# **Quantitative Detection of Myoglobin Content in Tan Mutton During Cold Storage by Near‑infrared Hyperspectral Imaging**

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# **Abstract**

The discolorations or abnormal colors in meat and meat products during processing and storage have negative efects on their commercial value. In this study, myoglobin content (MetMb and OxyMb) in Tan mutton was rapidly detected using near-infrared hyperspectral imaging (NIR-HSI) system (900–1700 nm), and built predictive models with full wavebands (FW) based on partial least squares regression (PLSR), least-squares support vector machines (LSSVM), and back propagation neuron network (BP). To reduce the computational complexity of calibration models, feature bands were obtained by bootstrapping soft shrinkage (BOSS), variable combination population analysis coupled with iteratively retains informative variables (VCPA-IRIV), and competitive adaptive reweighted sampling (CARS), respectively. The optimized BP model based on feature wavebands with BOSS method selection displayed the best capability for predicting MetMb level ( $R^2$ <sup>-</sup>C=0.8340,  $R^2$ <sub>P</sub> = 0.8253 *RMSEC* = 3.1592, *RMSEP* = 3.2918). In addition, the simplified VCPA-IRIV-BP model was significant in predicting OxyMb content with  $R^2_{\text{C}}$ ,  $R^2_{\text{P}}$ , *RMSEC*, and *RMSEP* values of 0.8024, 0.8680, 3.4676, and 2.7605, respectively. Results provided a theoretical reference for rapid evaluation of myoglobin content in other animal products via NIR-HSI.

**Keywords** Tan mutton · Quantitative detection · Near-infrared hyperspectral imaging · Myoglobin content · Variable selection · Back propagation neuron network

# **Introduction**

Besides its commercial value, mutton is a wealth source of protein, vitamins, and minerals (Yu et al. [2021a](#page-12-0)). Tan sheep, an important local sheep breed in Ningxia, China, is famous for its high-quality mutton. With the improvement of consumers' awareness of food safety, there is a high demand for the quality of meat products. As a traditional meat-preservation method, low-temperature storage delays

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the deterioration of meat and thus prolongs shelf life and maintains meat quality (Coombs et al. [2017](#page-11-0)). Although activities of the microorganism can be restrained by low temperature during cold storage, some reactions, such as psychrophilic microbial activity and enzymatic hydrolysis reaction, are still active (Cheng et al. [2021\)](#page-11-1), which directly lead to the discoloration of Tan mutton in the process of processing, transportation, and sales. Meat color usually infuences consumers' appetites for merchandise; the discoloration or abnormal color in meat and meat products during shelf life has negative efects on their commercial value (Maria and Luis. [2014](#page-11-2); Mancini and Hunt [2005](#page-11-3); Mancini and Ramanathan [2020](#page-11-4)).

Meat color is closely related to the relative content of three diferent chemical forms of myoglobin: deoxymyoglobin (DeoMb, amaranth), oxygenated myoglobin (OxyMb, bright red), and metmyoglobin (MetMb, grayish brown), respectively (Mancini and Hunt [2005](#page-11-3); Nguyen et al. [2016,](#page-12-1) [2019](#page-12-2)). In general, DeoMb predominates in meat before slaughter (Shin et al. [2021\)](#page-12-3). As meat is exposed to air after slaughter, the DeoMb on the meat surface is quickly transformed into OxyMb in the oxygenation process. Besides,



oxidation occurs from the interior of the meat, which transforms DeoMb and OxyMb into MetMb (Nguyen et al. [2016](#page-12-1)). Meanwhile, the contents of MetMb and OxyMb have dominant position after slaughter. The myoglobin redox state for the surface of postmortem muscle is shown in Fig. [1.](#page-1-0)

