Rapid Determination of Minced Beef Adulteration Using Hyperspectral Reflectance Spectroscopy and Multivariate Methods

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Abstract. This work aimed to assess the potential of hyperspectral reflectance spectroscopy for minced beef adulteration. For this, 30 samples of pure minced beef along with 90 samples adulterated beef at different levels were prepared and analyzed. Multivariate methods were used for spectral analysis to classify adulteration types. The best result was obtained by random forest (RF), where the accuracy of classification in prediction set was 87.78%. Considering the redundant information of reflectance spectra, the optimal wavelengths were selected by successive projection algorithm (SPA) to improve robustness. Selected important wavelengths models have a better classification effect than full wavelengths models. The optimal model developed by RF for detecting adulteration types achieved an accuracy of 96.87% in prediction set with selected wavelengths. Accordingly, hyperspectral reflectance spectroscopy with multivariate methods can provide the accurate and rapid detection of minced beef adulteration.

1. Introduction
Food adulteration has been widespread for a long time. It is one of the main problems of food quality and safety [1]. In 2013, beef burgers sold in some supermarkets in the British and Ireland were mixed with horse meat and other meats. The horse meat scandal caused great concern about the global adulteration of meat [2]. The adulteration of minced beef with cheap meat is a current problem, the basic principle of adulteration is to replace valuable meat with cheap meat in order to increase the economic return [3]. This kind of adulteration not only constitutes fraudulent abuse to consumers, but also seriously violates the rights and interests of consumers [4, 5].

Adulterated ingredients are usually similar to real products. For minced meat, it is difficult to identify one kind of minced meat from another, because meat choping removes the difference in muscle morphology, which is difficult to detect with the naked eyes [6]. Traditional analytical methods, such as chromatography, electrophoretic separation of proteins, and DNA-based techniques have been used for adulteration of minced meat [7, 8, 9, 10]. While these techniques provide powerful tools, they may not be practical choices for fast-paced processing environments because they are slow and disruptive, time consuming, need expensive experimental equipment and highly qualified staff [11]. They cannot be effectively evaluated in real time. In a word, the fast and convenient methods are needed.

Spectroscopy has been widely recognized as a fast, low cost adulterated detection technology for meat products [12]. Near infrared (NIR) spectroscopy is a fast and relatively easy technology to use [13]. It is simple, accurate, and does not consume chemical reagents. Because of these advantages, detection techniques have been widely used to classify chicken breasts and detect adulterated species.
in minced lamb and beef [14, 15]. However, the NIR spectra are limited to the near-infrared band, which can lead to the loss of important information. Hyperspectral imaging technology can detect both image information and spectral information [16]. However, collecting hyperspectral cube data is time consuming and usually contains the redundant information. Therefore, hyperspectral reflectance spectroscopy technology is more suitable for meat adulteration detection of the averaged visible-near-infrared (Vis-NIR) reflectance spectrum of analyte in the perceptual region. In the literature, few papers have reported the use of spectral technology to detect adulteration of minced beef. Therefore, the purpose of this study was to explore the feasibility of detecting minced beef adulteration by hyperspectral reflectance spectroscopy, and establish classification models using support vector machine (SVM) and RF to evaluate the adulteration types qualitatively.

2. Materials and Methods

2.1. Preparation of Samples
Fresh beef, beef hearts and pork were purchased from local supermarkets, and all beef samples were minced by a grinder. The beef hearts and pork were crushed in the same way as beef. The minced beef and other minced meat were weighed separately and mixed well, each sample weighing 20 g. It was hand-made into a disk and placed in a dry petri dish.

Minced beef samples were mixed with potential adulterants including pork, beef heart, with a range of 10%, 20% and 30% (w/w) for these adulteration ratios are more common. Types of adulteration include beef with pork, beef with beef hearts, beef, and pork with beef hearts. Then, 10 samples were prepared for each adulteration type for each adulteration proportion. Among them, 30 pure beef samples were prepared, and a total of 120 samples were obtained in this experiment.

