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Original Article

The potential use of infrared spectroscopy and multivariate analysis for differentiation of beef meatball from dog meat for Halal authentication analysis

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ABSTRACT

Objective: The objective of this research was to assess the suitability of FTIR spectroscopy coupled with multivariate analysis of partial least square regression (PLSR) along with pattern recognition technique of principal component analysis (PCA) for rapid quantitative and qualitative (identification) analysis of dog meat in beef meatball formulation.

Materials and Methods: The lipid fraction of meatball was obtained by employing two different extraction techniques, namely Bligh-Dyer and Folch method. FTIR spectral bands correlated with beef fat, pork fat, chicken fat and rat fat were measured, interpreted, and qualitatively analyzed. The small variations among spectra were exploited as a basis tools to differentiate between dog fat and other animal fats.

Results: PCA at combined wavenumber regions of 1700-700 cm^{-1} was capable of identifying dog meat in meatball. These wavenumbers were also used for quantitative analysis of dog meat in meatball using PLSR model. Based on statistical parameters used, namely R^2, RMSEC and RMSEP, Folch extraction method offered higher R^2 and lower RMSEC and RMSEP than Bligh-Dyer. PCA is succesfully applied for classification between meatball containing dog meat and other meats.

Conclusion: FTIR spectroscopy coupled with multivariate analyses of PLSR and PCA was effective means for rapid screening of dog meat in meatball products.

KEYWORDS

Bligh-Dryer; Chemometrics; Dog meat; Folch; FTIR spectroscopy

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INTRODUCTION

The identity of meat used in meatball products is very important to assure the halalness of meat products, because Muslim community is prohibited to consume any products containing non-halal components such as pork and dog meat (Fajardo et al., 2007; Abdel-Rahman et al., 2009; Rohman and Che Man, 2012). Because of the price difference between beef and other non-halal meat such as pork and dog meat, some unethical seller replaced beef intentionally with pork or with dog meat (DM) to get economical profits. Dog meat (DM) is considered as non-halal meat according to shariah law (Regenstein et al., 2003). The replacement of beef with DM in meatball can be taken into account as the adulteration action because unethycal producers labeled dog meatball with beef meatball.

With the increase awareness among Muslim community, especially in Islamic countries, Muslim consumers are concerned about meat consumed (Ballin, 2010; Mursyidi, 2013). It is not surprising if countries whose Muslim communities dominate such as Indonesia and Malaysia makes the regulation that any meat products present in market must be halal. Indonesia has stipulated Act No. 33 (2014) related to the assurance of halal products. As a consequence, studies on halal authentication including detection of meat adulteration in meatball products increased significantly. The misdeclaration of beef with DM is a serious issues due to some reasons, namely halal and kosher issues (religion), profit gain (economical), and health-related problems such as allergy (Regenstein et al., 2003; Rohman and Che Man, 2012).

Halal certification has become to be mandatory for meat and meat-based products like meatball, especially in the Muslim countries,. Therefore, the verification action requires reliable analytical techniques capable of detecting non-halal components in the products, even in small quantities (Nakyinsige et al., 2012; Mursyidi, 2013). Some analytical methods (physico-chemicals and molecular biology) have been developed, validated, and impleented for qualitative and quantitative analyses of non-halal components of pig derivatives such as lard, porcine gelatine, and pork in food products, which included real-time polymerase chain reaction (Erwanto et al., 2011; Maryam et al., 2016; Rahmawati et al., 2016), differential scanning calorimetry (Nurrulhidayah et al., 2015a), electronic nose (Mansor et al., 2011), chromatographic-based techniques (Indrasti et al., 2010), and nuclear magnetic resonance (NMR) spectroscopy (Nurrulhidayah et al., 2015b). These methods were time consuming with complex preparation samples, involving toxic reagents, using sophisticated instruments and skillful analyst. As a consequence, rapid and simple methods such as Fourier transform infrared (FTIR) spectroscopy due to its capability as fingerprint technique, especially in combination with chemometrics, has been developed to overcome these drawbacks (Kumar and Karne, 2017; Pallone et al., 2018).