Traditional analytical methods for assessing myoglobin in meat include sensory evaluation and chemical determination (Mancini [2013\)](#page-11-5). However, the disadvantages of these methods are that they require cumbersome pre-processing, complicated operation, copious consumption of reagents, destructive detection for samples, and so forth, failing to meet the rapid, accurate, and nondestructive requirements of modern food industry. Moreover, the diferent levels of experiment profciency may cause errors in measurement (Kuswandi and Nurfawaidi [2017;](#page-11-6) Shin et al. [2021](#page-12-3)). In recent years, hyperspectral imaging (HSI) technology has been widely applied in agricultural product detection because of its fast and non-destructive characteristics, and has become promising in evaluating and monitoring food quality (Ma and Sun [2020](#page-11-7)). HSI could simultaneously acquire spectral and spatial information at each pixel of an object. Spectral images are three-dimensional (3-D) in nature, with two spatial dimensions and one spectral dimension [hypercubes (x, y, λ)] (Qin 2013). The most important application of HSI in meat is line-scanning, which is signifcant for fundamental food research owing to its high resolutions and rapid scanning speed (Ma et al. [2019](#page-11-8)). Recently, HSI has been utilized to measure the parameters related to the internal and external qualities of meat, such as pH (Barbin et al. [2012\)](#page-10-0), fat content (Lohumi et al. [2016](#page-11-9)), thiobarbituric acid reactive substances (TBARS) (Cheng et al. [2019\)](#page-11-10), meat tenderness (Knight et al. [2019\)](#page-11-11), total volatile basic-nitrogen (TVB-N) (Baek et al., [2021](#page-10-1)), bacterial food-borne pathogens (Bonah et al. [2020](#page-10-2)), and fatty acid (Wang et al. [2020a](#page-12-4)). The detection of meat

muscle

color by using hyperspectral imaging was mainly focused on L\*, a\*, and b\* values (ElMasry et al. [2012](#page-11-12); Jiang et al. [2018](#page-11-13); Kamruzzaman et al. [2016](#page-11-14); Wu et al. [2012](#page-12-5)). Regarding the content of myoglobin, Yuan et al. [\(2020](#page-12-6)) and Cheng et al. ([2021](#page-11-1)) found that Vis/NIR and NIR hyperspectral imaging could be employed to detect myoglobin. Wan et al. ([2020\)](#page-12-7) used the HSI (900–1700 nm) for a non-destructive detection of the contents of DeoMb, OxyMb, and MetMb in nitrite-cured mutton during refrigerated storage. It could be inferred from these studies that although hyperspectral imaging has shown the potential for myoglobin prediction, the predictability of MetMb content by Yuan et al. ([2020\)](#page-12-6) was obtained with  $R^2$ <sub>P</sub>=0.7654 and *RMSEP* = 2.9306, the model performances are still inadequate and much improvement is required. It could also be drawn from the studies mentioned above that this modality needs to further develop robust algorithms for the analysis of aberrations from desirable characteristics and improving computational strategies of predictive model before application to meat products. For instance, Wan et al. ([2020](#page-12-7)) found that a total of 36 and 33 feature wavelengths for OxyMb and MetMb were selected by CARS, respectively. Nevertheless, it is still necessary to reduce the dimensionality of band and improve the band selection performance by BOSS and VCPA-IRIV algorithm.

Therefore, the aims of the current study were to (a) evaluate the efectiveness of diferent pre-processing methods and select the optimal method; (b) perform BOSS, VCPA-IRIV, and CARS as reference technologies to identify and extract key wavebands that were closely related to the myoglobin of Tan mutton to simplify predictive models; and (c) analyze and optimize quantitative strategies (such as PLSR, LSSVM, and BP) to predict the content of myoglobin (MetMb and OxyMb) based on key wavebands and full wavebands. Figure [2](#page-2-0) presents the schematic description of

<span id="page-1-0"></span>

IV(CarboxyMb):DeoMb+CO=COMb



<span id="page-2-0"></span>**Fig. 2** The schematic description of the HSI method depicting the whole experimental processes

the HSI detection method toward myoglobin content of Tan mutton during cold storage.

# **Materials and Methods**

#### **Sample Preparation**

The experiment was conducted in the laboratory of agricultural products non-destructive testing, School of Food & Wine, Ningxia University. Samples from the longissimus dorsi of Tan sheep were purchased from a local marketplace in Ningxia, China. All these carcasses were individually placed in a portable refrigerated incubator covered with ice, which was similar to the storage conditions of the refrigerator and then transported to our lab within 2 h and stored at 4℃. After the chilling and aging of the carcasses, samples were removed of fat and connective tissue, and then cut into a size of  $40 \times 40 \times 10$  mm; finally, 200 pieces obtained were collected for investigation. All samples were randomly divided into 10 groups, individually packaged by a polythene bag, labeled, and stored in a refrigerator at 4℃. During experiment, each mutton sample was wiped off the surface moisture to obtain its spectral image and then to measure the myoglobin contents during diferent storage times (1, 4, 7, 10, 13, 16, 19, 22, 25, and 28 days). The spectral and experimental measurements were conducted at a room temperature of 21℃ during winter.

