FTIR Spectroscopy in Combination with Chemometrics for Analysis of Wild Boar Meat in Meatball Formulation

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FTIR Spectroscopy in Combination with Chemometrics for Analysis of Wild Boar Meat in Meatball Formulation

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ABSTRACT

Bakso or meatball is one of the Indonesian favorite foods made from beef. This food is quite popular among Indonesian societies. Due to the high price of beef, unethical producers adulterate beef with wild boar. In this research, we developed Fourier Transform Infrared (FTIR) spectroscopy and multivariate calibration of Partial Least Square (PLS) calibration to analyze wild boar in beef meatball. Meanwhile, the chemometrics of Principle Componen Analysis (PCA) is used to classify wild boar and beef in the meatball. The relationship between actual value of wild boar (x-axis) and FTIR predicted value (y-axis) at optimized wavenumbers region is y = 0.9749x+1.4658 with coefficient of determination (R^2) of 0.988 and Root Mean Square Error of Calibration (RMSEC) of 2.0%. Furthermore, PCA was successfully used for the classification of wild boar meatball and beef meatball.

Key words: Partial least square, halal authentication, wild boar, meatball, principal component analysis

INTRODUCTION

Meatball is one of the favorite meat products for Indonesian community. Some countries have their own name to meatball, namely Bakso (Indonesia), Nem nuong (Vietnam), Bebola (Malaysia), Kofta (India), Konigsberger klopse (Germany), Koefte (Turkey), Kung-Wan (Taiwan and China) and Polpette (Rohman *et al*., 2011a). Bakso is mainly made from beef, however, the increase of beef price forces meatball producers to push down the production costs by substituting beef with low price meat such as Wild Boar Meat (WBM) in order to gain maximum profit. The Moslem scholar considered WBM as haram or forbidden thing to eat (Rohman and Man, 2012). The substitution of beef with WBM is considered as a fraud, because the producer still labeled their product as halal.

Meatball is processed meat which can be classified as restructured meat. The meat used for making meatball can come from beef, pork, fish, or chicken. The most popular one used in meatball and widely met in Indonesia market is beef (Kurniawati *et al*., 2014). The labelling of meat used in the meatball formulation is a an obligation for the sake of consumers' protection, religious credence regarding the consumption of non allowed components and hard-earned fortunes (Doosti *et al*., 2014; Aparicio *et al*., 2013). Currently, interest in meat authenticity has increased. Many consumers are concerned about the meat used and accurate labeling in the product they eat (Ballin, 2010; Reid *et al*., 2006). Some countries make a regulation to assure that the food products available in the market are safe. For centuries, whose Moslem community dominate, they obligate

that the products are halal (Riaz and Chaudry, 2012; Ali *et al*., 2013). Therefore, detection of species identity fraud in meat product including meatball is important for costumer protection and for the verification of non-halal products (Rohman and Man, 2012).

Differentiation of Wild Boar Meat (WBM) and beef is necessary for two reasons. First, economical reasons, because WBM has the lower value that beef and second, to assure the halalness of food because WBM is considered as nonhalal components (Regenstein *et al*., 2003). In some countries such as Indonesia, Malaysia and Middle East countries, halal certified food is compulsory for all kinds of food (Nakyinsige *et al*., 2012). Thus, it is important to create a reliable verification system to determine whether a food is halal or not. As a consequence, some analytical methods have been developed, proposed and used for identification and quantification of non-halal component in the food products (Mursyidi, 2013).

Due to its capability as a finger print technique, FTIR spectroscopy combined with a powerful chemometric technique is an emerging reliable analytical method to identify meat types present in meatball (Rahmania *et al*., 2015). The FTIR spectroscopy is rapid, non-destructive and not involving laborious sample preparation, therefore, FTIR spectroscopy is an ideal technique for supporting green analytical chemistry (Man *et al*., 2005). In the field of halal analysis, the combination of FTIR spectroscopy and chemometrics has been used for analysis of lard in the binary mixture with other animal fats (Man and Mirghani, 2001), analysis of lard in some vegetable oils (Rohman *et al*., 2011b), analysis of lard in chocolate and cake formulation using partial least square calibration (Man *et al*., 2005) and analysis of pork fat in meatball products and meatball broth (Rohman *et al*., 2011a; Kurniawati *et al*., 2014). Using literature review from several databases, there is no available publication regarding the use of FTIR spectra for analysis of WBM in beef meatball. Therefore in this study, we developed FTIR spectroscopy in combination with Partial Least Square (PLS) regression for quantitative analysis and Principal Component Analysis (PCA) for classification of wild boar meat in beef meatball.

