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Analysis of lard in meatball broth using Fourier transform infrared spectroscopy and chemometrics

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ABSTRACT

Meatball is one of the favorite foods in Indonesia. For the economic reason (due to the price difference), the substitution of beef meat with pork can occur. In this study, FTIR spectroscopy in combination with chemometrics of partial least square (PLS) and principal component analysis (PCA) was used for analysis of pork fat (lard) in meatball broth. Lard in meatball broth was quantitatively determined at wavenumber region of 1018–1284 cm^{-1}. The coefficient of determination (R^2) and root mean square error of calibration (RMSEC) values obtained were 0.9975 and 1.34% (v/v), respectively. Furthermore, the classification of lard and beef fat in meatball broth as well as in commercial samples was performed at wavenumber region of 1200–1000 cm^{-1}. The results showed that FTIR spectroscopy coupled with chemometrics can be used for quantitative analysis and classification of lard in meatball broth for Halal verification studies. The developed method is simple in operation, rapid and not involving extensive sample preparation.

1. Introduction

Transparency in meat speciation used in food products is an ever increasing demand and is essential for the protection of consumers' right, religious credence and hard-earned fortunes (Ali, Hashim, Mustafa, Che Man, Dhadi et al., 2012; Doosti, Ghasemi Dehkordi, & Rahimi, 2011; Fajardo, González, Rojas, García, & Martín, 2010). Verification of declared components in food products is also necessary for the prevention of adulteration practice. For this purpose, some countries make regulation for assuring that food products available are safe and authentic. Therefore, detection of species fraud in meat products including meatball is important for consumer protection and food industries (Doosti et al., 2011).

In Indonesia, one of the favorite foods consumed is meatball, or known as '*bakso*' (Rohman, Sismindary, Erwanto, & Che Man, 2011). Currently, in Indonesia, due to the high different prices between pork and beef, the adulteration practice of beef meatball with pork meatball is occurring. Meatball is a processed comminuted meat which can be classified as restructured meat. It can be prepared using beef, chicken, pork, or fish meat, and the one that is very popular and widely found in the Indonesian market is beef meatball (Purnomo & Rahardiyan, 2008). Sometimes, unscrupulous seller replaces beef meatball with pork meatball in order to gain economical profits.

The substitution of beef with pork is a serious problem not only for economic reason but also for religious point of view. Muslim and Jew communities are not allowed to consume food products containing pig derivatives like pork. In Islamic and Jews, pork is not halal and not kosher (Regenstein, Chaudry, & Regenstein, 2003; Rohman & Che Man, 2012). In the Middle East and other Islamic countries, especially in East Asia, halal certification has been made mandatory for all meat and meat based imported food products like meatball. Halal verification needs a reliable method assuring that non-halal items like pork are absent in food products (Nakyinsige, Che Man, & Sazili, 2012). As a consequence, some analytical methods have been developed, proposed and used for analysis of pork in food products (Mursyidi, 2013).

Analysis of pork in meatball and other food products can be performed by DNA amplification present in pork using polymerase chain reaction using different targets of amplification (Ali, Hashim, Dhahi, et al., 2012; Ali, Hashim, Mustafa, & Che Man, 2012; Ali, Hashim, Mustafa, Che Man, Dhahi, et al., 2012), electronic nose and gas chromatography–mass spectrometry by detection of the aroma and volatile compounds of lard present in meatball (Nurjuliana, Che Man, Mat Hashim, & Mohamed, 2011), and Fourier transform infrared (FTIR) spectroscopy by analyzing pork fat or lard as a whole of matter extracted from pork (Rohman, Sismindary, et al., 2011).

FTIR spectroscopy coupled with chemometrics is promising analytical techniques to be used in the halal verification studies. It is fast, not destructive and not involving laborious sample preparation. In halal authentication, FTIR spectroscopy has been exploited for analysis of lard in the binary mixture with other animal fats with the aid of multivariate

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calibration in combination with discriminant analysis (Che Man & Mirghani, 2001; Jaswir, Mirghani, Hassan, & Mohd Said, 2003), as well as for analysis of lard in cake and chocolate formulation using partial least square calibration (Che Man, Syahariza, Mirghani, Jinap, & Bakar, 2005; Syahariza, Che Man, Selamat, & Bakar, 2005). Previously, we have analyzed pork fat in meatball products (Rohman, Sismindary, et al., 2011). In that research, we extracted lard from pork fat contained in meatball in which some other components may be present in extracted lard. In this study, we develop FTIR spectroscopy in combination with partial least square and principal component analysis for quantification and classification of lard in broth of beef meatball. Due to the different components that may be present in lard extracted from meatball broth and lard extracted from pork fat in meatball, indeed, the calibration model should be developed.

2. Materials and methods

2.1. Preparation of animal fats

The pork fat (lard) and beef fat were obtained by rendering process of corresponding animal. The procedure of rendering follows with that reported in Rohman and Che Man (2009). The adipose tissues of pig and cattle were cut into small pieces using commercial cutter in order to effectively extract lard and beef fat. Using Beaker glass, the cut tissue was introduced into conventional oven during 3 h at 100 °C. The melted fat was strained with filter paper. The residue of water was removed using anhydrous sodium sulfate. The fat obtained was further used for analysis of fatty acid composition and FTIR spectra analysis.

