Sains Malaysiana 51(8)(2022): 2573-2582
http://doi.org/10.17576/jsm-2022-5108-17

Determination of Pork Adulteration in Roasted Beef Meatballs using Fourier Transform Infrared Spectroscopy in Combination with Chemometrics

(Penentuan Pencampuran Daging Babi dalam Bebola Daging Lembu Panggang menggunakan Spektroskopi Inframerah Transformasi Fourier dalam Gabungan dengan Kemometri)

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Received: 21 September 2021/Accepted: 8 February 2022

ABSTRACT

The pork adulteration in food is a matter of concern for Muslims worldwide. This study aimed to quantitatively analyze and classify pork and beef in roasted meatballs using the Fourier Transform Infrared (FTIR) spectroscopy method combined with chemometrics. To obtain the FTIR spectral data, pure beef meatballs and beef meatballs added with pork at various concentrations were analyzed in the mid-infrared region corresponding to wavenumbers ranging from 4000 to 400 cm^{-1}. Samples of beef meatballs randomly selected from the market were analyzed in the optimum condition in terms of wavenumbers capable of providing best modeling. Qualitative and quantitative analyses were performed on the optimum wavenumbers of 1100-900 cm^{-1} using chemometrics of Partial Least Square (PLS) and Principal Component Analysis (PCA), respectively. The PLS calibration model for the relationship between actual values of pork (x-axis) and predicted FTIR values (y-axis) of roasted meatballs resulted in the equation of $y = 0.99965x + 0.0221$, with a coefficient of determination (R^2-value) of 0.99965 and root mean square error of calibration (RMSEC) of 0.7137%. The PCA indicated differences in quadrants between pure beef and pork roasted meatballs. Most samples of marketed beef meatballs lied in the beef meatball quadrant. However, one sample of marketed beef meatballs was in the pork meatball quadrant; thus, it contained pork. To conclude, the FTIR spectroscopy method combined with chemometrics could qualitatively and quantitatively identify the ingredient of pork in roasted beef meatballs.

Keywords: Beef meatball; commercial products; *halal* analysis; meatballs; multivariate calibration

ABSTRAK

Pencabulan daging babi dalam makanan menjadi perhatian masyarakat Islam di seluruh dunia. Kajian ini bertujuan untuk menganalisis dan mengelaskan daging babi dan daging lembu dalam bebola daging panggang menggunakan kaedah spektroskopi Fourier Transform Infra-Red (FTIR) yang digabungkan dengan kemometri. Untuk mendapatkan data FTIR, bebola daging lembu murni dan bebola daging dengan pencemaran daging babi pada pelbagai kepekatan sebagai rujukan dianalisis dalam bilangan gelombang antara 4000 hingga 400 cm^{-1} untuk mendapatkan bilangan gelombang yang optimum untuk analisis. Sampel bebola daging lembu yang dipilih secara rawak dari pasar dianalisis dalam bilangan gelombang optimum. Analisis kualitatif dan kuantitatif dilakukan pada bilangan gelombang optimum 1100-900 cm^{-1} menggunakan teknik analisis multivariat kuasa dua terkecil separa (PLS) dan analisis prinsip komponen (PCA) dalam aplikasi Horizon MB. Model penentukuran PLS untuk bebola panggang menunjukkan persamaan $y = 0.99965x + 0.0221$, dengan R2 0.99965 dan RMSEC = 0.7137%. PCA menunjukkan perbezaan kuadran antara bebola daging lembu tulen dan daging babi. Sebilangan besar sampel bebola daging lembu yang dipasarkan berbohong di kuadran bakso daging lembu dengan satu sampel bebola daging lembu yang dipasarkan berada dalam kuadran bakso babi; mengandungi daging babi. Dapat disimpulkan bahawa kaedah FTIR dalam gabungan dengan kemometri dapat digunakan untuk mengenal pasti keberadaan kandungan daging babi dalam bebola daging lembu panggang secara kualitatif dan kuantitatif.