Infrared-Fourier transformed (FTIR) spectroscopy is green analytical method due to minimum chemical reagents and solvents needed, fast, non-destructive, and in some cases without any sample preparation, thus, FTIR spectroscopy can be regarded as green analytical techniques (Rohman et al., 2011a; Rohman et al., 2014). In halal authentication analysis, the combination of FTIR spectroscopy and chemometrics of multivariate calibration has been aplied for analysis of lard mixed with other animal fats of beef fat, schicken fat and lamb fat (Che Man and Mirghani, 2001), qualitative and quantitative analyses of lard in the mixture with some vegetable oils such as corn, soybean and sunflower oils (Rohman et al., 2011a), quantitative analysis of lard present in chocolate products and cake formulation (Che Man et al., 2005; Syahariza et al., 2005), and quantitative analysis of lard extracted from pork in beef meatball products and meatball broth (Rohman et al., 2011b; Kurniati et al., 2014). The lipid components extracted from food samples was different if solvents used for extraction was different in polarity, as a consequence, FTIR spectra obtained were slightly different. Therefore, there is a need to use different solvents for lipid extraction in lipid containing food such as meatballs (Pérez-Palacios et al., 2008; Waskitho et al., 2016). From literature review, no publication reported the employing of FTIR spectra combined with chemometrics for analysis of DM in meatball by comparing lipid profiles extracted using two different techniques (Bligh-Dyer method and Folch method) having different polarity. Therefore, the specific objective of this study was to develo FTIR spectroscopy combined with multivariate analysis for qualitative and quantitative analyses of DM in beef-based meatball for halal authentication purpose.

MATERIALS AND METHODS

Dog meat (DM) was obtained from some dog slaughterhouses in Purwokerto, central Java, Indonesia. Rat's meat was obtained from farmer's land in Purwokerto, while beef, chicken, and pork were purchased from several local markets. All samples were transferred into chilled condition (4°C) and stored at -20°C until being used for making meatball products.

Preparation of laboratory meatball samples: The laboratory meatball samples, used in either calibration or validation models, were prepared in the according to Rohman et al. (2011a). Meatball was mad by emulsification process of 10% starch and 90% of corresponding ground meat (DM or beef). The mixture was mixed vigorously with spice including salt,cumin powder, garlic powder, black pepper and chopped onion . All components used for meatball preparation were blended using vigorous mixing and the homogenous mixture of emulsified meat was shaped like balls. The meatball was further subjected to cooking in boiling water (100°C) for 20 min.

Construction of calibration and validation models: For making calibration and validation models, the corresponding meatball samples with certain concentration of DM were prepared. Calibration samples were prepared by mixing of DM and beef at concentration ranges of 0, 2.5, 5, 10, 20, 40, 50, 75, and 100% (wt/wt) of DM in beef. The validation samples, independent meatball samples with known concentration of DM, were also prepared with similar concentration level of DM. The meatball was subsequently cut into small with blender and taken to extraction procedure using Bligh-Dryer method and Folch method.

Fat extraction using Bligh-Dyer method: Fat extraction was performed using Bligh-Dyer method as described by Waskhito et al. (2016). An approximately of 4.0 g of meatball in a screw-capped polypropylene centrifuge tube was mixed with addition of 10 mL chloroform–methanol (1:2 v/v), heated at 60°C for 10 min, shaken vigorously for 1 min, subjected to vortex mixing for 5 min, followed by centrifugation at 3000 rpm for 10 min. After that, the supernatant was decanted into a separatory funnel, added with 5 mL of distilled water and shaken vigorously. Chloroform layer was drained and dried using anhydrous Na_2SO_4. After being filtered through Whatman filter paper, the extract was evaporated until solvent was completely removed. The extracts obtained during lipid extraction were subsequently analyzed using FTIR spectrophotometer.

Fat extraction using Folch method: An approximately of 5 gm of meatball samples were added with 100 mL of chloroform: methanol (2:1, v/v). The sample mixture was homogenized and subjected to centrifugation at 3000 rpm for 10 min, and filtered. After that, the filtrate was taken, added with 5 mL of distilled water andshaken vigorously. The mixture was centrifuged at 3000 rpm for 10 min, and the aqueous phase (upper layer) was removed. The organic phase (lower layer) was subjected to filtration through anhydrous Na_2SO_4 and collected (Waskitho et al., 2016). The lipid extracts obtained were further used for analysis using FTIR spectrophotometer.

