Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 14, 2020, p. 1042-1046
https://doi.org/10.5219/1412
Received: 6 June 2020. Accepted: 10 November 2020.
Available online: 28 November 2020 at www.potravinarstvo.com

ISSN 1337-0960 (online)

ANALYSIS OF PORK ADULTERATION IN THE CORNED PRODUCTS USING FTIR ASSOCIATED WITH CHEMOMETRICS ANALYSIS

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ABSTRACT

Meat-based foods such as beef corned became one of the targets of counterfeiting with pork because relatively cheaper. This becomes a serious problem for Muslims, especially in Indonesia. One method that can be used to detect fat was Fourier transform infrared (FTIR) spectrophotometry. The purpose of this study was to quantitatively analyze and a group of corned beef and corned pork using FTIR spectrophotometry combined with chemometrics. Reference samples corned pork-beef made of 7 various concentration (0%, 25%, 35%, 50%, 65%, 75%, 100%) and 6 product samples purchased in the Umbulharjo, Yogyakarta. Extraction was carried out by the soxhlet apparatus using n-hexane technical solvent for 4 – 5 hours at 69 – 70 °C. Fat analyzed using FTIR spectrophotometry for generating infrared spectral data then processed with Partial least square (PLS) chemometrics for quantitative analysis and Principal component analysis (PCA) for grouping. Results of quantitative analysis chemometrics PLS, selected areas fingerprints for analysis corned pork-beef was 1180 – 730 cm^{-1} with R^2 0.9833; RMSEC 2.06%; RMSEP 1.65% and RMSECV 2.22%. The results of PCA showed groupings in different quadrants between corned pork 100% and corned beef 100%. Results showed that FTIR spectrophotometry combined with chemometrics can be used for quantitative analysis and grouping of pork corned and beef corned on the market but it can not identify pork in corned after choking process.

Keywords: corned; beef fat; FTIR; lard; PCA; PLS

INTRODUCTION

Muslims must pay attention to eat food due to it will be part of his body. Therefore, halal food is an obligation for Muslims. Halal food is defined as zero non-halal components including pork. The pork adulteration in food is used because it is cheaper than beef. This is the economic aspect reason, pork can reduce production costs **(Rohman and Che Man, 2010; Guntarti et al., 2017)**. However, we do not only consider the economic aspects. The religious aspect is an important judgment because Muslims regard pork components in processed food products is a serious problem. Islam forbade its followers to consume food products that contain pork **(Regenstein, Chaudry, and Regenstein, 2003)**.

One of the products that allow pork adulteration is beef corned. Therefore, many researchers tried to develop a halal analytical method. The triglyceride (TGA) analysis by HPLC has detection limitations because the hydrolyzed TGA can be detected by HPLC and will interfere with halal authentication **(Rohman et al., 2012[a]; Ahda, Guntari, and Kusbandari, 2016)**. FTIR spectrophotometric has been chosen because it can be combined with chemometrics to detect lard in a mixture of chicken, mutton, and veal **(Nurrulhidayah et al., 2013)**, also to detect lard in CPO and meatball product **(Ahda and Safitri, 2016; Ahda et al., 2020)** and the fat rat in beef meatballs **(Rahmawati et al., 2016)**.

The advantages of FTIR spectroscopy is an efficient analysis for detecting components in a mixture containing animal fat **(Che Man and Rohman, 2011)**. Hence, the presence of pork in processed corned beef products needs an analytical method that is accurate and precise. The use of FTIR as a halal analytical method of corned has not been reported/published. Therefore, this study was performed to determine and distinguish infrared spectra combined with chemometrics for the analysis of lard in the corned beef.

Scientific hypothesis

Lard adulteration in corned beef products can cause different vibration of the FTIR spectrum because lard contains a different TGA composition compared to beef

MATERIAL AND METHODOLOGY

Materials

Samples of pork and beef purchased at a traditional market in Yogyakarta. Spices and other additives for the manufacture of reference samples purchased in supermarkets in the District of Umbulharjo, Yogyakarta. Materials n-hexane (p.a) (Merck), n-hexane (technical) (Merck), acetone (Merck) and anhydrous Na_2SO_4.

Standard Corned Products

The corned beef was made by mixing meat, sugar, pepper, and flour. The meat was steamed for 40 minutes then packed in cans. Standard corned samples were prepared with corned beef containing 7 concentrated levels of pork in beef **(Table 1)**.

Table 1 Reference composition of Corned from beef and pork mixtures.