MATERIALS AND METHODS

Wild boar meat (*Sus scrofa*) is obtained from wild boar hunter in Kalimantan, Indonesia, while beef was purchased from several local markets in Yogyakarta Indonesia. In order to anticipate the variation among meat composition, the wild boar and beef used are mixture from different farmer's land and local market. All the collected samples were transported under ice-chilled condition (4°C) and were stored at -20°C for further processing and for the preparation of meatball formulation.

Preparation of meatball samples: Meatball was prepared according to Purnomo and Rahardiyan (2008). It is made by emulsifying 90% of beef and or wild boar meat, then added with 10% of starch and mixed vigorously with salt and certain ingredient (garlich powder, cumin powder, chopped onion and black pepper). The meat and all other ingredients were blended by vigorous mixing and the emulsified homogenous meat mixture was shaped into ball. The meatball is then cooked in boiling water (100°C) for 10-20 min.

Preparation of calibration and validation standard: During the preparation of calibration samples, a set of standards consisting of Wild Boar Meat (WBM) and beef was prepared by mixing of both meat at concentration ranges of 0, 10, 15, 20, 35, 50, 65, 80, 85 and 100% (wt/wt) of WBM in beef. To check the accuracy and precision of a calibration model, some other samples known as validation samples were also prepared for making a validation model. The validation sample refer

to meatball samples prepared independently in the laboratory with known amount of WBM composition. Meatball was further subjected to lipid extraction according to the traditional Soxhlet method using hexane as an extraction solvent (AOAC., 1995). The hexane containing lipid fraction was evaporated under a vacuum rotary evaporator at 60°C and dried with anhydrous sodium sulfate. The lipid fraction obtained was subjected to FTIR measurements.

FTIR spectral acquisition: The FTIR spectra of all evaluated samples were acquired in the mid infrared region (650-4000 cm^{-1}) using an ABB MB3000 FTIR spectrophotometer (Clairet Scientific, Northampton, UK). The instrument was equipped with a deuterated triglycine sulfate (DTGS) detector and KBR as the beam splitter. The spectra were scanned at a resolution of 4 cm^{-1} with 32 scanning. The FTIR spectra were processed using FTIR software of Horizon MB version 3.013.1 (ABB, Canada). The samples were placed in good contact with Horizontal Attenuated Total Reflectance (HATR) element (ZnSe crystal) at controlled ambient temperature (20°C). All spectra scan, a new reference air background spectrum was taken. All FTIR spectra were recorded as absorbance values at each data point in triplicate.

Statistical analysis: Quantitative analysis of fat extracted from meatball containing WBM was performed using Partial Least Square (PLS) calibration. Accuracy of PLS model was evaluated by coefficient of determination (R^2), while the precision of analytical method was assessed using root Mean Square Error of Calibration (RMSEC) and Root Mean Square Error of Prediction (RMSEP). The classification among meatball samples was carried out using chemometrics of Principal Component Analysis (PCA). The PLS and PCA are carried out with the aid of Horizon MB software (Canada) included in the used FTIR spectrophotometer.

RESULTS AND DISCUSSION

FTIR spectral analysis of wild boar fat and beef fat: Figure 1 is FTIR spectra of wild boar's fat and beef's fat at wave numbers of 650-4000 cm^{-1}, corresponding to the stretching and bending vibrations of functional groups present in evaluated fats but the complicated absorption profiles were observed in the finger print region at a wave number of <1500 cm^{-1} (Smith, 2011). In the fingerprint region, all samples have different profiles of peaks and shoulders as indicated in circle. As shown in Fig. 2, there is a bit difference in selected fingerprint region for both spectra in which the absorption of certain peaks is slightly different, especially marked with e, j, k and l. The functional group responsible for IR absorption is compiled in Table 1.