FTIR spectra acquisition: FTIR spectra of all lipid extracts were scanned in mid IR region corresponding to wavenumbers of 4000-500 cm^{-1} using instrument FTIR spectrophotometer ABB MB3000 (Clairet Scientific, Northampton, The Unted Kingdom). Instrument used detector of deuterated triglycine sulfate (DTGS) detector. The FTIR spectra were acquired at resolution of 8 cm^{-1} using 32 scanning. All FTIR spectra were treated to data analysis using Horizon MB software version 3.0.13.1 (ABB, Canada), included in instrument. The samples were placed in the crystal ZnSe, as a part of horizontal attenuated total reflectance (HATR) at controlled temperature (20°C). The FTIR spectrum of air was used as a background. After each scanning, the background spectrum of air was taken. To facilitate qualitative and quantitative analyses, all FTIR spectra were recorded as absorbance values.

Statistical data analysis: Partial least square calibration (PLSR) was emplowed as regression model during qquantitative analysis of lipid fractions containing DM. The accuracy of PLS model was assesed using coefficient of determination (R^2). In addition, root mean square error of calibration (RMSEC) and prediction (RMSEP) were used as parameter in precision evaluation. Principal component analysis (PCA) was employed for classification among animal fats. Data analysis using PLSR and PCA was carried out using software of Horizon MB.

RESULTS AND DISCUSSION

FTIR spectra of fats extracted from dog meat, pork, beef meat, chicken meat, and rat meat using Bligh and Dyer method, scanned at mid IR region (4000-500 cm^{-1}) at 4 cm^{-1} resolution using HATR as sampling handling technique was shown in **Figure 1**. Each peaks and shoulders corresponding to functional groups responsible for infrared absorption in each evaluated fats. Each fats can be differentiated by means of FTIR spectra, especially in fingerprint region (1500-650 cm^{-1}).

Figure 2 revealed that FTIR spectra of fats extracted by Bligh-Dyer and Folch methods showed a similar profile, but not significant different ($P>0.05$). Principal component analysis (PCA) at wavenumbers of 1700-700 cm^{-1} could classify animal fats evaluated, as shown in **Figure 3**.

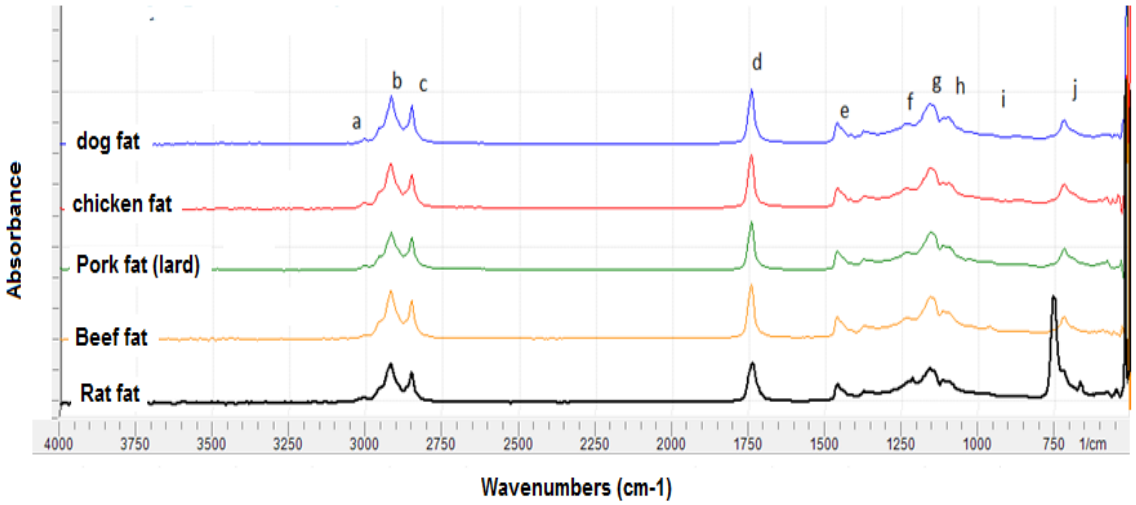

Figure 1. FTIR spectra of fats extracted from dog meat, pork, beef meat, chicken meat, and rat meat using Bligh and Dyer method, scanned at mid infrared region (4000-500 cm^{-1}) at resolution of 4 cm^{-1} using attenuated total reflectance (ATR) as sampling handling technique.

