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Pork Adulteration in Beef and Mutton Detection Using Visible Near-Infrared (Vis-NIR) Spectroscopy and Chemometrics

Rudiati Evi Masithoh[1]*, Aridatuz Zahroh[1], Muhammad Fahri Reza Pahlawan[2] and Laila Rahmawati[3]

[1]Department of Agricultural and Biosystems Engineering, Faculty of Agricultural Technology, Universitas Gadjah Mada, Jl. Flora No. 1 Bulaksumur, Yogyakarta 55281, Indonesia
[2]Department of Smart Agriculture Systems, College of Agricultural and Life Science, Chungnam National University, Daejeon 34134, Republic of Korea
[3]Research Center for Food Technology and Processing, National Research and Innovation Agency, Gunung Kidul, 55861 Yogyakarta, Indonesia

ABSTRACT

The addition of pork to beef and mutton is a potentially fraudulent practice. This study aims to detect adulteration of pork in beef and mutton using visible near-infrared (Vis-NIR) spectroscopy. Classification models for Beef+Pork and Mutton+Pork were developed using principal component analysis (PCA) and linear discriminant analysis (LDA). PCA demonstrated that respiratory pigments and the Soret band influenced the classification of meat based on Vis-NIR spectra. The most effective model was achieved by combining PCA with linear discriminant analysis (PCA-LDA), utilizing the original Vis-NIR spectra. The PCA-LDA model achieved a calibration accuracy of 99% for Beef+Pork and 82.4% for Mutton+Pork, with prediction accuracies of 91.3% and 73.7%, respectively. These results demonstrate that Vis-NIR spectroscopy can be utilized to authenticate minced meat, providing a promising approach for on-site screening or halal verification.

Keywords: Beef, food authentication, halal, mutton, PCA-LDA, pork, Vis-NIR

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E-mail address:
Evi@ugm.ac.id (Rudiati Evi Masithoh)
aridatuzzahroh@mail.ugm.ac.id (Aridatuz Zahroh)
mfrezap@mail.ugm.ac.id (Muhammad Fahri Reza Pahlawan)
laila.rahmawati53@gmail.com (Laila Rahmawati)
*Corresponding author

INTRODUCTION

Meat is one of the most widely traded processed foods, available in various forms, including cuts and minced meat. In minced form, meat is often prone to accidental or intentional mixing, as it is difficult to distinguish between different types of meat. Deliberately mixing two or more ingredients without the consumer's consent constitutes

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adulteration. Such practices can be economically detrimental when low-quality products are sold as high-quality ones at inflated prices. The mixing of pork, beef, and mutton is a particular concern due to price discrepancies and religious dietary restrictions; pork consumption is prohibited for Muslims, and beef is forbidden for Hindus. Adulteration also poses health risks if the additives are allergens or have toxic effects (Kucharska-Ambrożej & Karpinska, 2019). Hence, a reliable method must be established to detect the presence of undeclared ingredients in minced meat.

Meat types can be differentiated through sensory evaluation of color, taste, or aroma. DNA analysis and PCR tests offer another approach (Hrbek et al., 2020; Hu et al., 2021). However, these chemical methods are expensive, time-consuming, and require expert handling. Moreover, PCR tests cannot quantify adulteration levels in meat (Mabood et al., 2020). Conversely, non-destructive methods using infrared (IR) spectroscopy have been employed to detect products with similar colors (Masithoh et al., 2020) and to identify adulteration (Nobari-Moghaddam et al., 2021). IR spectroscopy offers a rapid, accurate, inexpensive, and easy-to-use detection method.

The successful application of Fourier Transform IR (FTIR) and Visible Near-Infrared (Vis-NIR) spectroscopy in determining meat quality has been reported (Siddiqui et al., 2021; Zhang et al., 2022). Vis-NIR spectroscopy operates within the near-infrared and visible light spectrums (400–2500 nm). Vis-NIR spectra contain information about color pigments in the visible region and C-H-N-O molecules in the NIR region (Cortés et al., 2019). Meat spectra in the visible regions contain information related to myoglobin (Mb) (Cozzolino & Murray, 2004; Weng et al., 2020), while the NIR region provides information about protein and lipids (Barbin et al., 2013).