Myoglobin was extracted from Tan mutton by following the method of Krzywicke ([1982](#page-11-15)) with minor modifcations. Firstly, 5-g Tan mutton samples were chopped in 25 mL phosphate bufer (0.04 mol/L, pH 6.8), evenly mixed, and then homogenized at 10,000 r/min for 25 s by an ultrafne homogenizer. Secondly, the homogenized solution was stored at 4℃ for 1 h in the dark, centrifuged at 6000 g at 4℃ for 15 min, and the supernatant was fltered. Finally, the absorbance of the fltrate was read at 525, 545, 565, and 572 nm using a UV-2401PC spectrophotometer (Shimadzu Inc., Columbia, MD, USA) with phosphate buffer as blank, respectively. The contents of MetMb and OxyMb were calculated as follows:

 $[MetMb] = (-2.514R<sub>a</sub> + 0.777R<sub>b</sub> + 0.800R<sub>c</sub> + 1.098) \times 100$ 

$$
[OxyMb] = (0.882R_a + 1.267R_b + 0.809R_c - 0.361) \times 100
$$

Here,  $R_a$ ,  $R_b$ , and  $R_c$  were absorbance ratios of  $A_{572 \text{ nm}}/$  $A_{525 \text{ nm}}$ ,  $A_{565 \text{ nm}}/A_{525 \text{ nm}}$ , and  $A_{545 \text{ nm}}/A_{525 \text{ nm}}$ , respectively.

## **Hyperspectral Acquisition**

Hyperspectral data was obtained by InGaAs camera (Models XC-130 100 Hz, Ophir Optronics Solutions Ltd., Jerusalem,

Israel) with a spectral range from 900 to 1700 nm and four 35 W halogen lamps (ViP V-light, Lowel Light Inc., NY, HSIA-LS-TDIF, Zolix instruments Co., Ltd., Beijing, China) in a dark box. Each mutton sample was placed on a mobile platform and scanned with a linear array push-scan (ImSpector N17E, Specim, Spectral Imaging Co., Ltd., Oulu, Finland). HSI system was preheated for 30 min before image acquisition (Cheng et al. [2021\)](#page-11-1). The spectral images of samples were collected by SpectraCube software.

To reduce noises and invalid information caused by dark current and uneven illumination in the InGaAs device of HSI system, it was necessary to calibrate with dark  $(R_D)$ and white  $(R_W)$  references before getting the hyperspectral images (Liu et al., [2019\)](#page-11-16). The bright and dark currents were corrected before measurement. The correction formula was as follows:

$$
R = \frac{R_O - R_D}{R_W - R_D} \times 100\%
$$

*R* is the relative spectral reflectance after correction, and  $R_{\text{O}}$ ,  $R_{\text{D}}$ , and  $R_{\text{W}}$  are the experimental spectral reflectance, dark current spectral refectance, and bright current spectral reflectance, respectively (Wu et al., [2013\)](#page-12-8).

## **Spectral Data Analysis**

#### **Pre‑processing of Spectral Data**

The spectral refectance of each mutton sample was extracted via ENVI 4.8 software, and the region of interest (ROI) was used to manually operate and extract the average spectrum of the target region.

Original spectral data may be afected by the signal noise from the uneven surface of Tan mutton samples, as well as the baseline drift, scattered light, and other factors during the hyperspectral image collection. Therefore, it is necessary to perform the spectral pre-processing on hyperspectral images for increasing computation speed and improving the stability and robustness of model. In the present study, some pre-processing methods of spectral data including baseline (Yang et al. [2021\)](#page-12-9), normalize (Feng et al., [2019\)](#page-11-17), Gaussian flter (GF) (Yuan et al. [2021\)](#page-12-10), moving average (MA) (Tao and Peng [2015\)](#page-12-11), Savitzky-Golay (SG) (Chen et al. [2011](#page-11-18)), and combinations thereof were used to improve model prediction performance.

