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Chemometric approach to characterizing and comparing the quality of buffalo meat from Nakhon Phanom and Khammouane provinces

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Abstract The results indicated that a chemometric approach could effectively characterize different attributes in quality between buffalo meat from Nakhon Phanom (NP) province, Thailand and Khammouane (KM) province, Laos. Neither the unsupervised principal component analysis (PCA) model nor the supervised partial least squares-discriminant analysis (PLS-DA) model completely separated the NP and KM groups. However, the sparse PLS-DA model was able to successfully distinguish between the meat samples originating from KM versus NP. Interestingly, orthogonal projections to latent structures discriminant analysis (OPLS-DA) exhibited superior discriminatory performances between regional meat samples. The robust OPLS-DA model used an orthogonal and a predictive factor, demonstrating a strong fit with $R^2X = 0.715$, $R^2Y = 0.877$ ($P<0.001$), and $Q^2Y = 0.803$ ($P<0.001$). Consequently, two crucial variables were identified based on the selection criteria (VIP>2, $P<0.05$, FDR<0.05). Meat odors from sensors 1 (AUC=0.936, 95% CI: 0.841-0.989) and 4 (AUC=0.948, 95% CI: 0.843-1.000) could effectively distinguish between the NP and KM meats. In conclusion, the chemometric analysis successfully discerned regional quality differences and identified key discriminatory variables.

Keywords: Buffalo, Meat quality, Odor, Chemometric, Sustainability

Introduction

Meat quality and authentication have become important issues in the food industry driven by consumer demand for assurances about the provenance, safety, and the integrity of meat (Zhang *et al*., 2021a; 2023). Authenticating animal-derived food products such as meat, dairy, honey, eggs, and fats relies on the sophisticated integration of advanced analytical techniques and multivariate statistical approaches (Ye *et al*., 2023). Chemometrics refers to mathematical and statistical techniques used to extract meaningful information from complex chemical datasets (Héberger, 2008). Principal component analysis (PCA) enables

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data exploration and dimensionality reduction (Kang *et al*., 2022). Partial least squares discriminant analysis (PLS-DA), sparse PLS-DA (sPLS-DA), and orthogonal PLS-DA (OPLS-DA) are supervised techniques for classification and discrimination. PLS-DA handles complex data structures (Kang *et al*., 2022), whereas sPLS-DA selects key discriminatory variables, improving model interpretation (Lê Cao *et al*., 2011). OPLS-DA effectively separating predictive and non-predictive variations (Kang *et al*., 2022). These chemometric methods, which are often combined with spectroscopy, have become prevalent to ensure meat quality and authenticity, thus, providing a complementary approach to robust analysis. In food and meat processing, chemometrics has become an indispensable set of tools with diverse applications. One major use is to detect food fraud by identifying chemical markers of authenticity *(*Putnik *et al.*, 2019). These powerful tools are also used for the characterization and comparison of meat samples (Arvanitoyannis and van Houwelingen-Koukaliaroglou, 2003; Vlachos *et al*., 2016). Chemometrics is now an integral component in food authentication, helping to differentiate and prove the identity of products through a combination of chemically analytical methods. Therefore, this study aimed to use the chemometric approach to discriminate buffalo meat from two regions of Nakhon Phanom and Khammouane.

Materials and methods

Experimental design

Forty loin (*longissimus lumborum*, LL) muscles were obtained from swamp buffaloes 24 hours post-mortem. Samples were purchased from a local market in Nakhon Phanom province (NP, n=20), Thailand (13.8140°N, 100.0373°E) and Khammouane province (KM, n=20), Laos PDR (17.6366°N, 105.1861°E) in February 2023. The samples were transported on ice at approximately 4°C to the laboratory for analysis. Prior to analysis, the meat was trimmed of fat and connective tissue. The LL muscles were cut into 1-inch-thick steaks to be used for meat quality assessments. A chemometric approach was used for data analysis.

pH

The pH of fresh loin samples was measured in triplicate using a handheld pH meter (HI99163, Hanna Instruments, USA) equipped with an FC2323 probe (Phoemchalard *et al.*, 2022). Before sample analysis, the pH probe was calibrated with standard buffer solutions at pH 4 and pH 7 to ensure accurate functionality.

Color

Instrumental color analysis was performed to evaluate the visual attributes of the buffalo meat samples (AMSA, 2012; Phoemchalard *et al.*, 2021a). The CIELAB color space system was utilized, in which the L* parameter represented lightness, the a* parameter indicated red/green, and the b* parameter measured yellow/blue. Color measurements were obtained in quintuplicate for each sample using a Konica Minolta CR-400 colorimeter (Konica Minolta Sensing Inc., Japan). The instrument was calibrated against a white reference standard prior to analysis.

