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Rapid detection of meat adulteration in chicken and rabbit meat based on spectra information by using a shortwave near infrared spectroscopy technique

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ABSTRACT

The food industry is increasingly concerned about meat adulteration due to its detrimental effects on food safety and consumer trust. The rapid detection of adulteration in minced chicken and rabbit meat was the focus of this study, which examined the potential of shortwave near-infrared (SW-NIR) spectroscopy. Spectral preprocessing techniques, such as Multiplicative Scatter Correction (MSC), Standard Normal Variate (SNV), Savitzky–Golay derivatives (SG1, SG2), and smoothing, were employed to analyze adulterated samples at six substitution levels (0%, 20%, 40%, 60%, 80%, and 100%). The water-related (970, 1450, 1940 nm) and fat-related (1200, 1720, 2300 nm) regions exhibited distinct absorption features that varied systematically with the extent of adulteration. The separation of pure and mixed samples was clearly defined by Principal Component Analysis (PCA), while Partial Least Squares Regression (PLSR) demonstrated robust predictive performance (R^2cal > 0.90; R^2pred ≈ 0.85–0.88) following preprocessing. Most misclassifications occurred between adjacent levels of adulteration, with a classification accuracy that surpassed 80%. These results indicate that SW-NIR spectroscopy, when applied in conjunction with suitable chemometric modeling, provides a rapid, non-destructive, and dependable approach to the identification of adulteration in rabbit and chicken meat. This is primarily due to variations in fat and water composition.

Introduction

Traditional foods, such as rabbit satay, are widespread in Indonesia's highlands. Beyond being a gastronomic attraction, rabbit satay is also regarded as an alternative traditional medicine in local communities, particularly in cultures where food is closely linked to tradition, faith, and religion (Dalle Zotte, 2002). Rabbit meat is increasingly recognized as a potential functional food due to its unique nutritional profile. It contains low allergenic proteins, low fat, and low cholesterol (Luo *et al*., 2022), alongside high levels of essential minerals (P, K, Ca, Se, and Co) and the highest concentration of iron compared with other meats (Długaszek and Kopczyński, 2013). In addition, rabbit meat provides important vitamins, including B3, B6, and B12 (Dalle Zotte and Szendro, 2011).

Given its nutritional and functional value, the demand for rabbit meat has been increasing. However, this growth also raises concerns about meat adulteration, in which rabbit meat may be substituted or mixed with cheaper alternatives such as chicken. Adulteration typically occurs for economic reasons—producers may use low-cost meats to reduce production expenses or increase profit margins. Such practices, however, present significant health risks. Consumers may unknowingly be exposed to allergens, harmful microorganisms, or chemical residues. Long-term exposure to adulterated products can contribute to nutritional deficiencies and chronic illnesses, while in some cases, it may also conflict with religious or cultural dietary requirements, thereby eroding consumer trust.

Chicken is the most common adulterant because it is inexpensive and widely available. Although chicken is a valuable protein source, it has a higher fat and cholesterol content compared to rabbit meat, which diminishes the nutritional and functional benefits expected from rabbit consumption. Substituting rabbit with chicken therefore not only misleads consumers but also reduces the potential health value associated with rabbit meat. Recent reports highlight the prevalence of meat adulteration worldwide. In Indonesia, undeclared chicken DNA was detected in beef sausage products at levels of around 9% (Prasetyaningrum and Praptyana, 2019). Similar findings have been reported globally, with studies in China and Iran identifying species substitution—including chicken in processed and minced meat products (Naghizadeh *et al*., 2022; Liu and Xing, 2023). These cases emphasize the need for reliable authentication tools, particularly for high-value or culturally significant meats such as rabbit.

Conventional methods for meat authentication, such as physical, chemical, and microbiological analyses, often require long processing times, skilled personnel, and large amounts of chemical reagents. By contrast, rapid, real-time, non-invasive, and environmentally friendly techniques are increasingly recognized as more suitable alternatives. Spectroscopy, particularly in the visible and near-infrared (Vis/NIR) range, has been widely studied for evaluating food quality and authenticating meat (Falcone *et al*., 2006; Zahroh *et al*., 2023; Rahmawati *et al*., 2024).