Spectroscopy and chemometrics are typically used together to extract relevant information from spectra and relate it to the target variable (Li et al., 2022). Chemometrics employs multivariate analysis techniques, such as partial least squares regression (PLSR) for prediction and principal component analysis (PCA) for classification. PCA and linear discriminant analysis (LDA) have been successfully used to discriminate between tea samples (Esteki et al., 2023) and coffee (Silva et al., 2021). Combining dimension reduction with PCA and classification using LDA has been shown to perform better for discrimination (Zhao et al., 2011). Therefore, this study aimed to use Vis-NIR spectroscopy (400–1000 nm) to detect pork adulteration in minced mutton and beef. A predictive model for quantifying pork adulteration levels in minced mutton and beef was developed using PCA and LDA.

MATERIALS AND METHODS

Samples

Beef, mutton, and pork samples were purchased from the local markets in Yogyakarta, Indonesia. The meat was refrigerated at 10 °C within 1 hour of collection to maintain freshness. Each meat type was minced separately using a mince-machine. Minced pork was used as the adulterant, while minced beef and mutton were the adulterated meats. Eleven pork adulterant concentrations were prepared in beef and mutton: 0%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, and 100%. For each concentration, 100 g mixtures of beef and pork (Beef+Pork) and mutton and pork (Mutton+Pork) were manually mixed for 20 minutes by two individuals to ensure homogeneity. All samples were stored at 10 °C until spectral acquisition to maintain sample freshness.

Spectra Acquisition

Reflectance spectra of visible near-infrared spectroscopy (Vis-NIRs) were obtained using a Vis-NIR spectrometer (Flame-T-VIS-NIR, Ocean Optics, 350-1000 nm). The system had a fiber optic reflection probe (QR400-7-Vis-NIR, Ocean Optics, USA) and a light source (tungsten halogen lamp, HL-2000-HP-FHSA, Ocean Optics, USA). Before spectra acquisition, the meat sample was removed from the refrigerator and cooled to room temperature. Each meat sample was divided into five small samples (each weighing approximately 20 g) and placed in an aluminum vial (7 cm diameter and 1 cm height) to ensure similar sample density. Twenty spectra were measured for each small sample.

Chemometrics Analysis

The reflectance spectra collected from Vis-NIR were imported into The Unscrambler®X software (CAMO, Oslo, Norway) for multivariate analysis, which contained independent variables (x-variables) and predictors (y-variables). The Vis-NIR spectra consisted of 3188 independent variables. Samples consisted of eleven variations of pork adulterant concentrations (0%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, and 100%) and were grouped into five classes as the predictors (y-variables): Pure (100% beef or mutton), Low (10–30% pork), Medium (40–60% pork), High (70–90% pork), and Pork (100% pork).

Principal component analysis (PCA) was conducted to classify Beef+Pork and Mutton+Pork using Vis-NIR spectra. PCA is an unsupervised multivariate analysis used for dimension reduction, pattern analysis, important wavelength identification, and outlier selection. PCA-LDA was a well-justified choice for this study due to its ability to handle high-dimensional spectral data, classify categorical variables, and balance performance and computational efficiency well. The combination of PCA for dimension reduction and LDA for classification, along with appropriate preprocessing techniques, enabled the development of a robust and accurate model for detecting pork adulteration in beef and mutton. Using the PCA algorithm, the multivariate dimensions of the spectra are reduced into new variables, namely principal components (PCs). Pattern analysis was conducted based on the score plot, and essential wavelengths were identified based on the loading plot. Sample outliers were identified based on T2 and leverage. Eight hundred (800) spectra of Vis-NIR were divided into a calibration and prediction set using the Kennard-Stone algorithm. The calibration set consisted of 500 spectra, while the prediction dataset consisted of 300 spectra.

PCA-LDA was used to build calibration models for predicting pork adulteration levels in beef and mutton. This supervised classification method is suitable for categorical variables, such as adulteration levels. Models were developed using spectra (x-variables) and adulteration levels (y-variables) from the calibration set. The development of the PCA-LDA model involves two essential steps. The first is the dimension reduction of multivariable spectra into 7 PCs using PCA. The second step uses the Quadratic method to construct an LDA model from the seven pre-built PCs. Model performance was evaluated using accuracy and misclassification rate using Equations 1 and 2. The ideal model has a high accuracy value and a low misclassification rate. Preprocessing techniques such as normalization and Savitzky-Golay first derivative were applied to the spectra.

$$Accuracy = \frac{n_{correct}}{n_{total}} \times 100\% \quad [1]$$

$$Misclassification\,rate = \frac{n_{false}}{n_{total}} \times 100\% = 100 - Accuracy \quad [2]$$

