Analysis of beef meatballs with rat meat adulteration using Fourier Transform Infrared (FTIR) spectroscopy in combination with chemometrics

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ABSTRACT

Indonesia is a country with a majority Muslim population, and the halal food consumed should be assured. One type of food that must be considered halal is food from meat products. Due to the price difference between halal meat and non-halal meat, some unethical producers try to adulterate beef with non-halal meat of rat meat. The research objective was to employ FTIR spectroscopy in combination with chemometrics for the analysis of rat meat adulteration in beef meatballs. Lipid components in meatballs containing beef, rat meat, or its binary mixture were extracted using three extraction methods, namely Bligh and Dyer, Folch, and Soxhlet methods. The lipid components extracted were then analyzed using FTIR spectroscopy and FTIR spectra obtained were used as variables during chemometrics modeling. The absorbance values of FTIR spectra of lipid components extracted by Bligh and Dyer, Folch, and Soxhlet methods at selected wavenumbers regions of 3100–800 cm⁻¹ were selected for discrimination between beef meatballs and meatballs adulterated with rat meat using chemometrics of linear discriminant analysis (LDA). LDA was successfully used to classify lipid components extracted by three lipid extraction methods from beef meatballs and rat meatballs with accuracy levels of 100%. The prediction of rat meatballs was successfully determined using multivariate calibrations of Partial Least Square (PLS) and Principle Component Regression (PCR) using optimized conditions. The difference in lipid composition, as indicated in FTIR spectra profiles of the analyzed samples, is used as a fingerprint technique for the analysis of rat meat in beef meatballs for halal authentication purposes.

INTRODUCTION

Currently, Indonesia is the largest Muslim community globally, and along with the increased awareness of Indonesian Muslim to consume only safe and halal foods, the halal authenticity of food products in Indonesia is mandatory according to Indonesia Act No. 33, the year 2014 Halal Products Assurance.¹,² One of the favorite meat-based foods of the Indonesian Muslim community is meatballs typically made of chicken, beef, or fish. As protein sources needed for human growth, meatballs must be subjected to Halal certification because the main component used in preparing meatballs is meat, which could come from halal meat sources or non-halal ones. Due to price discrepancy between halal and non-halal meats, some producers try to substitute or blend halal meat with non-halal meat. This study was aimed to analyze beef meatballs with rat meat adulteration using FTIR spectroscopy in combination with chemometrics.

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such as beef with non-halal meats such as pork, canine meat, or rat meat.\textsuperscript{3,4} Rat meat (RM) is potential meat to be used as a meat adulterant in beef meatballs because RM can be obtained free from local farmers; therefore, the identification of RM in meatballs is must for halal authentication analysis of beef meatballs.\textsuperscript{5}

Several analytical methods have been proposed, developed, and validated for the authentication of meatball products based on meat components, such as polymerase chain reaction (PCR), Real-time PCR using specific,\textsuperscript{6} and other techniques including multiplex PCR has been applied successfully for the analysis of rat’s DNA with specific and accurate results,\textsuperscript{6,7} gas chromatography equipped by flame ionization detector\textsuperscript{8,9} and mass spectrometer\textsuperscript{10} are a useful technique for RM identification through fatty acid compositional analysis, while protein-based methods using enzyme-linked immunosorbent assay (ELISA) offered RM detection utilizing the specific reaction of an antigen with an antibody.\textsuperscript{11} However, these methods typically involve extensive sample preparation steps and need some expensive reagents; therefore, rapid, simple, and reliable methods based on molecular spectroscopic methods are currently evolving as emerging analytical techniques for meat authentication.\textsuperscript{12}