## **Feature Waveband Selection**

Hyperspectral data contain a large amount of information (e.g. spectral bands), causing the analysis to be time-consuming. Additionally, most bands are independent of the fnal prediction. Therefore, feature waveband selection is a core procedure to reduce the dimension of calibration data and complexity of calibration model for improving calculation speed (Lorente et al. [2012\)](#page-11-19). In this study, competitive adaptive reweighted sampling (CARS), bootstrapping soft shrinkage (BOSS), and variable combination population analysis coupled with iteratively retaining informative variables (VCPA-IRIV) were employed to select feature wavebands.

BOSS algorithm is used to generate random combination of variables and construct sub-models by combined bootstrap sampling (BBS) and weight bootstrap sampling (WBS) techniques (Jiang et al. [2021](#page-11-20)). Model population analysis (MPA) is adopted to extract variable information from submodels (Deng et al. [2016](#page-11-21)). CARS is a variable selection method which simulates the "survival of the fttest" principle of Darwin's evolution theory. The bands with larger absolute value of regression coefficient are selected from partial least squares (PLS) model by adaptive reweighted sampling (ARS) technique in an iterative and competitive manner (Shao et al. [2021](#page-12-12)). N subsets of variables are screened out through N samplings, and the subset with the lowest *RMSECV* value is referred to optimal feature bands (Wang et al. [2020b](#page-12-13)). VCPA-IRIV is used to minimize the variable space and maintain a specifc number of variables for further computation of IRIV (Yun et al. [2019\)](#page-12-14). IRIV is implemental to optimize the variable space created by VCPA. A mixed-methods approach of VCPA utilizes its advantages in constantly lessening the variable space and retaining signifcant variables to overcome the disadvantages of IRIV in calculation with a large number of variables (Yu et al. [2021b](#page-12-15)).

## **Model Development**

In this study, partial least squares regression (PLSR), back propagation neuron network (BP), and least-squares support vector machines (LSSVM) were employed to build quantitative models to predict the myoglobin content in Tan mutton after slaughter.

As a linear multivariate regression method, PLSR has been widely used to construct the relationships among spectral measurements, including correlation variables, chemical composition determination indexes and so on. In this study, the model was utilized to predict the content of myoglobin in Tan mutton. Latent variables (LVs) were extracted, and the calibration model was validated by leave-one-out crossvalidation to avoid overftting or underftting and to achieve good performances (Feng et al. [2018a\)](#page-11-22). LSSVM algorithm is an extension of the support vector machine (SVM), which can be used in approximate nonlinear systems with higher accuracy. There are two major parameters that determine the accuracy of model on LSSVM: kernel function parameter ( $\sigma^2$ ) and regularization parameter (γ). The generalization capacity of the model augments with the reduction of the  $\gamma$ , but the training error increases, and the smaller the  $\sigma^2$ , the higher the complexity of the model and the larger the  $\sigma^2$ , which can easily lead to under-learning (Mo et al. [2020](#page-11-23)). There are three layers in a BP model called the input layer, the hidden layer, and the output layer (Ma et al. [2019](#page-11-8)). The model is trained by comparing the diferences between the network output and the reference property and by minimizing their error with changing the linkage weights (ElMasry et al. [2009](#page-11-24)). Then, the BP is built with acceptable errors by continuously adjusting the weights.

## **Model Evaluation**

Some statistical parameters including the coefficients of determination in calibration  $(R^2_C)$ , prediction  $(R^2<sub>p</sub>)$ , full cross-validation  $(R^2_{CV})$ , the root mean square errors in calibration (*RMSEC*), prediction (*RMSEP*), and cross-validation (*RMSECV*) (Cascant et al. [2016](#page-11-25)) were used to evaluate model performance. While  $R^2$  between 0.66 and 0.81 was acceptable, values between 0.82 and 0.90 and above 0.90 represented good and excellent results (Karoui et al. [2006](#page-11-26)). RMSE was calculated between predictive and observed values (Feng et al. [2018b](#page-11-27)). Therefore, a good model has low values of *RMSEC* and *RMSEP*, the diferences between these two values are extremely small, and high values of  $R^2c$ ,  $R^2cv$ , and  $R^2p$  (Feng et al. [2019](#page-11-17)). The formulas for  $R^2$  and  $RMSE$ were as follows:

$$
R^{2} = \frac{\sum_{j=1}^{n} (y_{j} - \overline{y}_{j})^{2} - \sum_{j=1}^{n} (y_{j} - \hat{y}_{j})^{2}}{\sum_{j=1}^{n} (y_{j} - \overline{y}_{j})^{2}}
$$

$$
RMSE = \sqrt{\frac{1}{n} \sum_{j=1}^{n} (\overline{y}_j - y_j)^2}
$$

where  $y_j$  and  $\hat{y}_j$  are the practical measured value and predictive estimated value, respectively;  $\bar{y}_j$  is the mean of reference measurement; and *n* is the number of samples.