2.2. Fatty acid composition

The composition of fatty acids composed of lard and beef fat was carried out using gas chromatography with flame ionization detector (GC-FID). The GC condition was as reported in Rohman et al. (2012). As standard of fatty acid methyl esters (FAMEs), we used 37 compounds (C4 to C24) from Sigma Chemicals (St. Louis, MO, USA) to identify the retention times of FAME in lard and beef fat. Quantification analysis of FAMEs was performed using internal normalization technique.

2.3. Preparation of calibration samples

A set of standards consisting of lard in beef fat was prepared by mixing of both at concentration ranges of 0–100% (v/v) of lard in beef fat. Selected samples, which were different from calibration samples, were used as validation samples. Lard, beef fat along with their blends were measured using FTIR spectrophotometer. The spectral regions in which the variations among analytes were observed were selected for developing multivariate calibration and principal component analysis.

2.4. Analysis of lard in meatball broth

The meatball broth was taken from several markets in Yogyakarta. An approximately 100 mL of meatball broth was taken, added with 100 mL hexane, and subjected to liquid–liquid extraction in a separatory funnel. The mixture was shaken vigorously and the hexane phase containing fats was taken. The water phase was further extracted using 50 mL of hexane. The hexane phases were collected and evaporated under vacuum rotary evaporator at 60 °C, and the residue of water was dried with anhydrous sodium sulfate. The fats obtained were subjected to FTIR measurements.

2.5. FTIR spectral acquisition

FTIR spectra of all samples was scanned in the mid infrared region of 400–4000 cm^{-1} using ABB MB3000 FTIR spectrophotometer (Clairet Scientific, Northampton, UK) equipped with deuterated triglycine sulfate (DTGS) detector and KBR as beam splitter, with a resolution of 8 cm^{-1} and 32 scanning. Spectra were processed using Horizon MB FTIR software version 3.0.13.1 (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 were rationed against a background of air spectrum. After every scan, a new reference air background spectrum was taken. These spectra were recorded as absorbance values at each data point in triplicate.

2.6. Statistical analysis

Quantitative analysis of lard was performed using partial least square calibration with the aid of Horizon MB software (Canada) included in FTIR spectrophotometer. While, the classification among meatball broth samples was carried out using principal component analysis with the software of Minitab (version 16, USA).

3. Results and discussion

3.1. Fatty acid analysis

Fatty acid composition is one of the parameter used in the quality control of edible fats and oils. The fatty acid profiles can be used for differentiation of edible fats and oils due to its capability to provide the fingerprint profiles of studied fats and oils.

Table 1 revealed the fatty acid composition of lard and beef fat. Beef fat contained more palmitic and oleic acids, while lard contained more oleic and stearic acids, respectively. The composition of these fatty acids in lard and beef fat was in agreement with those specified in Codex Allimentarius (2003).

3.2. FTIR spectra of lard and beef fat

The importance of IR spectroscopy in identification of samples originates from the much information content obtained, and the possibility to assign certain absorption bands related to functional groups (Bendini et al., 2007). Fig. 1 is FTIR spectra of lard and beef fat. Each peaks and shoulder indicated the functional groups responsible for infrared absorption at wavenumbers of 4000–400 cm^{-1}, corresponding to stretching and bending vibrations of functional groups. Peak assigned with (a) at 3007 cm^{-1} attributed from the stretching vibration of *cis* C═CH. Table 2 described the assignment peaks and shoulders present in lard and beef fat (Guillen & Cabo, 1997; Lerma-Garcia, Ramis-Ramos, Herrero-Martinez, & Simo-Alfonso, 2010).

FTIR spectra of beef fat and lard are difficult to be differentiated, however, due to the capability of FTIR spectra as fingerprint tools, both spectra revealed bit differences in peak/shoulder intensities and the exact wavenumbers at which the maximum absorbance was observed for each fat and oil, due to the different nature and composition of the both lard and beef fat, especially at wavenumber regions of 3007 (a), 1117 (l) and 1098 cm^{-1} (m). The wavenumber of 3008 cm^{-1} originates from *cis*-olefinic C═H, which can be used as an indicative of unsaturation degree. The more unsaturation degree of fats and oils, the higher the peak intensities in that wavenumber. Meanwhile, wavenumbers of 1117 and 1098 cm^{-1} originate from the stretching vibrations of C – O in triacylglycerols. As shown in Table 1, lard has more unsaturated fatty acid than that in beef fat. As a consequence, lard revealed higher peak intensity than beef fat at wavenumber of 3007 cm^{-1} (Che Man, Rohman, & Mansor, 2011). This wavenumber region in which lard and beef fat showed some differences was further optimized to be selected as wavenumber used for quantitative analysis of lard in meatball broth.