The concentration of corned pork (%)	Pork (grams)	Beef (grams)	Seasoning and Other Ingredients (grams)	
			Salt	Flour, Sugar, Pepper
100	90.0	0	3.0	7,0
75	67.5	22.5	3.0	7,0
65	58.5	31.5	3.0	7,0
50	45.0	45.0	3.0	7,0
35	31.5	58.5	3.0	7,0
25	22.5	67.5	3.0	7,0
0	0	90.0	3.0	7,0

Fat Extraction of Corned Products

Fat extraction was done with Soxhlet. The solvent used was n-hexane as solvent extraction. A total of 100 grams of corned beef was extracted **(Guntarti et al., 2015)**.

Fat Analysis by FTIR Spectrophotometry Method

Infrared spectra of reference samples and product samples were read by the FTIR spectrophotometer (ABB MB3000, Canada)

Data Analysis and Statistical analysis

Analysis of lard (extracted from corned beef) was carried out by spectrophotometry FTIR and processed by PLS multivariate and PCA analysis. The spectra region that showed the difference spectra of lard with other components were selected to create a model of PLS and PCA **(Miller and Miller, 2010)**.

The accuracy of the calibration models was indicated by the RMSEC and R^2 value obtained from the Horizon MB software (Philadelphia, USA). While the validation models produce RMSEP, RMSECV, and R^2 are calculated following equation below:

$$\mathrm{RMSECV} = \sqrt{\sum_{a=1}^{n} \left(\hat{\mathrm{Y}}\mathrm{b} - \mathrm{Ya}\right)^2 \Big/ \mathrm{N}} \qquad (1)$$

$$\mathrm{RMSEP} = \sqrt{\sum_{a=1}^{n} \left(\hat{\mathrm{Y}}\mathrm{a} - \mathrm{Ya}\right)^2 \Big/ \mathrm{N}} \qquad (2)$$

Where Ŷa is the actual value, Ya is the predictive value, N is the sample number, and The Ŷb is the calculated value for Ya (predictive value) based on the calibration equation with sample a **(Naes, et al., 2004)**.

RESULTS AND DISCUSSION

Design Model for Quantitative Analysis of pork in Corned

Based on the scanning, the infrared spectra showed that both pork and beef corned products contain different vibration of functional groups. The vibration C = O as the ester group was shown at wavenumber 1747 cm^{-1}. These peaks arise because the fat structure is a triglyceride consisting of the carbonyl ester group. Besides, the stretching vibration C – O group was identified at wave number 1238 cm^{-1} for beef, while pork has stretching vibration C – O group at 1234 cm^{-1}. The indication ester vibration was also illustrated at wavenumbers 1157 cm^{-1} and 1099 cm^{-1} **(Jaswir et al., 2003)**.

The vibration of the alkenes group (C = C) was illustrated at wavenumbers 3008 cm^{-1} and 1654 cm^{-1}. The different spectra produced between both pork and beef corned products showed that they have a different composition **(Figure 1)**. It can be said pork contains more unsaturated fatty acids level compared with beef. **Belitz, Grosch, and Schieberle (2009)** reported that pork contains high unsaturated fatty acids (double bonds) including oleic acid (43%) and linoleic acid (9%), while beef contains less unsaturated fatty acid than pork. Detailed vibration of all functional groups is given in **Table 2**.

However, quantitative analysis of lard in the corned products showed that the optimum difference of both pork and beef corned products was obtained at the wavenumber range of 1180 – 730 cm^{-1}. For meat discriminant, pork, beef, and mutton can be seen at 2925 cm^{-1}, 2855 cm^{-1}, and 1745 cm^{-1} with strong peaks and weak peaks at 750 cm^{-1} and 1800 cm^{-1} as fingerprint regions **(Yang et al., 2017)**. Besides, lard detection in cake formulation can be performed by FTIR at 1117 – 1097 and 990 – 950 cm^{-1} **(Syahariza et al., 2005)** and lard identification in steamed sausages and grilled sausages at wavenumbers of 1000 – 791 cm^{-1} and 1070 – 796 cm^{-1}, respectively **(Guntarti, Ahda, and Sunengsih, 2019)**. Even, lard adulteration in palm oils can be detected at 3006 and 1117 cm^{-1} (**Sim, Chai and Kimura, 2018)** or 3006 cm^{-1}, and 1120 – 1095 cm^{-1} **(Che Man, et al., 2013)** or 1481.22 – 999.05 cm^{-1} and 1793.67 – 1650.95 cm^{-1} **(Ahda and Safitri, 2016)**. Therefore, each sample type will change a marker region for lard detection. Based on the wavenumber range of 1180 – 730 cm^{-1}, the result of regression equation is y = 0.95x + 1.32, with R^2 value of 0.983 and RMSEC value of 2.055. The calibration equation is also validated using external validation and cross-validation. The results of cross-validation curve y = 0.9721x - 0.5334 with R^2 values 0.9995 and RMSECV value 2.22%. Besides, external validation has R^2 values of 0.9984, RMSEP of 1.65%, with the equation y = 0.9940x - 0.7061. the higher R^2 value and lower error (RMSEC and RMSECV) are the good indication of the obtained equation **(Rohman et al., 2012[b]; Rohman, Setyaningrum, and Riyanto, 2014; Ahda et al., 2020)**.

