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Authentication of Processed Beef Sausage Products Using Chemometric Analysis Based on FTIR Spectrophotometry Data

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Abstract

This study aims to detect chicken meat contamination in beef sausage products distributed in Bandar Lampung City, Lampung Province. The selection of chicken and beef for this research is based on economic factors, as the higher price of beef compared to chicken drives the adulteration of meat products. The sausage fat was obtained using the Soxhlet extraction method with n-hexane solvent. Subsequently, the sausage fat extract was analyzed using FTIR (Fourier Transform Infrared) spectrophotometry to obtain infrared spectral data. This data was then analyzed using chemometric methods PCA (Principal Component Analysis) and PLS (Partial Least Squares). The PCA analysis results indicated that commercial sausages (AP, BP, CP, DP, and EP) and pure beef sausages showed closely clustered samples, suggesting similar physical and chemical properties with pure beef sausages. The PLS calibration set analysis yielded a model with a coefficient of determination (R^2) value of 0.970409 and a root mean square error of calibration (RMSEC) parameter value of 0.09%, while the PLS validation set analysis produced a model with a coefficient of determination (R^2) value of 0.963486 and a root mean square error of prediction (RMSEP) parameter value of 0.13%. Based on the PLS model predictions, it was determined that the percentage of chicken meat mixed in beef sausages circulating in the market ranged from 0.0281% to 0.1106%. This indicates a small but notable adulteration in beef sausages with chicken meat.

1. Introduction

Food authenticity is a critical concern in the food industry, impacting consumers, producers, and regulatory authorities. The detection of food adulteration is essential for safeguarding consumer health, especially due to the risks associated with undisclosed ingredients that may trigger allergies [1].

Sausages are a commonly adulterated processed food product derived from the Latin term "*salsus*" meaning salted or cured meat [2]. Sausages are widely consumed and favored food globally, particularly among children. Typically, sausages consist of minced or ground meat mixed with spices and encased in cylindrical casings [3]. Beef is the primary raw material for sausages; however, escalating beef prices have incentivized traders to substitute or mix with cheaper meats such as chicken to maximize profit margins.

The current high market price of beef has led to widespread adulteration practices, where chicken meat is surreptitiously blended with beef sausages to reduce production costs and increase profitability [4]. This blending of meats makes it challenging to visually distinguish products, as the meat is often finely ground and homogenized with other ingredients. Consequently, ongoing efforts are focused on developing reliable techniques to identify the specific types of meat used in processed foods.

FTIR spectrophotometry has emerged as a powerful tool for analyzing food fats and detecting adulteration. This technique leverages unique absorption patterns in the infrared spectrum, known as fingerprint regions, where different animal fats exhibit distinct characteristics that differentiate them from other components [5]. FTIR spectrophotometry offers rapid and accurate chemical analysis with minimal sample preparation [6].

One limitation of FTIR spectrophotometry in analyzing the presence of chicken adulteration in beef sausage is its sensitivity to overlapping spectral features from different components within the sample matrix [7]. FTIR spectra may exhibit complex patterns influenced by various molecular vibrations in both chicken and beef fats, potentially making it challenging to distinguish between them based solely on spectral data. This complexity can lead to ambiguity in the interpretation and accurate quantification of adulterants. Therefore, employing chemometric analysis becomes essential to extract meaningful information and discern subtle differences in spectral patterns that indicate the presence and extent of chicken adulteration in beef sausage samples [8].

Chemometric techniques like Principal Component Analysis (PCA) and Partial Least Squares (PLS) offer robust tools to analyze complex FTIR data, enhance discriminatory power, and provide accurate quantitative assessments, ultimately addressing the limitations associated with direct spectral interpretation alone. Chemometric analysis plays a pivotal role in interpreting FTIR data to identify adulteration. As a scientific discipline, chemometrics applies mathematical and statistical principles to process and analyze chemical data effectively [9].

PCA facilitates qualitative analysis by reducing complex multivariate data and identifying correlations among variables [10]. Samples sharing similar principal components (PCs) exhibit comparable physical and chemical properties, enabling effective clustering. On the other hand, PLS serves as a quantitative calibration method to establish linear relationships between spectral data (X) and reference values (Y), producing robust models for analysis [11]. Many researchers have explored the application of FTIR spectrophotometry and chemometrics for food analysis, particularly in detecting food adulteration. Saputra *et al.* [12] conducted identification of pig meat adulteration in various mixtures of fat samples and selected foods, Siddiqui *et al.* [13] detected pig fat adulteration, and Jamwal *et al.* [14] studied the detection of edible oil adulteration.

