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Research Article

Detection of adulteration of cattle and buffalo meat through NIRS and chemometric analysis

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Abstract

The objective of this study was to evaluate the efficacy of near-infrared (NIR) reflectance spectroscopy in detecting adulteration in cattle and buffalo meat. A total of 16 samples were tested, 2 of which were pure and 14 were adulterated. The beef samples were adulterated by mixing buffalo meat in the range of 0-28% (w/w) at approximately 2% increments according to weight. To detect adulteration, DLP® NIRscan™ Nano Software was used to gather spectra. The Unscrambler X program was used to develop calibration and validation models utilizing principal component regression and partial least squares. Root mean square error of calibration (RMSEc), root mean square error of cross-validation (RMSEcv), coefficient of calibration (R^2_c), and coefficient of cross-validation (R^2_{cv}) were used to assess the accuracy of the calibration models. The R^2 value of 0.90 or above indicates that the regression model is excellent. For the PCR model, the predicted R^2_{cv} value was 0.73 and for the PLSR model, the predicted R^2_{cv} value was 0.98 through leverage correction. In cross-validation, the R^2_{cv} value was 0.65 for both the PCR and PLSR models. According to the findings, it is suggested that NIR spectroscopy is a reasonably efficient method for detecting adulteration in cattle meat with buffalo meat.

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Introduction

Meat is a cornerstone of human nutrition, providing essential proteins, vitamins, and minerals, and is highly valued in diets worldwide (Hashem et al., 2023a; Hossain et al., 2023a and 2023b). Despite its importance, meat authenticity and traceability have become significant concerns (Premanandh, 2013) due to adulteration, with fraud becoming increasingly sophisticated and widespread (Cawthorn et al., 2013). Advances in meat processing have transformed it into various forms, such as minced meat, sausages, and patties, which complicate the detection of species and quality. This processing often masks the morphological characteristics of the meat, making it easier for fraudsters to substitute higher-quality or different species of meat with inferior grades (Downey et al., 2000; Alamprese et al., 2016; Rahman et al., 2023, Islam et al., 2018, 2019 and 2022). Such fraud not only leads to economic losses but also raises issues related to food safety, allergies, and compliance with dietary restrictions (Dean et al., 2006). Another prevalent form of meat fraud involves mislabeling frozen-thawed meat as fresh. Distinguishing between fresh and frozen-thawed meat is challenging due to their similar appearance, complicating the identification of fraudulent practices (Barbin et al., 2013; Rima et al., 2019). Various traditional analytical methods have been suggested to prevent the sale of fraudulent meat products by retailers, including immunological detection and DNA-based techniques such as ELISA and PCR, electrophoretic and chromatographic methods, and various advanced techniques such as NMR and SEM (Kamruzzaman et al., 2015 and 2016; Jha et al., 2003; Vasconcellos et al., 2003; Vallejo-Cordoba et al., 2010; Alam et al., 2024). However, these methods often require significant time, labor, and expertise, limiting their feasibility for routine, online analysis. There is thus a need for cost-effective, efficient, rapid, and reliable methods, with particular focus on non-destructive optical technologies, with near-infrared (NIR) spectroscopy emerging as a promising tool. NIR spectroscopy offers a rapid, non-destructive, and efficient approach for detecting adulteration and ensuring meat authenticity when combined with chemometric models (Osborne, 1993; Hashem et al., 2021, 2022, and 2023b; 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). An increase in the risk of fraud could be used to reduce fraud opportunities (Spink and Moyer, 2011). 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. Several research studies have been investigating non-destructive technologies for assessing the safety and quality of pork and beef. These technologies include Hyper spectral Imaging (HSI) (Bonah et al., 2020), Magnetic Resonance Imaging (Lee et al., 2015), visible near-infrared (Vis-NIR) spectroscopy (Leng et al., 2020), and Raman Spectroscopy (Yang et al., 2020). However, there has not been a comprehensive review summarizing the promising applications of imaging and spectroscopic techniques specifically for evaluating pork and beef quality. In various studies, NIR technology has been proven to be useful

