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ORIGINAL ARTICLE

Identification of Adulterated Sausage Products by Pork using FTIR and GC-MS Combined with Chemometrics

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ABSTRACT: A technique for halal food analysis, especially sausage products, can be performed based on the lard content in the products. In this study, we compared both Fourier transforms infrared (FTIR) and Gas chromatography Mass spectroscopy (GC-MS) instruments to detect lard in sausage products. FTIR spectroscopy revealed differences in the vibration of functional groups, while GC-MS detected Fatty acid methyl ester (FAME) compositions in sausage products. The difference in data obtained was processed by PCA to distinguish beef sausage. The use of FTIR spectroscopy is simpler in handling samples than GC-MS. However, FTIR spectroscopy can not explain the chemical compositions that distinguish between halal and haram products. Therefore, halal food analysis using GC-MS confirmed and clarified the products adulterated by pork. Discriminant analysis of commercial sausage products using FTIR was performed at wavenumbers 1200 – 1000 cm^{-1} and all sausage samples did not contain pork. It was also clarified with GC-MS to ensure their halal-ness based on the FAME compositions. The loading plot showed that pork sausage has lauric acid, myristic acid, and palmitoleic acid as fatty acids that distinguish it from beef sausage. Based on these results, FTIR spectroscopy and GC-MS combined with chemometrics can be performed for halal detection in sausage products and classified successfully between pork sausage and beef sausage.

INTRODUCTION

Halal food is one of the great goals discussed and investigated because it is the basic information needed for human welfare, mainly for Muslims. In the modern era, food production has been created based on great intelligence, science, and modern technology [1]. Hence, the halal and haram components in food are absolutely difficult to identify with our eyes directly [2]. In general, non-halal is usually associated with pig derivatives; for example, pork, lard, and pork gelatin. The pork is less expensive than the beef [3]. Therefore, the use of these pig

derivates can cause a profit increase. This phenomenon also occurs in sausages because they are usually processed using meat. Sausage is one of the food products in demand by consumers because it has practical value.

Currently, producers will blend the haram meat into the sausage product to increase their business profit. Therefore, halal certification in food products is very important to preserve the consumer from haram foods. Hasan and Awang [4] informed that both producers and consumers are concerned to understand the benefits of halal certification

in food products. Hence, halal has recently increased and it is everyone's responsibility including the government. Even the government and companies have to ensure that food produced has halal certification [5]. Nakyinsige [6] also said that the leaders of the meat industry should know and accept the rules of sharia in halal meat production. Therefore, the halal food industry contributes to community development and national economic growth [7].

Halal foods also encourage many researchers to develop several methods for halal food inspection. The development of the analytical method is a challenge to look for the appropriate method for detecting non-halal compounds because they are hard to detect [8]. Several analytical methods used to identify non-halal products include FTIR spectroscopy [9, 10], HPLC [11, 12], gas chromatography [13], differential scanning calorimetry with certain detection limits, and polymerase chain reaction [14]. Che Man [15] reported that FTIR can identify lard, which is mixed in cocoa flour, from a chocolate product, quickly, accurately, inexpensively, and also environment friendly. Moreover, GC-MS was also used for fatty acid analysis in food or meat. The fatty acid contents resulting can be used as a differentiator in each sample [13]. One obstacle to the halal analysis issue is ensuring that this method is a truly valid method for analyzing non-halal substances in food ingredients [2]. Therefore, this study conducted a comparative analysis using FTIR spectroscopy and GC-MS for halal food analysis on sausage products.

MATERIALS AND METHODS

Chemical and reagents

$NaOCH_3$ 0.2 N, N-hexane, anhydrous Na_2SO_4, sausage components (beef, pork, salt, and flour), and commercial sausages were bought in the market, Yogyakarta.

Sausage producing

Sausage products were made by containing 90% of meat (adulterated sausage with several concentrations of lard around 0%, 25%, 35%, 50%, 75%, and 100%) and 10% flour, salt, and seasonings. All components were mixed by

machine or a blender until homogeneous. The blended material was put into plastic and then steamed for 10 minutes. The weight of each sausage is around 250 grams.

Oil extraction of sausages

50 grams of sausages are extracted with n-hexane at 70°C for 6 hours using the Soxhlet apparatus. The oil obtained is added with anhydrous Na_2SO_4 to absorb water in the oil. Furthermore, the oil was separated from N-hexane by evaporation in a fume hood. The obtained oil is stored at a temperature of around 100°C.