Table 1: Functional groups and modes of vibration of fats extracted from 100% wild boar's meatball and 100% beef meatball. The spectra can be seen in Fig. 1

Assignment	Wavenumber (cm^{-1})	Functional groups	Intensity
a	3005	Cis C=CH stretching	Medium
b	2953	C-HCH_3 stretching vibration	Medium
c and d	2926 and 2854	C-H-CH_2 asymmetric and symmetric stretching vibrations	Very strong
e	1744	Carbonyl C = O from ester	Very strong
f	1457	C-HCH_2 scissoring bending	Medium
g	1417	Cis = C-H bending	Weak
h	1377	C-HCH_3 scissoring bending	Medium
i	1236	C-HCH_2 scissoring bending	Weak
j	1157	-CH in plane	Medium
k and l	1118 and 1097	C-O from ester	Medium
m	966	CH = CH trans	Medium
n	719	-CH = CH-bending out of plane	Medium

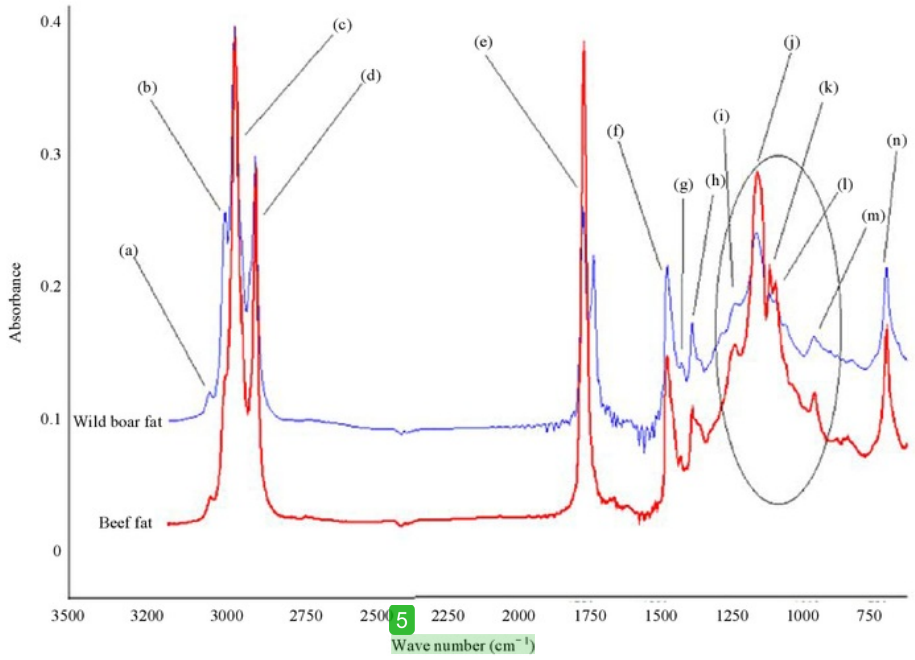

Fig. 1: FTIR spectra of fats extracted from the meatballs containing 100% wild boar meat and 100% beef at 4000-650 cm^{-1}