#### **Software**

PLSR and pre-processing methods were implemented via Unscrambler X10.4 software (Version 10.4, CAMO, Oslo, Norway). The procedures of SPXY, CARS, BOSS, VCPA-IRIV LSSVM, and BP were executed via MATLAB R2019a software (The Mathworks Inc., Natick, MA, USA). Identifcations of a region of interest (ROI) and extraction of spectral data were achieved via ENVI 4.8 software (ITT Visual Information Solutions, Boulder, Co., Ltd., USA).

# **Results and Discussion**

# **NIR Spectral Features of Samples**

The original and mean refectance spectra extracted from Tan mutton samples in the range of 900–1700 nm were depicted in Fig. [3.](#page-5-0) Figure [3b](#page-5-0) shows the diferences in the refectance of the curves in the visible region. Samples of refectance in the range of 910 to 1400 nm difered in the magnitude of refectance but not in trends. Generally, the peaks were observed in the NIR region of meat components broad bands arising from highly overlapping bands of the chemical bonds C-H, O–H, and N–H groups (Kamruzzaman 2012; Osborne [2000](#page-12-16)). In the NIR band, the major absorption bands were observed in 1005, 1241, and 1443 nm. The absorption wavelengths near 1200 nm (1241 nm) and 1600–1700 nm were due to C-H stretching the 1st and 2nd overtone, respectively (Wang et al. [2020a](#page-12-4)), while those near 1400–1660 nm (1443 nm) were due to the 1st overtone of O–H and the 2nd overtone of N–H stretching modes of water-bonded groups (Pu et al. [2015](#page-12-17)).

## **Outliers**

Samples that afected model performance were examined as abnormal samples. Based on the distributions of means and standard deviation (SD) of prediction errors for MetMb and OxyMb in each sample, Monte Carlo outlier method (Zhang 2017) was used to detect anomalous samples. The outlier values were removed from the original data, and the remaining data were used to build PLSR detection model. The overall performance of model was improved for the detection of MetMb and OxyMb upon the removal of outliers, with the  $R^2c$  increased from 0.7993 to 0.8164 and 0.7785 to 0.8183, respectively, and the *RMSECV* decreased from 5.2565 to 4.9425 and 4.4732 to 3.9735, respectively. As shown in Table [1,](#page-5-1) calibration set and prediction set were divided by SPXY algorithm, and the means and SD for the calibration and prediction set were compared, which signifed an analogous sample spread between those two sets. The advantage of SPXY can effectively bestow the multidimensional vector space and thus enhance the robustness of the model (Wei et al., [2020](#page-12-18)).



<span id="page-5-0"></span>**Fig. 3** Spectral curves of samples with diferent storage periods. **a** Original spectral curves; **b** mean spectral curves

<span id="page-5-1"></span>**Table 1** Statistics of relative MetMb and OxyMb contents of samples

<b>Subsets</b>	Calibration set							Prediction set					
	N	Max	Min	Mean	SD.	$CV(\%)$	N	Max	Min	Mean	<b>SD</b>	$CV(\%)$	
MetMb	145	54.0224	16.3248	33.4178	9.5920	28.70	49	50.6423	17.8136	36.5319	9.4420	25.85	
OxyMb	142	53.8122	24.0138	38.4713	7.7360	20.11	47	50.6403	24.7749	35.5443	7.5919	21.36	

## **Pre‑processing of Spectral Data**

The original spectra can be rectifed by pre-processing method to enhance the resolution of overlapping data and reduce the instrument drift scattering and system noise. Figure [4](#page-6-0) shows the results of performance of full wavelength model with references MetMb and OxyMb values based on diferent pre-processing methods. MA-SG preprocessing displayed the highest accuracy for prediction. The  $R_{\text{p}}^2$  values of the optimal models for the contents of MetMb and OxyMb were 0.7217 and 0.7911, respectively, which were higher than the bound with raw data. And the  $R^2$ <sub>C</sub> = 0.8355, 0.8152, *RMSEC* = 3.8906, 3.3256, and *RMSEP*=5.2304, 4.3763, respectively. Therefore, the most appropriate pre-processing method of predicting the MetMb and OxyMb contents of the mutton samples was MA-SG. Compared with original spectra, the results were improved by the MA-SG pre-processing, which might be explained by decreased scattering efects or eliminating signal noises. To reduce interference information, MA and SG have been widely used in HSI research (Vaiphasa [2006](#page-12-19)). Weng (2020) and Tao and Peng ([2015](#page-12-11)) found that SG and MA obtained good results in hyperspectral scattering imaging.