Sausage analysis using FTIR Spectroscopy

The obtained oil was analyzed using FTIR spectroscopy. 20 microliters of oil were placed on the ATR crystal at a controlled temperature (20°C) and measured on 32 scans and at a resolution of 4 cm^{-1}. The range of FTIR wavenumber in this analysis is 4000-400 cm^{-1}, and recorded as absorbance.

Derivatization of fatty acids from sausages

10 mg of oil was dissolved in N-hexane as much as 10 ml in a 10 ml measuring flask. This solution was taken as much as 3 mL and mixed with 0.2 ml $NaOCH_3$ catalyst as much as 3 ml and also mixed for 2 minutes under a temperature of 80°C and repeated once. To separate the oil, it was added 1.5 ml of saturated NaCl and centrifuged for 10 minutes. The upper layer of the solution is FAME (fatty acid methyl ester). Moreover, FAME was injected into the Gas Chromatography-Mass Spectrometer (GC-MS) instrument with the controlled system such as coloum used is capiler RTX-5 (30 m x 0,25 mm, width 0,25 μm, i.d 0,25 μm containing mixed silica (diphenyl and dimethyl polysiloxane) with a total flow of mobile phase is 60 mL min^{-1}. Injector temp: 300°C and oven temp: 50°C (hold for 5 min), and increase up to 260°C (5°C/menit) and final temperature is 260°C for 8 min. Detector is Mass Spectrometer detector (IonSourceTemp: 200.00°C; Interface Temp.:305.00°C; Detector Gain: 0.92 kV +0.00 kV).

Chemometrics analysis of FTIR spectrum and chromatogram of GC-MS

Horizon ABB MB 3000 software version 3.0.13.1 (ABB, Canada) is applied to create the classification model of sausage products from the FTIR spectrum, the discrimination of sausage products based on the fatty acid content is performed using Minitab software version 15.

RESULTS AND DISCUSSION

Identification of adulterated lard in sausage products based on the vibration of functional groups

Halal authentication in food products has been developed because it helps consumers to ensure their safety and security. Previous research showed that FTIR is one of the instruments used in halal authentication due to it is a friendly and fast method for halal authentication. Rohman and Che Man [16] reported that the FTIR spectrum at wavenumbers 1500-1000 cm^{-1} can discriminate the lard with goat, beef, and chicken fat. Besides, Ahda [10] reported that the use of FTIR can be applied for the identification of adulteration of wild boar in meatballs at wavenumbers 999 - 1481 cm^{-1} and 1650 - 1793 cm^{-1} at 1st derivative. Figure 1 showed that the difference between pork sausage, beef sausage, and commercial sausage is 1,200-1,000 cm^{-1}. To find the best correlation of pork in beef sausage was determined, and the highest determination and the lowest error score are explained in Table 1.
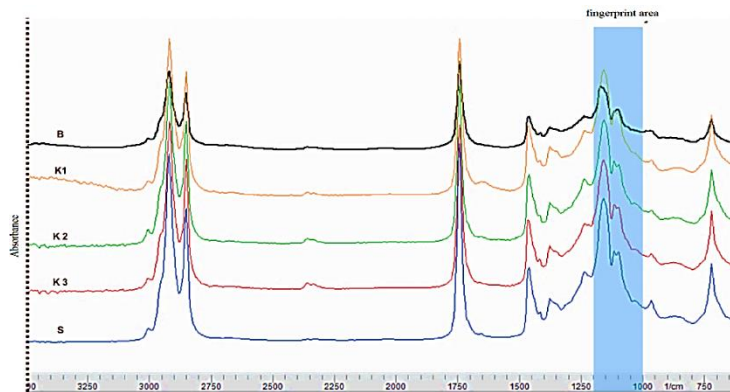

Figure 1. FTIR spectrum of fat form sausage: B is lard; S is beef; K1-3 are Commercial products

Table 1. The determination coeficient value and error score at several wavelenght of FTIR spectrum.

wavelenght (cm^{-1})	R2 Value	RMSEC (%)
1000 – 900	0.58	4.50
1200 – 1000	0.99	2.09
1200 – 900	0.94	2.80
1200 – 750	0.90	3.18
1200 – 650	0.91	3.05

Table 1 showed that the best calibration equation is performed at 1,200-1,000 cm^{-1} because it has the highest R2 value and smallest of the smallest root-mean-standard error for calibration (RMSEC). Previous research reported that FTIR spectroscopy at wavenumbers 1200-1000 cm^{-1} can be applied for analysis of lard adulteration in commercial products such as cream products [17], sausage products [18], and imported chocolate products [19], and meatball products [20, 21]. The research from Rohman [21] also identified successfully pork in beef meatballs using FTIR spectroscopy combined with principal component analysis (PCA) at wavenumbers 1200-1000 cm^{-1}. The determination coefficient (R2) is near 1 indicating a good correlation between the x and y axis, while a smaller RMSEC illustrates smaller deviations from the real data or illustrates the preciseness of the model [22]. Both R2 and

RMSEC values can explain the resulted calibration criteria [16]. Hence, a high value of R2 and the low value of RMSEC explained that it is an acceptable method for halal authentication [23].