Drip loss and cooking loss

The loss of dripping was determined by weighing meat samples (W_i) and then placing them in a refrigerator suspended in plastic bags at 4°C for 24 hours (Honikel, 1998). After this time, the samples were removed, blotted, and reweighed (W_f). The drip loss was calculated using the following equation:

$$\text{Drip loss (\%)} = [(W_i - W_f)/W_i] \times 100$$

For cooking loss analysis (Honikel, 1998), 2.54-cm thick meat samples were weighed (W_i), vacuum sealed, and cooked by boiling in a water bath at 80°C until an internal temperature of 75°C was reached. The samples were then cooled, refrigerated overnight at 4°C, blotted, and reweighed (W_f). The cooking loss was calculated using the following equation:

$$\text{Cooking loss (\%)} = [(Wi - W_f)/W_i] \times 100$$

All water-holding capacity (WHC) analyses, including that of drip loss and cooking loss, were performed in triplicate.

Textural properties

The shear force analysis was performed following the standard procedure (AMSA, 2016). Prior to analysis, samples from the cooking loss study were cooled to 4°C overnight. The 1.27-cm diameter cylindrical core samples were extracted from each meat sample using a coring device. The measurements were then performed at room temperature. The shear force parameters were obtained using a Warner-Bratzler V-blade attached to a TA-XT *plus* Texture Analyzer (Stable Micro System Ltd., Surrey, UK). The blade was set to travel at a

crosshead speed of 4 mm/s using a 50 kg load cell. The maximum force (kg/cm^2) and shear work (kg/s) were determined from the force-deformation curve replicated 6 times on each sample. All instrumental texture profiling was conducted in accordance with AMSA guidelines to ensure a standardized and reproducible assessment of meat shear forces.

Texture profile analysis (TPA) was conducted using a texture analyzer equipped with a P/50 cylindrical probe. The meat samples remaining from the cooking loss analysis were cut into $1\times1\times1$ cm^3 pieces and analyzed in triplicate. The cooked meat samples were compressed twice to 75% of their original height at a crosshead speed of 1 mm/s, following the two-bite simulation method (Bourne, 1978). Textural parameters including hardness, adhesiveness, gumminess, cohesiveness, chewiness, springiness, and resilience were determined from the force-time curves using the instruments Exponent software, version 6.1.16.0. This standardized TPA methodology allowed a quantitative characterization of meat texture attributes.

Proximate composition

Loin samples were collected from each group of buffaloes. Before proximate analysis, all external fat and connective tissues were removed. The meat was then cut into cubes and ground. Approximately 150 g of each sample was placed on a round plate and moisture, protein, fat, ash, and collagen were analyzed using a FoodScan™ 2 Meat Analyzer (Fossanalytics, Hillerod, Denmark) approved by the AOAC method (Anderson, 2007).

Electronic nose (E-noses)

The meat odor profiles were analyzed using an e-noses system equipped with a metal oxide semiconductor (MOS) sensor technology (Electronic Nose Co., Ltd., Bangkok, Thailand). The e-noses contained an array of eight different MOS sensor types, including TGS 816 (sensor 1), TGS 2600 (sensor 2), TGS 823 (sensor 3), TGS 2603 (sensor 4), TGS 826 (sensor 5), TGS 2610 (sensor 6), TGS 2620 (sensor 7), and TGS 2444 (sensor 8) models, allowed for a broad volatile compound detection. Meat samples were prepared and the analysis parameters of the e-noses was optimized according to established protocols (Phoemchalard *et al.*, 2021b; Tathong *et al*., 2023) to maximize sensor responses. Odor analysis was carried out at a sensor temperature of 25°C using the CIM NOSE 2.0 software system (Electronic Nose Co., Ltd.) to record and process sensor responses.