In this study, we aim to build upon previous research by applying FTIR spectrophotometry and chemometric techniques to specifically detect chicken meat adulteration in beef sausage products circulating in Bandar Lampung markets. This research addresses a novel aspect of food authenticity by focusing on a common food adulteration issue in the local context, providing valuable insights for consumer protection and food quality assurance. Five samples sourced from both traditional and modern markets undergo FTIR spectrophotometric analysis, employing PCA and PLS methods for comprehensive adulteration detection and quantification.

2. Experimental

2.1. Tools and Materials

The glassware used in this research included beakers of various capacities, a fat flask, and a Soxhlet extractor, all sourced from Borosil (India). The filter paper was obtained from Whatman (United Kingdom).

The chemicals used in this study included n-hexane, supplied by Merck (Germany), as well as distilled water, salt, pepper powder, powdered broth, powdered garlic, and cooking oil, all purchased from local suppliers in Indonesia.

The instruments utilized included an analytical balance (capacity up to 220 g, Sartorius, Germany), a condenser (IKA, Germany), a heating mantle (IKA, Germany), knives, a blender (Philips, Indonesia), an Agilent Cary 630 FTIR Spectrometer (Agilent Technologies, United States), and a Buchi Rotary Evaporator R-100 (Buchi, Switzerland).

Minitab 19 software, licensed by Minitab Inc. (United States), was used for data analysis, which was performed on an HP Pavilion laptop (United States).

The research also involved standard sausages composed of beef, chicken, tapioca flour, ice cubes, eggs, salt, pepper powder, powdered broth, powdered garlic, cooking oil, and sausage casings. Additionally, five sausage samples available in Bandar Lampung were analyzed.

2.2. Standard Sausage Preparation

Ground beef and ground chicken were mixed with eggs, salt, pepper powder, powdered garlic, powdered broth, and cooking oil and then kneaded until well combined. The mixture was then blended, and some ice cubes were added. After blending for a while, tapioca flour was incorporated along with the remaining ice cubes, and the mixture was blended again until a uniform dough was formed. Tapioca flour accounted for 10% of the total mixture by weight.

Ice cubes were added to maintain a low temperature during mixing, preventing the fat from melting and ensuring a smooth, cohesive texture. They also contributed moisture, aiding in ingredient binding and resulting in a juicier, well-textured final product. The prepared dough was then stuffed into sausage casings and tied with string.

Water was heated until boiling, after which the stove was turned off. The water was left to cool to approximately 80°C before submerging the sausages. The stove was then turned back on at a low flame, and the sausages were boiled for about 30 minutes. Five minutes before removing them from the water, a basin filled with ice water was prepared to accelerate the cooling process. Rapid cooling in ice water prevented overcooking, preserved texture and helped maintain freshness by

inhibiting bacterial growth and stopping the cooking process immediately. After boiling, the sausages were transferred to the ice water basin, then removed and drained once cooled.

2.3. Fat Extraction

Fat extraction followed the method described by Wirnawati *et al.* [15]. Thin slices of standard sausages were sun-dried until completely dehydrated. The dried sausages were then finely blended into a powder. A 15 g portion of the powdered sausages was wrapped in filter paper and extracted using the Soxhlet method for 2.5 hours with 150 mL of n-hexane as the solvent. The obtained fat extract was evaporated using a rotary evaporator at 69°C for 15 minutes per hour until all the solvent had evaporated. Finally, the fat extract was transferred into vial bottles.

The calibration set was prepared using six samples, including one sample of standard beef sausage fat, one sample of standard chicken sausage fat, and four samples of mixed fats from standard beef and chicken sausages with concentration ranges of 20, 40, 60, and 80%. The 0% concentration represented pure chicken sausage fat, while the 100% concentration represented pure beef sausage fat.

2.4. Application to Market Samples

The process of sampling market sausage samples started with a survey conducted at traditional markets and supermarkets in Bandar Lampung. Sampling was carried out using the purposive sampling method, where data on five sausage brands were collected. Subsequently, the collected market sausage samples were extracted using the Soxhlet method, following the earlier procedure. After extraction, the samples were characterized using FTIR spectrophotometry and analyzed using PCA and PLS methods.

