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[1]Department of Animal Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh.
[2]Department of Poultry Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh.
[3]Scientific Officer
BLRI, Savar, Dhaka

***Corresponding Author:**

MA Hashem
E-mail: hashem_as@bau.edu.bd

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Research Article

Detection of adulteration of goat and sheep meat through NIRS and chemometric analysis

MA Hashem[1*], J Ambia[1], N Mia[1], MH Ali[3] MM Rahman[1], MS Ali[2]

Abstract

This research aimed to assess the capability of near-infrared (NIR) reflectance spectroscopy coupled with chemometric analysis in detecting adulteration in Goat and Sheep meat. A total of 16 samples were prepared, consisting of 2 pure samples and 14 adulterated samples. Spectra were collected using DLP® NIRscan™ Nano Software to detect adulteration. Partial least square and principal component regression models were developed for calibration and validation using The Unscrambler X software. The accuracy of the calibration models was assessed using root mean square error of calibration (RMSEc), root mean square error of cross-validation (RMSEcv), coefficient of calibration (R^2c), and coefficient of cross-validation (R^2cv). Typically, a regression model is considered excellent when $R^2 \geq 0.90$. For the PCR model, the predicted R^2cv was 0.62, and for PLSR, it was 0.99 after leverage correction. Conversely, through cross-validation, the R^2cv for the PCR model was 0.29, and for PLSR, it was 0.18. The results suggest that NIR spectroscopy coupled with chemometric analysis was reasonably efficient in detecting adulteration in goat meat with sheep meat.

Introduction

The significance of meat in the human diet, its high demand, and consumption in developed countries (Hossain et al., 2023a and 2023b). Concerns about fraudulent mislabeling have heightened awareness about the quality and safety of meat products across various stakeholders in the food industry (Hossain et al., 2023b; Mia et al., 2023; Mobin et al., 2022). Recent years have seen increased attention to meat authenticity and the importance of accurate labeling to align with consumer preferences, lifestyle choices, religious considerations, and health concerns. Adulteration of meat poses multifaceted challenges, including economic, quality, safety, and socio-religious implications, with substitution being a particularly prevalent issue at various stages of the supply chain, from production to retail (Hossain et al., 2023b; Rahman et al., 2023). Various analytical methods have been proposed to prevent the sale of fraudulent meat products by retailers. These methods include immunological detection and DNA-based techniques such as ELISA and PCR, as well as electrophoretic and chromatographic methods (Alam et al., 2024). Enzyme activity determination, nuclear magnetic resonance, and scanning electron microscopy have also been used to differentiate between fresh and frozen-thawed meat. However, these techniques are invasive, time-consuming, and require highly skilled personnel, making them unsuitable for routine analysis and online application. There is thus a need for cost-effective, efficient, rapid, and reliable methods, with particular interest in non-destructive optical technologies capable of real-time assessment. NIR spectroscopy is a promising, non-destructive technique for detecting meat adulteration due to its simplicity and rapidity (Hashem et al., 2021, 2022 and 2023a; Miah et al., 2024). By utilizing NIR spectroscopy in conjunction with chemometrics, prediction models can be developed to identify adulterated meat supplies, whether in industrial or retail settings (Mishra and Passos, 2021). This technique, well-established in meat science research and the food industry, operates by analyzing the interaction of NIR light with molecular vibrations in samples. Despite challenges such as spectral overlap and variability, NIR spectroscopy offers valuable insights into sample composition (He et al., 2024). In Bangladesh, where meat adulteration is prevalent, there is a need for affordable techniques to address this issue; making spectroscopy coupled with multivariate techniques a suitable option for species identification. The research aims to develop a rapid NIR spectroscopy-based method for detecting adulterated goat meat with sheep meat, with specific objectives including building prediction models using PCR and PLSR.

Materials and Methods

Experimental site

The experiment was conducted in the laboratory of Animal Science, Bangladesh Agricultural University, Mymensingh.

Sample collection

Two selected spices meat such as goat meat and sheep meat were collected from a trusted butcher in a nearby market.

Preparation of adulterated sample

To detect the adulterate a total of 16 samples were prepared each of 30 g. The Chevon samples were adulterated by mixing mutton in the range of 2-28 at approximately 2% increments according to weight. The minced Chevon and mutton were individually weighed, thoroughly mixed, and homogenized (Yan et al., 2023).