Chemometrics analysis

Physicochemical data on buffalo loin meat from the Nakhon Phanom and Khammouane provinces were normalized using the median and Pareto scaling prior to statistical analysis. Both univariate analysis and multivariate chemometric approaches were utilized, including PCA, PLS-DA, sPLS-DA, OPLS-DA, together with a receiver operating characteristic (ROC) curve analysis. The predictability and robustness of the OPLS-DA models were evaluated using R^2X, R^2Y, and Q^2Y values. R^2X values evaluated predictor variable variation, while R^2Y values evaluated response variable variation. Q^2Y values measured predictive ability by cross-validation. Values near 1 signified excellent predictive and explanatory power. The permutation test (1000x) yielded $P<0.05$, further validating the accuracy of the model. Large $R^2Y–Q^2Y$ gaps (>0.3) or Q^2Y intercepts less than 0.05 indicated overfitting; therefore, because the gaps did not exceed 0.3 it indicated that there was no overfitting (Eriksson *et al*., 2003). Variables with VIP>2, $P<0.05$, and FDR<0.05 were considered the most distinct and influential characteristics differentiating sample groups in the models. In addition, a permutation test (1000x) was performed, resulting in P-values less than 0.05, further validating the accuracy of the models. A large difference between R^2Y and Q^2Y (greater than 0.3) or Q^2Y intercepts below 0.05 indicated model overfitting (Eriksson *et al*., 2003). To identify the most important variables from the models, variable importance in projection (VIP) scores from OPLS-DA were applied above 2 along with P-values less than 0.05 and FDR values below 0.05. Variables exceeding these thresholds can be considered as the most distinct and influential variables differentiating the sample groups in the models. For ROC curve analysis, the evaluation of the machine learning model performance for binary classification was measured by plotting the sensitivity against the 1-specificity across the discrimination thresholds. Models with ROC curves that shifted closer to the upper-left plot corner exhibited higher true positives rather than lower false positives, therefore reflecting better discrimination. The area under the ROC curve (AUC) provided a quantitative metrication of overall model performance, with a higher AUC indicating a greater ability to correctly distinguish positive and negative instances (Omar and Ivrissimtzis, 2019; Pendrill *et al*., 2023). All chemometric modelling and evaluation was performed using MetaboAnalyst 5.0 (Chong *et al*., 2019; Pang *et al*., 2021). The combined use of univariate and multivariate statistical techniques allowed a comprehensive comparative analysis of the quality parameters of buffalo meat between the regions.

Results

The differential expression analysis between meat groups using the fold change analysis (FC) (Figure 1A) and the visualization of the volcano plot (Figure 1B) revealed that sensor 4 was significantly down-regulated and sensor 1 was significantly up-regulated. Consequently, the FC analysis demonstrated that sensor 4 had a large, statistically significant negative log2 (FC) between the meat groups, indicating that its expression was lower in one group than in the other. Meanwhile, sensor 1 exhibited a large and significant positive log2 (FC), which meant that its expression was higher in one group compared to the other. The volcano plot visualized these findings, with sensor 4 falling in the significantly downregulated quadrant (lower left) and sensor 1 plotted in the significantly upregulated quadrant (upper right). The positions in the volcano plot indicated that sensors 4 and 1 have substantial magnitude FC combined with high statistical significance between the two meat groups.

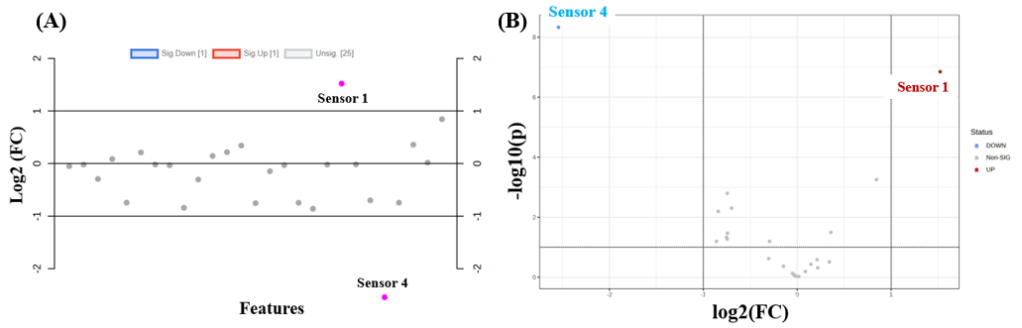

Figure 1. Fold change (A) analysis and Volcano plot (B) between meat groups

T-tests were conducted to compare 28 variables between the two groups (Figure 2). Using a significance level of 0.05 for both the P-value and FDR, the results indicated that 7 of the 28 variables showed statistically significant differences between the groups. Sensor 4 (Methyl mercaptan and trimethylamine), sensor 1 (butane, methane, propane), sensor 8 (ammonia), sensor 5 (isobutane, ethanol, ammonia), sensor 3 (organic solvent vapors), adhesiveness and fat content were found to differ significantly between the two meat groups according to the results of the t-test results ($P<0.05$ and $FDR<0.05$). The meat odor responses detected by sensors 3, 4, and 5, as well as the fat content, were significantly higher in NP meat compared to the KM meat. In contrast, the

responses of sensors 1 and 8, along with adhesiveness, were greater in KM meat than in that of NP meat.