Kata kunci: Analisis halal; analisis kualitatif dan kuantitatif; bakso lemak babi; bakso lembu; produk komersial

INTRODUCTION

Halal food is a major concern for Muslims worldwide (Mursyidi 2013). One of the recent problems for Indonesian Muslims is the adulteration practice of meat-based food products by non-halal meat; one of the types of meat typically used as an adulterant in beef meatballs is pork (Nurrulhidayah et al. 2013). Meatball is one of the favorite foods for most Indonesian because it tastes good and the price is affordable. Meatball products are usually made from meat typically originating from chicken or beef for Muslim consumers or from pork for non-Muslim consumers.

The increased demand for meatballs results in wide innovation meat products; one of the currently popular products is roasted meatballs. Roasted meatballs are boiled meatballs that are reprocessed by baking and giving certain additional ingredients to enhance the taste. The unethical traders or producers of beef meatballs who want to get greater profits may blend or substitute pork for beef (Guntarti et al. 2015) because pork is cheaper than beef in Indonesia.

The heating process can cause damage to the fat contained in foodstuffs. The fat contained in meatball products can suffer damage resulting from the process of steaming (dehydration) and burning (oxidizing) (El-Gindy et al. 2006; Guntarti & Amidin 2018). Roasted meatballs subjected to heating can stimulate oxidation reactions which lead to fat damage. In the oxidation process, most of the unsaturated fatty acids will be damaged and the results of the damage can largely evaporate (Che Man et al. 2011).

Several analytical methods were developed to analyze beef adulteration with pork, including high-performance liquid chromatography-tandem mass spectrometry (Salamah et al. 2019), two-dimensional GC x GC (Gorst-Allman et al. 2012), differential scanning calorimetry (DSC) (Marikkar et al. 2002), electronic nose (EN) (Nurjuliana et al. 2011) & real-time polymerase chain reaction (Kurniasih et al. 2019). However, these methods still need sophisticated instruments (MS-based methods) and lack specificity (DSC and electronic nose). Therefore, simple and specific methods based on FTIR spectroscopy must be developed and used for routine analysis.

Due to its nature as a fingerprint technique, the FTIR spectroscopy combined with multivariate data analysis (MDA) was developed and validated to detect meat adulteration, especially the adulteration of beef with pork subjected to cooking processes (Rohman et al. 2016). The combination of FTIR spectra and chemometrics was successfully used to analyze pork as an adulterant in beef meatballs (Rohman et al. 2011), *dendeng* (Kuswadi et al. 2015; Maryam et al. 2015) and corned meat (Guntarti et al. 2019). Edible fats and oils are members of the lipid group. Animal fats contained in these food products can be damaged because they were exposed to free air or oxidation. Che Man et al. (2010) discovered that the difference between lard and beef tallow was in the wavenumber of 3010 cm^{-1}. Fatty acid samples at wavenumbers of 1165 and 1111 cm^{-1} are overlapping. However, to date, no research has analyzed pork in roasted beef meatball samples using the Fourier Transform Infrared (FTIR) with chemometrics of Partial Least Square (PLS) and Principal Component Analysis (PCA) (Rahayu et al. 2018). Therefore, this study aimed to develop the FTIR spectrophotometry combined with chemometrics to analyze the pork adulteration in roasted beef meatballs.

MATERIALS AND METHODS

SAMPLES

Reference meatballs made from a mixture of beef, pork and seasonings from various levels were boiled. After that, the meatballs were roasted at 80 °C according to Więk and Tkacz (2017). The compositions of meats in meatballs with other components are shown in Table 1. The marketed samples of commercial beef meatballs were obtained from local markets and supermarkets around Yogyakarta. Finally, the meatballs were roasted.

TABLE 1. Various concentrations of reference meatball formula

Meatballs Concentration (%)	Weight of Beef	Weight of Pork	Spices
Pork Meatballs (0%)	45.00 g	0 g	5.00 g
Pork Meatballs (75%)	7.50 g	37.50 g	5.00 g
Pork Meatballs (65%)	12.50 g	32.50 g	5.00 g
Pork Meatballs (50%)	20.00 g	25.00 g	5.00 g
Pork Meatballs (35%)	27.50 g	17.50 g	5.00 g
Pork Meatballs (25%)	32.50 g	12.50 g	5.00 g
Pork Meatballs (100%)	0 g	45.00 g	5.00 g

EXTRACTION OF LIPID COMPONENTS IN MEATBALLS

Lipid components from the roasted meatballs were extracted using the Soxhlet method by Guntarti and Zelinda (2019); this method employed the *n*-hexane as extracting solvent. Briefly, the extraction process was carried out for ±6-7 h with a temperature of ≤ 70 °. The obtained fat was added to anhydrous Na_2SO_4 powder to remove water. The obtained lipid fractions were then measured using the FTIR spectral measurement (Naquiah et al. 2017).