in distinguishing between kangaroo and beef meat (Ding and Xu, 1999); pork, chicken, and duck meat (Rannou and Downey, 1997); different cuts of chicken meat (Fumière et al., 2000); mixtures of lamb and beef meat (McElhinney et al., 1999); beef, pork, and chicken (Downey et al., 2000); as well as in identifying and authenticating raw meat from various species such as pork, chicken, lamb, and beef (Cozzolino and Murray, 2004); turkey meat (Alamprese et al., 2013); or pork, texturized vegetable protein, chicken, and wheat gluten (Rady and Adedeji, 2018). In Bangladesh, where meat adulteration is prevalent, there is a need for affordable techniques to address this issue; spectroscopy combined with multivariate approaches might be a way to solve the challenge of species identification. The research objective is to build a rapid NIR spectroscopy-based method for detecting adulterated cattle meat with buffalo meat, with specific objectives including building prediction models using PCR and PLSR.

Materials and Methods

Experimental site

The experiment was conducted at the Animal Science Laboratory under the Department of Animal Science, Bangladesh Agricultural University, Mymensingh.

Sample collection

Buffalo meat was collected from Nandina bazar Jamalpur, and cow meat was collected from KR market, BAU. Samples of meat were promptly taken to the Animal Science Laboratory at Bangladesh Agricultural University, Mymensingh.

Preparation of adulterated sample

To detect the adulterate, a total of 16 samples were prepared, each of 30 g. The beef samples were adulterated by mixing buffalo meat in the range of 0-28% (w/w) at approximately 2% increments according to weight. Each piece of minced beef and buffalo was weighed separately, then thoroughly mixed and homogenized. Some pure spectra of each beef and buffen were also acquired to see the spectral differences among the tested samples. The samples were scanned to collect spectra.

NIR spectra acquisition

Near-infrared spectroscopy (NIRS) is a spectroscopic technique that utilizes the near-infrared portion of the electromagnetic spectrum. It is an extensively used, convenient, rapid, and non-destructive technique that requires minimal sample preparation before analysis (Nicolai et al., 2007). The method relies on radiation interacting with the target or sample, whether through absorption, reflection, transmission, or scattering (Narsaiah et al., 2020; Cheng et al., 2013). In 1800, F.W. Herschel discovered Infrared (IR) radiation, which is classified into near-infrared (NIR), mid-infrared (MIR), and far-infrared (FIR). The regions of the electromagnetic spectrum known as NIR, MIR, and FIR range from 780-3 µm, 3-50 µm, and 50-1000 µm. In food analysis, the NIR and MIR regions are frequently utilized due to the absorption of organic molecules in this range. Since these chemical bonds undergo overtone vibrational and rotational transitions, The NIR region (780 to 2500 nm) provides information about the relative proportions of C–H, O–H, N–H, and S–H bonds, which are the primary structural components of organic molecules (Osborne, 1993). Near-infrared spectra can be challenging to interpret directly due to the overlap of weak overtones and combinations of vibrational bands. Therefore, quantitative analysis of sample constituents by NIRS requires multivariate calibration. NIR spectroscopy, when utilized alongside chemometric techniques, is emerging as an effective tool for both the quantitative and qualitative analysis of food components (Bevilacqua et al., 2013; Gonzalez-Martín et al., 2014). Furthermore, near-infrared (NIR) light has better penetration capabilities than infrared light, allowing for the detection of information deep within samples. Currently, numerous studies have been conducted by integrating chemometrics, such as image processing, preprocessing, and modeling, with different optical non-destructive detection techniques (Jiang et al., 2019; Huang et al., 2022; Xie et al., 2023; Liu et al., 2021). This integration has led to improved quantitative and qualitative performance.

The sample spectra will be recorded using a DLP NIR scan Nano EVM spectrometer (Texas Instruments DLP® NIRscan™ USA USA). NIR spectra will be collected from 900–1700 nm. Two aliquots from each sample will be analyzed, with spectra recorded in duplicate to account for instrumental or sampling variability. The diffuse reflectance mode (900-1700 nm) will be used to scan both intact and minced samples. Samples will be placed steadily on the sample holder during spectra collection. Three different scan locations will be performed for each intact sample. Three scans will be conducted on minced samples in a 50 × 30 mm circular cup with a quartz window. Reflectance data will be saved at 2 nm intervals as log (1/R), where R represents reflectance.