Despite the growing concerns over meat adulteration, research on the authentication of rabbit meat remains limited compared to other livestock products such as beef, pork, or lamb. Conventional methods for detecting adulteration are often time-consuming, costly, and require skilled expertise, making them less practical for routine screening. While Vis/NIR spectroscopy has shown great promises for rapid and non-destructive meat authentication, its application to rabbit meat adulteration particularly substitution with chicken has not been systematically explored.

Shortwave Near-Infrared (SW-NIR) spectroscopy (970–1700 nm) presents significant advantages for meat analysis, offering richer chemical information via overtones and combination bands of O–H, C–H, and N–H bonds compared to the visible spectrum's color-based differences. SW-NIR targets moisture, fat, and protein, crucial for distinguishing rabbit from chicken meat. It surpasses mid-infrared (MIR) spectroscopy by requiring minimal sample preparation and allowing deeper tissue penetration for rapid, non-destructive, and representative measurements, unlike MIR's surface-limited analysis. Furthermore, SW-NIR signals are more stable and reproducible than Raman spectroscopy, which is prone to fluorescence interference in biological samples. The technology is also char-

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acterized by compact, cost-effective instruments suitable for in-depth or industrial use. However, the complexity of overlapping absorption features in the SW-NIR range necessitates the integration of chemometrics for multivariate pattern recognition to discern subtle differences, particularly for detecting adulteration, such as chicken in rabbit meat. This study therefore explored SW-NIR spectroscopy coupled with chemometrics as an effective method for rapid, non-destructive, and reliable detection of chicken adulteration in rabbit meat.

Materials and methods

Sample preparation

Chicken (*Gallus gallus*) and rabbit (*Oryctolagus cuniculus*) meat used in this study were purchased from a local market in Kaliurang, Sleman, Yogyakarta, Indonesia. The samples were stored at a controlled temperature of 4–10°C until measurement. Chicken meat was used as an adulterant in rabbit meat at six different levels: 100% (AY100), 20% (AY20KL80), 40%(AY40KL60), 60%(AY60KL40), 80%(AY80KL20), and 0% (KL100). For each adulteration level, approximately 100 g of meat was weighed using an analytical balance (Fujitsu FS-AR210) and thawed at room temperature (22–24°C). The samples were then homogenized with a Mitochiba chopper (CH-300) for 30 s to ensure uniform blending at each adulteration level.

SW-NIR spectroscopy

Shortwave near-infrared (SW-NIR) spectroscopy was used to obtain the reflectance value of each level of adulteration. The SW-NIR reflectance spectrometer (Flame-NIR Ocean Optics, Orlando, FL, USA; 970-1700 nm) with a tungsten halogen (360–2,400 nm, HL-2000-HP-FHSA Ocean Optics) as a light source and a Fiber optic probe was equipped. Before measuring the spectra, the spectroscopy was calibrated using a calibration plate (CR-A43, Konica Minolta Inc.). The dark reference was obtained by turning off the light source, while the white reference was obtained by turning on the light source. The SW-NIR spectroscopy was used for the range 900-1700 nm. Approximately 15.0±0.5 g samples were placed into the sample holder. The samples were scanned by SW-NIR with 600 m/s for integration and 17 times of scanning. The samples were divided into 70% for calibration and 30% for validation.

Spectral preprocessing and model performance evaluation

Five preprocessing techniques were applied to reduce noise in the spectral data: multiplicative scatter correction (MSC), standard normal variate (SNV), Savitzky–Golay first derivative (SG-1), Savitzky–Golay second derivative (SG-2) and smoothing. Principal Component Analysis (PCA), an unsupervised multivariate technique, was used to identify grouping patterns in the data. A multivariate analysis was conducted utilizing partial least squares regression (PLSR), a recognized chemometric technique. PLSR was selected for its capacity to effectively handle the high dimensionality and multicollinearity characteristics of spectral data. The model operates by converting predictor and responder variables into latent variables (LVs) that exhibit optimal covariance with the response. The initial phase of this PLSR-based model involves determining the number of LVs. To mitigate overfitting, a comprehensive leave-one-out cross-validation strategy was employed during model training. The LVs were initially established at 10, and the predicted residual error sum of squares (PRESS) was assessed iteratively. For model validation, the optimal number of LVs was identified and applied.

