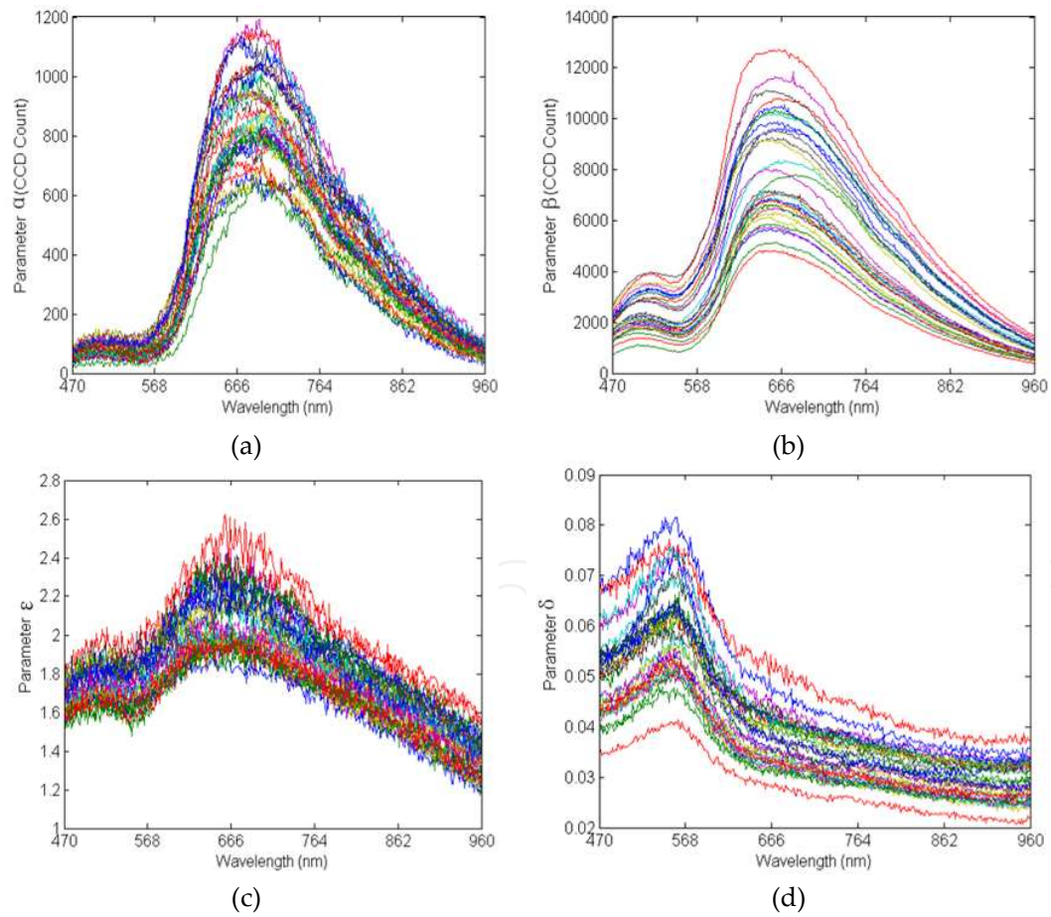

Figure 21. Gompertz parameters extracted from pork samples: (a) parameter α, (b) parameter β, (c) parameter δ, (d) parameter ε

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For the Lorentzian function fitting, the models developed using parameter *a* and integrated "*a&b&c*" variables determination method can give good validation result with Rv of 0.841. For the Gompertz function fitting, the model developed by parameter δ is superior to other parameters. In addition, the method of integrated parameter was also implemented to improve the model performance, and better validation results was obtained with Rv of 0.939 for pork E. coli contamination.

4. Conclusions and Future Outlook

The existing researches showed that when predicting the quality and safety attributes by NIR spectroscopy, minced samples present better results than intact tissue. It is mainly because minced samples are more homogeneous.. For intact samples, spectrum data obtained were affected by surface texture and inner component content. Meanwhile the spectrum data collected by surface diffuse method only represented the surface information. It was difficult to obtain internal spectrum because of the limit of depth, so it had great influence on the precision and stability of the model.The results of the present study verify that NIR spectroscopy is a suitable method to predict simultaneously several meat chemical properties. NIR shows superiorities not only in time and money consumption, but also in the detriment to body health or the environment. However, NIR spectroscopy didn't predict well when estimating some attributes of meat, for example WHC (water-holding capacity), mainly due to the low precision of the reference method. Therefore, better NIR models for such characteristics are required, mainly with respect to a better sampling procedure and improvement of the precision of the reference methods. Based on published results, NIR spectroscopy shows only limited ability for assessing the physical quality of meat.

Taking this limitation into consideration, it is difficult in this regard to envisage implementation at industrial level because of the low precision of the reference methods. More precise method and better control of other influential factors may be the fundamental way achieve improvements to this parameters.

NIR spectroscopy will be more and more popular in the meat detection industries. Nowdays, more effort has been made to reduce errors in reference methods, and more reliable models are developed by using larger sample sets which show wide ranges in reference values. In addition, the use of fiber optic probes may improve the ability of NIR to monitor and control meat processing using remote on-line detection. Furthermore, this technique does not require any consumables or supporting equipment once optimized and put into use, which has great advantage over the traditional techniques on solving the challenging quality control problems. More effort needs to be made to realize the implementation from off-line application to on-line approach [38].

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