FTIR SPECTRAL SCANNING

The meatballs were analyzed using the FTIR spectrophotometer ABB MB 3000 (Canada) and scanned at min infrared region corresponding to wavenumbers of 4000-650 cm^{-1}. The samples were placed on ATR crystal plates at a controlled temperature (25 °C). The measurements were made on 32 scans and at 4 cm^{-1} resolutions using absorbance mode to facilitate quantitative analysis based on Lambert-Beer law.

CHEMOMETRICS ANALYSIS

FTIR spectra were subjected to the chemometrics analysis, including the PLS for quantification and the PCA for classification. The software used was Horizon MBTM software. Microsoft Excel 2007 (Microsoft Inc., USA) was used to validate and calibrate the modeling. Some statistical parameters, such as the coefficient of determination (R^2), root mean square errors of cross-validation (RMSECV) and root mean square error of prediction (RMSEP), were used as criteria to calibrate and validate models (Miller & Miller 2010; Zhao et al. 2014).

RESULTS AND DISCUSSION

The FTIR spectroscopic analysis was based on the characteristics of the functional groups in lipid fractions that were extracted from beef meatballs and roasted pork meatballs. The FTIR spectra of lipid fractions were extracted from 100% of roasted beef meatballs and 100% roasted pork meatballs, as presented in Figure 1. Each peak and shoulders in FTIR spectra corresponded to functional groups in evaluated samples that were absorbing infrared radiation, as shown in Table 2.