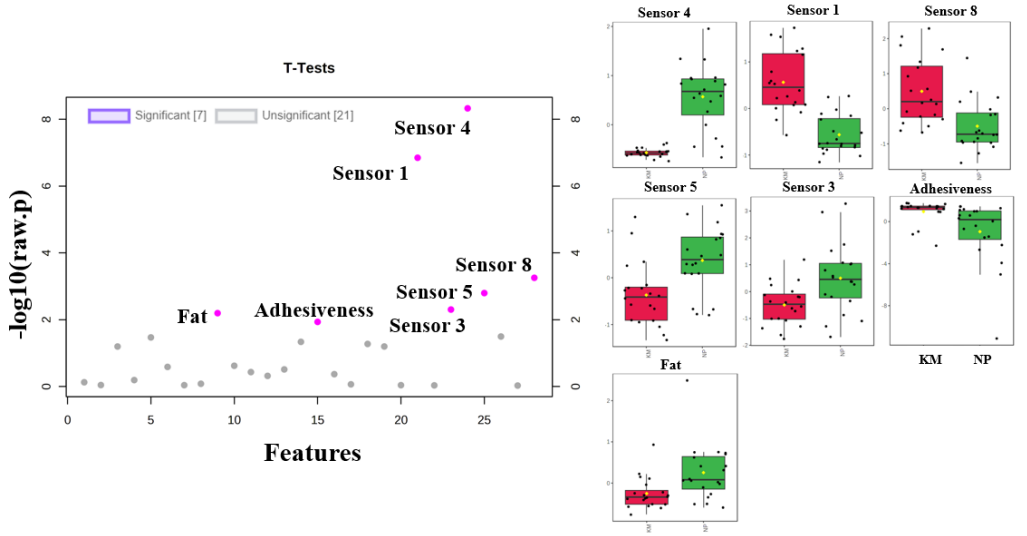

Figure 2. Two-sample t-tests (P-value and FDR<0.05)

Pattern search analysis was conducted to identify variables that correlated significantly with the readings of sensors 1 and 4 (Figure 3). Using a significance level of 0.05 for both the P-value and FDR, 5 variables were found to have statistically significant correlations with sensor 1. Sensor 8 showed a strong positive correlation of 0.76 (t=7.18, P<0.001, FDR<0.001). Sensor 4 displayed a moderate negative correlation of -0.69 (t=-5.95, P<0.001, FDR<0.001). Sensor 3 demonstrated a moderate negative correlation of -0.48 (t=-3.38, P<0.01, FDR<0.01). Collagen exhibited a moderate positive correlation of 0.48 (t=3.34, P<0.01, FDR<0.01). Finally, cooking loss revealed a weak positive correlation of 0.43 (t=2.95, P<0.01, FDR<0.05). These results indicated strong predictive relationships between sensor 1 and sensors 8, 4, and 3, together with moderate associations with collagen content and cooking loss. These findings were based on the analysis of the pattern search that met the significance criteria.

For sensor 4, four variables showed statistically significant correlations. Sensor 3 uncovered a strong positive correlation of 0.76 (t=7.07, P<0.001, FDR<0.001). Sensor 1 exhibited a moderate negative correlation of -0.69 (t=-5.95, P<0.001, FDR<0.001). Sensor 8 revealed a moderate negative correlation

of -0.59 (t=-4.46, P<0.001, FDR<0.001). Finally, sensor 2 demonstrated a weak positive correlation of 0.44 (t=3.00, P<0.01, FDR<0.05).

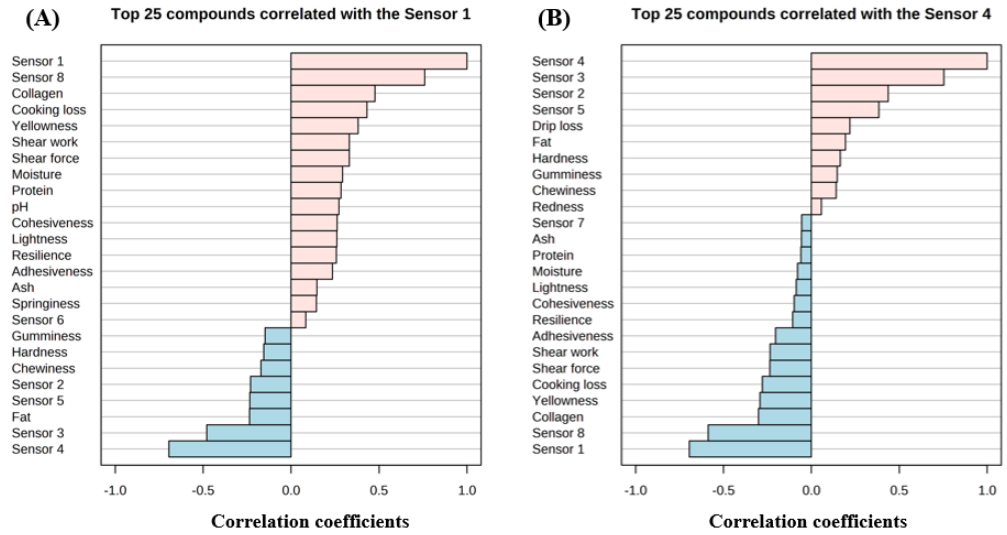

Figure 3. Pattern search of top variables correlated to sensors 1 (A) and 4 (B)

Multivariate techniques, including PCA, PLS-DA, sPLS-DA, and OPLS-DA, are shown in Figures 4 and 5. The PCA revealed that the first two principal components (PC) accounted for 66.5% and 13.5% of the total variance, respectively. The PLS-DA further indicated that the first two PCs explained 14.1% and 60.4% of the variation. Furthermore, sPLS-DA improved sample modeling by identifying key variables that contributed to group separation; The first two PCs from sparse PLS-DA accounted for 14.7% and 39.99% of the variance.