Vibrational spectroscopy based on the interaction of electromagnetic radiation and analyte(s) including Fourier transform infrared (FTIR) spectroscopy has emerged as a powerful instrumental technique applied in food analysis because of its property as fingerprinting.\textsuperscript{13} Combined with multivariate data analysis or chemometrics FTIR spectra have been successfully applied for analysis of meatball adulterations by analyzing lipid components using Soxhlet, Folch, and Bligh & Dyer methods.\textsuperscript{14} The combination of FTIR spectra-chemometrics is reported to authenticate beef meatballs from pork,\textsuperscript{15} wild boar meat,\textsuperscript{16} dog meat,\textsuperscript{14} and rat meat.\textsuperscript{17} However, the performance of FTIR and chemometrics for the analysis of rat meat adulteration in beef meatballs using different extraction techniques has not been reported yet. Therefore, the objective of this study was to optimize FTIR spectroscopy and chemometrics of classification and multivariate calibrations for authentication analysis of beef meatball from rat meat through analysis of lipid components extracted from different extraction techniques.

\textbf{Materials and methods}

\textbf{Materials}

Rat meat (Rattus norvegicus) was obtained from Godean, Sleman, Yogyakarta. Beef meat, spices, and other meatball additives were obtained from Colombo Market, Kaliurang, Yogyakarta, Indonesia. The entire samples were stored at \(-18^\circ\text{C}\) before being used to make reference meatballs. The reagents and solvents used were of pro-analytical grade.

\textbf{Preparation meatballs}

Preparation of reference meatballs of beef, rat meat, and the mixture of beef-rat meat.\textsuperscript{15} The prepared meatballs consisted of 90% meat and 10% additional ingredients (starch, garlic, salt, pepper, and sugar). The content of rat meat in beef meatballs was varied in 0%, 10%, 20%, 30%, 40%, 50%, 75%, and 100%. Meatballs are made by emulsifying 90% meat and 10% additional ingredients into the mixture. The meat dough is formed into balls and cooked for 10–20 minutes in boiling water (100°C).

\textbf{Preparation calibration and validation samples}

Calibration and validation standards were prepared by mixing rat meat into beef meatballs at concentrations of 0%, 10%, 20%, 30%, 40%, 50%, 75%, and 100% to observe the difference in lipid spectra. The fat from the meatballs (0–100%) was extracted in triplicate using three different lipid extraction methods, namely the Bligh and Dyer, Folch, and Soxhlet methods.
Acid hydrolysis
To improve extraction efficiency, acid hydrolysis could be used to release the bound lipids attached to protein and carbohydrates.[18] Twenty grams of reference meatball was hydrolyzed using 200 mL of 1 N Hydrochloric Acid and heated for 15–25 minutes in a water bath (60–65°C), and then the sample was filtered.[19]

Extraction of lipid components using Bligh Dyer
Extraction of lipid using Bligh and Dyer was carried out according to[8,20,21] with slight modification. The acid hydrolyzed samples were then put into two centrifuge tubes, each of the tubes contained approximately 10.0 g of sample. 30 mL of dichloromethane-methanol (1:2 v/v) was added to each tube. The mixture was vortexed for 15 min, centrifuged (10 min, 3000 rpm), and filtered by Whatman filter paper. The filtrate from each tube was collected together in a separating funnel. The residue that remained in the tube was added with 10 mL dichloromethane, vortexed for 15 min, centrifuged (10 min, 3000 rpm), and filtered by Whatman filter paper. The filtrate was collected with the previous filtrate. The filtrate was mixed with 20 mL of distilled water and shaken vigorously. The mixture was allowed to stand until the biphasic system appeared. The upper aqueous phase was discarded. The lower phase (dichloromethane) was separated through anhydrous sodium sulfate. The dichloromethane was evaporated using a vacuum rotary evaporator at 40°C. The lipid extract was then transferred into the vial and evaporated at 40°C using an oven until the solvent was completely removed.