RESULTS AND DISCUSSION

Spectral Profiles of Pure and Pork-adulterated Samples Based on Vis-NIR Spectra

Figure 1 shows the profiles of minced pork, mutton, and beef developed using original Vis-NIR spectra. The overall spectral shapes and trends are similar, and the difference lies in the absorbance intensities, which distinguish the meat. The highest reflectance belongs to mutton, followed by pork and beef, while the highest absorbance belongs to beef, followed by pork and mutton. All pure meat spectra have similar patterns showing several peaks between 430 nm (hemoglobin pigments), 500–600 nm (respiratory pigments, and around 940–990 nm (related to OH and CH) (Ayaz et al., 2020; Peyvasteh et al., 2020).

Original Vis-NIR spectra of Beef+Pork and Mutton+Pork in various concentrations are shown in Figures 2a and 2b. Both figures exhibit similar trends, showing absorbance at 400-600 nm related to the Soret, oxymyoglobin, and myoglobin bands (Cozzolino & Murray, 2004). After being preprocessed using the Savitzky-Golay first derivative method, the spectra were altered, as shown in Figures 3a and 3b. The reflectance peaks are more distinct compared to the original spectra.

In the Vis-NIR region (400–600) nm, distinct respiratory pigment bands are related to meat myoglobin and heme absorption. This region (Figure 2a) shows the decrease in absorbance of those bands with the decrease of red pigments (Alamprese et al., 2013);

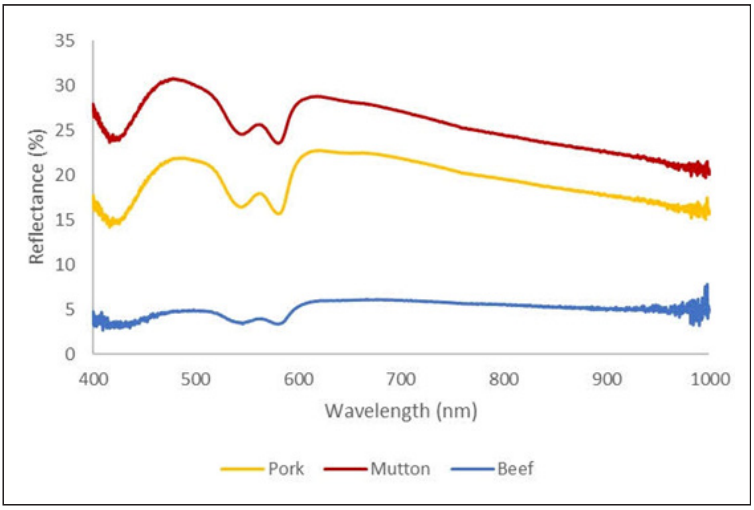
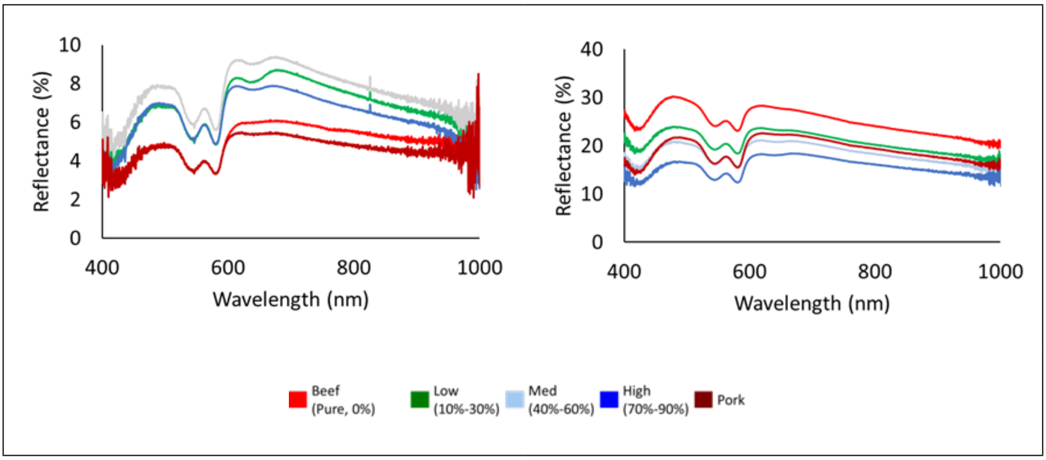