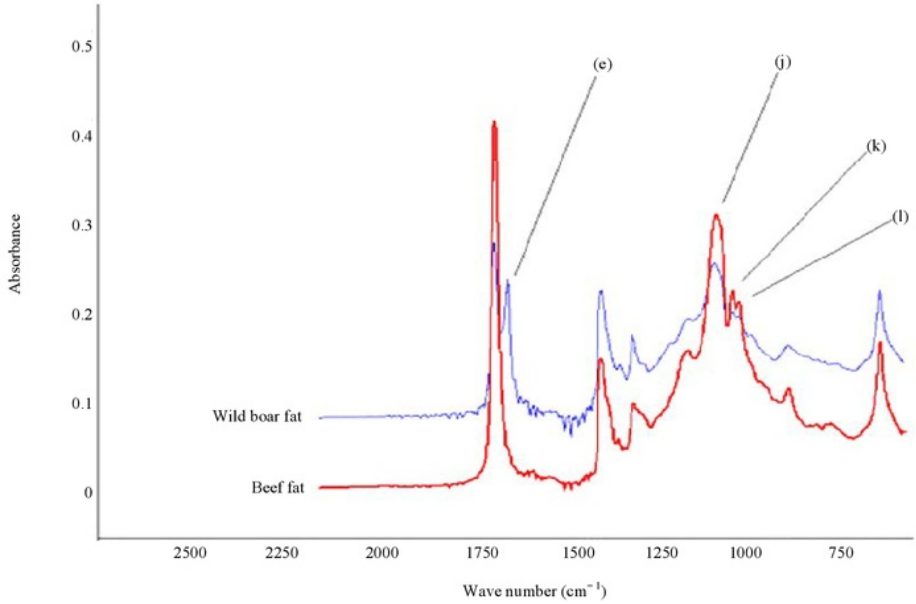

Fig. 2: Enlarged region showing the subtle difference for both spectra

The FTIR Spectra from all samples are represented by fat spectrum of meatball extraction which contains wild boar and beef fats. Spectrum is measured in mid infrared area of (650-4000 cm^{-1}). Spectroscopy FTIR is a very good method to analyze the authenticity of fat and oil because it performs finger print characteristics (Rohman and Man, 2012). The fingerprint technique can be understood that the FTIR spectra of wild boar meat and beef are different in terms of peak intensities (absorbencies) and exact wave numbers for each peak (Man *et al.*, 2011). The FTIR spectra are effective means for the qualitative and quantitative analyses, including fats and oils analysis, coming from the much information gathered and the feasibility to mark the certain absorption bands and shoulders related to the functional groups contained in fats and oils responsible for IR absorption (Bendini *et al.*, 2007).

Quantitative analysis of wild boar's fat in meatball formulation: The quantitative analysis of wild boar's fat in meatball samples containing 100% WBM, 100% beef and the mixture of both meat was performed using Partial Least Square (PLS) regression. The wavenumber region used for quantitative analysis was selected based on the ability to offer the best prediction model for the relationship between actual value and FTIR predicted values of WBM fat in meatball formulation. Finally, after optimization of wavenumbers region, the wavenumbers of 1000-1250 cm^{-1} were used for quantitative analysis. Besides, this wavenumber gave the highest coefficient of determination (R^2) and low errors.

Figure 3-4 showed the relationship between actual values of wild boar's fat (x-axis) and FTIR calculated values (y-axis) with R^2 and RMSEC values of 0.998 and 2.00% (wt/wt), respectively. Furthermore, this calibration model was used for the prediction of validation samples. The R^2 and RMSEP obtained were 0.986 and 5.84 % (v/v), respectively. Based on this result, it can be stated that FTIR spectroscopy at wavenumbers of 1000-1250 cm^{-1} assisted with PLS calibration model can give the accurate results (high R^2) with low errors (low RMSEC and RMSEP) for the determination of WBM in meatball formulation.

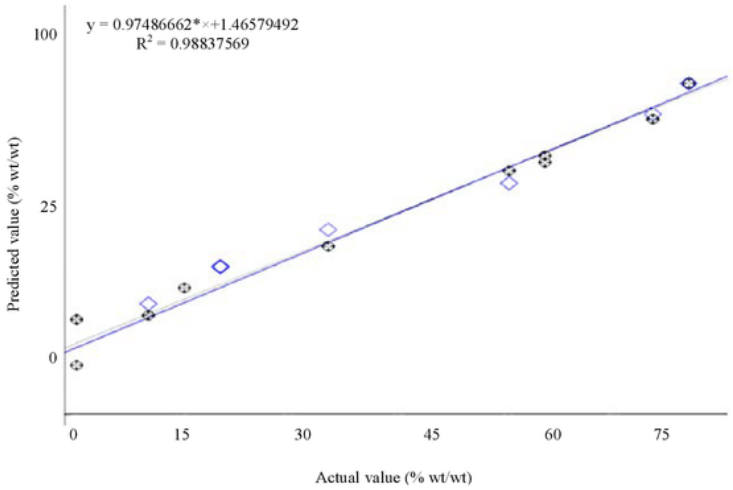

Fig. 3: Relationship between actual values of wild boar's fat (x-axis) and FTIR calculated values (y-axis) modeled using PLS wavenumbers of 1000-1250 cm^{-1}