Extraction of lipid components using Folch
Extraction of lipid components using Folch was carried out according to[8,21,22] with slight modification. Samples of meatballs as much as 20.0 g, which had been acid hydrolyzed, were mixed with 400 mL of dichloromethane-methanol (2:1 v/v). The mixture was homogenized in a Turrax homogenizer (10 min, 13,500 rpm) and filtered by Whatman filter paper. The filtrate was placed in a separating funnel, mixed with 20 mL of distilled water, and shaken vigorously. The mixture was allowed to stand until the biphasic system was formed. The upper (aqueous) phase was discarded. Anhydrous sodium sulfate was added into the lower phase (dichloromethane), mixed, and filtered by Whatman filter paper. The dichloromethane was evaporated by a rotary evaporator under a vacuum at 40°C. A thick lipid extract was then transferred into the vial and evaporated at 40°C using an oven until the solvent was completely removed.

Extraction using Soxhlet
Samples of meatballs that had been acid hydrolyzed as much as 50.0 g were wrapped with filter paper and placed into the Soxhlet apparatus. 438 mL of petroleum ether was used as extracting solvent. The extraction was performed for 8 h at 100°C (±50 cycles). The lipid extract was added with anhydrous sodium sulfate, mixed, filtered by Whatman filter paper, and then evaporated by vacuum rotary evaporator at 40°C. The lipid extract was then transferred into the vial and evaporated at 40°C using an oven until the solvent was completely removed.[8]

FTIR spectral measurement
Fourier Transform Infrared Spectroscopy (FTIR Nicolet iS20) using detector DTGS (deuterated triglycerine sulfate) was connected to software OMNIC. The samples were directly placed into multibounce attenuated total reflectance (ATR) crystal and scanned using resolution of 8 cm-1 and number of scanning 32. All spectra were measured at mid infrared region (4000–650 cm-1) using air as background. All spectra were recorded as absorbance mode for facilitating quantitative analysis according to Lambert-Beer law. The data obtained were managed using the software of TQ Analyst.
**Linear Discriminant Analysis (LDA)**

Linear Discriminant Analysis is used to discriminate between adulterated beef and rat meat. The sample consisted of beef mixed with rat meat at different concentrations covering 10–75%. The Coomans plot is built to discriminate between beef and rat meat.

**Chemometrics analysis**

FTIR spectra of the lipid obtained from three different techniques were subjected to chemometrics of LDA and multivariate calibrations (PLS and PCR). Based on series FTIR spectra, LDA is used to find mathematical models capable of detecting the membership of each object to its proper class. After obtaining a classification model, it is possible to predict if unknown items belong to one of the defined classes. The precision of PLS and PCR calibration model for quantification of rat meat in meatball was evaluated using coefficient of determination, while the precision during calibration and validation models were evaluated using root mean square error of calibration (RMSEC) and root mean square error of prediction (RMSEP).

**Results and discussion**

Extraction of lipid components in meatballs containing beef and rat meat using different methods revealed that Soxhlet extraction provide better results than using the Bligh Dyer method or the Folch method because Soxhlet extraction was more straightforward, and the yield of lipid components obtained by Soxhlet was also higher than those in Bligh Dyer and Folch methods\(^{[21,23]}\). In addition, the yield of lipid components extracted by the Folch method is higher than the Bligh dyer method because the ratio of solvent to sample in Folch is higher than that in Bligh dyer. The color of the lipid components obtained using the Bligh Dyer and Folch method is dark brown, while the Soxhlet method is yellowish-white.\(^{[8]}\)

The IR spectroscopy technique is a fast method in identifying samples, providing important information, and establishing certain absorption bands associated with functional groups.\(^{[24]}\) Fats and oils are chemically triglycerides that can be analyzed using FTIR spectroscopy, and IR spectroscopy is an ideal technique for the analysis of edible fats and oils because they could be applied directly in a neat shape to ATR crystals or passed through a flow cell.\(^{[15]}\) FTIR spectroscopy is a powerful analytical method for authentication analysis of fats and oils due to its nature as a fingerprint technique.\(^{[5]}\)

This study uses reference meatballs because for the development of predictive models using FTIR; therefore, it can be used to categorize beef meatballs and rat meatballs or a mixture of beef and rat meatballs. Lipids obtained from the extraction of meatballs containing beef and rat meat were analyzed using FTIR spectroscopy in the middle infrared region (4000–650 cm\(^{-1}\)) combined with chemometrics of multivariate calibration and supervised pattern recognition of linear discriminant analysis (LDA) to discriminate between adulterated beef and rat meat.