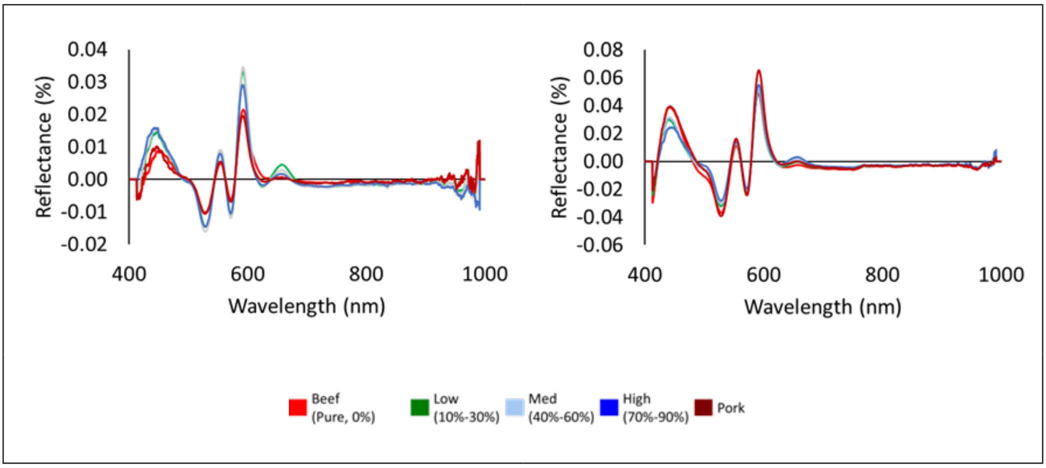
Figure 1. Original Vis-NIR spectra of pure minced pork, mutton, and beef

beef absorbances decrease if more pork concentration is added. More absorbances at 650 nm and 950–1000 nm are noticed for Beef+Pork than for Mutton+Pork. In Figure 2a, pure beef has no distinct absorbance, but a peak absorbance at 650 nm is observed after pork is added. The absorbance at 650 nm might be due to metmyoglobin (Bekhit et al., 2019) present in Beef+Pork. Peaks around 950–1000 nm correspond to O-H (Morsy & Sun, 2013) and are more distinct in Beef+Pork than Mutton+Pork, either for original spectra (Figure 2) or Savitzky-Golay first derivative spectra (Figure 3). These peaks are due to the higher average water content in beef compared to mutton and pork (Lee et al., 1995).



(a) (b)

Figure 2. Original Vis-NIR spectra of (a) Beef+Pork and (b) Mutton+Pork at varying pork concentrations



(a) (b)

Figure 3. Savitzky-Golay 1st derivative Vis-NIR spectra of (a) Beef+Pork and (b) Mutton+Pork at varying pork concentrations

Principal Component Analysis (PCA) of Vis-NIR Spectra

The PCA for beef adulterated with pork (Beef+Pork) using Vis-NIR spectra is shown in Figure 4. The total variance can be explained by 99% using the first three PCs, specifically PC-1 (96%), PC-2 (2%), and PC-3 (1%). The score plot of PC-2 vs. PC-3 effectively differentiated the meat samples into four distinct groups. Category I, the pure beef samples, are placed in the fourth quadrant of the graph. The combination samples of low and medium levels of adulteration (Category IV) are placed in the third quadrant of the graph. The highly contaminated samples (Category III) are in the second quadrant of the graph, while the pork samples (Category I) are in the first quadrant. The loading plot of PC-2 in Figure 2d shows that the Soret band influences PC-2 at around 400 nm and water content at around 975 nm (Alamprese et al., 2013; Weng et al., 2020). The PC-3 loading plot shows that the Soret band influences PC-3 at around 440 nm and respiratory pigments at 535–590 nm (Ayaz et al., 2020; Weng et al., 2020).