# **Feature Waveband Selection**

After being pre-processed by MA-SG, a total of 256 wavebands for MetMb and OxyMb were obtained in the NIR range (900–1700 nm). To decrease the dimensionality of spectral data and computation time, CARS, VCPA-IRIV, and BOSS were employed to select feature wavebands from the full spectra range. Each variable selection method had randomness in the operation process and influenced the reliability of the prediction model. Each of the above algorithms was calculated 50 times. Finally, the minimum value of *RMSECV* was selected as the final feature wavelength selection result of each variable selection method. *RMSECV* values reduced with the deletion of irrelevant bands. When *RMSECV* reached its minimal value, the optimal bands were obtained (Shao et al. [2021](#page-12-12); Xu et al. [2019\)](#page-12-20).

Figure [5](#page-7-0) presents the extraction of feature wavelengths by using CARS algorithm; the CARS weight peaks or valleys for reflectance were mainly in the spectral range from 1050 to 1150 nm or from 1300 to 1670 nm for MetMb (900 to 1250 nm or from 1350 to 1600 nm for OxyMb), indicating that this algorithm could explicate most of the damage information (Fig. [5a\)](#page-7-0). The peaks or valleys of 1050 to 1250 nm were assigned smaller weights than 1350 to 1650 nm, but they still contained some valid information (Fig. [5b](#page-7-0)), so some effective information of the these bands were extracted. According to the mean weight of every wavelength, 22 and 20 characteristic variables were selected for MetMb and OxyMb (Table [2\)](#page-7-1) accounting for 8.6% and 7.8% of the total wavelengths, respectively. The positions of feature variables are shown in Fig. [5c](#page-7-0). Figure [6](#page-8-0) depicts the distribution of feature bands selected by BOSS algorithm. WBS is applied to generate the submodel by weight and MPA is used to analyze the submodel to update the weights of features (Deng et al. [2016](#page-11-21)). The procedure of optimization feature by BOSS, in which less important variables are not eliminated directly but are assigned smaller weights. There were 15 and 21 feature bands for MetMb and OxyMb, respectively, accounting for 5.9% and 8.2% of the total bands. Figure [7](#page-8-1) shows the selection of feature wavelengths by using VCPA-IRIV algorithm; Fig. [7](#page-8-1) [a](#page-8-1) and [c](#page-8-1) show that when the number of sampling runs was 49 and 39, *RMSECV*



<span id="page-6-0"></span>**Fig. 4** Performances of full wavelength model based on diferent pre-processing methods of **a** MetMb and **b** OxyMb



<span id="page-7-0"></span>**Fig. 5** Running process of selecting optimal variables by CARS algorithm for: mean weight of CARS runs for refectance (**a** 900–1700 nm; **b** 900–1350 nm); **c** optimal characteristic wavelengths

attained the lowest value of 4.0123 and 3.5706 for MetMb and OxyMb, respectively. As presented in Fig. [7](#page-8-1) [b](#page-8-1) and [d](#page-8-1), a total of 23 and 18 feature wavelengths for MetMb and OxyMb were selected by VCPA-IRIV from full wavelengths, respectively, accounting for 9.0% and 7.0% of the total wavebands. Those feature wavebands selected

<span id="page-7-1"></span>**Table 2** Feature waveband selection based on diferent algorithms