Pre-processing of the NIR spectra

Pre-processing of the NIR spectra is a crucial step (Liland et al., 2016; Arianti et al., 2023). Different substances that affect the NIR spectra and make it more difficult to analyze the data are mostly linked to the phenomenon of light scattering. The light scattering effect can be largely linked to a variety of interferents that affect the NIR spectra and make data analysis more difficult. In the literature, several algorithms have been introduced to partially separate the physical effects from the chemical signals observed due to light scattering. The standard normal variate (SNV) is the most frequently utilized pre-processing method for correcting the multiplicative and additive impacts of scattered light. There have been numerous efforts to enhance the basic pre-processing algorithms, extract relevant chemical information from spectra, and accurately represent the complex nature of samples (Bi et al., 2016; Martens et al., 2003). In our study, we utilized the SNV (Standard Normal Variate) and First Derivatives + S. Golay methods to correct the spectra and assess the impact of the pre-processing technique on the model, as indicated by the selected performance metrics. Additionally, recent algorithms like EMSC (Extended Multiplicative Scatter Correction) and EISC (Extended Inverse Scatter Correction) are polynomial expansions of the traditional MSC and ISC algorithms (Miguel-Espinar et al., 2023).

Model development and spectral data analysis

Calibrations and predictions for adulteration in spice samples were established using two linear chemometric algorithms, partial least-squares regression (PLSR), and principal component regression (PCR), based on full spectra (228 variables) (Ambrożej and

Karpinska, 2020). The calibration models should include the ideal number of latent variables (LV) or principal components (PCs), which is determined as the minimum value for the root mean square error of cross-validation (RMSECV) in order to avoid overfitting or underfitting issues with the model (Soyeurt et al., 2020). The calibration models were constructed using the calibration dataset and then validated using the cross-validation technique. In this study, the leave-one-out (i.e., full) cross-validation method was utilized to validate the PLSR and PCR models. This involved removing one sample (test sample) from the data set, establishing a PLSR/PCR model for the remaining samples (training sample), and using the model to predict the sample left out. This process was repeated for each sample in the data set, providing a more realistic measure of the predictive errors of the model (Hong et al., 2023). The model's precision and predictive capabilities were assessed using coefficients of determination (R^2), root-mean-square error of calibration (RMSEC), and root-mean-square error estimated by cross-validation (RMSECV).

The best model for each attribute was chosen based on the highest determination coefficient (R^2), and the lowest standard error of calibration (RMSEC) and validation (RMSECV). The R^2 and RMSEC or RMSECV are defined as follows:

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

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

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, When the R^2 is 0.90 or higher, a multivariate calibration model is considered to have excellent accuracy (Cuadrado et al., 2005). However, it is always desirable to obtain an R^2 value as close to 1 as possible, along with errors as close to 0.

Software

All spectral transformations, PCR, and PLSR analysis were conducted 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 cattle meat and buffalo meat in the spectral range of 900–1700 nm is shown in Fig 1. Spectra of pure cattle, pure buffalo, and cattle meat adulterated with buffalo meat (2%) are shown in Fig 2. The spectral patterns of the pure and adulterated samples of different types of meat exhibited similar trends across the entire spectral range. However, the original spectra varied in terms of absorbance values.

The NIR range is crucial for analyzing meat and offers insight into its chemical composition. In this spectral region, the observed peaks are linked to overtones and combinations of fundamental vibrations of C-H, N-H, and O-H functional groups, which are the main structural components of meat molecules. Upon examining the NIR spectra of the species under investigation, it was found that the primary absorption bands appeared at 1375 nm and 1493 nm. (Fig. 1).

