Fourier-transform infrared spectroscopy combined with chemometrics for detection of pork in beef meatball formulation

By ANY GUNTARTI

ORIGINAL ARTICLE

Fourier-transform infrared spectroscopy combined with chemometrics for detection of pork in beef meatball formulation

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Abstract

Objective: This research focused to analyze the pork content in beef meatball. Pork analysis in beef meatball using Fourier-transform infrared (FTIR) spectroscopy combined with chemometrics. **Methods:** Beef meatball and pork meatball were determined by FTIR spectroscopy. The FTIR spectrum was used to detect pork in beef meatball combined with chemometrics method such as partial least square (PLS) and principal component analysis (PCA). **Results:** In this study, we developed FTIR spectroscopy using multivariate chemometrics. Chemometrics which is used is PLS to analyze pork in beef meatball, and PCA is used to classify pork and beef in meatball. The result PLS is linear regression equation which is a relationship between actual value and FTIR predicted value, y = 0.9984x + 0.0758 with a determination coefficient (R^2) 0.9984. Root mean square error calibration (RMSEC) is 1.09%, root mean square external error calibration is 0.04%, and root mean square error external cross-validation is 0.48%. PCA successfully used to classify meatball in the market between beef meatball and pork meatball. **Conclusion:** Result of pork detection in meat meatballs using FTIR has determination coefficient of 0.9984 with RMSEC of 1.09%. Pork meatball and Beef meatballs can be distinguished using the chemometrics method.

Key words: Halal authentication, meatball, partial least square, principal component analysis

INTRODUCTION

Meatball is one of the most Indonesia's beloved dishes. Meatball is a favorite food in many countries such as Indonesia, Vietnam, Malaysia, and India. Several countries have a specific name for meatball such as Bakso (Indonesia), Nemnuong (Vietnam), Bebola (Malaysia), Kofta (India), Konigsberger klopse (Jerman), Koefte (Turki), Kung-Wan (Taiwan and Cina), and Polpette.[1,2] The meatball is mainly made from beef but to keep production cost due to increases producer prices of beef meatball, producers usually replace beef with lower price meat such as pork and wild boar. Its main aims to gain more profits. Pork is considered as haram food or prohibited food.[3]

Counterfeiting beef with pork is regarded as deception because the producers still give label halal in their product.[4] Meatballs are made from virtually all species, including pork, fish, beef, chicken, and shellfish. Some meatballs also contain to added collagen. Because collagen is converted into gelatin through hydrolysis during cooking, it could be contribute to the smooth texture of meatballs by biding both meat particles and water. Meatballs, one of the most popular street foods in Indonesia's market.[5] The correct labeling of meat which is used in the manufacture meatball is an obligation for the sake of consumers' protection and religious reason concerning the using up of the banned component.[6,7] Nowadays, most of the consumers are concerned about using and labeling meat that is less accurate in food product information.[8,9] Some countries make a rule to ensure that the food products available at the market are save. For centuries, Muslim dominating, they require that the products are halal.[10,11] Therefore, the species identify deception detection

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in meat product include meatball is important for consumer protection and non-halal product verification.[3] Pork differentiation (PM) and beef are needed for two reasons. First, economic reason, due to PM has a lower value than beef.

Second, ensure halal food because PM is considered as a non-halal component.[12] Some countries such as Indonesia, Malaysia, and Middle East countries, halal certified food is an obligation for all types of food.[13] Thus, it is important to make a verification system for a determination whether the food is halal or not. As a result, some sophisticated methods have been developed, proposed, and used to identify and qualify non-halal component in food products.[14] Due to the ability as fingerprint technique, Fourier-transform infrared (FTIR) spectroscopy combined with chemometric technique a reliable analysis method to identify the type of meat which is present in meatball.[15] In addition, rapid of FTIR method, non-destructive, and easy preparation sample make FTIR spectroscopy can be an ideal technique for supporting chemical analysis.[16-18]

In the field of halal analysis, combination FTIR spectroscopy and chemometrics have been used to evaluate pig lard in a binary mixture with other fat animal,[19,20] lard analysis in some vegetables,[21] lard analysis in chocolate formulation and cake using partial least square (PLS) calibration,[4] and lard analysis in meatball product and meatball soup.[1,5] Based on the literature of the present investigation, it has been envisioned that FTIR spectrum for analyzing pork in a beef meatball. The focus of this study, we developed FTIR spectroscopy combined with PLS regression to analyze quantitative and principal component analysis (PCA) to classify pork in a beef meatball.

MATERIALS AND METHODS

Chemical and Reagents

The chemicals used in all experiments were obtained from Sigma and Merck (Indonesia). All of other chemicals and reagents were obtained from Sigma-Aldrich, Yogyakarta.

Sample Collection

Pork and beef are obtained from several local markets in Yogyakarta, Indonesia. For anticipating the variation between meat composition, wild boar and beef which is used are mixture from different yield and local market. All samples which are collected were transported in ice cold condition (4°C) and stored at −20°C for further processing and preparation of formulation meatball.