Identification of the 3 commercial sausages (CS 1, 2, and 3) is nearest and is clustered with 100% beef sausage. Hence, the predicted meat used in commercial samples is created from beef and is not pork. The discriminant analysis was able to classify and separate clearly pork and beef in sausage so halal and non-halal products can be identified (Figure 2). This result showed FTIR spectroscopy combined with chemometrics can be applied for halal authentication in sausage products.
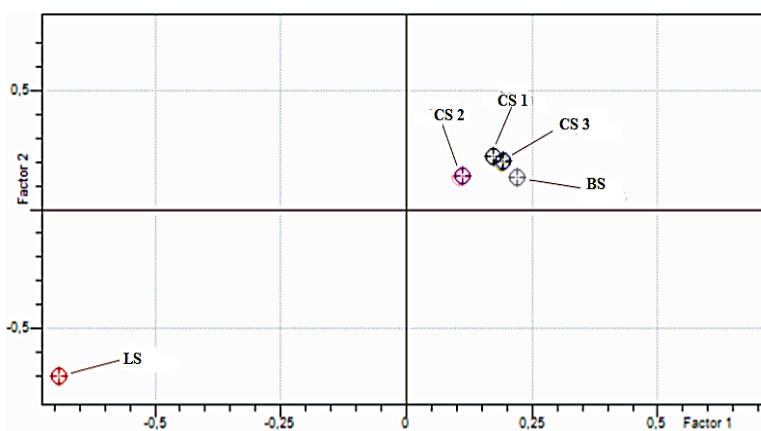

Figure 2. The Discrimination of Sausages by FTIR combined with PCA at wavenumbers 1200-1000 cm^{-1}; LS: Pork Sausage; BS: Beef Sausage; CS 1, 2, and 3 are Commercial Samples

Identification of adulterated lard in sausage products

based on the FAME compositions

To ensure halal authentication by FTIR, the analysis of fatty acid compositions in sausage products can be conducted. Figure 3 describes the different fatty acid compositions in sausages between 100% pork sausage standard and 100% beef sausage standard. Besides, the fatty acid compositions in lard and beef are not the same compared with both sausage standards. Lard contains 9 fatty acid types, beef contains fatty acid types. The difference between lard and beef is lard contains Linoleic acid but pork sausage does not have it. This case could be degraded due to the production process of sausage. The change in linoleic acid is not significantly different at 35°C and 75°C but it decreases markedly at 105oC and 135°C [24]. Another research from Raba [25] said that the high temperature (up to 200°C) will decrease unsaturated fatty acids because they may be oxidized.

This problem caused difficulty to direct investigation because it needed a statistical tool for calculating and predicting more impler. The discriminant analysis was performed to classify and distinguish between lard, beef, and both sausage standards (Figure 4). Figure 4 showed that beef sausage and beef are neighboring because both materials have similar fatty acid compositions. However, lard and pork sausages have been located far from each other. Because lard has a higher Polyunsaturated Fatty Acids (PUFA) content than beef fat [26]. The high unsaturated fatty acid in lard will cause degradation to occur. Nizar [27] reported that lard contains unsaturated fatty acid content (MUFA+PUFA) of around 60.6% while beef has about 44.9%. This result explained that the adulteration of sausage by lard can be distinguished using GC-MS combined with PCA. Hence, commercial sausages were also discriminated against based on fatty acid compositions (Figure 5). Figure 5 shows that both principal components are PC1 and PC2, which are 53% and 34.6%, respectively. It means that both PCs have explained 87.6% of nature variables. Commercial sausages are far from 100% pork sausage due to; they can be said, free pork or