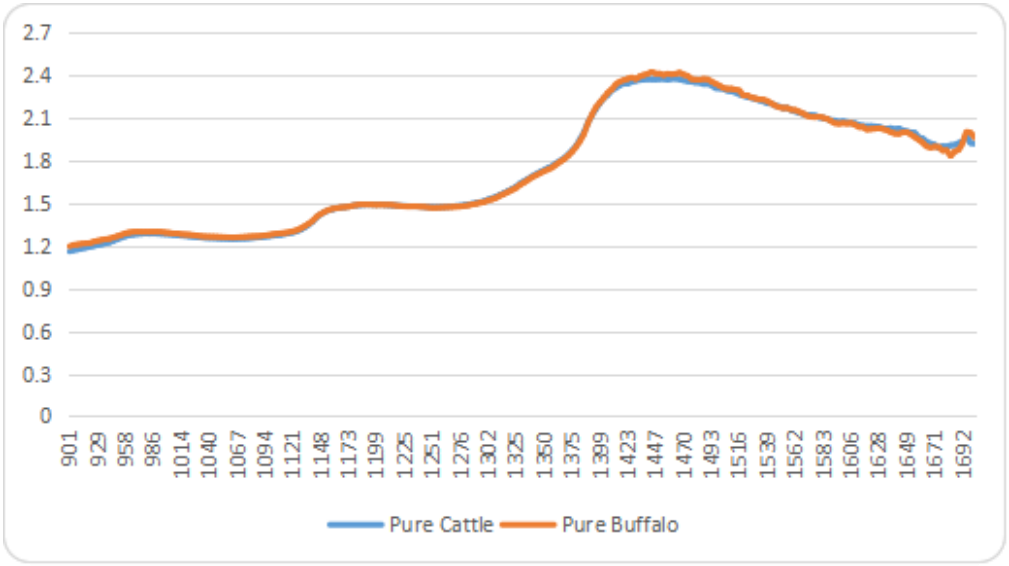

Figure 1. NIR spectra in the spectral range of 900–1700 nm for pure cattle meat and pure buffalo meat.

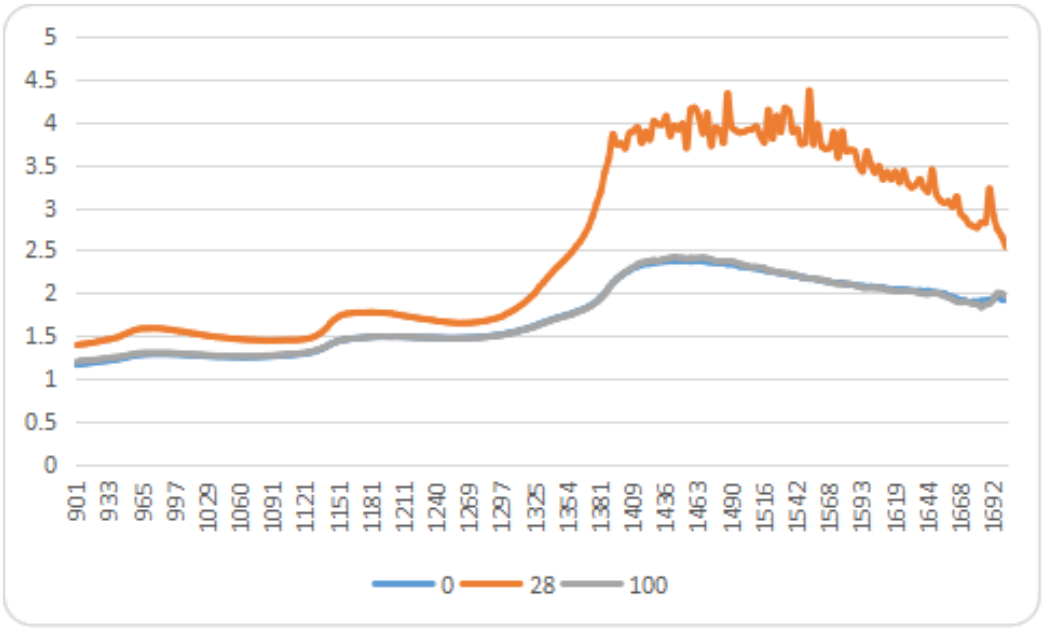

Figure 2. NIR spectra in the spectral range of 900-1700 nm for 100% cattle meat, cattle and buffalo meat mixture (2-28) and 100% buffalo meat.

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 PCR and PLSR, 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 models 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. Based on the data in Table 1, it is evident that PLSR outperformed PCR and consistently used less LV. As a result, PLSR is more economical than PCR for identifying meat adulteration (Mousa et al., 2022). This outcome is not surprising given that PCR maximizes the explained variance of the spectral matrix (X) without utilizing the response variable (Y). Consequently, there is no assurance that the calculated Principal Components (PCs) are significant for predicting the response variable. In contrast, PLSR decomposes both X and Y to compute LV that are crucial for improved prediction.