Preparation of Meatball Samples

Sample meatball preparation: Meatball is made according to reference.[22] This is made by emulsifying 90% of beef or pork, then added with 10% starch and mixed with salt and certain materials (garlic powder, cumin powder, chopped onion, and black pepper). Meat and all ingredients are mixed with hard stirring and mixture homogeneous emulsion meat formed into a ball. The meatballs are then cooked in boiling water (100°C) for 10–20 min.

Preparation of Calibration and Validation Standard

Calibration preparation and validation standards: During preparation of calibration sample, a standard set consisting of pork (PM) and beef made by mixing the meat in the concentration range 0, 25, 35, 50, 65, 75, and 100% (wt/wt) of the PM in the beef. Validation sample refers to a meatball sample which is prepared independently in the laboratory with a known amount of PM composition. Meatball subsequently is extracted lipid according to the Soxhlet method using n-hexane as a solvent.[23] Lipid fraction is evaporated under the temperature at 60°C and dried with anhydrous sodium sulfate.

Process of Samples Analysis using FTIR Spectrophotometer

FTIR spectral acquisition: FTIR spectrum is read at wavenumber mid-infrared (650–4000 cm^{-1}) using ABB MB3000 FTIR spectrophotometer (ClairetIlmiah, Northampton, UK). Instrument is equipped with a detector deuterated triglycine sulfate (DTGS) and KBR as a beam splitter. The spectrum is scanned at a resolution 4 cmG1 with 32 scanning. FTIR spectrum is processed using software from Horizon MB version 3.013.1 (ABB, Canada). Samples are placed in good contact with horizontal attenuated total reflectance (ATR) element (ZnSe crystal) at controlled temperature ambient (20°C). All FTIR spectrums are recorded as absorbance values, and the readings were taken 3 times.

Statistics Analysis

Statistics analysis: Quantitative analysis of extracted fat meatball which is containing PM is done using PLS calibration. PLS model accuracy is evaluated by the coefficient determination (R^2), while the accuracy analysis method is assessed using root mean squared error calibration (RMSEC), root mean squared error of prediction (RMSEP), and root mean square error cross-validation. Classification between meatball samples done using chemometrics from PCA. PLS and PCA are done with Horizon MB software (Canada) included in the FTIR spectrophotometer which is used.

RESULTS

In the present resulted revealed that spectra FTIR of lard and beef fat in meatball have a different result. FTIR spectrum indicated any different of functional groups between lard and beef fat. Functional groups are responsible for the IR absorption shown in Table 1 and FTIR spectrum in Figure 1.

Quantitative analysis of lard and beef fat in meatball make a correlation about true value (lard content) versus predicted value. The good correlation give coefficient determination higher and error lower (see RMSEC). This result can be showed in Figure 2.

However, FTIR spectroscopy combined with chemometrics can use for detection the lard in a beef meatball. For qualitative analysis combined the FTIR spectrum and PCA. The PCA can classify between 100% lard and 100% beef fat. The result of this research is showed in Figure 3.

DISCUSSION

FTIR-spectroscopy measurements were carried out to identify a range of functional groups and are sensitive to changes in molecular structure.[24] FTIR provide information on the basis of chemical composition and physical state of the meatball.

FTIR spectral analysis of lard and beef fat: Figure 1 is FTIR spectrum from lard and beef fat at wave number 650-4000 cm^{-1}, in accordance with the stretching and bending vibrations group functional present in the fat [Table 1]. Measurements on the wavenumber for the optimum result are in the fingerprint area in some wave's <1500 cm^{-1}. In fats and oils, most of the peaks and shoulders of the spectrum are attributable to the specific functional groups.[25]

FTIR spectroscopy is one method for analyzing fats authenticity and oils with functional groups characteristic/fingerprint.[3] For each FTIR spectrum peaks of pork and beef are different in terms of peak intensity (absorbance) and wavenumber.[26] FTIR spectrum is an effective tool to qualitative and quantitative analysis, including the fats and oils analysis.[28]

Quantitative analysis of lard in the meatball formulation: Quantitative analysis of Lardin the meatball samples containing 100% PM, 100% beef, and mix of both meats are done using PLS regression [Figure 2]. Quantitative analysis on the optimum wave number is chosen based on their ability to provide the best prediction model for the relationship between actual value and prediction fat value of FTIR in meatball formulation. Optimization result wave number area is 1022–883 cm^{-1}. These results are used for quantitative analysis. In this wave number gives the highest coefficient determination (R^2) and low error. Figure 2 showed the relationship between the actual values from lard (x-axis) and calculated FTIR values (y-axis) with R^2 and RMSEC values are 0.9984 and 1.09% (wt/wt). Furthermore, this calibration model is used to predict the validation sample. R^2 and RMSEC values for this model are 0.986 and 0.04% (v/v), respectively. Based on this results show that FTIR spectroscopy in 1022–833 cm^{-1} wavenumber with PLS calibration model can provide accurate results (high R^2) with low error RMSEP and RMSEC for PM determination in the meatball formulation.[29]