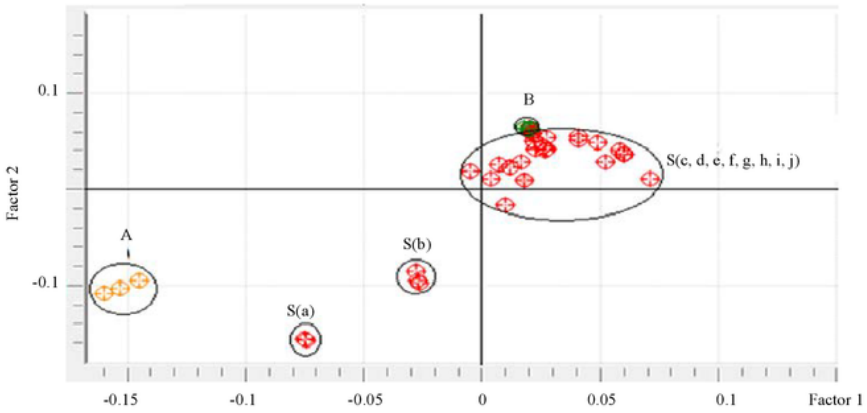

Fig. 4: PCA score plot of wild boar's fat and beef fat in the meatballs, representing the projection of samples defined by the first principle component and the second principle component, (A) 100% wild boar meatball, (B) 100% beef meatball; S(a), S(b), S(c, d, e, f, g, h, i, j) = commercial samples

Classification of meatball containing wild boar's fat and beef fat: The meatball formula containing 100% Wild Boar Meat (WBM) and 100% beef was classified using chemometrics of Principal Component Analysis (PCA). The PCA is unsupervised pattern recognition techniques widely used for the classification of different samples (Miller and Miller, 2005). The wavenumber regions for PCA were also optimized. Finally, the same wavenumbers used for quantitative analysis, namely 1000-1250 cm^{-1}, were chosen for PCA modeling due to its capability to provide good separation among the evaluated samples.

The PCA score plot is a latent variable. Samples with same PC values are similar in terms of chemical composition and so on. The commercial samples used during this study as represented by S(a), S(b) and S(c, d, e, f, g, h, i, j). The S(c, d, e, f, g, h, i, j) have not different profiles with beef meatballs, so the score plot of commercial samples of S(c, d, e, f, g, h, i, j) have same composition with beef meatball. However, the score plot of samples S(a) and S(b) is far away from that of meatball with 100% beef fat and 100% WBM meatball. Thus, PCA failed to classify whether the samples S(a) and S(b) are beef meatball or WBM meatball. There is also the possibility that samples S(a) and S(b) originated from the meatball composed of different meats.

CONCLUSION

The FTIR spectroscopy combined with PLS and PCA at wavenumber of 1000-1250 cm^{-1} can be successfully used for the quantitative analysis of wild boar meat in meatball formulation. The R^2 and RMSEC values obtained for quantification were 0.998 and 2.00%, respectively. The PCA was successfully used for the classification of wild boar meat and beef meat in meatball formulation.

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REFERENCES

AOAC., 1995. Official Methods of Analysis. 16th Edn., Association of Official Analytical Chemists, Washington, DC., USA.

Ali, M.E., U. Hashim, S. Mustafa, Y.B.C. Man and T.S. Dhahi *et al*., 2012. Analysis of pork adulteration in commercial meatballs targeting porcine-specific mitochondrial cytochrome b gene by TaqMan probe real-time polymerase chain reaction. Meat Sci., 91: 454-459.