2.5. Measurement of FTIR Spectrum

The FTIR spectrum scanning of all standard sausages was conducted using an FTIR spectrophotometer. Drops of fat were placed on an attenuated total reflectance (ATR) crystal in the wavenumber range of 4000-650 cm^{-1}. Background spectrum recording was performed each time a sample measurement was taken to minimize spectrum variation over time due to ambient air effects. Before each initial scan and sample change, the ATR crystal was cleaned with n-hexane and acetone and dried with soft tissue. The cleanliness of the crystal surface was verified by scanning the background spectrum and comparing it to previous readings. Data acquisition was repeated three times. Subsequently, the obtained data consisted of specific peaks corresponding to functional groups present in the sample.

2.6. Chemometric Analysis

The FTIR spectrum data was processed and analyzed using PCA and PLS within Minitab 19 software. Significant spectral regions were selected for developing PCA and PLS models, as well as discriminant analysis, focusing particularly on the fingerprint region. The optimal number of PCA and PLS factors was determined through cross-validation, where factors were systematically removed and assessed against the root mean standard error of cross-validation (RMSECV) to identify the minimum required factors. Model predictability was assessed by calculating the root mean square error of prediction (RMSEP), following the approach described by Gurdeniz and Ozen [16].

3. Results and Discussion

3.1. Spectrum of Standard Sausage Fat

Pure beef and chicken sausage fat were analyzed using FTIR spectrophotometry. The IR spectra of standard sausage fat can be seen in Figure 1. The spectra of pure beef sausage fat and pure chicken sausage fat appear relatively similar as both spectra are characteristic of edible oil fats [17]. However, there are differences in the peak wavelengths, where the peak of pure beef sausage fat is relatively higher than that of pure chicken sausage fat at certain specific wavelengths. The high absorbance peaks indicate the presence of double bonds (C=C), suggesting the presence of unsaturated fats in the sample. The resulting spectra exhibit consistent frequencies in certain regions because both samples are triglyceride components [18]. The differences in wavenumber for standard sausage fat are observed in the regions of 1700 cm^{-1} and 900 cm^{-1}.

The first difference is observed at the wavenumber region of 1700 cm^{-1}. Pure beef sausage fat shows absorption at 1707 cm^{-1}, while pure chicken sausage fat is at 1714 cm^{-1}. These absorptions indicate stretching vibrations of aliphatic ketone C=O groups. The second difference is observed at the wavenumber region of 900 cm^{-1}. Pure beef sausage fat shows absorption at 939 cm^{-1}, while pure chicken sausage fat is 961 cm^{-1}. These absorptions indicate out-of-plane bending vibrations of C-O-H groups [9]. The FTIR spectra exhibit distinctive absorption patterns indicative of molecular vibrations within the analyzed samples. Notably, strong absorbances at 2922 cm^{-1} and 2855 cm^{-1} correspond to asymmetric and symmetric stretching vibrations of C-H (CH_2) groups, respectively. A strong absorbance at 1744 cm^{-1} is due to the stretching vibrations of C=O groups from triacylglycerol ester bonds commonly found in fats.

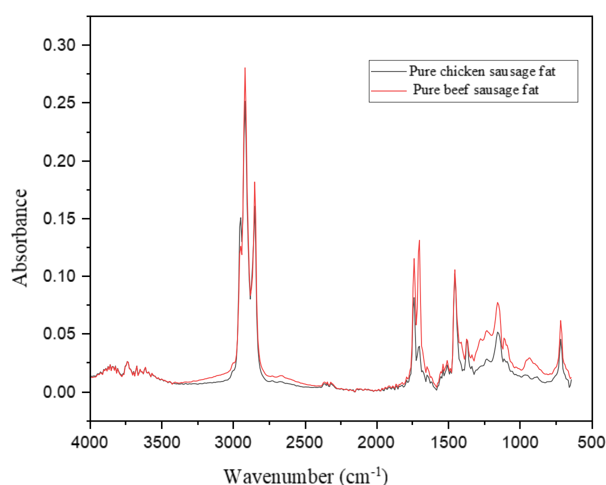

Figure 1. FTIR spectra of standard sausage fat

Moderate absorbances at 1461 cm^{-1} and 1379 cm^{-1} represent scissoring bending vibrations of C-H (CH_2) groups and symmetric bending vibrations of C-H (CH_3) groups, respectively, with a less distinct band likely attributed to symmetric bending vibrations of methyl groups. Additionally, strong absorbances at 1237 cm^{-1}, 1162 cm^{-1}, and 1118 cm^{-1} indicate stretching vibrations of C-O ester groups, particularly aliphatic ones. Finally, a moderate absorbance at 723 cm^{-1} corresponds to rocking vibrations of CH_2 groups. These absorption features provide valuable insights into the chemical composition and structure of the analyzed fats [12].