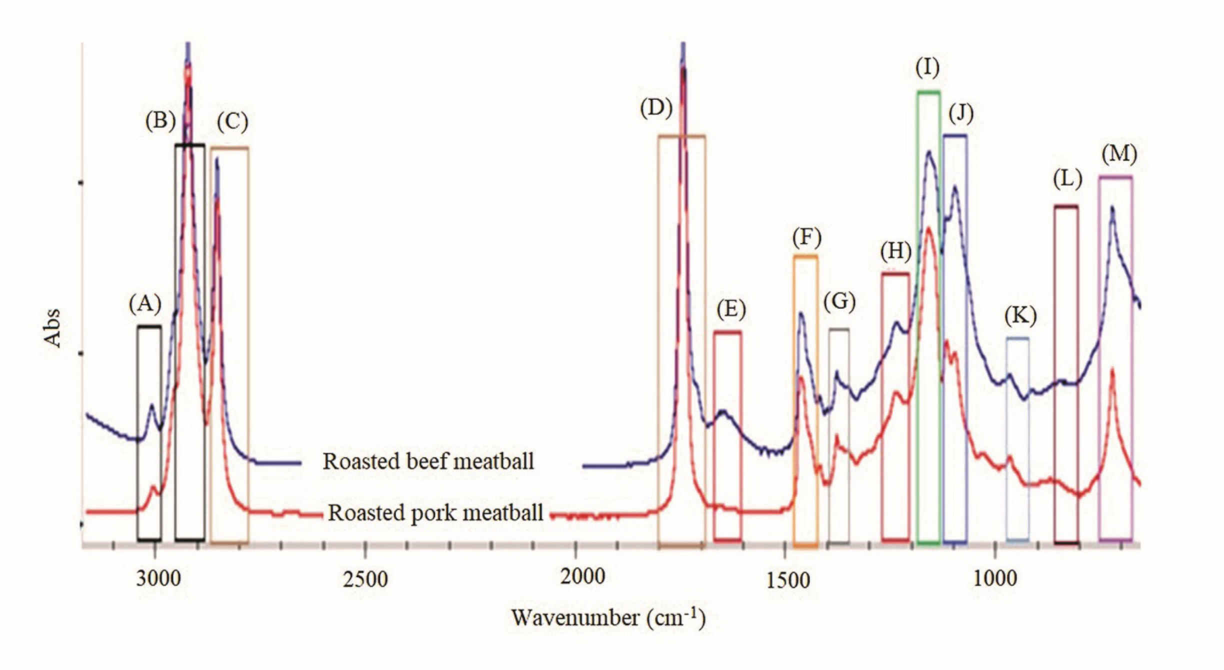

FIGURE 1. FTIR spectra results of 100% of roasted beef meatballs and 100% of roasted pork meatballs

TABLE 2. Functional groups and vibration models in roasted beef meatballs and roasted pork meatballs (Pavia et al. 2001)

Sign	Wavenumber (cm^{-1})		Reference	Group Vibration Functions	Intensity
	Pork	Beef			
(A)	3008.14	3004.46	3006	Cis C = CH stretches	Weak
(B)	2922.17	2921.67	2924	Asymmetrical stretching vibration of the methyl group (CH_3)	Strong
(C)	2852.73	2852.42	2852	Symmetric and asymmetric stretching vibrations of methylene groups ($-CH_2$)	Medium
(D)	1743.32	1743.47	1743	The carbonyl functional group (C = O) of the ester connected to triacylglyceril	High
(E)	1656.72	-	1654	Cis C=C	Weak
(F)	1463.90	1464.42	1465	Symmetrical bending vibrations in aliphatic CH_2 and CH_3 groups	Medium
(G)	1377.44	1377.32	1377	Symmetrical bending vibrations in the $-CH_3$ group	Medium
(H)	1235.98	1235.57	1228	Vibrations of C-O group stretches on esters	Weak
(I)	1158.02	1158.82	1155	Vibrations of C-O group stretches on esters	Medium
(J)	1096.24	1097.08	1097	Vibration bending –CH and changes in vibrational forms of –CH fatty acids	Weak
(K)	967.43	965.56	962	Vibration bending of the -CH functional groups in isolated olefin trans	Low
(L)	844.20	869.78			Weak
(M)	720.79	721.47	721	Overlap vibrations of methyl group shake vibrations ($-CH_2$) with out-of-plane vibrations in the cis-substituted olefin	Medium

Both spectra (lipid fractions of roasted beef meatballs and pork meatballs) look very similar. However, detailed investigation enabled this research to observe some differences, especially in the wavenumbers 1656 cm^{-1} €. Therefore, this region could be used as a target to optimally select the wavenumbers providing the best quantification and classification. These wavenumbers were only observed in FTIR spectra of lipid components extracted from pork meatballs with the weak intensity in the mode of stretching vibrations *cis* C = C. The heating process caused the loss of this functional group in lipid components extracted from roasted beef meatballs. It was suspected that the loss of this group occurred because the thermal oxidation process cleaves the double bonds corresponding to unsaturated fatty acids (Amaral et al. 2018). Linoleic acid (C18:2) in beef fat tended to be less concentrated when compared to lard; thus, the spectra of roasted beef acid linoleic were possibly released. Linoleic acid contained 2 cis-double bonds that had unstable properties during the fat storage process and the burning process (Garcia 2012).

Hermanto et al. (2008) reported that linoleic acid contained in beef was only 1.17%, which is smaller than that in pork (24.94%). Epoxy compounds were also formed in roasted meatball spectra. Epoxy compounds due to symmetric stretching in the epoxy ring occurred around the wavenumber region of 1250 cm^{-1}. Another band appeared in the wavenumber region between 950-810 cm^{-1} and the third band appeared in the wavenumber region between 840-750 cm^{-1} (Pavia 2001). The epoxy compound could occur due to the oxidation of HC = CH- bonds during the combustion process. The oxidation of these triglycerides could produce hydroperoxides, epoxy, hydroxy compounds, keto groups, and dimers (Zhang et al. 2012). The epoxy compounds were deduced from epoxy acid which gave an unexpected aroma and changed the colors of fat to be darker (Gerzt et al. 2014; Papuc et al. 2016).