2.2. Reflectance Spectra
The reflectance spectra of meat samples were collected using the PSR-3500® portable ground spectrometer (spectrum evol, Lawrence, MA, USA) and DARWIN SP software (Canal Street Lawrence, MA, USA). The instrument is equipped with three detectors, a 512Si array detector and two 256 InGaAs array detectors, measuring the reflection spectrum of 350-2500 nm. During the spectral acquisition process, the fiber optic probe is perpendicular to the sample at a distance of approximately 2 cm. Spectral acquisition is carried out in a black box, to the extent that external light interference is avoided and the dish is placed in the middle of the black box. Five reflection spectra were acquired for each individual sample, and the average reflection spectrum for each sample was used for the next analysis with a spectral resolution of 1 nm. The resulting reflectance spectrum is affected by environmental factors such as differences in illumination and system physical structure. Whiteboard calibration is used to eliminate or minimize these side effects.

The relative reflectance of each sample is calculated as follows:

\[ R_{ci} = \frac{D_{ci}}{D_{ri}} \cdot R_{ri} \]

Where, \( R_{ci} \) is the reflectivity of the meat samples, \( R_{ri} \) is the reflectivity the whiteboard. \( D_{ci} \) and \( D_{ri} \) are the initial radiation value of the meat samples and whiteboard, respectively.

2.3. Spectral Analysis Method

2.3.1. Data Processing. In spectral measurement, the original spectra not only contains the chemical composition information of the samples, but also is easily interfered by various effects such as random noise and background information, which makes the spectral data appear baseline drift or spectral overlap, thus affecting the accuracy of the prediction model. In this study, standard normal variate (SNV) and multiplicative scatter (MSC) were used to process spectral data, MSC is usually used for baseline correction, and SNV is used to eliminate the influence of noise.
2.3.2. Selection of Important Wavelengths. The acquired reflectance spectra contain a large amount of redundant data due to irrelevant variables. Therefore, the selection of important wavelengths is important for the establishment of a simplified and robust model. This study used SPA to choose representative wavelengths.

2.3.3. Model Building and Performance Evaluation. Classification models for detecting beef adulterants were established using SVM and RF. All the samples were divided into two groups. According to the Kennard-Stone algorithm, 80 samples were selected as the calibration set to build the model, and the remaining 40 samples were used as prediction set to estimate model performance. The classification model performance was assessed using the accuracy of calibration (ACCc) and prediction (ACCp). The above processing is performed in MATLAB (Version: R2014a, Mathworks, Inc. MA, USA).

3. Results and Discussion

3.1. Relative Reflectance for Pure and Adulterated Samples
The average reflectance spectra of minced beef with and without adulterants were measured, as shown in Figure 1. Although the spectral patterns of pure beef and adulterated beef are very overlapping, some of the prominent features can be attributed to individual chemicals. As for the visible region, the absorption band at 430nm was associated with the Soret absorption band of erythrocyte hemoglobin, and the bands at 540 and 560 nm were attributed to respiratory pigments [17]. The first absorption peak (525nm) was related to metmyoglobin, whereas the peak around 570 nm was due to the absorption of oxymyoglobin [18]. Absorption peak in the NIR region at 700 nm is due to O-H first stretching overtone. The spectrum at 856 nm and 1098 nm were assigned to the C–H third overtone. The spectrum at around 1225 nm and 1440 nm were attributed to O-H first stretching overtone and water absorption, respectively, and the peak at 980 nm was assigned to the second overtones of O-H bending. In the end, a small valley at around 1950 nm for the absorption bands of proteins related to the N-H overtones [19].

In the Vis-NIR region, beef samples adulterated with pork displayed obvious reflectivity at 450-2500 nm, while beef samples adulterated with beef heart showed obvious regional reflectivity at 750-1300 nm. The reflectivity of the beef with the pork and the heart is between the reflectivity of the beef with pork and beef heart.