The OPLS-DA model evaluation metrics of R^2X, R^2Y, and Q^2Y indicated strong model performance and predictive ability. Two orthogonal factors and one predictive factor were used to formulate the model. The R^2X value of 0.715 showed a good explanatory capacity of the predictors of the X variable space. The R^2Y of 0.877 (P<0.001) suggested that it was an excellent model fit, with the predictors accounting for a substantial proportion of variance in the response variables. Finally, the Q^2Y of 0.803 (P<0.001) demonstrated excellent predictive relevance, as the cross-validated predictions significantly exceeded the null model.

Using VIP scores along with statistical significance testing, the most distinct variables that differentiated buffalo meat originating from NP and KM were identified. Interestingly, variables with VIP>2, P<0.05, and FDR<0.05 were considered the most critical variables. Based on meeting these criteria (Figure 5C), sensor 1 and sensor 4 emerged as the variables most influential in distinguishing meat from the two provincial origins according to the multivariate model.

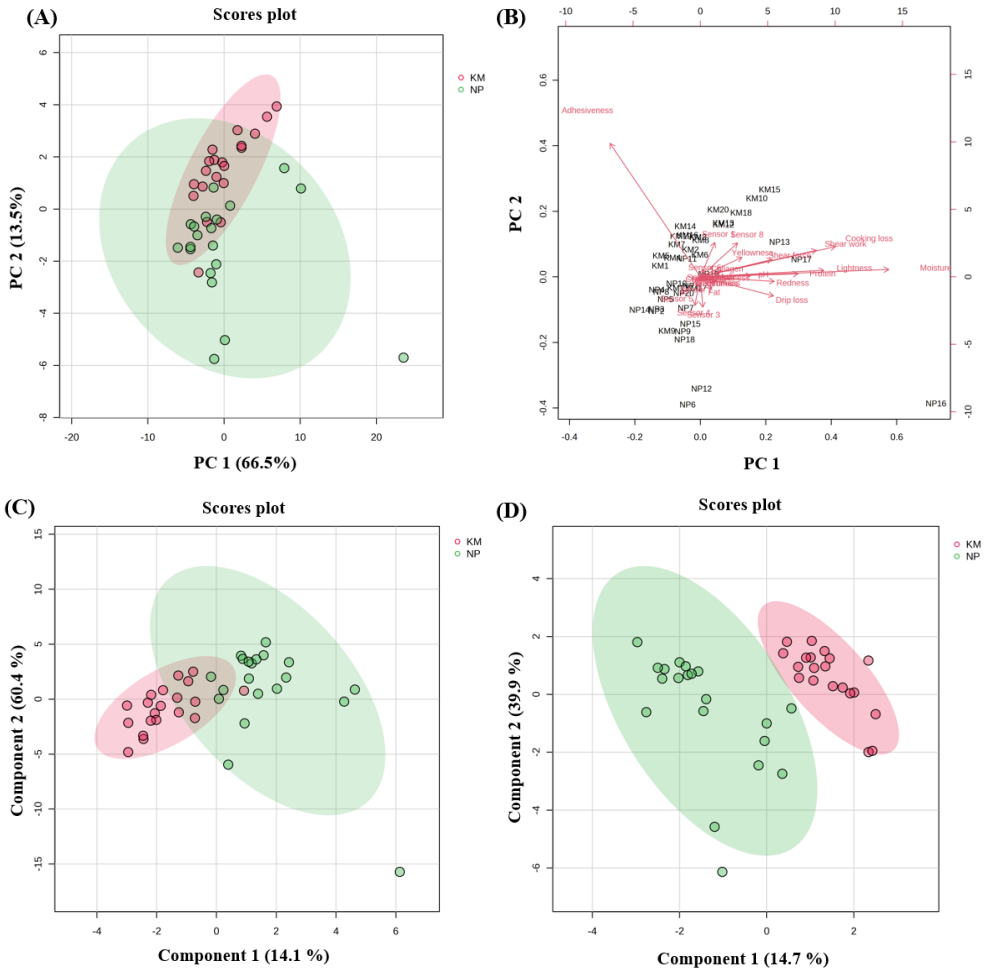

Figure 4. 2D plot (A) and biplot (B) of principal component analysis (PCA), 2D plot (C) of partial least squares discriminant analysis (PLS-DA), and 2D plot (D) of sparse PLS-DA (sPLS-DA) of buffalo meat originating from Nakhon Phanom (NP) province, Thailand and Khammouane (KM) province, Laos