The multivariate calibration of PLS was selected to predict pork in beef meatballs by analyzing the extracted lipid components. Figure 2 showed some wavenumber region optimization. The optimization results in terms of R^2, RMSEC and RMSEP values were compiled in Table 3. The wavenumber region was selected by considering the highest value of R^2 and the lowest values of RMSEC and RMSEP. Finally, the wavenumber region of 1100-900 cm^{-1} was selected for the PLS modeling between actual values of pork and predicted values of FTIR. FTIR spectra of lipid fractions were extracted from meatballs with different levels of pork and beef. These spectra are depicted in Figure 3.

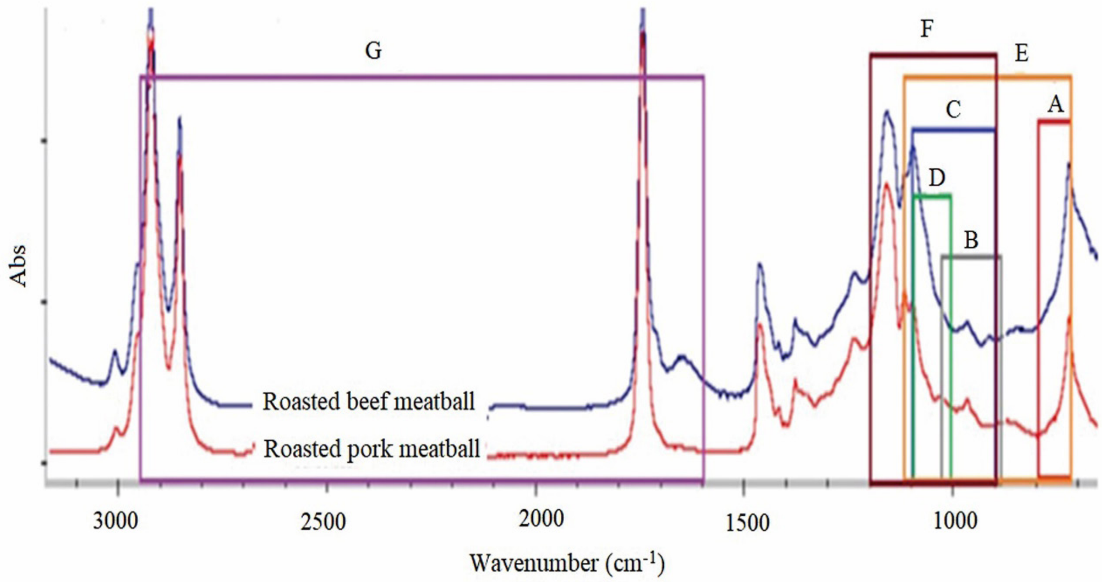

FIGURE 2. Optional areas of roasted meatball wavenumber optimization

TABLE 3. Roasted pork meatballs wavenumbers of 100% reference

Image Code	Wavenumber (cm^{-1})	R^2	RMSEC (%)
A	800-750	0.59	4.38
B	1099-898	0.91	2.73
C*	1100-900	0.99	0.70 *
D	1100-1000	0.90	9.57
E	1110-750	0.96	6.76
F	1200-900	0.96	2.32
G	2920-1600	0.96	2.34