Model spectral performance evaluation and software

The parameters of model performance evaluation are the coefficient of determination (R^2), standard error (SE), and the ratio of prediction to deviation (RPD) (Nie *et al*., 2009). The best model was conducted with the higher value of R2 and the lower value of SE. The dataset for model spectra performance evaluation is SW-NIR which include 0%= 56 samples; 20%= 55 samples ; 40%= 55 samples ; 60%= 55 samples; 80%= 55 samples and 100%= 55 samples (331 samples x 128 wavelengths). The chemometric analysis in this study was carried out with Python 3.12 and Visual Studio Code (Microsoft Corporation, Redmond, WA, USA; version 1.90.0) software.

Results

Spectra interpretation

Fig 1. show the original spectra of rabbit meat which adulterated with chicken meat in 0%, 20%, 40%, 60%, 80%, and 100%. To allow easy interpretation of the result, Fig1a was divided into two plot graphs with range 975-1150 nm (Fig. 1b) and 1180-1340 nm (Fig. 1c). Samples which contain of 100% rabbit meat had a lower reflectance compared with the adulterated samples. Fig. 1a is clearly identify the difference spectra than other preprocessing analysis (Fig. 2). MSC and SNV result on Fig. 2a and 2b had no difference in the spectra's reflectance result for all samples, but there is smoother line graph that identify the lower noises detected. Fig. 2c and 2d with SG-1 and SG-2 as preprocessing did not gave clearly spectra information.

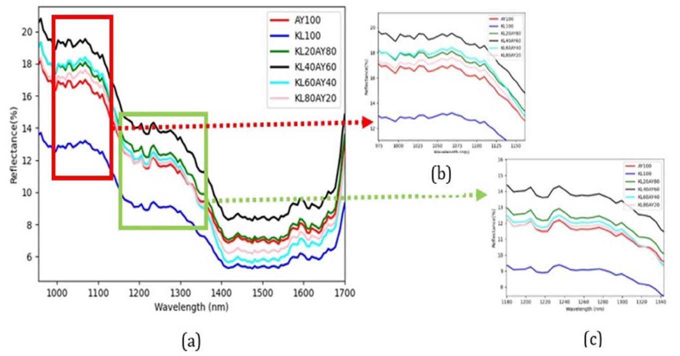

Fig 1. Original spectra of rabbit meat adulteration with chicken meat. a) Full original spectra; b) Original spectra with range of 975 nm- 1150 nm; c) Original spectra with range of 1180-1340. AY = Chicken meat; KL= Rabbit meat.

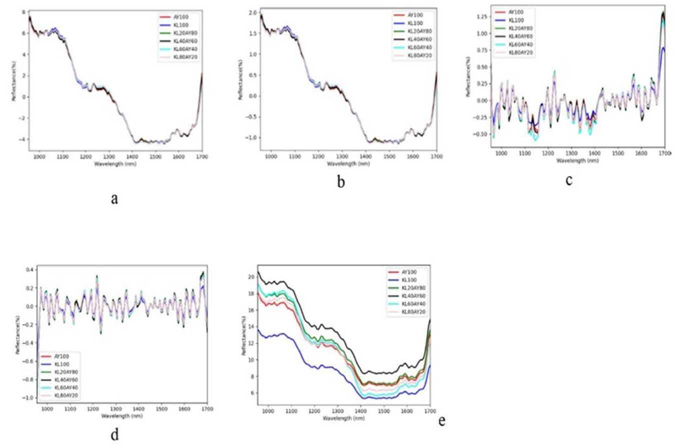

Fig 2. Spectra plot on preprocessing method. a) MSC; b) SNV; c) SG-1; d) SG-2; e) Smoothing. AY = Chicken meat; KL= Rabbit meat

PLSR

Table 1. shows the PLSR values for rabbit meat contaminated with chicken meat at levels of 0%, 20%, 40%, 60%, 80%, and 100%. Based on Table 1, five preprocessing and original data gave the R^2P and standard error prediction (SEP) ranging from 0.85-0.88 and 11.74-13.18, respec-

tively. The best model was conduct by applying original data which gave the higher R^2P (0.88) and the lowest SEP value (11.74).