Classification of fat and beef in meatball containing pork: Meatball formulae containing 100% pork (PM) and 100% beef are classified using chemometrics from PCA. PCA model is built for each group of samples using the model for different sample classification.[30,31] The data processing of FTIR spectrum results in the wave number1022–883 cm^{-1}. Commercial samples which are used are represented by P (1, 3, 4, 5). P (1, 3, 4, 5) located in the beef meatball quadrant area, so that commercial sample P (1, 3, 4, 5) has the same composition with beef meatball. However, P (2) sample far away from 100% beef and 100% pork meatball. Thus, PCA failed to classify whether P (2) sample is beef meatball or pork meatball (PM). The possibility P (2) sample derived from meatball with different meat [Figure 3].

Table 1: Functional groups and modes of vibration of fats extracted from 100% pork's meatball and 100% beef meatball

Assignment	Wavenumber (cm^{-1})	Functional groups	Intensity
(a)	3009	*cis* C=C-H stretching	Weak
(b)	2955	stretching vibration asymmetric (-CH_3)	Medium
(c) and (d)	2924 and 2851	CH-CH2 asymmetric and symmetric stretching vibrations	Strong
(e)	1747	Carbonyl C=O from ester	Strong
(f)	1659	*cis* C=C stretching	Weak
(g)	1462	Bending vibration CH_2 and CH_3 alifatik	Strong
(h)	1377	Bending vibration symmetric CH_3	Medium
(i) and (j)	1234 and 1161	Stretching vibration C-O	Medium
(k) and (l)	1095 and 1034	Bending Vibration–CH	Weak
(m)	964	Bending Vibration –CH	Weak
(n)	721	CH=CH-bending out of plane	Strong

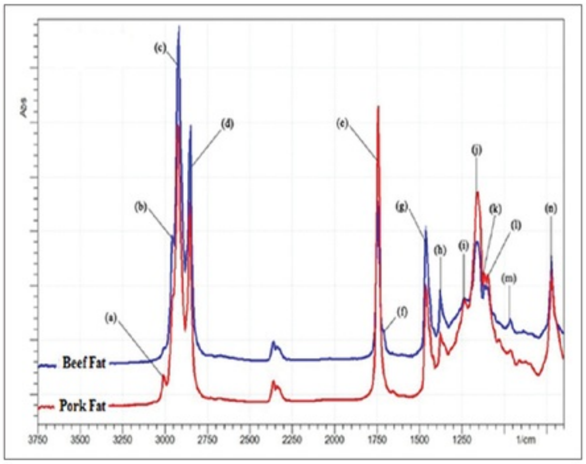

Figure 1: Fourier-transform infrared spectra of fats extracted from the meatballs containing 100% pork meat and 100% beef at 4000–650 cm^{-1}

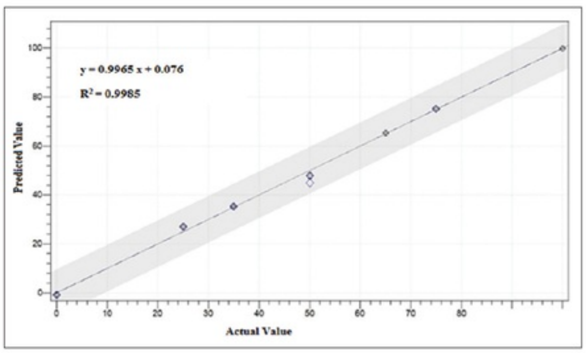

Figure 2: Relationship between lard actual values (x-axis) and calculated Fourier-transform infrared values (Y-axis) modeled using partial least square for 1022–883 cm^{-1} wavenumber

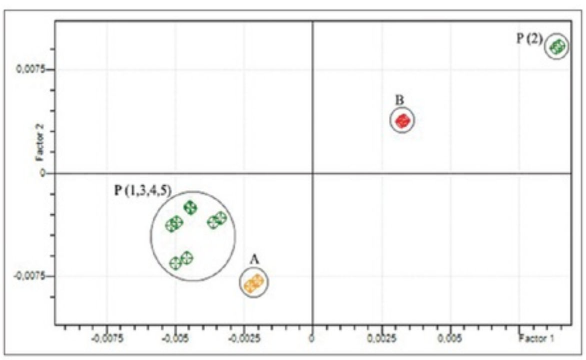

Figure 3: Score plot of principal component analysis for lard and beef fat in the meatball, which represents the projected sample is determined by the first and second principal component, (a) 100% beef meatball, (b) 100% pork meatball;

CONCLUSION

FTIR spectroscopy combined with PLS and PCA on 1022-833 cm^{-1} wavenumber successfully used for the quantitative analysis of pork in meatball formulation. R^2 and RMSEC values obtained for quantification are 0.9984 and 1.09%. PCA has **been successfully used** for pork and beef classification in the meatball formulation.

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