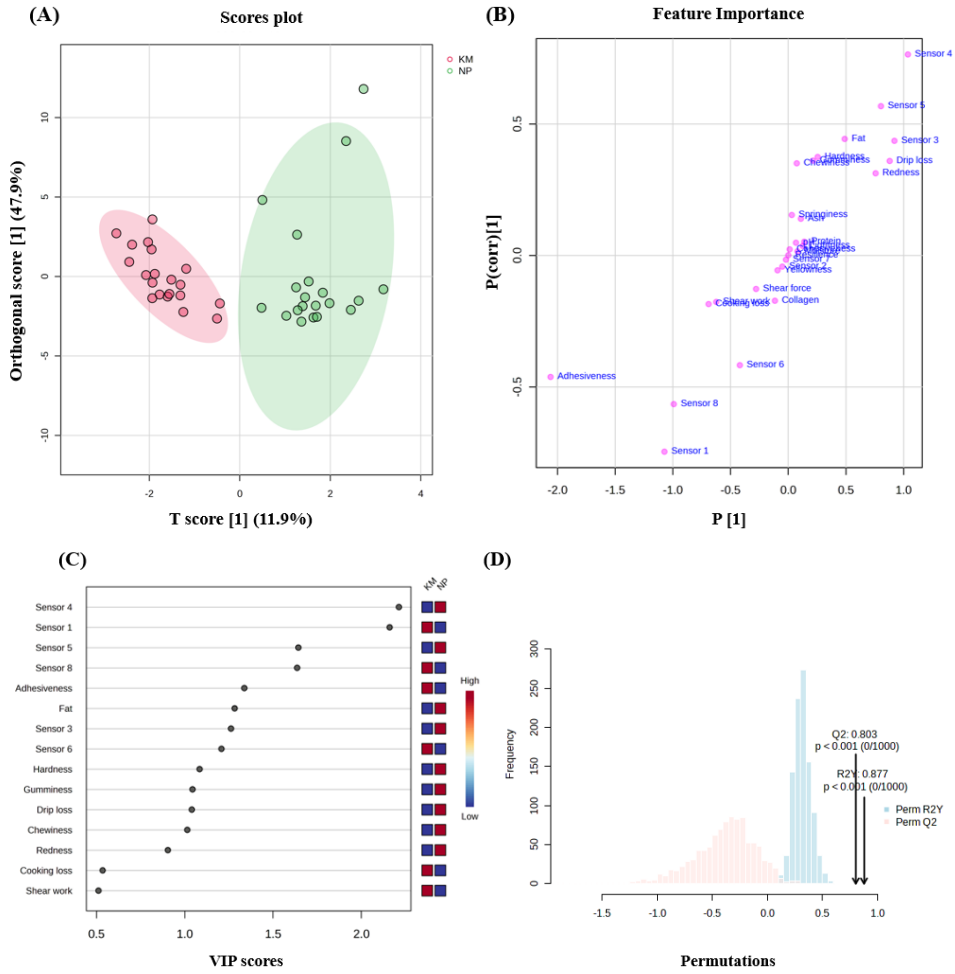

Figure 5. Multivariate modelling of physicochemical variations using OPLS-DA: 2D score plot (A), S-plot (B), VIP-plot (C), and permutation (D)

The ROC curve analyzes of sensors 1 and 4 demonstrated strong discriminative abilities (Figure 6A-B). Sensor 1 had an AUC of 0.936 (95% CI: 0.841–0.989), while sensor 4 had a superior AUC of 0.948 (95% CI: 0.843–1.000). The high AUC values for sensors 1 and 4 indicated excellent and outstanding differentiation capacities and furthermore were perfect classifiers having an AUC of 1.0. The narrow confidence intervals signified the high precision of the AUC estimates and also showed that sample sizes were adequate. For sensor 1, the CI suggested that the true AUC would be 0.841–0.989 according to the new data. The CI of sensor 4 indicated that its true AUC would be between 0.843–1.000, the upper boundary reaching 1.000. Accordingly, this implied a significant statistically perfect classification. Thus, the ROC analyzes of sensors

1 and 4 validated the strong discriminative powers in differentiating between the positive and negative classes.