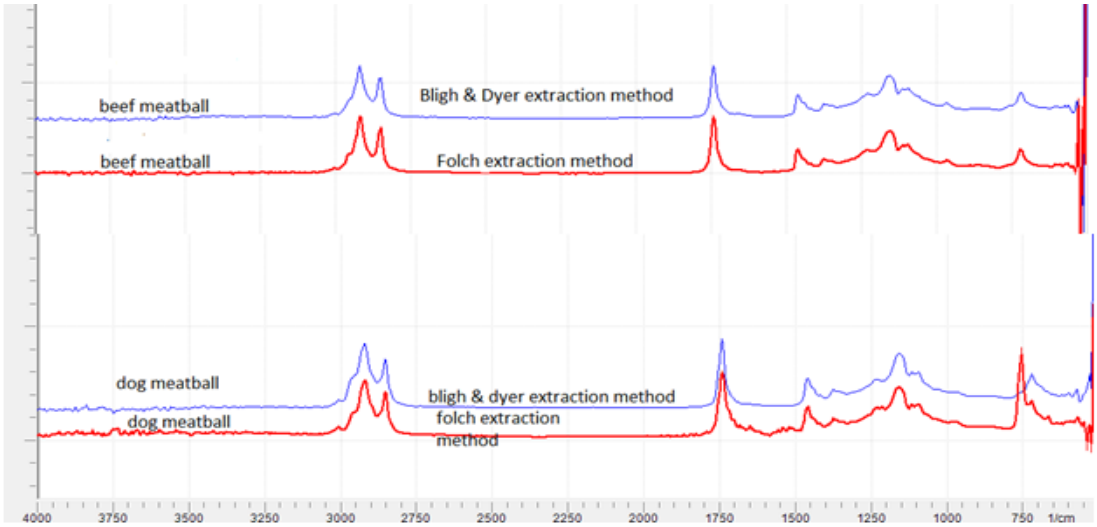

Figure 2. FTIR spectra from dog meatball (100% dog meat) and beef meatball (100%) extracted with Bligh and Dyer as well as Folch extraction method.

Table 1. Functional group and modes of vibration of FTIR spectra of fats from dog, pork, beef, chicken and rat meat.

Assignment	Wavenumber	Functional group	Intensity
a	3005	Cis C=CH stretching	Medium
b	2912	C-HCH stretching vibration	Very strong
c	2861	C-HCH stretching vibration	Very strong
d	1746	Carbonyl C=O from ester	Very strong
e	1468	C-HCH scissoring bending	Medium
f	1226	C-HCH scissoring bending	Weak
g	1167	-CH in plane	Medium
h	1096	C-O from ester	Medium
i	956	CH=CH trans	Weak
j	723	-CH=CH bending out of plane	Medium -strong

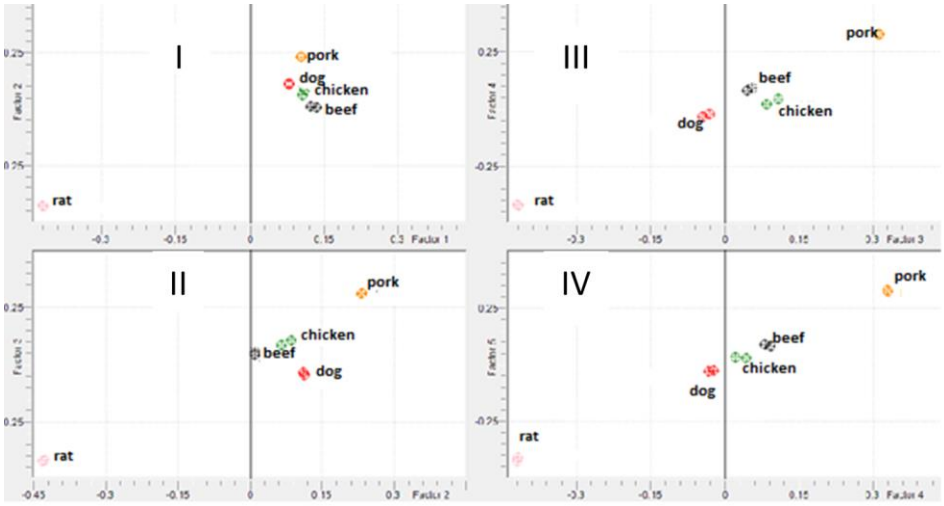

Figure 3. PCA score plot of fats extracted from pork meat, dog meat, chicken meat, beef and rat meat, representing the projection of samples defined by the 1st and 2nd principle component (I), 2 nd and 3th principle component (II), 3th and 4th principle component (III), and 4th and 5th principle component (IV).