As shown in Figure 5, the PCA using Vis-NIR spectra was developed using raw spectra for mutton adulterated with pork (Mutton+Pork). The first 3 PCs explained 100% of the variance, namely PC-1: 99%, PC-2: 1%, and PC-3: 0%. The PC-1 has the highest explained variance. However, based on Figure 3a, PC-1 could not differentiate between Mutton+Pork samples. On the other hand, PC-2 and PC-3 could classify Mutton+Pork samples into four categories. The PC-2 could discriminate pork (Category IV) and highly adulterated beef (Category III) from pure mutton (Category I) and low and medium-adulterated mutton (Category II). Samples in Category IV and Category III have negative score values of PC-2, while samples in Category II and Category I have positive values of PC-2. The

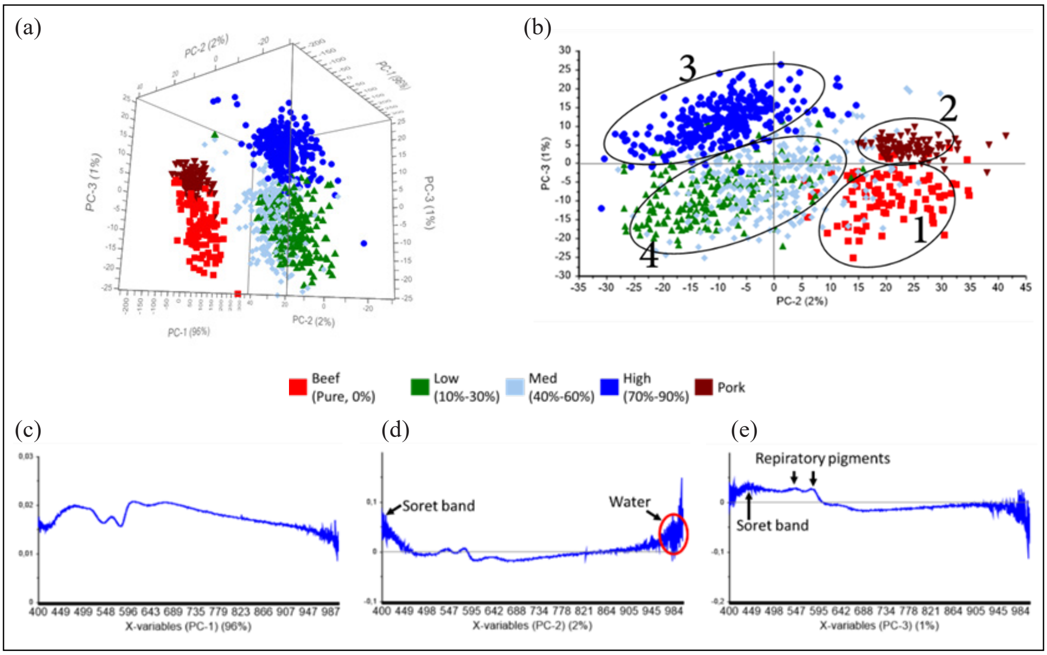


Figure 4. PCA score and loading plot of Beef+Pork using Vis-NIR spectra

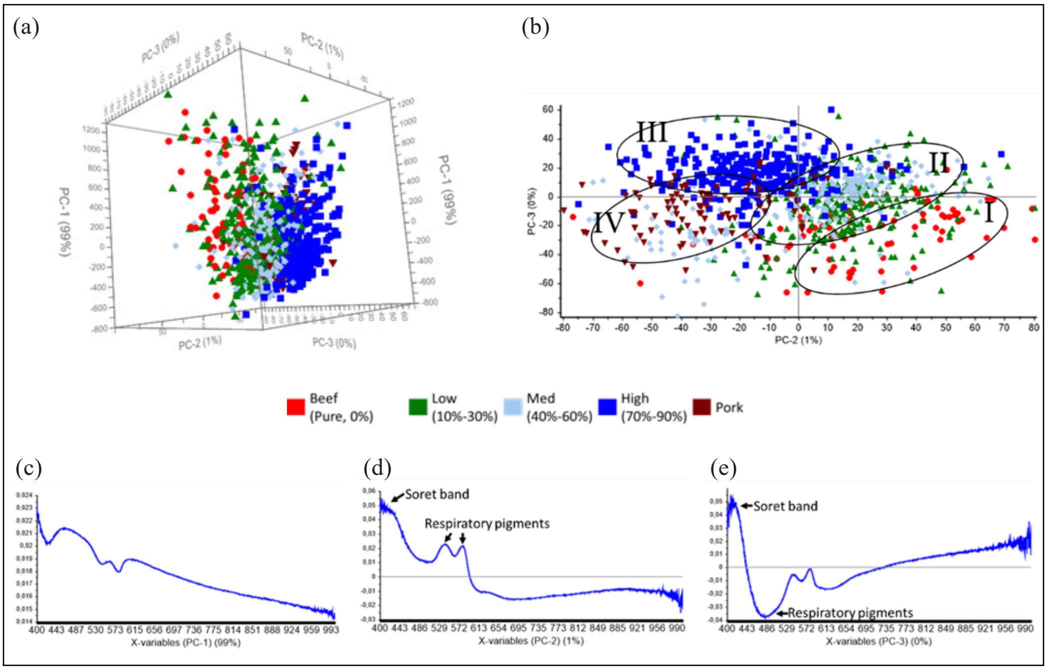


Figure 5. PCA score and loading plot of Mutton+Pork using Vis-NIR spectra

PC-3 could discriminate Category IV from Category III and Category II from Category I. Samples in Category III and Category II had positive values of PC-3, while samples in Category IV and Category I had negative values of PC-3. On the score plot of PC-2 vs. PC-3, Category I is in the fourth quadrant of the graph, Category II in the third quadrant, Category III in the second quadrant, and Category IV in the fourth quadrant of the graph. The loading plot of PC-2 (Figure 5d) and PC-3 (Figure 5e) shows that the Soret band influenced the classification at 400–440 nm in both PCs (Ayaz et al., 2020). Respiratory pigments influenced the PC-2 at 530–580 nm and 480 nm in the PC-3 (Weng et al., 2020).

Principal Component Analysis—Linear Discriminant Analysis (PCA-LDA) Using VisNIR Spectra

The PCA-LDA performances built using Vis-NIR spectra are shown in Table 1. The highest accuracies for Beef+Pork were 99.0% (calibration) and 91.3% (prediction), while for Mutton+Pork, they were 82.4% (calibration) and 73.7% (prediction). Those models were both obtained from raw spectra. The failure of preprocessing to improve accuracy in this study highlights the importance of careful selection and optimization of preprocessing techniques. While preprocessing aims to remove noise and enhance relevant spectral features, it can inadvertently remove or distort information crucial for discriminating between different adulteration levels. The PCA-LDA of Beef+Pork developed using original spectra has a misclassification rate of 1.0% for calibration and 8.7% for prediction. Table 1 also showed that preprocessed spectra could not improve model performance compared to the model with raw spectra. Lower performances of preprocessed spectra could be obtained because sometimes the preprocessed spectra may even omit information essential to the model.