Category	Methods	Number	Wavelengths (nm)
MetMb	CARS	22	1082, 1115, 1118, 1121, 1151, 1154, 1303, 1323, 1359, 1389, 1392, 1401, 1431, 1437, 1544, 1568, 1571, 1574, 1577, 1601, 1645, 1663
	<b>BOSS</b>	15	981, 996, 1016, 1076, 1079, 1085, 1118, 1136, 1145, 1165, 1195, 1395, 1574, 1642, 1678
	<b>VCPA-IRIV</b>	23	1079, 1085, 1112, 1118, 1142, 1148, 1189, 1192, 1377, 1380, 1386, 1389, 1401, 1425, 1490, 1526, 1538, 1541, 1547, 1562, 1571, 1589, 1642
OxyMb	<b>CARS</b>	20	921, 930, 1097, 1112, 1118, 1148, 1192, 1204, 1356, 1371, 1389, 1398, 1401, 1404, 1428, 1431, 1493, 1496, 1568, 1678
	<b>BOSS</b>	21	918, 921, 927, 930, 933, 978, 981, 990, 993, 996, 1022, 1049, 1061, 1076, 1145, 1195, 1291, 1294, 1380, 1675, 1678
	<b>VCPA-IRIV</b>	18	981, 984, 996, 1040, 1061, 1076, 1118, 1198, 1374, 1398, 1401, 1493, 1496, 1577, 1580, 1672, 1675, 1678



<span id="page-8-0"></span>**Fig. 6** Running process of selecting optimal variables by BOSS algorithm for MetMb and OxyMb: each feature weight value and optimal characteristic spectrum (**a** for MetMb, **b** for OxyMb)



<span id="page-8-1"></span>**Fig. 7** The variables selected by VCPA-IRIV algorithm for MetMb and OxyMb: **a** the changes in *RMSECV* for MetMb; **b** optimal characteristic wavelengths for MetMb; **c** the changes in *RMSECV* for OxyMb; **d** optimal characteristic wavelengths for OxyMb

by VCPA-IRIV, CARS, and BOSS algorithms are shown in Table [2](#page-7-1).

In some cases, the characteristic wavelengths selected in the NIR spectral region were directly related to the molecular bonds of C-H, O–H, and N–H. Absorption bands in the NIR region were observed at 950 nm and at 1456 nm both related to O–H second and frst overtones respectively, mainly associated with water content (Barlocco et al. [2006](#page-11-28)). The extracted effective wavelengths selected in the range of 1100–1400 nm were attributed to combination bands of the C-H stretching and deformation modes (Pu et al. [2015](#page-12-17)). The key wavelengths selected between 1400 and 1600 nm were related to the frst overtones of the O–H and N–H stretching modes of water-bonded groups in meat components (Wan et al. [2020](#page-12-7)). In addition, feature wavelengths in the 1600–1700 nm were attributed to fat absorption bands related to C-H stretching frst overtones (Pu et al. [2015](#page-12-17)).

# **Prediction Models Based on Characteristic Wavelengths**

<span id="page-9-0"></span>**Table 3** Performances of

and OxyMb

predicting contents of MetMb

In general, PLSR and LSSVM had achieved good results in predicting MetMb and OxyMb contents. As Cheng et al. ([2020](#page-11-29)) found that it was feasible to utilize the prediction model of PLSR, LSSVM in combination with HSI to evaluate the quality of MetMb and OxyMb contents. Compared with PLSR and LSSVM models, BP method was utilized to improve accuracy and robustness of prediction model on the contents of MetMb and OxyMb. The prediction models of MetMb and OxyMb contents were performed by two nonlinear models (BP & LSSVM) and a linear model (PLSR), in which the independent variables of MetMb and OxyMb were predicted from full spectral variables and spectral variables selected by CARS, VCPA-IRIV, and BOSS. The optimal prediction results in PLSR, LSSVM, and BP models for the contents of MetMb and OxyMb in Tan mutton are listed in Table [3.](#page-9-0) The sequence of model perfor-mance was BP > LSSVM > PLSR. As shown in Table [3](#page-9-0), BP model based on BOSS emerged the best capability to predict MetMb  $(R^2_{\text{C}} = 0.8340, R^2_{\text{P}} = 0.8253 \text{ RMSEC} = 3.1592,$ *RMSEP*=3.2918). Besides, the VCPA-IRIV-BP model had a better predictive performance with a higher  $R^2$ <sub>C</sub> and  $R^2$ <sub>P</sub> of 0.8024, 0.8680 and a lower *RMSEC* and *RMSEP* of 3.4676 and 2.7605 for OxyMb, respectively. It is worth mentioning that CARS algorithms showed a good performance in predicting the contents of MetMb and OxyMb for the selection of feature wavebands. Nevertheless, the number of key wavebands extracted by BOSS and VCPA-IRIV algorithm



Bold entries indicating the best method and corresponding model performance

was relatively low for the contents of MetMb and OxyMb, respectively. Compared with corresponding full wavelengths models, the simplifed VCPA-IRIV-BP and BOSS-BP models indicated better predictive accuracy and robustness. Therefore, BP model was more efective in predicting the MetMb and OxyMb contents in Tan mutton during cold storage than PLSR and LSSVM models. Thus, the potential of NIR-HSI for a quantitative detection of myoglobin content of mutton was identifed in this study.