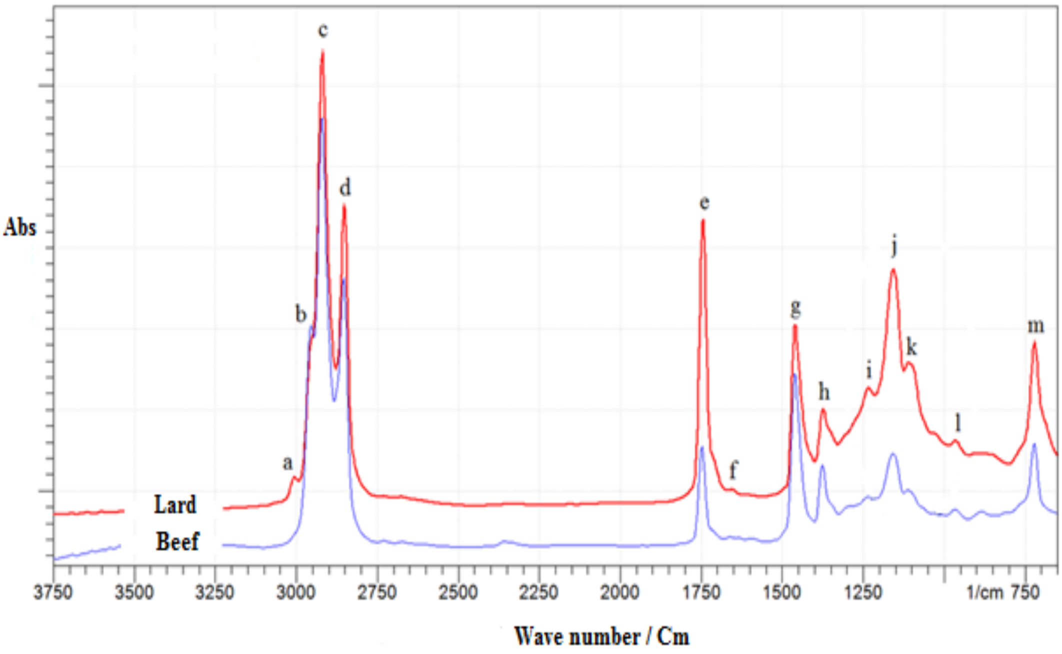

Figure 1 FTIR spectra of standard corneds from prok and beef at wave numbers 4000 – 400 cm^{-1}.

Table 2 Differences of function groups of standard corned from beef and pork.

Ribbon	Wavenumbers (cm^{-1})			Function Group	Vibration Model	Intensity
	Beef	Pork	Reference			
A	-	3008	3000	C=C-H (*cis-*)	Stretch	Weak
B	2954	2954	2960	C-H (CH_3)	Asymmetric Stretch	Medium
C	2920	2920	2930	C-H (CH_2)	Asymmetric Stretch	Strong
D	2854	2850	2850	C-H (CH_2)	Symmetrical Stretch	Strong
E	1747	1747	1750	C=O (esters)	Stretch	Strong
F	-	1654	1650	C=C (*cis-*)	Stretch	Weak
G	1461	1461	1470	C-H (CH_2, CH_3)	Cutout-Bend	Medium
H	1377	1377	1380	C-H (CH_3)	Symmetrical Stretch	Medium
I	1238	1234	1240	C-O (in esters)	Stretch	Medium
J	1157	1157	1160	C-O (in esters)	Stretch	Medium
K	1099	1099	1100	C-O (in esters)	Stretch	Medium
L	964	964	1000	C=C-H (*trans-*)	Out the Field-Bend	Medium
M	721	721	720	$-(CH_2)_n-$	Wobble-Bend	Medium