Table 1. PLSR models in the spectral range of 900-1700 nm for detecting adulteration in beef and buffen

Application	LV	Mathematical treatments/ Preprocessing	Model Process	Model	R^2c	RMSEC (%)	R^2cv	RMSECV (%)
Cattle meat adulterated with Buffalo meat	8	None	LC	PLSR	0.99	0.52	0.98	1.19
	1	None	CV	PLSR	0.69	4.76	0.65	5.51
	3	SNV	LC	PLSR	0.98	1.07	0.97	1.45
	1	SNV	CV	PLSR	0.95	3.90	0.57	6.05
	5	First derivatives +S. Golay	LC	PLSR	0.74	2.01	0.92	2.47

LV = latent variable, LC = leverage correction, CV = cross-validation, SNV = standard normal variate, and RMSEC and 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 beef and buffen

Application	LV	Mathematical treatments/ Preprocessing	Model Process	Model	R^2c	RMSEC (%)	R^2cv	RMSECV (%)
Cattle meat adulterated with Buffalo meat	14	None	LC	PCR	0.99	0.38	0.73	4.49
	1	None	CV	PCR	0.69	4.78	0.65	5.51
	13	SNV	LC	PCR	0.99	0.42	0.99	0.56
	6	SNV	CV	PCR	0.85	3.40	0.59	5.90

LV = latent variable, LC = leverage correction, CV = cross-validation, SNV = standard normal variate, and RMSEC and RMSECV are the root mean square errors in calibration and cross-validation, respectively.

The level of buffen adulterated in beef was predicted by the PLSR with R^2_c of 0.99, RMSEC of 0.52%, R^2_{cv} of 0.98, and RMSECV of 1.19%, while the level of buffalo meat adulteration in cattle meat was predicted by the PCR model with R^2_c of 0.99, RMSEC of 0.38%, R^2_{cv} of 0.73, and RMSECV of 4.49%. In this study, the calibration results were highly similar compared to cross-validation results. The similarity in model performance suggests that the models were not overfit, and the majority of the variance observed in the measured values was replicated in the reproduced 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 adulteration detection in cattle meat with buffalo meat. In general, a regression model's accuracy (i.e., the closeness between actual and predicted values) is considered excellent when the $R^2 \geq 0.90$ (Kamruzzaman et al., 2016). Therefore, the created model is deemed sufficiently precise for future applications. Kamruzzaman et al., (2015) conducted an

experiment utilizing visible near-infrared hyperspectral imaging and machine learning on rapid and non-destructive detection of chicken adulteration in minced beef. In this study, he found a root mean square error in prediction (RMSEP) of 2.62, but we found the root mean square is 4.49, which is higher. The accuracy result found in this study was higher (4.49) than those mentioned by Yang et al., (2018) for anticipating adulteration in beef. The author used PLS-DA with RMSECV 0.08. Zhang et al., (2013) conducted an experiment to detect adulteration in chicken meat using NIR hyperspectral imaging. In this study, he found RMSEP 0.48, but we found 1.19, which is higher. In our recent study, we observed that the PLSR models produced coefficients of determination (R^2cv) of 0.98, 0.65, 0.97, 0.57, and 0.92, along with root mean square errors of cross-validation (RMSECV) of 1.19%, 5.51%, 1.45%, 6.05%, and 2.47% for cattle meat with buffalo meat, respectively. Whereas, another study demonstrated that NIR spectroscopy with PLSR could effectively screen and quantify adulteration levels in goat and sheep meat. The PLSR models in this case 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% (Hashem et al., 2024). Partial least squares regression (PLSR) was developed to correlate the NIR spectra of different meat samples and their corresponding level of adulteration, and cross-validation was used during the calibration step. The results indicate that the near-infrared (NIR) technique, combined with PLSR regression analysis, can be used to quantitatively identify non-destructive adulteration of cattle meat with buffalo meat. The results demonstrate that spectral data collected from the NIR spectral region can be used with appropriate multivariate PCR and PLSR methods to detect adulteration in cattle and buffalo meat.

Conclusions

The study demonstrated that NIR spectroscopy combined with PLSR could efficiently screen and quantify adulteration levels in cattle and buffalo meat. From the established PLSR models, we obtained coefficients of determination (R^2cv) ranging from 0.57 to 0.98 and root mean square errors of cross-validation (RMSECV) ranging from 1.19% to 6.05%. Cross-validation was utilized during calibration to optimize the PLSR model. Overall, spectral data from NIR combined with appropriate multivariate methods proved effective in detecting adulteration in cattle meat with buffalo meat.

Conflict of interest

The authors do not have any conflicts of interest.

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