Aparicio, R., M.T. Morales, R. Aparicio-Ruiz, N. Tena and D.L. Garcia-Gonzalez, 2013. Authenticity of olive oil: Mapping and comparing official methods and promising alternatives. Food Res. Int., 54: 2025-2038.

Ballin, N.Z., 2010. Authentication of meat and meat products. Meat Sci., 86: 577-587.

Bendini, A., L. Cerretani, F. di Virgilio, P. Belloni, M. Bonoli-Carbognin and G. Lercker, 2007. Preliminary evaluation of the application of the ftir spectroscopy to control the geographic origin and quality of virgin olive oils. J. Food Qual., 30: 424-437.

Doosti, A., P.G. Dehkordi and E. Rahimi, 2014. Molecular assay to fraud identification of meat products. J. Food Sci. Technol., 51: 148-152.

Kurniawati, E., A. Rohman and K. Triyana, 2014. Analysis of lard in meatball broth using Fourier transform infrared spectroscopy and chemometrics. Meat Sci., 96: 94-98.

Man, Y.B.C. and M.E.S. Mirghani, 2001. Detection of lard mixed with body fats of chicken, lamb and cow by fourier transform infrared spectroscopy. J. Am. Oil Chem. Soc., 78: 753-761.

Man, Y.B.C., Z.A. Syahariza, M.E.S. Mirghani, S. Jinap and J. Bakar, 2005. Analysis of potential lard adulteration in chocolate and chocolate products using Fourier transform infrared spectroscopy. Food Chem., 90: 815-819.

Man, Y.B.C., A. Rohman and T.S.T. Mansor, 2011. Differentiation of lard from other edible fats and oils by means of fourier transform infrared spectroscopy and chemometrics. J. Am. Oil Chem. Soc., 88: 187-192.

Miller, J.N. and J.C. Miller, 2005. Statistics and Chemometrics for Analytical Chemestry. 5th Edn., Pearson Education Ltd., Edinburgh, UK., ISBN: 0-13-129192-0.

Mursyidi, A., 2013. The role of analytical chemistry in Halal certification. J. Food Pharm. Sci., 1: 1-4.

Nakyinsige, K., Y.B.C. Man and A.Q. Sazili, 2012. Halal authenticity issues in meat and meat products. Meat Sci., 91: 207-214.

Purnomo, A. and D. Rahardiyan, 2008. Review Article: Indonesian traditional meatball. Int. Food Res. J., 15: 101-108.

Rahmania, H., Sudjadi and A. Rohman, 2015. The employment of FTIR spectroscopy in combination with chemometrics for analysis of rat meat in meatball formulation. Meat Sci., 100: 301-305.

Regenstein, J.M., M.M. Chaudry and C.E. Regenstein, 2003. The kosher and halal food laws. Compr. Rev. Food Sci. Food Saf, 2: 111-127.

Reid, L.M., C.P. O'Donnell and G. Downey, 2006. Recent technological advances for the determination of food authenticity. Trends Food Sci. Technol., 17: 344-353.

Riaz, M.N. and M.M. Chaudry, 2003. Halal Food Production. 1st Edn., CRC Press, Boca Raton, FL, USA., pp: 58-65.

Rohman, A. and Y.B.C. Man, 2012. Analysis of pig derivatives for halal authentication studies. Food Rev. Int., 28: 97-112.

Rohman, A., Y. Erwanto and Y.B.C. Man, 2011a. Analysis of pork adulteration in beef meatball using fourier transform infrared (FTIR) spectroscopy. Meat Sci., 88: 91-95.

Rohman, A., Y.B.C. Man, A. Ismail and H. Puziah, 2011b. FTIR spectroscopy combined with chemometrics for analysis of lard adulteration in some vegetable oils. Cyta-J. Food, 9: 96-101.

Smith, B.C., 2011. Fundamentals of Fourier Transform Infrared Spectroscopy. 2nd Edn., CRC Press, USA., pp: 74, 1281-1286.

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