Table 1. PLSR model performance for predicting chicken meat as adulterant on rabbit meat

Preprocessing	R^2_C	SEC	R^2_{CV}	SECV	R^2_P	SEP	RPD	LVs
MSC	0.96	6.98	0.89	11.05	0.85	13.18	2.59	14
SNV	0.96	6.92	0.89	11.04	0.86	12.62	2.7	14
SG-1	0.95	7.94	0.91	10.02	0.88	11.74	2.9	7
SG-2	0.94	8.43	0.9	10.91	0.85	13.07	2.61	7
ORI	0.96	6.83	0.91	10.36	0.88	11.74	2.9	14
Smoothing	0.92	9.84	0.88	11.52	0.87	12.01	2.84	16

MSC, SNV, SG1, SG2, and Smoothing are multiplicative scatter corrections, standard normal variate, Savitz\ky–Golay first and second derivatives and smoothing respectively. R^2C and R^2P denote the coefficient of determination for calibration and prediction. SEC and SEP refer to standard error calibration and prediction, respectively. LV represents the number of latent variables used in the PLSR. Numbers in bold indicate the best result.

PCA

PCA was performed to visualize the spectral results of rabbit meat adulterated with chicken meat in groups. Fig 3a shows that rabbit meat (100%) is grouped in negative quadrant (negative PC2), meanwhile rabbit meat which adulterated by chicken meat is grouped into positive PC2 and positive PC1. Two principal components (PC) explained that 94.05% are effect on PCA grouping. This result was plotted into 3D figure (Fig. 3b) which could perfect visually identification on sample grouping. Fig. 3c is PC loading plot denotes the PC that affects the distribution and classification of samples. The wavelength contribution was plotted by beta coefficient on Fig. 4.

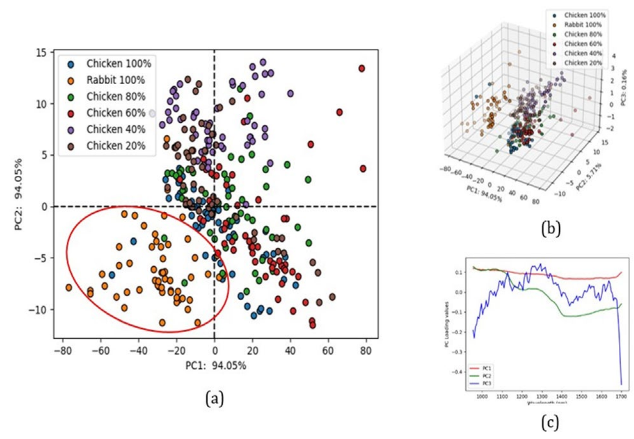

Fig 3. PCA result plot on original spectra. AY = Chicken meat; KL= Rabbit meat.

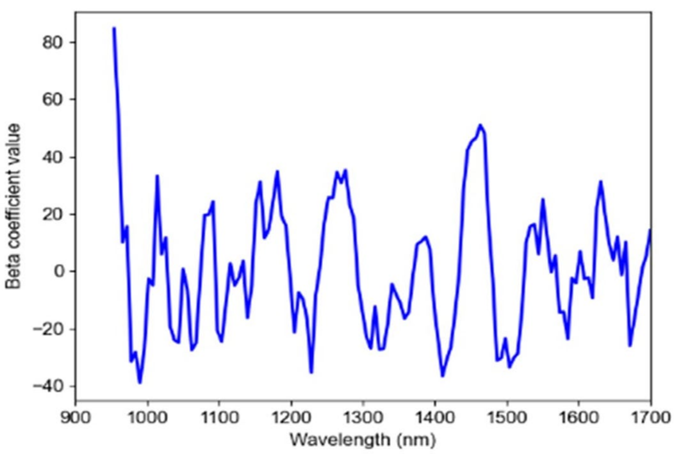

Fig 4. Beta coefficient curves-based SW-NIR fingerprints.

Discussion

The SW-NIR spectra obtained in this study (900-1700 nm) clearly revealed the presence of water (O-H), proteins (N-H), and fat (C-H) (Barbin *et al.*, 2015). The reflectance spectra in Fig 1 demonstrate clear differences between pure rabbit meat (KL100) and the fully adulterated chicken sample (AY100). The adulterated samples (KL20AY80, KL40AY60, KL80AY20) exhibited intermediate spectral profiles, positioned between the two pure species, suggesting that the spectral response correlates with the level of adulteration. Luo *et al.* (2022) mentioned that rabbit meat has a lower fat compared with others meat, it could be seen on the Fig 1a that KL100 has a significant different than other samples. This highlights that fat-related absorption features play an important role in differentiating pure rabbit meat from adulterated mixtures.