3.2. Calibration Set Spectrum

The calibration set, consisting of 6 samples with varying concentrations of pure beef sausage fat (0, 20, 40, 60, 80, and 100%), was analyzed using an FTIR spectrophotometer. The spectra from the calibration set exhibit nearly identical absorption patterns and closely resemble the spectrum of standard sausage fat. However, there are differences in absorbance values among the spectra. The calibration set spectrum is depicted in Figure 2.

In the calibration set spectrum, the peak at 100% concentration (pure beef sausage fat) is notably higher than at other concentrations. Specifically, at 100% concentration, the peak is observed at the wavenumber of 2922 cm^{-1}, with moderate absorbance at 2855 cm^{-1} and 1461 cm^{-1}, indicating strong asymmetric stretching vibrations, symmetric stretching vibrations, and scissoring bending vibrations of C-H (CH_2) groups, respectively. Additionally, the strong absorbance at 1744 cm^{-1} indicates stretching vibrations of aliphatic C=O ester groups.

The absorbance at 1237 cm^{-1} indicates stretching vibrations of C-O groups, while the absorbance at 1379 cm^{-1} suggests symmetric bending vibrations of C-H (CH_3) groups. Furthermore, the strong absorbance at 1162 cm^{-1} and 1118 cm^{-1} represents stretching vibrations of aliphatic C-O ester groups. Finally, the strong absorbance at 939 cm^{-1} signifies out-of-plane bending vibrations of =CH-H groups, while the moderate absorbance at 723 cm^{-1} corresponds to rocking vibrations of CH_2 groups [5].

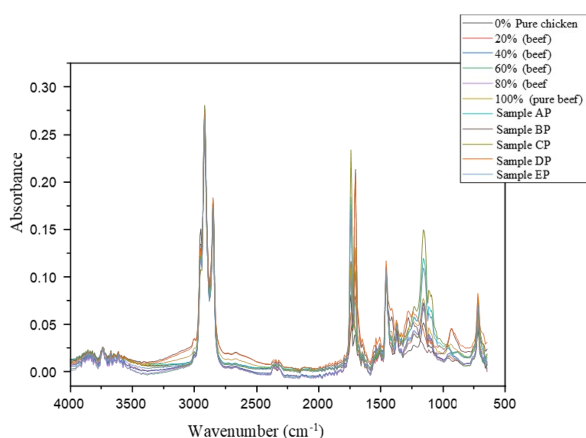

Figure 2. The FTIR spectra of the calibration set and samples

Table 1. List of sausage brands available in traditional markets and supermarkets

Location	Brand name	Code
Traditional Market	Muantab	AP
Traditional Market	Umia-mi	BP
Supermarket	So Nice	CP
Supermarket	Champ	DP
Traditional Market	Dosuka	EP

The sampling process began with a survey to identify 5 sausage brands from both traditional markets and supermarkets in Bandar Lampung. This study employed a non-probability sampling technique, specifically purposive sampling, which involves selecting samples based on specific criteria [19]. The criteria for sample selection included proximity to Bandar Lampung and availability in traditional markets or supermarkets. The obtained market sausage samples were extracted using the Soxhlet method with n-hexane solvent and subsequently analyzed using FTIR and chemometrics.

The spectra data from the calibration set was used for calibration model development and classification. Subsequently, this data was analyzed using PCA and PLS. The spectra dataset used for model analysis included selected wavenumber regions within the spectrum range (4000-650 cm^{-1}). The spectrum data analyzed comprised 11 specific wavenumbers: 2922 cm^{-1}, 2855 cm^{-1}, 1744 cm^{-1}, 1707 cm^{-1}, 1461 cm^{-1}, 1379 cm^{-1}, 1237 cm^{-1}, 1162 cm^{-1}, 1118 cm^{-1}, 939 cm^{-1}, and 723 cm^{-1}, which represent the major peaks of the FTIR spectra. The PCA model was developed from 6 calibration sets, while the PLS model was developed from 6 calibration and 7 validation sets.