NIR spectra acquisition

Near-infrared spectroscopy (NIRS) operates within the electromagnetic spectrum range of 780 nm to 2500 nm, analyzing material chemistry through diffuse reflectance radiation (Chelladurai and Jayas, 2014). This technique, discovered by F.W. Herschel in 1800, categorizes infrared radiation into near-infrared (NIR), mid-infrared (MIR), and far-infrared (FIR) regions based on wavelength. NIR and MIR regions are preferred in food analysis due to organic molecule absorption. NIR spectroscopy boasts rapidity, affordability, non-destructiveness, high penetration, and minimal sample preparation, making it widely applicable and termed Near-Infrared technology. Enhanced by advancements in light-fiber optics and detector technologies since the 1980s, NIR spectroscopy has become a powerful tool for scientific research. Near-infrared spectra primarily reveal weak overtones and combinations of fundamental bands for specific bonds, influenced by material chemistry and physical structure (Czarnecki et al., 2015). Multivariate calibration is necessary for quantitative analysis due to spectral overlap. Coupled with chemometric methods, NIR spectroscopy facilitates quantitative and qualitative determination of food components by measuring light intensity before and after sample interaction, calculating diffuse reflectance caused by sample absorption and scattering at specific frequencies corresponding to molecular vibrational frequencies.

The sample spectra will be recorded using a DLP NIR scan Nano EVM spectrometer (Texas Instruments DLP® NIRscan™ USA). Each sample will be analyzed in duplicate to mitigate instrumental or sampling variability. Both intact and minced samples will be scanned in diffuse reflectance mode within the specified wavelength range. Intact samples will undergo scanning in three different positions, while minced samples will be placed in a circular cup with a quartz window and scanned three times. Reflectance data will be stored as log (1/R) at 2 nm intervals.

Pre-processing of the NIR spectra

Pre-processing of the NIR spectra is an essential step (Arianti et al., 2023). Various interferents, which influence the NIR spectra and complicate further data analysis, can primarily be associated with the light scattering effect. In the literature, a few algorithms have been introduced that permit a partial separation of the physical effects from the chemical signals that are observed due to light scattering. The most common pre-processing techniques that are used to correct the multiplicative and additive effect of scattered light are the standard normal variate (SNV). To date, there have been many efforts that have attempted to improve the basic pre-processing algorithms, extract the relevant chemical information from the spectra and reflect the complex nature of samples as well as possible (Bi et al., 2016). Recent algorithms, such as the extended multiplicative scatter correction (EMSC) and the extended inverse scatter correction (EISC), are the polynomial extensions of the classic MSC and ISC algorithms (Miguel-Espinar et al., 2023). In our study, SNV and First derivatives + S. Golay were used to correct the spectra and evaluate the influence of the preprocessing method on the model as expressed by the selected figures of merit.

Model development and spectral data analysis

Calibrations and predictions of adulteration in spices samples based on full spectra (228 variables) were established using two linear chemometric algorithms, namely partial least-squares regression (PLSR) and principal component regression (PCR) (Ambrożej and Karpinska, 2020). The optimum number of latent factors (LFs) or principal components (PCs) to be included in the calibration models was selected at the minimum value of the root mean square error of cross-validation (RMSECV) to avoid either over- or under-fit problem of the model (Soyeurt et al., 2020). The calibration models were strictly built using the calibration dataset and validated with cross-validation technique. In this study, leave-one-out (i.e. full) cross-validation method was employed to validate the PLSR and PCR models. This was done by removing one sample (test sample) from the data set and PLSR/PCR model was then established for the remaining samples (training sample). Finally, the model was used to predict the sample left out. This procedure was repeated for every sample in the data set, giving a more realistic measure of the predictive errors of the model (Hong et al., 2023). The precision and the predictive capabilities of the models were evaluated by the coefficients of determination (R^2), root-mean-square error of calibration (RMSEC) and the root-mean-square error estimated by cross-validation (RMSECV). The best model was selected for each attribute on the basis of the highest determination coefficient (R^2) and lowest standard error of calibration (RMSEC) and validation (RMSECV). The R^2 and RMSEC or RMSECV are defined as follows:

$$R^2 = 1 - \frac{\sum_{i=1}^{N}(\hat{y}_i - y_i)^2}{\sum_{i=1}^{N}(\hat{y}_i - \bar{y}_i)^2} \quad (1)$$

$$RMSEC \text{ or } RMSECV = \sqrt{\frac{\sum_{i=1}^{N}(\hat{y}_i - y_i)^2}{\mathrm{N}}} \quad (2)$$

Where $\hat{y}_i$ = predicted value of the i^{th} sample, y_i = measured value of the i^{th} sample, N = number of samples.

Generally, the accuracy of multivariate calibration model is considered as excellent when the R^2 is 0.90 or higher (Cuadrado et al., 2005). However, it is always expected to obtain R^2 as close as 1 with errors as close as 0.

Software

All spectral transformations, PCR and PLSR analysis was carried out in The Unscrambler X (CAMO AS, Trondheim, Norway).