The major absorbtion bands are observed around 980-1000nm, 1200nm, and 1450-1600nm, which are typically associated with water (O-H stretching overtones) and protein or lipid absorptions (C-H and N-H bonds). The spectral range 975-1150 nm is the sensitive range which related to water content and protein absorbtion (second overtone of O-H stertching). The chicken spectra (AY100) show slightly lowet reflectance compared to rabbit meat (KL100), suggesting difference in muscle water distribution an d structural composition. Adulterated samples fall progressively between the two, confirming that this window is useful for distinguishing substitution levels.

The spectra with range 950-1100 nm identify as fat compound (Tang *et al.*, 2004), this research is in line with (Grau *et al.*, 2011) shows that fat compound (C-H bounds) on chicken is located around 1040 nm. The peak around 1180-1340 nm in Fig 1c. indicated as second overtone of C-H stretching overtones which associated with fat (ElMasry and Nakauchi, 2016). This result also in line with Wang (2000) which reported that chicken could be differentiated using SW-NIR spectra in the 1000-1400 nm region due to fat and proteon differences. The wavelength around 1400-1450 nm is refers to the first O-H overtone from water-bounded group (ElMasry and Nakauchi, 2016). The wavelength on 1414-1490 nm presence as water and 1490-1567 nm as protein content (Peyvasteh *et al.*, 2020).

The result of PLSR model is shown in Table 1. Preprocess treatment has been done such as MSC, SNV, SG-1, SG-2, orignal data, and smoothing. The PLS method generates new predictors, such as latent variables (LV) or principles components (PC) (Lee *et al.*, 2018). The LV value affects the result of the PLSR model developed; the higher the LV values, the more overfitting the final model presents. Internal validation is required in the form of a Cross-validation value to counteract the resultant model's overfitting. The best model was conducted by applying original data which gave the higher R^2P (0.88) and the lowest RMSEP value (11.74). In addition to R^2 and RMSEP, an RPD was recommended to enhance model performance. In general, the RPD readings range from 2.59 to 2.9. The model was successfully executed in this investigation, as indicated by the higher RPD results, which were equivalent to three (Sowmya and Ponnusamy, 2021). This result indicated that the target parameter could be successfully using the original data without preprocessing data. The best model was plotted into PCA in Fig 3.

Adulterated and pure chicken samples were clearly distinguished from pure rabbit meat samples by Principal Component Analysis (PCA). The rabbit was positioned in the negative quadrant, while the adulterated samples were shifted to the positive quadrant. The distinctive spectral features that are associated with distinctions in chemical composition are the driving force behind this separation. In contrast to chicken, rabbit meat has higher levels of protein and minerals and lower levels of fat and cholesterol (Dalle Zotte and Szendro, 2011; Luo *et al.*, 2022). The spectral regions that were previously identified reflect these compositional differences. In particular, the negative quadrant separation of rabbit meat is significantly impacted by protein-associated absorptions at 1400–1500 nm, which are more severe in rabbits. In contrast, adulterated and chicken-rich samples shift toward the positive quadrant because of stronger signals in the 950–1150 nm and 1200–1300 nm regions, which correspond to water (O–H bonds) and lipid-related C–H harmonics, respectively (Ozanich *et al*., 1992; ElMasry and Nakauchi, 2016). The gradual consolidation of adulterated samples between the rabbit and chicken groups implies a compositional gradient: the spectra are drawn toward the positive quadrant as the proportion of chicken increases, as water and fat contributions become more significant. This emphasizes PCA's capacity to identify the fundamental biochemical factors that contribute to adulteration, as opposed to merely statistically categorizing samples.

Conclusion

The rapid detection of meat adulteration in rabbit meat with chicken meat as an adulterant (0, 20, 40, 60, 80, and 10%) using shortwave near-infrared (SW-NIR) spectroscopy demonstrates a highly effective, non-invasive method for ensuring meat authenticity and quality based on the spectra identification. This technique utilizes spectral information to identify discrepancies in meat composition, offering fast and accurate results without the need for extensive sample preparation. The PLSR model gave the best result on original spectra, which provide R^2C and R^2P in 0.96 and 0.88, respectively. The standard error (SE) values are 6.83 and 11.74 for SEC and SEP, while the RPD value is 2.90 with the LV value of 14. By applying SW-NIR spectroscopy as a screening method, food safety authorities and manufacturers can potentially detect adulteration, thus preventing food fraud and safeguarding consumer trust. Moreover, laboratory tests are necessary to prove and validate the spectroscopy screening result.

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Conflict of interest

The authors declare that they have no conflict of interest regarding the publication of this article.

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