The representative spectra of lipid obtained from the extraction of beef and rat meatballs using the Bligh and Dyer, Folch, and Soxhlet methods are demonstrated in Figure 1. the spectra physically similar when an observation using the naked eyes and show the general characteristics of the absorption bands for triglycerides in which the main component is composed of these fats are triglycerides.\(^{[15]}\) The fingerprint technique means that there are no two compounds or samples having the same spectra in terms of amount and intensity of peaks, FTIR spectroscopy could be used to extract a difference among these fats. Table 1 contains the assignment of prominent peaks. The peak at about 3002–3009 cm\(^{-1}\) is due to the C-H strain vibration at = C-H cis. The – CH\(_2\) functional group peaks at 2920–2921 cm\(^{-1}\) and 2852 cm\(^{-1}\), respectively, due to asymmetric and symmetrical vibrations. A peak indicates the carbonyl group (C = O) of the triglyceride ester at 1743 cm\(^{-1}\). Methylene and methyl groups can also be observed in the 1462 cm\(^{-1}\) and 1375–1377 cm\(^{-1}\) regions due to their bending vibrations. The absorption of carbonyl (C = O) ester bonds was observed at a frequency of 1743 cm\(^{-1}\) with strong intensity
due to the large difference in electronegativity of carbon and hydrogen atoms. The bands at 1235, 1158, 1117, 1097, and 721 cm\(^{-1}\) result from overlapping methylene shake vibrations and out-of-plane bending vibrations of cis substituted olefins.

In this study, LDA is used to predict the class membership of unknown samples (beef meatball, rat meatball, and beef-rat meatball mixture) using FTIR spectra measurements at specific wavenumber regions as variables.\[^{12}\] **Figure 2** clearly shows that both groups are separated, without classification objects observed. The absorbance values at wavenumbers regions of 3100–800 cm\(^{-1}\) were used to discriminate lipid components extracted by Folch, Bligh Dyer Method, and Soxhlet Method. These absorbances were then converted to Mahalanobis distance and used as variables for grouping beef meatball, rat meatball, and mixture beef-rat meatball to form the Cooman’s plot. This indicated that

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**Table 1.** The functional groups and modes of vibration of lipids extracted from 100% rat meatball and 100% beef meatball\[^{26–32}\]

| Assignment | Wavenumber (cm\(^{-1}\)) | Functional Group | Intensity |
|------------|---------------------------|------------------|-----------|
| a          | 3002–3009                 | Cis C = CH stretching | Medium    |
| b          | 2920–2922                 | C-HCH stretching vibration | Very strong |
| c          | 2852                      | C-HCH stretching vibration | Very strong |
| d          | 1743                      | Carbonyl C = O ester | Very strong |
| e          | 1462                      | C-HCH stretching vibration | Medium |
| f          | 1375–1377                 | C-CH\(_3\) scissoring bending | Medium |
| g          | 1196–1235                 | C-C Alkane | Medium |
| h          | 1158–1172                 | C-H in plane | Medium |
| i          | 1097–1117                 | C-O from ester | Medium |
| j          | 965–969                   | CH = CH (trans) | Medium |
| k          | 719–721                   | C-H = CH\(_{-}\) bending (out of plane) | Medium |
LDA successfully distinguished between beef meatball and beef-rat meatball mixture. Misclassification occur due to close chemical composition similarities between groups or the incorrect selection of wavenumbers.
Two multivariate calibrations of PLS and PCR were used to facilitate the quantitative analysis of meat adulterants. PLS and PCR are the most common multivariate used in chemometrics. In this study, both PLS and PCR models were used to generate calibration. The factors used in the PLS model were automatically selected by the TQ software when method calibrated. Both models represented higher R2 values (close to 1), in comparison PLS model showed superiority over PCR model in terms of lower RMSEC and RMSEP. The statistical parameters used for accuracy evaluation were coefficient of determination (R2) between actual values, and FTIR predicted values. For precision evaluation, RMSEC and RMSEP were used. Tables 2, 3, and 4 showed the summary results of PLS and PCR during the analysis of lipid components extracted from beef–rat meatballs using three different lipid extraction method statistics parameters used as criteria were coefficient of determination (R2) between actual values and FTIR predicted values for accuracy evaluation and RMSEC and RMSEP for evaluation of precision. The selection of FTIR spectral condition was based on its capability to provide high and low values of RMSEC and RMSEP. The FTIR spectral condition was optimized during PLS and PCR modeling to provide high R2 values and low RMSEC and RMSEP values. Linearity was indicated by the