lard. This study also concluded that both FTIR and GC-MS are suitable methods for halal food analysis if they are combined with chemometrics for simpler observation or decision-making.
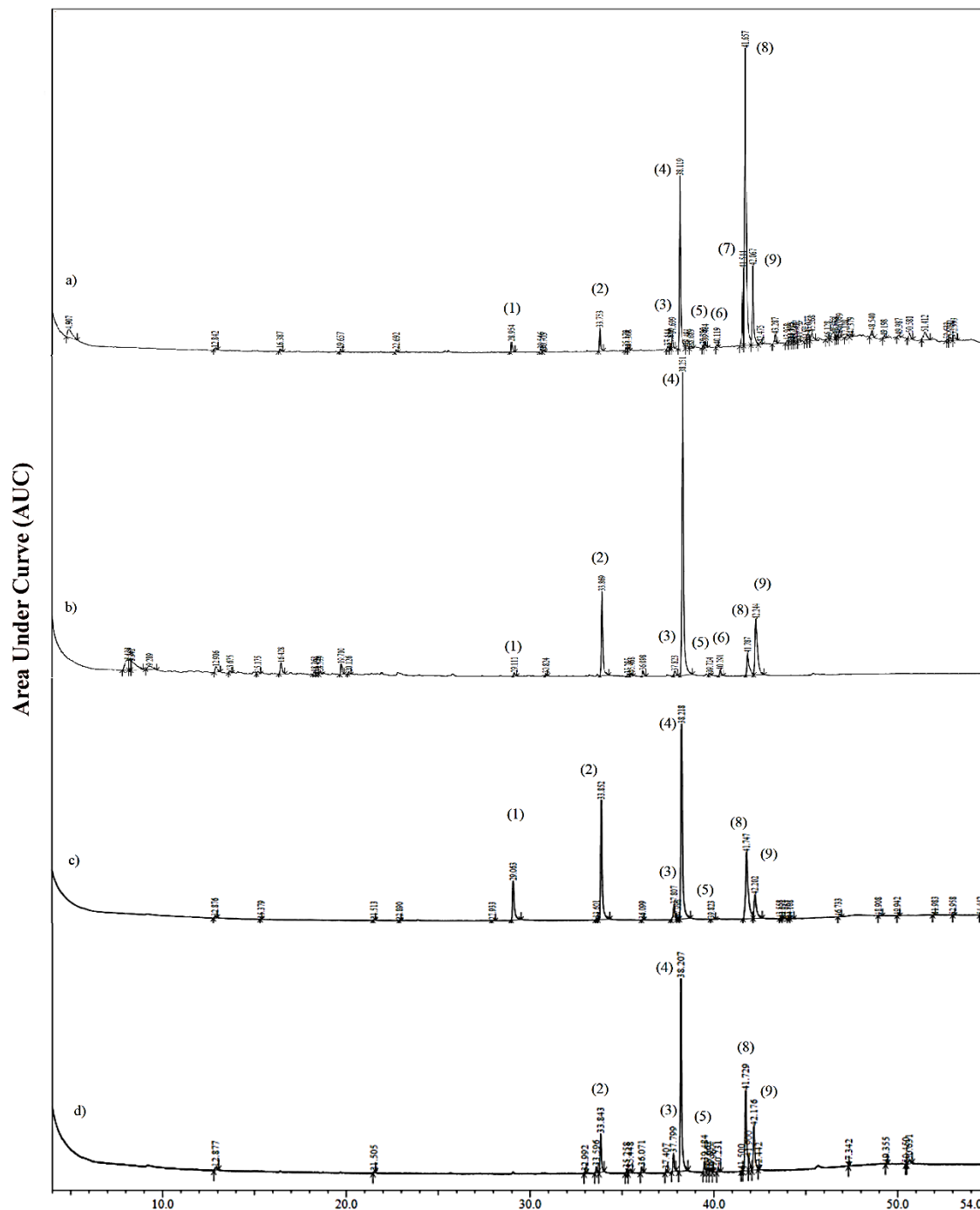

Figure 3. FAME compositions in lard (a); beef (b); pork sausage standard (c); and beef sausage standard (d). 1) Lauric acid (tr: 28.95 min); 2) Myristic acid (tr: 33.75 min); 3) Palmitoleic acid (tr: 37.69 min); 4) Palmitic acid (tr: 38.16 min); 5) 9,10-methylenehexadecanoic acid (tr: 39.69 min); 6) Margaric acid (tr: 40.11 min); 7) Linoleic acid (tr: 41.54 min); 8) Oleic acid (tr: 41.66 min); and 9) Stearic acid (tr: 42.06 min)