Table 1
PCA-LDA Performances to classify adulteration levels in meat

Sample	Performance	Original		Normalization		SGD1	
		C	P	C	P	C	P
Beef+Pork	Accuracy	99.0%	91.3%	97.0%	90.7%	97.2%	90.0%
	Misclassification rate	1.0%	8.7%	3.0%	9.3%	2.8%	10.0%
Mutton+Pork	Accuracy	82.4%	73.7%	82.4%	70.3%	77.0%	75.0%
	Misclassification rate	17.6%	26.3%	17.6%	29.7%	23.0%	25.0%

Note: SGD1 = 1st order of Savitzky-Golay derivative, C = calibration, P = prediction

CONCLUSION

Vis-NIR spectroscopy, coupled with PCA-LDA, provides a simple, fast, and accurate method for determining the levels of pork adulteration in Beef+Pork and Mutton+Pork mixtures. The best PCA-LDA model, utilizing the original Vis-NIR spectra, achieved a calibration accuracy of 99% for Beef+Pork and 82.4% for Mutton+Pork. SW-NIR yielded lower performance, with the best calibration accuracy of 91.4% and 86.2% for Beef+Pork and Mutton+Pork, respectively. The model built can predict adulteration levels in minced beef and mutton without requiring complex sample preparation and will be particularly beneficial to several stakeholders. Meat producers and processors can use it for quality control, ensuring product authenticity. Regulatory agencies can employ it for on-site screening and enforcing food labeling. Consumers, especially those with religious dietary restrictions, can use it to verify the absence of forbidden meats. Retailers can also leverage it to ensure product authenticity.

This study provides a strong foundation for future research, addressing a significant challenge in food authenticity and consumer protection. Further investigations could explore the application of this Vis-NIR PCA-LDA methodology to other meat types (e.g., poultry, fish) and processed food products. Furthermore, integrating this technique with emerging technologies, such as hyperspectral imaging, may offer enhanced analytical capabilities and potentially improve the accuracy and robustness of adulteration detection. Overall, this result shows the capability of using non-destructive methods for on-site testing, contributing to greater transparency and trust in the meat supply chain.

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