| Wavenumber (cm⁻¹) | Multivariate calibration | Spectra | Calibration | Prediction |
|-------------------|--------------------------|---------|-------------|------------|
|                   |                          |         | RMSEC       | R²         | RMSEP      | R²         |
| 1500–1000         | PLS                      | Normal  | 0.0835      | 0.9572     | 0.0815     | 0.9594     |
|                   |                          | 1st Derivative | 0.0589      | 0.9790     | 0.0537     | 0.9826     |
|                   |                          | 2nd Derivative | **0.0202**  | **0.9975** | **0.0514** | **0.9937** |
|                   | PCR                      | Normal  | 0.0766      | 0.9641     | 0.0788     | 0.9621     |
|                   |                          | 1st Derivative | 0.0479      | 0.9862     | 0.0437     | 0.9887     |
|                   |                          | 2nd Derivative | 0.0616      | 0.9770     | 0.0657     | 0.9783     |
| 1400–800          | PLS                      | Normal  | 0.0786      | 0.9623     | 0.0793     | 0.9615     |
|                   |                          | 1st Derivative | 0.0649      | 0.9744     | 0.0814     | 0.9603     |
|                   |                          | 2nd Derivative | 0.241       | 0.5493     | 0.256      | 0.4751     |
|                   | PCR                      | Normal  | 0.0618      | 0.9768     | 0.0660     | 0.9737     |
|                   |                          | 1st Derivative | 0.0590      | 0.9789     | 0.0625     | 0.9765     |
|                   |                          | 2nd Derivative | 0.0360      | 0.9922     | 0.172      | 0.8471     |
| 1800–1000         | PLS                      | Normal  | 0.0847      | 0.9560     | 0.0837     | 0.9572     |
|                   |                          | 1st Derivative | 0.107       | 0.9293     | 0.105      | 0.9324     |
|                   |                          | 2nd Derivative | 0.214       | 0.6707     | 0.218      | 0.6580     |
|                   | PCR                      | Normal  | 0.0810      | 0.9599     | 0.0790     | 0.9620     |
|                   |                          | 1st Derivative | 0.0518      | 0.9838     | 0.0523     | 0.9835     |
|                   |                          | 2nd Derivative | 0.0384      | 0.9911     | 0.0498     | 0.9832     |
| 2800–1800         | PLS                      | Normal  | 0.0208      | 0.9974     | 0.0494     | 0.9866     |
|                   |                          | 1st Derivative | 0.00256     | 1.0000     | 0.150      | 0.8953     |
|                   |                          | 2nd Derivative | 0.00143     | 1.0000     | 0.165      | 0.8823     |
|                   | PCR                      | Normal  | 0.0159      | 0.9985     | 0.0462     | 0.9891     |
|                   |                          | 1st Derivative | 0.0205      | 0.9975     | 0.153      | 0.8994     |
|                   |                          | 2nd Derivative | 0.0201      | 0.9976     | 0.168      | 0.8643     |
| 3700–3200         | PLS                      | Normal  | 0.0775      | 0.9634     | 0.0793     | 0.9616     |
|                   |                          | 1st Derivative | 0.0564      | 0.9807     | 0.0558     | 0.9814     |
|                   |                          | 2nd Derivative | 0.107       | 0.9293     | 0.132      | 0.9046     |
|                   | PCR                      | Normal  | 0.0791      | 0.9618     | 0.0812     | 0.9597     |
|                   |                          | 1st Derivative | 0.0490      | 0.9855     | 0.0500     | 0.9852     |
|                   |                          | 2nd Derivative | 0.0370      | 0.9917     | 0.0815     | 0.9881     |
| 3700–3200 dan 1500–1000 | PLS | Normal | 0.0658      | 0.9737     | 0.145      | 0.9124     |
|                   |                          | 1st Derivative | 0.0819      | 0.9590     | 0.174      | 0.8496     |
|                   |                          | 2nd Derivative | 0.0922      | 0.9477     | 0.181      | 0.8341     |