RMSEC: Root Mean Square Errors of Calibration

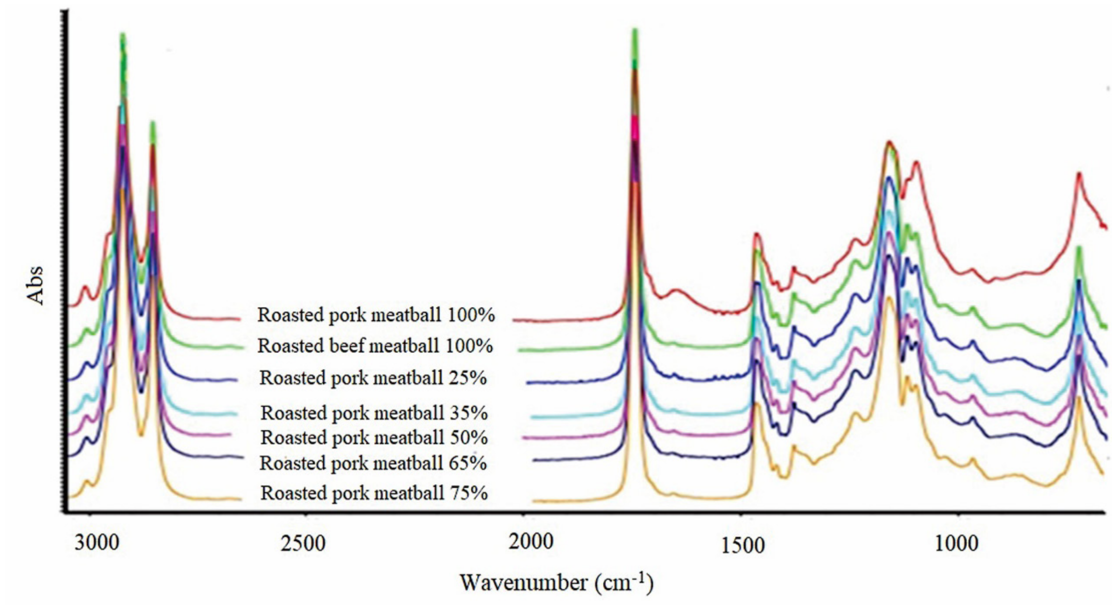

FIGURE 3. FTIR spectra in roasted reference meatballs with various concentration levels

The FTIR spectra of lipid fractions extracted from roasted beef and roasted pork meatballs are almost similar because the main component of these two types of fat is triacylglycerols (Rohman et al. 2011). However, because the property of triacylglycerols served as fingerprint analytical tools, FTIR spectra of both lipid fractions could be distinguished, especially in fingerprint regions (1500-650 cm^{-1}). After performing the optimization process, the selected fingerprint region of 1100-900 cm^{-1} was used to quantitatively analyze (predict) roasted pork in roasted beef meatballs. This condition showed that the R^2 value of the relationship between actual values (x-axis) and predicted FTIR values (y-axis) was 0.99 with the equation of y = 0.99+0.02 and RMSEC value of 0.71% (Figure 4).

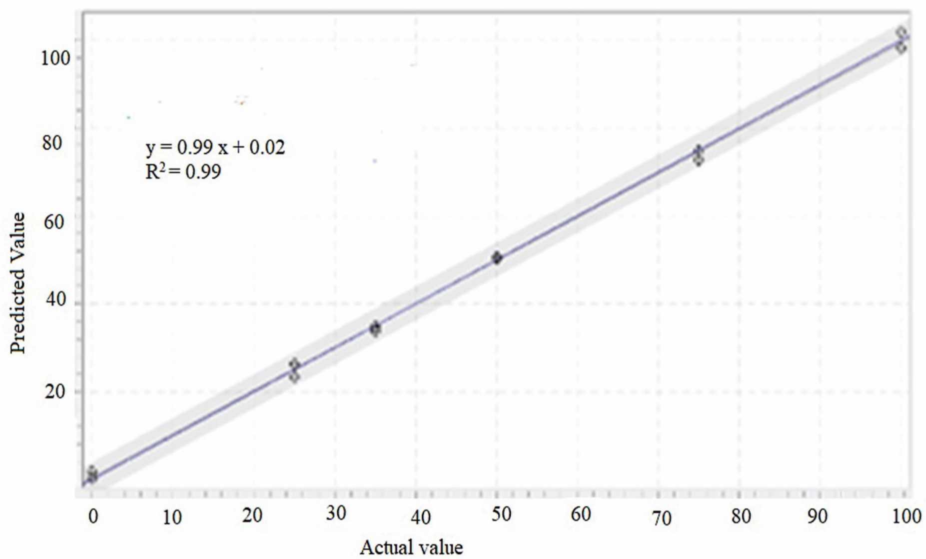

FIGURE 4. Relationship between actual values and predicted values of FTIR fat of roasted reference beef meatballs

The R^2 and RMSEC values can be used to describe the accuracy and precision of the analytical method. From a high value of R^2 and low values of RMSEC and RMSEV, FTIR spectra in combination with PLS can be regarded as an accurate and precise technique to predict pork in roasted beef meatballs (Rohman & Windarsih 2020). Table 4 compiles the performance of statistical parameters to validate and calibrate models. The selected model can be further used to analyze pork in marketed beef meatballs.