Table 2. Eigenmatrix correlation analysis

Eigenvalue	Proportion	Cumulative
5.9611	0.542	0.542
3.9129	0.356	0.898
0.9319	0.085	0.982
0.1455	0.013	0.996
0.0286	0.003	0.998
0.0164	0.001	1.000
0.0027	0.000	1.000
0.0006	0.000	1.000
0.0002	0.000	1.000
0.0001	0.000	1.000

Figure 3. Scree plot PCA

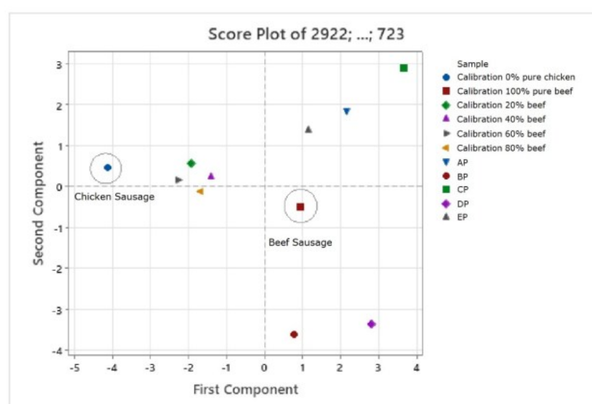

Figure 4. Score plot PCA

PCA aims to group correlated variables and replace them with new groups known as principal components [5]. This grouping is based on the absorbance values of each peak in the spectrum of fats within the wavelength range (4000-650 cm^{-1}). The principal component model or PCA is formed from 6 calibration sets with concentration ranges (0, 20, 40, 60, 80, and 100%) and 5 market sausage samples (AP, BP, CP, DP, and EP) measured in duplicate. Absorbance values at the main peaks of the FTIR spectrum are used for analysis. After PCA analysis, results such as eigen matrix correlation analysis, scree plot, score plot, and 3D plot are obtained.

Table 2 presents an eigenmatrix correlation analysis, which is pivotal in PCA computations for understanding the variance explained by each principal component. In PCA, each principal component is associated with an eigenvalue, representing the variance explained by that component. Higher eigenvalues signify greater variance, as explained by the respective principal components.

Proportion values indicate the proportion of total data variance explained by each principal component derived from PCA. These values help select and interpret the principal components used in data analysis. Higher proportion values signify a more substantial contribution of the principal component to explaining data variance, providing control over over-retained data dimensions. Principal components with higher proportion values are deemed more informative and relevant for explaining data variance.

Cumulative values gauge the extent to which a specific number of selected principal components elucidate total data variance. This concept aids researchers in understanding the retained information when utilizing a certain number of principal components in data analysis.

The eigenvalues showcased in Table 2 demonstrate the variance from each principal component. PC1, with the highest eigenvalue of 5.9611, is deemed the most critical principal component in explaining data variance, accounting for 54.2% of the total variation. PC2 has an eigenvalue of 3.9129, representing 35.6% of the variance. When combined, these values yield a cumulative value of 89.8%, indicating adequate information retention. Therefore, PCA analysis can condense 11 wavenumbers into 2 new variables.

PCA analysis necessitates correlated variables, as it initiates by computing correlation values between variables. Once correlation values are computed for each variable, they are organized into a correlation matrix and subsequently analyzed in PCA by examining the eigenvalues of each variable. The condition for forming principal components is having eigenvalues ≥1.

Figure 3 depicts a scree plot from the PCA analysis, illustrating principal components and eigenvalues. The scree plot is a graph used in PCA analysis to aid in selecting the number of principal components for data analysis. It is a line graph displaying eigenvalues on the vertical (y) axis and the principal component number on the horizontal (x) axis. The blue line on the screeplot represents the variation of principal components in PCA analysis, showing principal components arranged in descending order based on eigenvalues. A steeper decline in eigenvalues at the beginning of the scree plot indicates a greater contribution of principal components in explaining data variance. The criterion for forming principal components is having eigenvalues ≥1.