* The selection condition was assigned with bold.
Table 3. The optimization wavenumbers region of multivariate calibration for beef meatballs, rat meatballs, and mixed beef-rat meatballs using lipid extraction Folch Method.

| Wavenumber (cm\(^{-1}\)) | Multivariate calibration | Spectra | Calibration | Prediction |
|--------------------------|--------------------------|---------|-------------|------------|
|                          |                          | RMSEC  | R\(^2\)     | RMSEP      | R\(^2\)    |
| 1500–1000                | PLS                      | Normal | 0.159       | 0.8635     | 0.164      | 0.8554     |
|                          | 1\(^{st}\) Derivative     | 0.000404 | 1.000       | 0.0137     | 0.9994     |
|                          | 2\(^{nd}\) Derivative     | 0.00309  | 0.9952      | 0.0317     | 0.9950     |
|                          | PCR                      | Normal  | 0.140       | 0.8956     | 0.143      | 0.8911     |
|                          | 1\(^{st}\) Derivative     | 0.0315   | 0.9950      | 0.0307     | 0.9956     |
|                          | 2\(^{nd}\) Derivative     | 0.0591   | 0.9822      | 0.0603     | 0.9817     |
| 1400–800                 | PLS                      | Normal  | 0.296       | 0.3407     | 0.296      | 0.3406     |
|                          | 1\(^{st}\) Derivative     | 0.00188  | 1.000       | 0.0100     | 0.9997     |
|                          | 2\(^{nd}\) Derivative     |         |             |            |            |
| 1800–1000                | PLS                      | Normal  | 0.292       | 0.3746     | 0.292      | 0.3743     |
|                          | 1\(^{st}\) Derivative     | 0.0122   | 0.9992      | 0.0298     | 0.9955     |
|                          | 2\(^{nd}\) Derivative     | 0.128    | 0.9136      | 0.144      | 0.8903     |
|                          | PCR                      | Normal  | 0.112       | 0.9343     | 0.113      | 0.9340     |
|                          | 1\(^{st}\) Derivative     | 0.0391   | 0.9923      | 0.0512     | 0.9868     |
|                          | 2\(^{nd}\) Derivative     | 0.0600   | 0.9816      | 0.0672     | 0.9773     |
| 2800–1800                | PLS                      | Normal  | 0.310       | 0.1682     | 0.310      | 0.1730     |
|                          | 1\(^{st}\) Derivative     | 0.0888   | 0.9594      | 0.164      | 0.9475     |
|                          | 2\(^{nd}\) Derivative     | 0.0413   | 0.9914      | 0.153      | 0.9603     |
|                          | PCR                      | Normal  | 0.0712      | 0.9741     | 0.0925     | 0.9639     |
|                          | 1\(^{st}\) Derivative     | 0.119    | 0.9238      | 0.179      | 0.9006     |
|                          | 2\(^{nd}\) Derivative     | 0.748    | 0.8821      | 0.219      | 0.7760     |
| 3700–3200                | PLS                      | Normal  | 0.0576      | 0.9831     | 0.0747     | 0.9738     |
|                          | 1\(^{st}\) Derivative     | 0.00521  | 0.9999      | 0.115      | 0.9569     |
|                          | 2\(^{nd}\) Derivative     | 0.113    | 0.9339      | 0.155      | 0.8940     |
|                          | PCR                      | Normal  | 0.0693      | 0.9755     | 0.0825     | 0.9682     |
|                          | 1\(^{st}\) Derivative     | 0.189    | 0.7994      | 0.220      | 0.7236     |
|                          | 2\(^{nd}\) Derivative     | 0.169    | 0.8443      | 0.226      | 0.7389     |