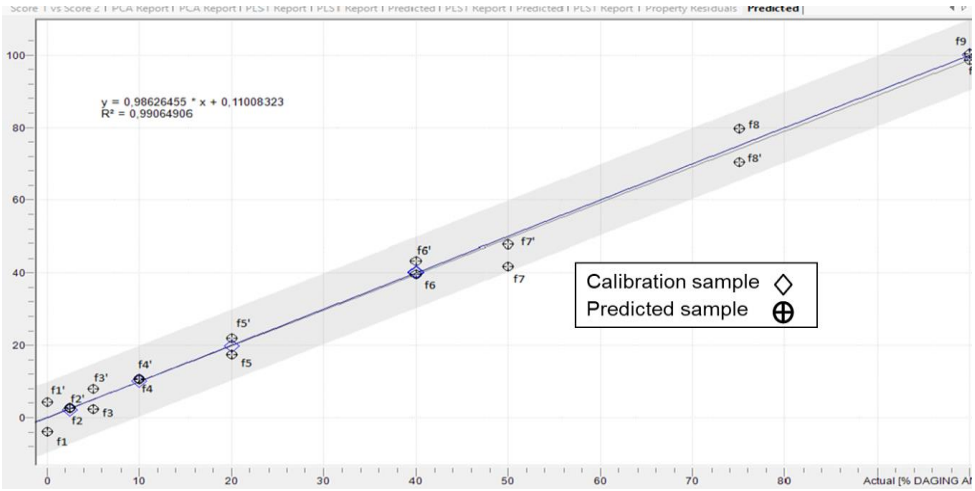

Figure 4. Relationship between actual values of dog's fat from Folch extraction method (x-axis) and FTIR calculated values (y-axis) modeled using PLS wavenumbers of 1700-700 cm^{-1}, f1 and f1' (0%), f2 and f2' (2.5%), f3 and f3' (5%), f4 and f4' (10%), f5 and f5' (20%), f6 and f6' (40%), f7 and f7' (50%), f8 and f8' (75%), f9 and f9' (100%).

Quantitative analysis of dog meat in beef meatball was performed by analyzing lipid fraction extracted using Bligh-Dyer and Folch method. The calibration models were facilitated by partial least square. The correlation between actual values of dog meat and predicted values of dog meat extracted with Bligh-Dyer (**Figure 4**) and Folch (**Figure 5**) using PLS calibration at wavenumbers of 1700-700 cm^{-1} revealed acceptable accuracy and precision, as shown by high R^2 values and low values of RMSEC and RMSEP, respectively.

All edible fats and oils are mainly composed from 95% triacylglycerols (TAG), as a consequence, functional groups attached to TAG would dominate FTIR spectra of edible fats and oils, including beef fat and DM fat. **Figure 1** showed FTIR spectra of fats extracted from dog meat, beef, pork, chicken meat and rat meat, revealing similar FTIR spectra profiles. Fortunately, FTIR spectra are fingerprint in nature, which allow analyst to differentiate among objects, by looking into detail the differences in peak intensity and exact wavenumber ($1/\lambda$) position of each peak and shoulder. **Table 1** compiled the functional groups of each peaks and shoulders responsible for IR absorption in **Figure 1**. Peak appeared in region of 3020 cm^{-1} corresponded to stretching vibration of C-H vinylic. The stretching vibrations of methylene group ($-CH_2-$) and methyl group ($-CH_3$) can be seen in $1/\lambda$ 2929 and 2866 cm^{-1} respectively. The bending