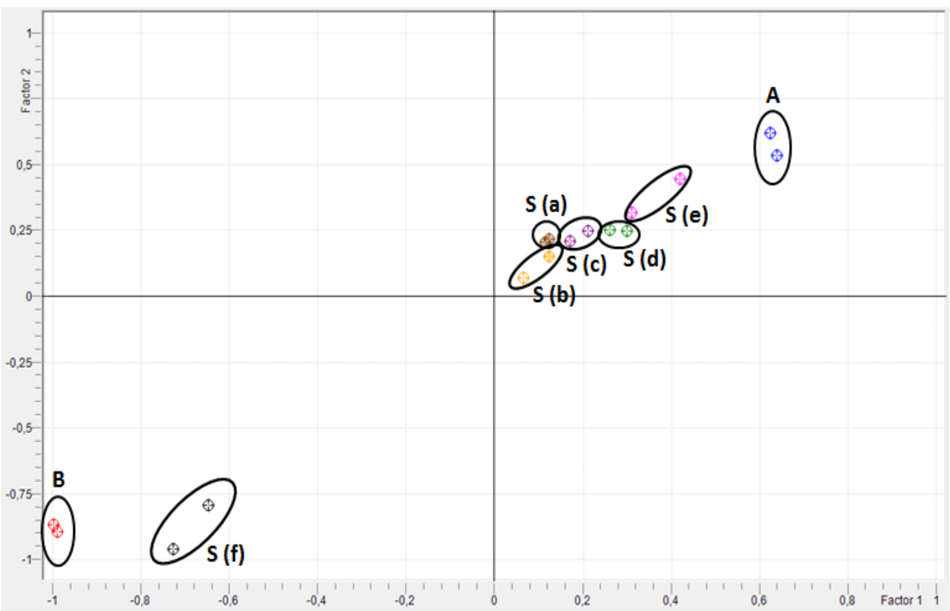

Figure 2 PCA *Score Plot* from Cornet Product Samples in the Market. Note: (A) Corned beef 100%; (B) Corned pork 100%; (S (a, b, c) Coocked commercial corned products; (S (d, e, f)) Raw commercial corned products.

Based on external validation and cross-validation were indicated that the calibration model at wave number 1180 – 730 cm^{-1} was able to give accurate results for the quantitative analysis of pork in the corned mixtures.

Analysis of Pork Adulteration in Commercial Corned Products

Analysis of pork in a food product can be performed and grouped by PCA analysis. It is an analytical technique to reduce the data when it found a correlation between the data **(Garcia, 2012)**. The PCA is unsupervised pattern recognition techniques widely used for the classification of different samples **(Nunes, 2014; Rahmania, Sudjadi and Rohman, 2015)**. PCA analysis will reduce the number of independent variables in the data to produce the new variables that are called the principal component or major components **(Che Man, Syahariza, and Rohman, 2010)**. Hence, the wavenumber regions for PCA were also optimized. Finally, the same wavenumbers used for quantitative analysis were chosen for PCA modeling due to its capability to provide good separation among the evaluated samples **(Rahayu et al., 2018; Gamperline, 2006)**.

In this study, pork analysis in the corned product is performed in optimum condition at wavenumbers of 1180-730 cm^{-1}. The discriminant analysis showed that 100% pork corned and 100% beef corned can be separated and distinguished **(Lumakso et al., 2015)**, it illustrated that both corned products have a different composition **(Figure 1)**. Therefore, analysis of pork adulteration in the commercial corned products can be performed in similar conditions **(Van der Spiegel et al., 2012)**.

In this research, we identify 3 commercial corned products and also observe the cooking effect in disrupting halal analysis. The result showed that 3 commercial corned products are not at all produced from beef. Sample (f) are grouped as pork corned, hence we can estimate that it is made from pork **(Figure 2)**. However, all samples are grouped in the beef corned product after the cooking process. It showed that the cooking process can affect the chemical properties of pork. The unsaturated fatty acids of pork may degrade during the heating process because pork corned is not separated from beef corned **(Bhaskar et al., 2012)**. Therefore, halal authentication using FTIR combined with chemometrics has a problem if the product is carried out by different process because they are possible to degrade during the process **(El-Gindy, Emara, and Mostafa, 2006)**.

CONCLUSION

FTIR spectrophotometry combined with chemometrics at wavenumbers of 1180 – 730 cm^{-1} resulted in a good correlation between the predicted value and actual value with R^2 of 0.9833, RMSEC of 2.06%, RMSEP of 1.65%, and RMSECV of 2.22%. Hence, it can be used for quantitative analysis of lard in the corned product. At wavenumbers 1180 – 730 cm^{-1}, halal authentication can be performed clearly and one of the commercial corned products was identified pork in its product. However, this method can not identify pork corned after it is cooked. Therefore, the cooking process will affect chemical compositions in commercial corned through the degradation process of unsaturated fatty acids.

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