* The selection condition was assigned with bold.

The value of R\(^2\) getting closer to 1.\(^{[1]}\) RMSEP value is used to determine the errors occurring in the calibration model. The smaller the RMSEC value, the smaller the error from the calibration process.\(^{[27]}\)

Based on the optimization, PLS in Bligh Dyer method using the 2\(^{nd}\) derivative spectrum in the wavenumber region 1500–1000 cm\(^{-1}\) provide the best model, with an R\(^2\) calibration value of 0.9975, RMSEC value of 0.0202, R\(^2\) validation value of 0.9937, and RMSEP value of 0.0514. PLS in Folch method using the 1\(^{st}\) derivative spectrum in the wavenumber region 1400–800 cm\(^{-1}\) was also preferred for quantifying rat meat in meatballs with an R\(^2\) calibration value of 0.99999, RMSEC value of 0.00188, R\(^2\) validation value of 0.9997, and RMSEP value of 0.0100. The lipid components extracted from meatballs using Soxhlet were also quantified using PLS applying normal spectrum in the wavenumber region of 1500–1000 cm\(^{-1}\) with R\(^2\) calibration value of 0.9985, RMSEC value of 0.0172, R\(^2\) validation value of 0.9972, and RMSEP value of 0.0284. Figure 3 reveals the correlation between the actual value of lipid components of meatballs extracted by Bligh Dyer Method [A], Folch Method [B], and Soxhlet Method [C] with FTIR predicted values facilitated by PLS. Based on high values of R2 and low values of RMSEC and RMSEP. These results suggested that FTIR spectroscopy in combination with LDA and PLSR is an effective means for authentication of beef meat from rat meatballs. A close relationship between actual values (x-axis) and FTIR predicted values (y-axis) existed in Figure 3 means that the PLSR method is adequate to detect and predict the level of rat meat.
Table 4. The optimization wavenumbers region of multivariate calibration for beef meatballs, rat meatballs, and mixed beef-rat meatballs using lipid extraction Soxhlet Method.