Because PC1 and PC2 have eigenvalues >1, these components significantly contribute to explaining data variance. The PCA grouping results in a score plot, a two-dimensional graph visualizing data in principal component space. Score plots are commonly used in PCA analysis to understand the structure and patterns in data after dimension reduction. Grouping in the score plot is based on PC1 and PC2, as these components have the largest number of variables and can represent all components. The two-dimensional grouping results for market sausage samples can be seen in Figure 4.

Figure 4 is a two-dimensional PCA score plot of the calibration set and market sausage samples, where 0% represents pure chicken sausage, 100% represents pure beef sausage, and 20, 40, 60, and 80% represent mixed sausages. Sausages labeled AP, BP, CP, DP, and EP are market sausage samples. The score plot helps identify patterns in the data and discover clusters or groups within the data. The PCA score plot shows each sample grouped into different distances from one sample to another. The similarity level between samples can be observed from the distance between them. The closer the distance between samples, the greater the similarity between those samples [20].

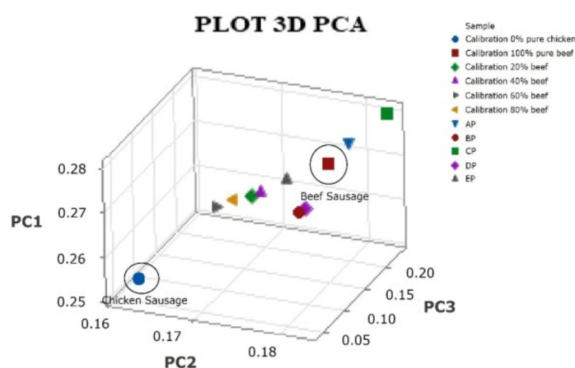

Figure 5. Score plot 3D PCA

In this plot (Figure 4), four separate quadrants differentiate between pure beef sausage, pure chicken sausage, and mixed sausages. Pure beef and chicken sausages are in different quadrants, while mixed sausages form a group. The score plot indicates that sausages labeled AP, BP, CP, DP, and EP and pure beef sausages show closely spaced samples. This suggests that these sausages share similar principal component values, thus exhibiting similar physical and chemical properties. In other words, sausages labeled AP, BP, CP, DP, and EP share similarities with pure beef sausage.

Figure 5 is a visual representation of PCA used to reduce data dimensions into the three most important principal components. In the 3D plot, we can observe samples scattered in three-dimensional space, with each axis representing one of the principal components and their grouping becoming more apparent. The plot shows that pure beef and pure chicken sausages are in different dimensional spaces, while mixed sausages and pure beef sausages are in the same dimensional space. The labeled AP, BP, CP, DP, and EP sausages share the same dimensional space as pure beef sausages. This indicates that these sausages share similarities with pure beef sausage.

The similarities in these properties can be found in their ability to assess sausage texture, stability, and nutritional content. For instance, pH and emulsion stability are often linked to texture and fat retention, while fatty acid profile and proximate estimation provide insights into the nutritional composition. These properties serve as indicators of the consistency and quality of sausages and can help in comparing different formulations