Results and Discussion

NIR spectra of the different pure and adulterated meat

The average NIR reflectance spectra extracted from tested pure goat meat and sheep meat in the spectral range of 900–1700 nm is shown in Fig. 1. Spectra of pure goat and adulterated sheep (2%) shown in Fig. 2. The spectra of the tested pure and adulterated samples of different meat showed similar trends throughout the whole spectral range. Despite the similarity, the studied original spectra were different in absorbance values.

The NIR range is an important spectral region in meat analysis and provides information about the chemical compounds. Generally, the peaks observed in the NIR region are related to overtones and combinations of fundamental vibrations of C–H, N–H and O–H functional groups, which are the primary structural components of meat molecules. By inspecting NIR spectra of the examined spices it was found that the major absorption bands were observed for pure goat and sheep from 1381 nm to 1542 nm (Fig. 1). But adulterated samples major spectral wavelength ranged from 1396 nm to 1536 nm (Fig. 2).

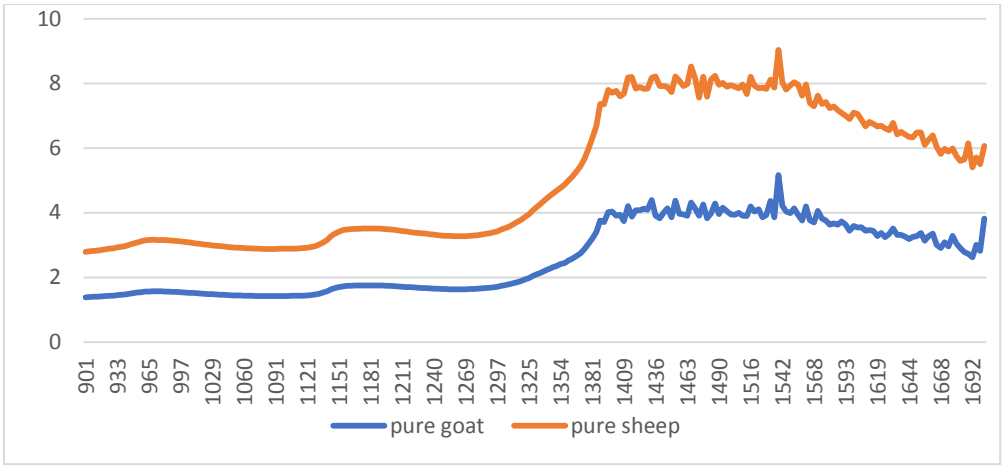

Figure 1. NIR spectra in the spectral range of 900-1700 nm for pure goat meat and pure sheep meat.

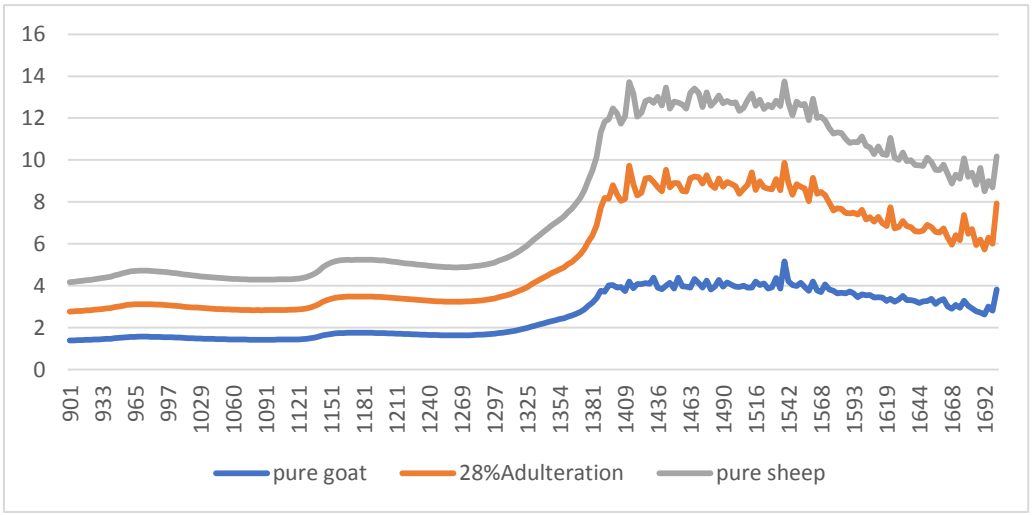

Figure 2. NIR spectra in the spectral range of 900-1700 nm for 100% goat meat, goat meat and sheep meat mixture (2-28) and 100% sheep meat.

Development of calibration model based on NIR spectra

Spectral data at full wavelength range (900-1700 nm) with (228) variables were modeled using two linear multivariate methods namely PLSR and PCR and the results were compared to determine the best calibration method. The performance of the calibration models was optimized by leverage correction and cross-validation (Yates et al., 2023). Different regression model of PCR and PLSR without and with pre-processing are shown in Table 1.