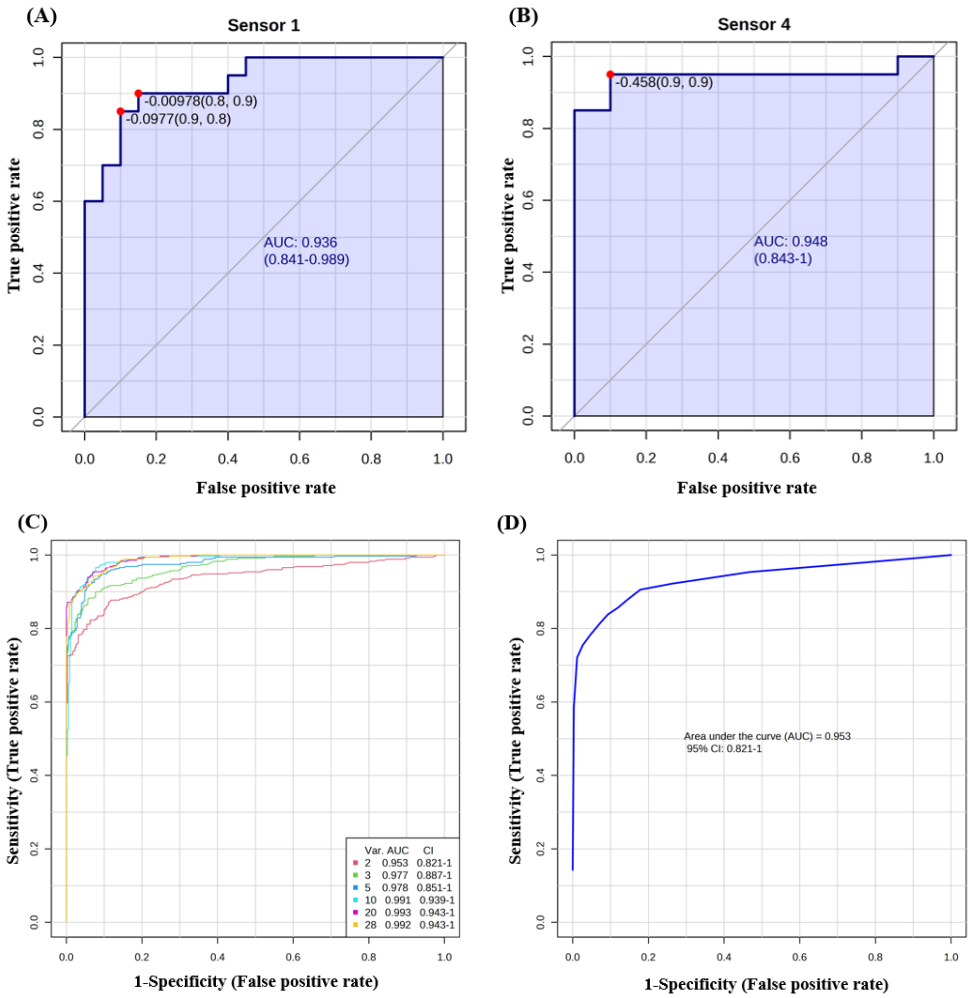

Figure 6. Classical univariate ROC curve analyses of sensor 1 (A) and sensor 4 (B) and multivariate ROC curve based on exploratory analysis (all models (C) and two features (D))

Exploratory analysis of the multivariate ROC curve incorporated all models (Figure 6C) and demonstrated an exceptional discriminative performance. The AUC values increased steadily with the input of additional models, ranging from 0.953 for the first 2 models increasing to 0.993 for 20 models. The AUC values were 0.953, 0.977, 0.978, 0.991, 0.993, and 0.992 for the top 2, 3, 5, 10, 20, and 28 ranked models, respectively. The upward trend in AUC with the inclusion of

more models indicated that the combination of multiple models leads to increased predictive accuracy and enhanced differentiation between positive and negative cases. Remarkably, the confidence intervals for all the model combinations ranged from 0.821 to 1.000, signifying a significant statistically perfect classification. This implied that the new data showed the true AUC values to have a 95% probability of reaching the maximum AUC of 1.0.

Additionally, the exploratory analysis of the multivariate ROC curve incorporating sensors 1 and 4 (Figure 6D) achieved strong discriminative ability, with an AUC of 0.953 (95% CI: 0.821–1.000). An AUC of 0.953 indicated excellent differentiation between positive and negative cases using the combined inputs of sensor 1 and sensor 4. The confidence interval ranging from 0.821-1.000 suggested that the true AUC was between 0.821–1.000. This would imply a 95% certainty if the analysis was repeated on new data. In particular, the upper boundary of the CI reaching 1.000 implied that the multivariate model attained a statistically significant perfect classification. This provided evidence that the combination of sensor 1 and sensor 4 yielded an optimized predictive performance similar to that of a flawless classifier.

Discussion

Chemometrics has been found to be helpful to discriminate and authenticate meat quality. This investigation has been carried out using chemometric techniques namely PCA and PLS-DA, which have shown partial separation among the NP and KM buffalo meat samples; however, large overlaps were recorded for NP (green) and KM (pink) samples in the score plots. This demonstrated that uniquely, physicochemical attributes in unsupervised and supervised models, did not provide a suitable means to classify different meat quality profiles. Conversely, sPLS-DA was able to classify KM and NP meat samples. However, OPLS-DA produced better performance criteria separating the different regional meat samples with 2 orthogonal and 1 predictor variables, with a high fitting (R^2X=0.715, R^2Y=0.877) and good predictively (Q^2Y=0.803) respectively. Therefore, two key factors emerged from this study that were capable of discriminating between NP or KM meat and that was selecting standard meat smells from sensor 1 (AUC=0.936) and sensor 4 (AUC=0.948).