![Figure 1. Mean Spectra of Various Minced Beef](image-url)
3.2. Classification Results Using the Full Wavelengths
SVM and RF were used to construct models for the adulteration of minced beef. The experiment results of the two models were shown in Table 1. The SVM model with raw spectra classified the samples with the accuracy of calibration set being 86.55% and the accuracy of prediction set being 84.17%. The classification result of the RF model obtained with raw spectra was better than that of SVM. The two pretreatment methods can improve analysis accuracy, and the improvement effect of MSC is the greatest. Therefore, the classification of adulterated beef using hyperspectral reflectance spectra is feasible and accurate with RF and MSC.

| Model | Pretreatment | ACC_{C} (%) | ACC_{P} (%) |
|-------|--------------|-------------|-------------|
| SVM   | RAW          | 86.55       | 84.17       |
|       | SNV          | 90.75       | 87.28       |
|       | MSC          | 92.96       | 90.14       |
| RF    | RAW          | 89.55       | 87.78       |
|       | SNV          | 91.12       | 89.13       |
|       | MSC          | 94.87       | 92.54       |

3.3. Classification Results Using the Selected Wavelengths
Eliminating redundant data unrelated to target attributes and specific wavelengths can improve robustness and simplify the model. SPA can select the spectra of important wavelengths to remove redundant information and improve analysis robustness. Meanwhile, the usage of important wavelength can be adapted to simplify the detection equipment and save time in the follow-up detection. For the spectral information, the model optimal parameters and results of different max parameters are obtained by changing the maximum effective wavelength number, as shown in Figure 1. As can be seen from the figure, the RMSEP decreases as the number of selected variables increases, and the curve remains stable when the number of variables is greater than 13. When the number of variables is 12 (marked in red), RMSEP reaches the optimal value. Figure 2 shows the 12 variables selected. The results show that the proposed variable selection method is feasible and effective, and the necessity of eliminating spectral redundant data is emphasized. In addition, a simplified spectral system can be designed to identify meat adulteration with SPA.

![Figure 2. Number of the Selected Variables](image)

![Figure 3. The Selected Twelve Variables](image)

SVM and RF were used to develop models by MSC. Table 2 showed the results of classification models for pure and adulterated samples using important wavelengths. RF performed best with the optimal wavelengths selected by SPA (509, 704, 738, 806, 956, 1126, 1198, 1285, 1348, 1406, 1498...
and 1519 nm) using MSC pretreatment (ACC$_C$ = 96.87% and ACC$_P$ = 95.04%). Moreover, using the selected wavelengths showed better performance than the full wavelengths models. In summary, after removing the effects of noncritical factors, the performance of the classification models with optimum wavelengths improved effectively.

The results indicate that the variable selection method is effective, and the necessity of eliminating spectral redundant data is emphasized. In addition, a simplified spectral system can be designed to identify meat adulteration with the optimum wavelength obtained.

### Table 2. Classification Results Derived from the SVM and RF Models Using the Optimal Wavelengths

| Method  | Wavelength (nm) | ACC$_C$(%) | ACC$_P$(%) |
|---------|-----------------|------------|------------|
| SPA-SVM | 509, 704, 738, 806, 956, 1126, 1198, 1285, 1348, 1406, 1498, 1519 | 94.96 | 92.50 |
| SPA-RF  | 96.87 | 95.04 |

### 4. Conclusion

This study shows that hyperspectral reflectance spectroscopy with multivariate methods, SVM and RF, have the potential for the rapid detection of minced beef adulteration. MSC and SNV were applied to remove disturbance, SPA was applied to reduce redundant information. SVM and RF were used for classification. With the optimal wavelengths, the RF model exhibited the best capability in the classification of adulteration. Accordingly, hyperspectral reflectance spectroscopy with multivariate methods led to the accurate and rapid detection of minced beef adulteration. It can be expected to discriminate other agricultural adulteration.

### 5. Acknowledgment

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