TABLE 4. The statistical results for validation parameters of the FTIR spectrophotometric method of roasted meatballs of FTIR-PLS

Step	Validation parameters	Roasted meatballs
Calibration	RMSEC	0.71
	R^2	0.99
	a(Intercept)	0.02
	b(*Slope*)	0.99
Internal validation	RMSECV	0.88
Cross-validation	R^2	0.99
	a (Intercept)	0.10
	b (Slope)	0.99
External validation	RMSEP	0.29
	R^2	1
	a (Intercept)	0.10
	b (Slope)	0.99

RMSEC: Root mean square errors of calibration, RMSEP: Root mean square errors of prediction, RMSCV: Root mean square errors of cross-validation

PCA was used to classify beef and pork meatballs by applying FTIR spectra of lipid fractions extracted from 100% of roasted pork meatballs and 100% of roasted beef meatballs as the variable. The wavenumber regions used by the PCA were equal to that used by the PLS modeling, i.e., 1100-900 cm^{-1}. Figure 5 exhibits the PCA reference sample of roasted beef meatballs and roasted pork meatballs. Moreover, Figure 5 denotes that the quadrants between roasted pork and roasted beef are far apart. The score plot of principal components (PCs) is also known as a latent variable in which the closer PC values, the more similar the physical-chemical properties are. The physical-chemical properties of pork meatballs and beef meatballs are significantly different; thus, they can be separated into different quadrants. These results suggest that the PCA method can be used to classify market-roasted beef meatballs containing lard or pork in the mixture (Rohman et al. 2016).

Furthermore, the lipid fractions extracted from the marketed beef meatballs were classified using the PCA model as shown in Figure 6. One sample of marketed meatballs with code CP 5 (non-branded meatballs) appeared in the same quadrant as that in roasted pork fat. Therefore, the marketed meatballs with Code CP 5 had similar physical-chemical properties to pork meatballs. In addition, the samples with codes CP 1, CP 2, CP 3, and CP 4 were in the same quadrant as that of lipid fraction extracted from reference beef meatballs. This indicated that they were in similar physical-chemical properties to that in beef meatballs.

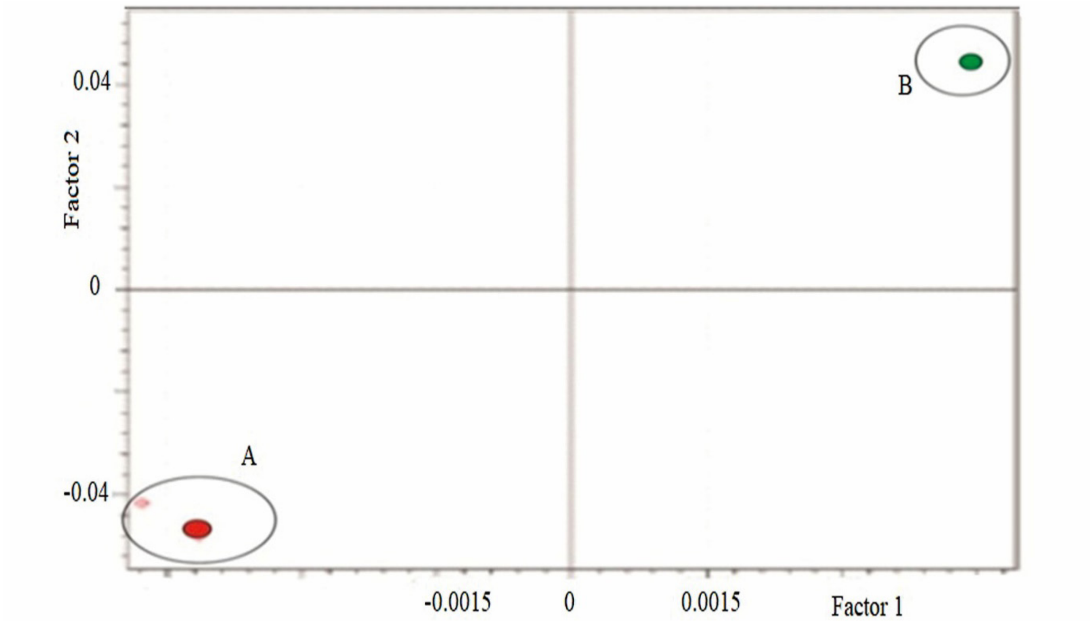

FIGURE 5. Results of PCA analysis on pure samples of roasted pork meatballs (A) and roasted beef meatballs (B)