The loading plot depicts that fatty acid types contribute to discriminating between lard sausage standards, beef sausage standards, and all commercial sausage products. Lard sausage contains lauric acid, myristic acid, and palmitoleic acid as discriminating compounds. Whereas beef sausage and all commercial sausage products have

some fatty acid compounds such as Oleic acid, Palmitic acid, Pentadekanoat acid, Heptadecanoic acid, and Stearic

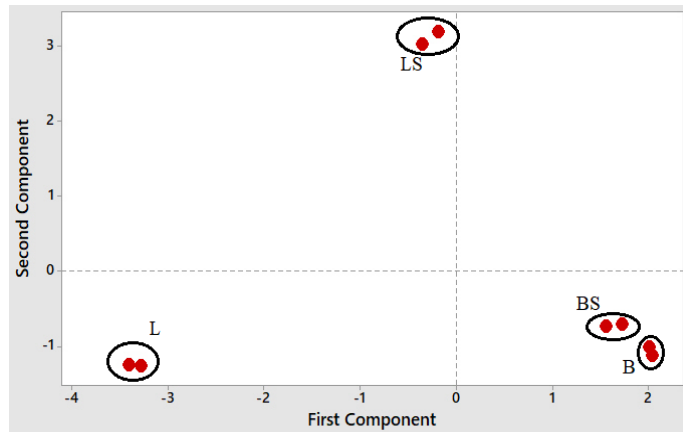acid, contributing to this discriminant analysis (Figure 6).

Figure 4. Discriminant analysis of lard, beef, and both pork sausage and beef sausage based on fatty acid compositions. L: lard; B: beef; LS: pork sausage; BS: beef sausage
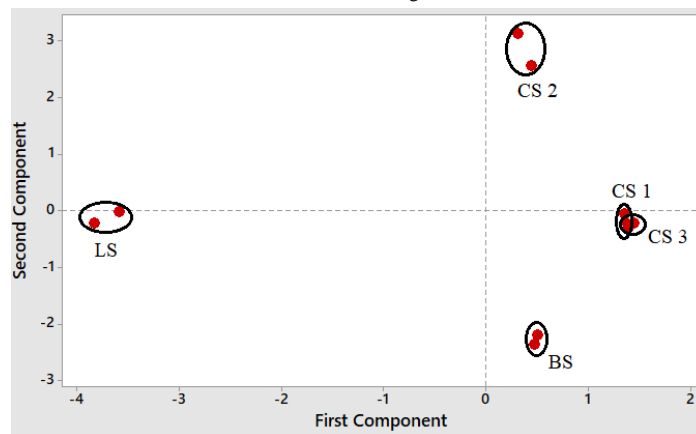

Figure 5. The Discrimination of Sausage products by GC-MS combined with PCA; LS: Pork Sausage; BS: Beef Sausage; CS 1, 2, and 3: Commercial Samples.
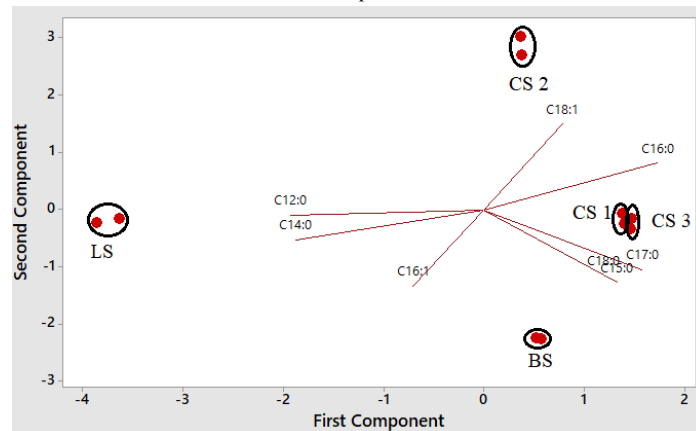

Figure 6. Biplot from the result of discriminant analysis of Sausage products by GC-MS combined with PCA; LS: Pork Sausage; BS: Beef Sausage; CS 1, 2, and 3: Commercial Samples

CONCLUSIONS

Commercial sausages can be identified based on the fingerprint of functional groups and also fatty acid types

from meat sources used in sausage products. FTIR combined with chemometrics can be used for the halal

analysis of commercial sausage products in optimum condition at wavenumbers 1200-1000 cm⁻¹. Besides, halal analysis using GC-MS identified fatty acids in the pork sausage such as pork sausage has lauric acid, myristic acid, and palmitoleic acid. Both results showed that FTIR and GC-MS are suitable instruments for halal authentication with a similar result.

ACKNOWLEDGEMENT

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