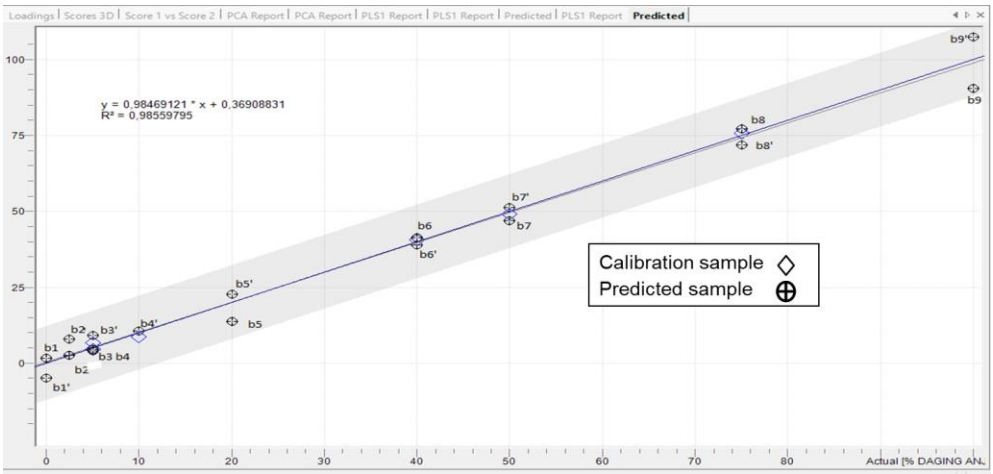

Figure 5. Relationship between actual values of dog's fat from Bligh & Dyer extraction method (x-axis) and FTIR calculated values (y-axis) modeled using PLS wavenumbers of 1700-700 cm^{-1}, b1 and b1' (0%), b2 and b2' (2.5%), b3 and b3' (5%), b4 and b4' (10%), b5 and b5' (20%), b6 and b6' (40%), b7 and b7' (50%), b8 and b8' (75%), b9 and b9' (100%).

vibrations of CH_2- and methyl -CH_3 groups were also observable at 1/λ 1490 cm^{-1} and 1375 cm^{-1}, respectively. The absorption of carbonyl group (C=O) from ester linkage was observed at 1/λ 1740 cm^{-1}. The bands at 1/λ 1235, 1160, 1117, 1098 and 721 cm^{-1} corresponded to the rocking vibrations of overlapping methylene and bending vibration of out of plane of *cis*-substituted olefins (Guillen and Cabo, 1997). It is very complicated to differentiate among FTIR spectra using naked eye, but a precise investigation in fingerprint region (1500 –700 cm^{-1}), there were visual differences at absorption peaks at 1492cm^{-1} (e), 1226 (f), 1167 (gm), 1096 (h) and 723 (j), in terms of peak intensity difference. These differences can be used as strategy during selection of wavenumber regions used for classification and quantification of DM level in beef meatball.

FTIR spectra analysis revealed that samples extracted by Bligh-Dyer and Folch methods showed a similar profile (**Figure 2**). Result of statistical analysis showed that no significant differences between Folch and BD extraction method (P=0.22; P>0.05) using peak intensities. The fats extracted from dog, pork, beef, chicken and rat meat were differentiated using PCA, one of the unsupervised pattern classification techniques commonly used for the classification of objects. The wavenumber regions exploited for PCA were also optimized, and regions of 1700-700 cm^{-1} were finally chosen for calssification using PCA because of its capability to offer good separation among fats extracted. **Figure 3** showed the score values of the first principle components known as PC1 (x-axes) and the second principle components known as PC2 (y-axes), accounting of 81.38% and 13.92% for PC1 and PC2, respectively using absorbances as predictor variables. Samples with similar score values were considered similar in terms of chemical composition (Miller and Miller, 2005).

The quantification of DM in the samples of meatball containing 0, 2.5, 5, 10, 20, 40, 50, 75 and 100% of DM was facilitated with PLSR. The selection of wavenumber region was relied on the ability to provide the best prediction model for the relationship between actual values and predicted values of DM in meatball samples. Finally, the optimized wavenumber of 1700-700 cm^{-1} was used for quantification of DM. This wavenumber gave high R^2 values and low errors. **Figure 4-5** showed the linear correlation between actual values of dog meat (x-axes) and calculated values (y-axes) using Bligh-Dyer and Folch extraction methods. Using Folch method, R^2 and RMESC values obtained were 0.9906 and 1.80% respectively, while using Bligh-Dyer method, R^2 of 0.9860 and RMSEC of 2.01% were obtained, respectively. Based on R^2 and RMSEC values, extraction method using Folch offered better prediction model for DM in beef meatball than Bligh-Dyer.

CONCLUSION

FTIR Spectroscopy using HATR sampling technique and chemometrics of PCA and PLSR could be successfully used for qualitative and quantitative analyses of DM in meatball formulation. The lipid fractions extracted using Folch and Bligh-Dyer extraction methods at wavenumbers of 1700-700 cm^{-1} can be employed for classification and quantification DM in meatball samples.

PCA at wavenumber regions of 1700-700 cm^{-1} was capable of classifying of meatball with DM and beef in its formulation. PLS regression using this wavenumber offered good prediction model for quantitative analysis of DM in meatball. FTIR spectroscopy coupled with chemometrics of PCA and PLSR was effective means for classification and quantification of DM in meatball products.

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CONFLICT OF INTEREST

There is no conflict of interest to declare.

AUTHORS' CONTRIBUTION

SRW and AR performed research activity, compiled data, and prepared manuscript. SM, S, and AR designed research, analyzed data, and prepared manuscript.

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