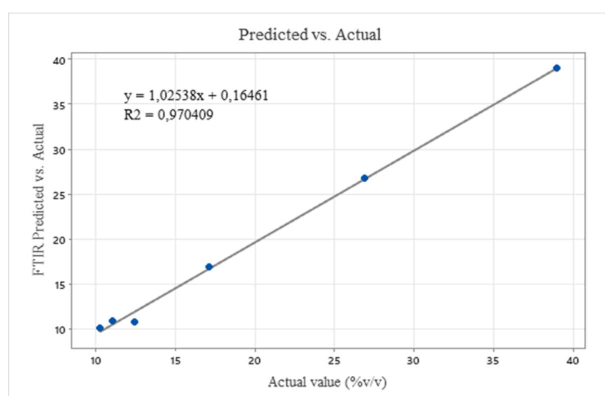

Figure 6. PLS models from the validation set

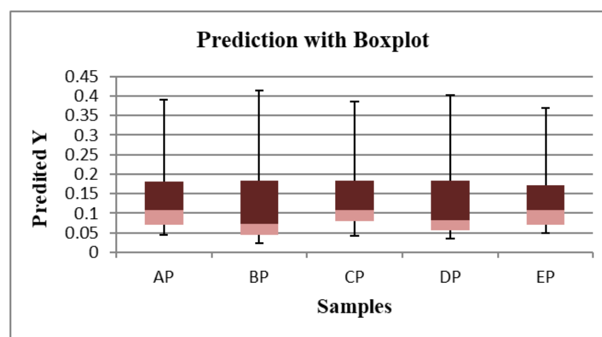

Figure 7. The mixture ratio of chicken sausage in beef sausage using Boxplot

PLS is used to reduce the influence of various irrelevant predictors on data variation to estimate prediction errors, thus improving model performance [21]. PLS identifies a linear relationship between the x variables obtained from spectral measurements (predictor variables) and the y variables representing chemical content or biological activity (response variables). This method aims to reduce data dimensions and maximize the linear relationship between x and y. In this study, the x variables are absorbance values, and the y variable is the concentration of sausage fat. The PLS model consists of 6 calibration sets covering concentrations (0, 20, 40, 60, 80, and 100%) and 7 validation sets covering concentrations (0, 10, 30, 50, 70, 90, and 100%). The absorbance values at the main peaks of the FTIR spectrum are used for analysis.

After conducting the PLS analysis, statistical parameters such as R^2 and prediction values for accuracy evaluation are obtained, including RMSEC and RMSEP. The R^2 value (coefficient of determination) describes the extent of the relationship between actual values and predicted values from the FTIR instrument. If the R^2 value approaches ±1, the relationship between independent and dependent variables becomes more linear. To indicate good prediction ability, the R^2 value should be high ($\geq$ 0.90), whereas if it is low ($\leq$ 0.64), it is unlikely to achieve high and consistent accuracy levels in FTIR spectrophotometry analysis [22].

RMSE (Root Mean Square Error) in PLS consists of RMSEC, which is the calibration error value in the model, and RMSEP, which is the prediction error value in the model. The RMSE value should be less than or equal to one. If the RMSE value approaches 0 or is low, it indicates a well-formed model due to low error rates [23]. Figure 6 shows the PLS model from the calibration set. In the PLS model above, the R^2 value (coefficient of determination) is 0.970409, and the RMSEC value is 0.09%. This indicates that the PLS model is effective because the R^2 value approaches 1, and the RMSEC value is low.

3.3. Sausage Authentication Detection Using Boxplots

Five market sausage samples were analyzed using FTIR spectrophotometry with sample scanning in duplicate. The PLS model can be integrated with boxplots as part of comprehensive data analysis. A boxplot is a statistical chart used to depict data distribution and display summary statistics. With the help of boxplot analysis in chemometrics, one can quickly grasp data

characteristics graphically, aiding in analytical decision-making and better interpretation. Figure 7 shows the PLS sample results using boxplots. The figure displays prediction outcomes using the selected PLS model, revealing that the percentage of chicken sausage mixture in beef sausages circulating in the market ranges from 0.0281% to 0.1106%.

4. Conclusion

The infrared spectrum analysis showed distinct differences between pure beef sausage fat and pure chicken sausage fat in specific wavenumber regions, notably at 1700 cm^{-1} and 930 cm^{-1}. The absorption peak of pure beef sausage fat was higher than that of pure chicken sausage fat in these regions. PCA effectively distinguished between pure beef sausage, pure chicken sausage, and mixed sausages. Market sausages labeled AP, BP, CP, DP, and EP exhibited similar physical and chemical properties to pure beef sausage. The PCA analysis yielded a first principal component (PC1) with an eigenvalue of 5.9611 and a second principal component (PC2) with an eigenvalue of 3.9129, demonstrating the reduction of 11 wavenumbers into 2 new variables. The PCA analysis results indicated that commercial and pure beef sausages showed closely clustered samples, suggesting similarities in physical and chemical properties. Specifically, these similarities include comparable spectral profiles in the regions associated with protein and fat content, which are key indicators of meat composition. This clustering suggests that commercial sausages have a composition more akin to pure beef sausages, likely due to the presence of similar types of fat and protein constituents. Additionally, the PLS models from the calibration set achieved strong performance, with an R^2 value of 0.970409 and RMSEC of 0.09%, while the validation set yielded an R^2 of 0.963486 and RMSEP of 0.13%. The boxplot method for detecting sausage purity revealed that the mixture ratio of chicken sausage in beef sausage circulating in the market ranged from 0.0281% to 0.1106%.

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