Similarly, chemometric techniques could be applied to classify specific meat types, e.g., by building up discriminant models using odor profiles of Tibetan pig meat (Garlito *et al*., 2023) or from metabolomic profiles (Ryu *et al*., 2019; Wang *et al*., 2020; Akhtar *et al.,* 2021; Phoemchalard *et al*., 2022). In addition, incorporating multivariate analysis techniques, regression modeling, and experimental study design (Zhang *et al*., 2021b) could be used as tools for quality control, by identifying various useful QC attributes such as the quality of

meat. Furthermore, this method in conjunction with FTIR spectroscopy has been used to verify and track meat and meat products, thereby offering an easier and faster substitution than conventional methods (Andre and Soukoulis, 2020). Moreover, a chemometric approach, such as the one-class partial least squares classification version (PLS) and the soft independent class analysis modeling (SIMCA), have been used to build quality, safety, and quality monitors of meat using the NIR spectral fingerprint (Rohman, 2019). Chemometrics provide a means for the extraction of signature chemical features and pattern recognition which are used to identify their properties (Granato *et al*., 2018). When coupled with spectroscopy and/or chromatography, chemical signatures help validate authenticity and quality through chemometrics (Magdalena Efenberger-Szmechtyk and Kregiel, 2018).

Hyperspectral imaging integrates spectroscopic and imaging techniques to obtain spatial and spectral information. This technology enables meat quality assessment and validation (Reis *et al*., 2018). Hyperspectral images provide chemical and physical data for chemometric analysis. Chemometrics is based on near-infrared spectra identify quality patterns in meat (Niu *et al*., 2014). Beyond meat, chemometrics and hyperspectral imaging can discriminate the geographical origins of food. For example, chemometric analysis of hyperspectral data successfully differentiated European beef samples based on characteristic spectral profiles (Vlachos *et al*., 2016).

In our data, the multivariate ROC analysis also showed a good classification capacity, where the sensors 1 (AUC=0.936) and 4 (AUC=0.948) were found to be highly discriminant measures. The excellent AUCs proved to be an excellent capacity of distinction between the positive and negative classes. Moreover Classifier 4 was also excellent regarding all three class labels, and we saw a very low CI in all of them (close to 0). Combined, the ROC results showed that sensors 1 and 4 served well as diagnostic or prognostic markers due to their high specificity. Additionally, the multivariate ROC curve showed that having more than 10 models accomplished a 72% accuracy rate that was an excellent classifier (AUC>0.99) of 72%. Furthermore, combining only the output of sensors 1 and 4 gave high accuracy with good differentiation (almost a perfect AUC with a narrow CI). The synergistic effect was supported in combination with prognostic values using multiple sensors or models to enhance classification performance and prediction capabilities.

Meat quality can also be distinguished through the sensing response of the e-noses as they can sense and differentiate multiple scents by employing an array of MOS sensors. This method allowed fast and non-destructive discrimination of complicated volatile meat flavor patterns using pattern recognition MOS sensor signals (Sarno and Wijaya, 2019; Anwar *et al*., 2023). Data from the e-noses

allowed comparison of various meat flavor signatures corresponding to different samples sources. Although the use of e-noses has proven to be potential in meat inspection and identification, there remains a challenge particularly in deployment. The main drawback of e-noses is their reliability, sensor consistency, and how to choose the best sensor array configuration (Sarno and Wijaya, 2019; Liu *et al*., 2023). The reliance on pattern recognition of volatile compounds makes the sample collection process and data analysis important using e-noses (Wojnowski *et al*., 2017; Sarno and Wijaya, 2019). Furthermore, because e-noses cannot differentiate single molecules, other methods such as GC–MS might be required to determine the presence of targeted biomarkers (Wojnowski *et al*., 2017; Jia *et al*., 2018). Moreover, using e-noses for quality assessment requires developing relationships between sensor responses and the meat properties (Ghasemi-Varnamkhasti *et al*., 2009; Górska-Horczyczak *et al*., 2016; Jia *et al*., 2018),. In addition another study showed that the potential of inexpensive colorimetric sensors used to build an electronic nose prototype coupled with chemometric data processing could be used to quickly identify pork-adulterated minced beef (Han *et al*., 2020).

In conclusion, although PCA and PLS-DA did not exhibit enough discrimination, sparse PLS-DA and, especially, OPLS-DA were successful in distinguishing regional variations in meat quality. The ROC curve results clearly indicated that sensor response, as in sensors 1 and 4, showed great promise and could clearly be used as a prediction tool due to it being highly sensitive and accurate. To achieve sustainability and food security, it is essential to verify the origin and quality of meat so as to; reduce fraud, ensure transparent labeling, support sustainable production, promote lower impact consumption, and improve food availability and security.

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