| Wavenumber (cm⁻¹) | Multivariate calibration | Spectra | Calibration | | |
|------------------|--------------------------|---------|-------------| | |
|                  |                          |         | RMSEC       | R²       | RMSEP | R²  |
| 1500–1000        | PLS                      | Normal  | 0.0172      | 0.9985   | 0.0284 | 0.9972 |
|                  |                          | 1st Derivative | 0.0863      | 0.9617   | 0.0861 | 0.9621 |
|                  |                          | 2nd Derivative | 0.0812      | 0.9661   | 0.0793 | 0.9682 |
|                  |                          | Normal   | 0.0355      | 0.9936   | 0.0418 | 0.9922 |
|                  |                          | 1st Derivative | 0.0572      | 0.9833   | 0.0592 | 0.9838 |
|                  |                          | 2nd Derivative | 0.0569      | 0.9835   | 0.0523 | 0.9886 |
| 1400–800         | PLS                      | Normal   | 0.0434      | 0.9905   | 0.0476 | 0.9894 |
|                  |                          | 1st Derivative | 0.0804      | 0.9668   | 0.0778 | 0.9692 |
|                  |                          | 2nd Derivative | 0.0778      | 0.9690   | 0.0792 | 0.9683 |
|                  |                          | Normal   | 0.0389      | 0.9923   | 0.0403 | 0.9924 |
|                  |                          | 1st Derivative | 0.0560      | 0.9840   | 0.0555 | 0.9849 |
|                  |                          | 2nd Derivative | 0.0583      | 0.9827   | 0.0584 | 0.9839 |
| 1800–1000        | PLS                      | Normal   | 0.0543      | 0.9850   | 0.0565 | 0.9841 |
|                  |                          | 1st Derivative | 0.0786      | 0.9683   | 0.0787 | 0.9683 |
|                  |                          | 2nd Derivative | 0.0905      | 0.9577   | 0.0885 | 0.9598 |
|                  |                          | Normal   | 0.0263      | 0.9965   | 0.0444 | 0.9919 |
|                  |                          | 1st Derivative | 0.0449      | 0.9898   | 0.0466 | 0.9890 |
|                  |                          | 2nd Derivative | 0.0430      | 0.9906   | 0.0443 | 0.9900 |
| 2800–1800        | PLS                      | Normal   | 0.0996      | 0.9486   | 0.102  | 0.9499 |
|                  |                          | 1st Derivative | 0.00336     | 0.9999   | 0.151  | 0.8967 |
|                  |                          | 2nd Derivative | 0.00677     | 1.000    | 0.153  | 0.9084 |
|                  |                          | Normal   | 0.0536      | 0.9854   | 0.732  | 0.9791 |
|                  |                          | 1st Derivative | 0.215       | 0.7317   | 0.240  | 0.6675 |
|                  |                          | 2nd Derivative | 0.169       | 0.8435   | 0.226  | 0.7410 |
| 3700–3200        | PLS                      | Normal   | 0.157       | 0.8673   | 0.171  | 0.8442 |
|                  |                          | 1st Derivative | 0.186       | 0.8070   | 0.218  | 0.7291 |
|                  |                          | 2nd Derivative | 0.0153      | 0.9988   | 0.139  | 0.9319 |
|                  |                          | Normal   | 0.0484      | 0.9881   | 0.0459 | 0.9908 |
|                  |                          | 1st Derivative | 0.207       | 0.7545   | 0.204  | 0.8187 |
|                  |                          | 2nd Derivative | 0.0939      | 0.9544   | 0.171  | 0.8968 |

* The selection condition was assigned with bold.

in beef meatball samples so FTIR spectroscopy in combination with multivariate calibrations is an accurate and precise method for simultaneous quantitative analysis of beef and rat meat in meatball formulation.

Conclusion

FTIR spectroscopy is a powerful analytical method for authentication analysis of fats due to its nature as a fingerprint technique. The FTIR spectral condition was optimized during PLS and PCR modeling to provide high R² values and low RMSEC and RMSEP values. Linearity was indicated by the value of R² getting closer to 1. The data obtained were managed using the software of TQ Analyst. FTIR spectra in combination with chemometrics of Linear Discriminant Analysis (LDA) have been successfully applied for the reliable classification of beef meat ball, rat meatball, and beef-rat meatball mixture. Multivariate calibrations of PLS and PCR offered a fast and dependable method for authenticating beef meatballs from rat meat, applying FTIR spectral absorbances as a variable with acceptable accuracy and precision. FTIR spectroscopy and chemometrics is a reliable technique for screening the presence of non-halal meat intended for halal authentication analysis.
Figure 3. The correlation between the actual value of lipid components of meatballs extracted by Bligh Dyer Method [A], Folch Method [B], and Soxhlet Method [C] with FTIR predicted values facilitated by Partial Least Square calibrations (PLS).

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Disclosure statement

No potential conflict of interest was reported by the authors.

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