The detailed results of PLSR are listed in Table 1 and PCR is listed in Table 2, where for each model R^2_c, R^2_{cv}, RMSEC, and RMSECV are reported. It is clear from the Table 1 that PLSR performed better and always required fewer LV than PCR. Therefore, PLSR is more parsimonious than PCR in predicting adulteration in meat (Mousa et al., 2022). It was not

surprising because PCR estimates each PC of the spectral matrix (X) to maximize the amount of explained variance without using the response variable (Y), so there is no guarantee that the calculated PCs are important concerning the response variable for prediction, while PLSR decomposes both X and Y to calculate LV that are important for better prediction.

The level of chevon adulterated with mutton was predicted by the PLSR with R^2_c of 0.99, RMSEC of 0.61%, R^2_{cv} of 0.99 and RMSECV of 0.89%, while the level of goat meat adulterated with sheep was predicted by the PCR model with R^2_c of 0.99 RMSEC of 0.24%, R^2_{cv} of 0.62, and RMSECV of 5.33%. In this study, the calibration results were highly similar compared to cross validation results. The similarity in model performance implied that the models were not over-fit, and the majority of the variance presented in the measured values was reproduced in the prediction model. Based on model performance in terms of LV, R^2_c, R^2_{cv}, RMSEC and RMSECV, it seems that, out of the two models tested, the PLSR model was the most appropriate for adulterate detection in goat meat with sheep meat (Hashem et al., 2021). Generally, the accuracy (i.e. the closeness between actual and the predicted values) of regression model is considered as excellent when the $R^2 \geq 0.90$ (Kamruzzaman et al., 2016). Therefore, the developed model can be considered sufficiently accurate for future application. The accuracy results found in this study were similar to those mentioned by Lohumi et al. (2014) for predicting beef adulteration with minced chicken in the range (1-35 w/w %) at approximately 25% intervals (Wang et al., 2018). The authors used PLSR and predicted adulteration determination (R^2_p) of 0.97, 0.97, and 0.96 with root mean square error in prediction (RMSEP) of 2.62, 2.45, and 3.18% (w/w) for R, A and KM spectra, respectively. The results clearly ascertain that hyperspectral imaging coupled with machine learning can be used to detect, quantify and visualize the amount of chicken adulterant added to the minced beef. The present findings are similar with Hashem et al. (2022). They found that (R^2cv) through PLS-DA was 0.99 and 0.99, RMSEC was 0.06, and both the RMSECV and RMSEP were 0.08. In contrast, in SVM, methods were 0.97 and 0.96. The RMSEC, RMSECV, and RMSEP were 0.15, 0.17, and 0.24, respectively to identify pork, beef and mutton adulteration.

Table 1. PLSR model in the spectral range of 900-1700 nm for detecting adulteration in chevon and mutton

Application	LV	Mathematical treatments/ Preprocessing	Model Process	Model	R^2c	RMSEC (%)	R^2cv	RMSECV (%)
Goat meat	4	None	LC	PLSR	0.99	0.61	0.99	0.89
adulterated with	5	None	CV	PLSR	0.99	0.25	0.30	7.76
Sheep meat	3	SNV	LC	PLSR	0.99	0.68	0.99	0.96
	5	SNV	CV	PLSR	o.99	0.13	0.19	8.34
	5	First derivatives +S. Golay	LC	PLSR	0.99	0.34	0.99	0.51

LV = Latent variable, LC = Leverage correction, CV = Cross validation, SNV = Standard normal variate and RMSEC, RMSECV are the root mean square errors in calibration and cross-validation, respectively.

Table 2. PCR model in the spectral range of 900-1700 nm for detecting adulteration in chevon and mutton

Application	LV	Mathematical treatments/ Preprocessing	Model Process	Model	R^2c	RMSEC (%)	R^2cv	RMSECV (%)
Mutton	14	None	LC	PCR	0.99	0.24	0.62	5.33
adulteration in	13	None	CV	PCR	0.99	0.69	0.29	7.81
Chevon	12	SNV	LC	PCR	0.99	0.42	0.52	5.99
	13	SNV	CV	PCR	0.99	0.42	0.18	8.40

LV=Latent variable, LC=Leverage correction, CV=Cross validation, SNV=Standard normal variate and RMSEC, RMSECV are the root mean square errors in calibration and cross-validation, respectively.

Conclusions

The study demonstrated that NIR spectroscopy coupled with PLSR could effectively screen and quantify adulteration levels in goat and sheep meat. PLSR models yielded coefficients of determination (R^2cv) ranging from 0.19 to 0.99 and root mean square errors of cross-validation (RMSECV) ranging from 0.51% to 8.34%. Cross-validation was used during calibration to optimize the PLSR model. Overall, spectral data from NIR combined with appropriate multivariate methods proved effective in detecting adulteration in goat meat with sheep meat.

Conflict of interest

There are no conflicts of interest among the authors.

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