The predictability of MetMb content obtained in this study was higher than that obtained by Yuan et al. [\(2020\)](#page-12-6) targeting at cooked Tan mutton with  $R^2$ <sub>P</sub> = 0.7654 and *RMSEP*=2.9306. However, the performances of the OxyMb and MetMb prediction models developed were inferior to that obtained by Cheng et al. [\(2020\)](#page-11-29) focusing on Tan mutton with  $R^2$ <sub>P</sub>=0.914 and 0.915 for OxyMb and MetMb, respectively. In addition, Wan et al. [\(2020](#page-12-7)) found that the optimal models for OxyMb and MetMb were CARS-PLSR (*Rp*=0.9661 and *RMSEP*=2.3762), and CARS-LSSVM (*Rp*=0.8931 and *RMSEP*=3.2743) by NIR-HSI system (700–1700 nm), respectively. Those diferences related to myoglobin prediction might be explained by diferent spectral ranges, data processing methods, sample preparations, and packaging methods. Despite the limitation on spectral data processing, this study is encouraging to further investigate the processing methods of hyperspectral images data. The minima of efective information are available to improve model efficiency.

# **Conclusions**

In this study, the NIR-HSI system (900–1700 nm) was adopted to evaluate the MetMb and OxyMb of Tan mutton during cold storage. PLSR, LSSVM, and BP models depended on variable wavelengths which produced superior performances in evaluating MetMb and OxyMb. In contrast, BP model was more appropriate for predicting the contents of MetMb and OxyMb in Tan mutton. CARS, VCPA-IRIV, and BOSS were adopted to extract the optimal wavebands from FW to simplify calibration models. The number of selected characteristic variables was 15 and 23 in BOSS and VCPA-IRIV for MetMb and OxyMb, respectively. As a result, the optimized BP models from characteristic wavelengths were selected by BOSS and VCPA-IRIV for predicting MetMb and OxyMb and exhibited better results than other variable selection methods. The simplifed BOSS-BP model yielded good results in assessing MetMb content ( $R^2$ <sub>C</sub> vs.  $R^2$ <sub>P</sub>=0.8340 vs. 0.8253, *RMSEC* vs. *RMSEP*=3.1592 vs. 3.2918), and the best predictive VCPA-IRIV-BP model showed a better performance with  $R^2_C$ ,  $R^2_P$ , *RMSEC*, and *RMSEP* values of 0.8024, 0.8680, 3.4676, and 2.7605 for OxyMb. As an emerging and sound technique, HSI can rapidly and non-destructively monitor the safety and quality of

mutton. However, some infuential factors such as sample types, packaging methods, and storage methods may impact experimental results. Future research might be conducted to augment sample type from diferent packaging and storage methods by using HSI. Furthermore, analysis methods of spectral data in this study still have a gap compared with classic methods, and it is signifcant to further explore the spectral data processing and modeling methods to improve data analytical efficiency in future.

**Author Contribution** Yourui Sun and Haonan Zhang: data collection and analysis; investigation; methodology; writing—original draft. Guishan Liu: supervision; writing—review and editing. Lijuan Cheng: conceptualization; validation; supervision. Yue Li: validation; software. Fangning Pu and Hao Wang: software.

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**Data Availability** The datasets analyzed during the current study are available from the corresponding author on reasonable request.

## **Declarations**

**Ethics Approval** This article does not contain any studies with human or animal subjects.

**Consent to Participate** Not applicable.

**Conflict of Interest** Yourui Sun declares that she has no confict of interest. Haonan Zhang declares that he has no confict of interest. Guishan Liu declares that she has no confict of interest. Jianguo He declares that he has no confict of interest. Lijuan Cheng declares that he has no confict of interest. Yue Li declares that she has no confict of interest. Fangning Pu declares that he has no confict of interest. Hao Wang declares that he has no confict of interest.

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