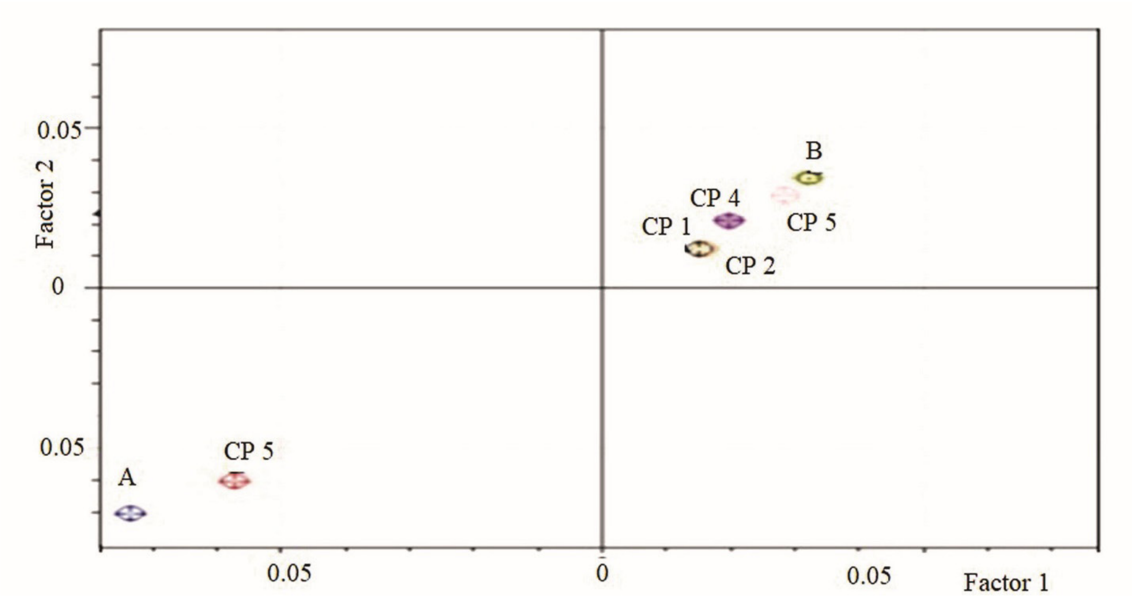

FIGURE 6. PCA scores of market roasted meatballs at wavenumber 900-1100 cm^{-1}: A) pork meatballs, B) beef meatballs, CP) marketed meatball

CONCLUSION

The combination of FTIR spectra at wavenumbers region 1100-900 cm^{-1} and the PLS calibration model could provide an accurate and precise analytical technique to predict pork levels in roasted beef meatballs. In addition, PCA was successfully applied to classify pork meatballs, beef meatballs and marketed meatballs samples. The FTIR spectroscopy and chemometrics provided a rapid and reliable technique to quantify and classify pork in roasted beef meatballs. This technique supports the implementation of Indonesian Act No. 33 (2014) concerning the Halal Assurance System.

ACKNOWLEDGEMENTS

All authors are grateful for the 2015 and 2016 fundamental grants from the Indonesian Ministry of Research, Technology and Higher Education. This research funding was awarded to Prof. Dr. Any Guntarti with a contract number 011/HB-LIT/III/2015. The authors also acknowledged the World Class Professor Program from the Director of Resources, Directorate General of Higher Education, Research and Technology of the Republic of Indonesia that awarded reference letter SK No. 2817/E4.1/KK.04.05/2021 to Dr. Laela Hayu Nurani.

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