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Short Communication

Halal food analysis using GC-MS combined with principal component analysis (PCA) based on saturated and unsaturated fatty acid composition

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Abstract

An analytical method using GC-MS for food authentication from haram meat is an important thing for Muslims. In this research, halal food authentication was based on detecting the fatty acid contents. The results show that a beef meatball product tainted by other than beef fat can be detected based on fatty acid compositions. Further, the food authenticity can also be detected based on the SFA:MUFA ratio which for pork meatball is approximately 1.0, for wild boar meatball around 1.2, and for beef meatball about 3.6. These results were also supported by PCA analysis, where the food products are grouped appropriately to match their meat mixture. The commercial meatball product tested was not adulterated by pork or wild boar, and did not contain beef meat.

Keywords: meatball, GC-MS, fatty acid types, food authenticity

1. Introduction

Meatball is a product that contains a meat protein mixture from beef, chicken, fish, and shrimp. Meatball is made from ground meat that is mixed with some additives, such as salt, tapioca flour, and seasonings; shaped to a ball weighing about 25-30 g/ball. The meatball has a chewy texture as a specific characteristics after cooking. Meatballs are among the favorite foods of many consumers. However, counterfeit meatballs are made by including non-halal ingredients, because of the high cost of halal meat, such as beef, fish, and shrimp.

For a Muslim, halal food is an obligation, because God told the Muslim society to consume halal food. Therefore, the Indonesian government has made a regulation relating to halal products, namely Law No. 33 in 2014 that guarantees the halal products. The government regulations support giving attention to halal food, including development of methods for detecting adulteration of halal food products. One prohibited source of meat is the pig, for meat used as a food ingredient. A method that can be used for the analysis of lard is the Fourier Transform Infrared (FTIR) spectro photometry (Rohman & Che man, 2011). Pork fat mixed with beef fat, sheep fat, and chicken fat can also be analyzed by FTIR spectroscopy, combined with partial multivariate quadratic calibration or Partial Least Squares (PLS) (Che Man & Mirghani, 2001). Counterfeit butter and chicken fat have also been successfully detected using FTIR spectroscopy with the help of a PLS calibration model (Nurrulhidayah, 2013). Other analytical methods used to detect fatty acids in meat are GC-MS (Nugraha *et al.*, 2018), SYBR green RT-PCR (Farrokhi & Joozani, 2011), and TaqMan RT-PCR probes (Ali *et al.*, 2012).

Aripin and Huda (2018) reported that meatball has proximate composition and physicochemical properties dependent on the meat it is made from. Hence, fat detection using GC-MS to detect the fatty acid composition can be used for differentiation (Nugraha *et al.*, 2018). Therefore, this study

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aimed to analyze the contents of fatty acids in various meat mixtures as meatball products, for use in halal studies. The analysis of the fatty acid components in meatballs by using GC-MS is based on transesterification, usually with acidic or basic catalysts. The weakness of an alkaline catalyst is that it will work well in the limit of free fatty acids (ALB) <0.5%, while with high free fatty acids saponification reactions with bases cause problems (Shu *et al.*, 2010).

2. Research Methods

2.1 Materials

Materials included NaCl, Na_2SO_4 anhydrate, Boron trifluoride (BF_3) as an acid catalyst, methanol, Pig Meatball, Wild boar Meatball, Beef Meatball, lard and beef fat, and commercial meatball.

2.2 The extraction process for meatball

100 g of crushed meatballs is put in a Soxhlet and extracted using n-hexane for 6 hours. After that, the n-hexane solution was added with Na_2SO_4 anhydrate and then evaporated until the oil/fat was obtained.

2.3 Identification of Methyl Esther using GC-MS throughout the Transesterification process

10 mg of oil was put in a 25 ml measuring flask and dissolved in 10 ml of n-hexane and then added n-hexane to the mark. 3 ml of the solution was taken, then 100 ml of BF_3 solution was added and homogenized. After that, the solution was added with 3 mL of methanol and homogenized, and then heated for 40 minutes at ± 80ºC. After the heating, the solution was added with 1.5 ml saturated NaCl, then centrifuged for 10 minutes. The n-hexane layer formed is collected and then analyzed using GC-MS.

2.4 Data processing analysis

The discriminant analysis of fatty acid content used PCA performed using Minitab 15.

2.5 Results and Discussion

The transesterification process of fats or oils usually uses one of 2 types of catalysts, namely acidic or basic catalyst. A basic catalyst, such as a metal alkoxide, has many advantages because it is a reactive catalyst, achieving yields above 98%, with fast reaction time. However, the use of a basic catalyst requires avoiding water, because water will hydrolyze the triglycerides to produce fatty acids that can react with a basic catalyst. Furthermore, the base catalysts such as metal hydroxides (KOH and NaOH) are slightly less reactive than metal alkoxides, but conversion results are as high as with metal alkoxides (Ejikeme *et al.*, 2009). However, hydrolysis of triglycerides into fatty acids causes the formation of soap if the fatty acids react with a base catalyst. Shu *et al.* (2010) reported that free fatty acids at (ALB) <5% are good for alkaline catalysts, while a higher ALB will cause saponification. In addition, Batti *et al.* (2008) reported that transesterification of animal fat in tallow meat using H_2SO_4 acid catalyst reaches the yield of 98.4 ± 2.3%, while a base catalysts gave 2.06 ± 0.11%. However, it should be noted that acidic catalysts produce slower reactions than basic catalysts (Asakuma *et al.*, 2011). Therefore, this study uses an acid catalyst, namely boron trifluoride and the transesterification mechanism is shown in Figure 1.

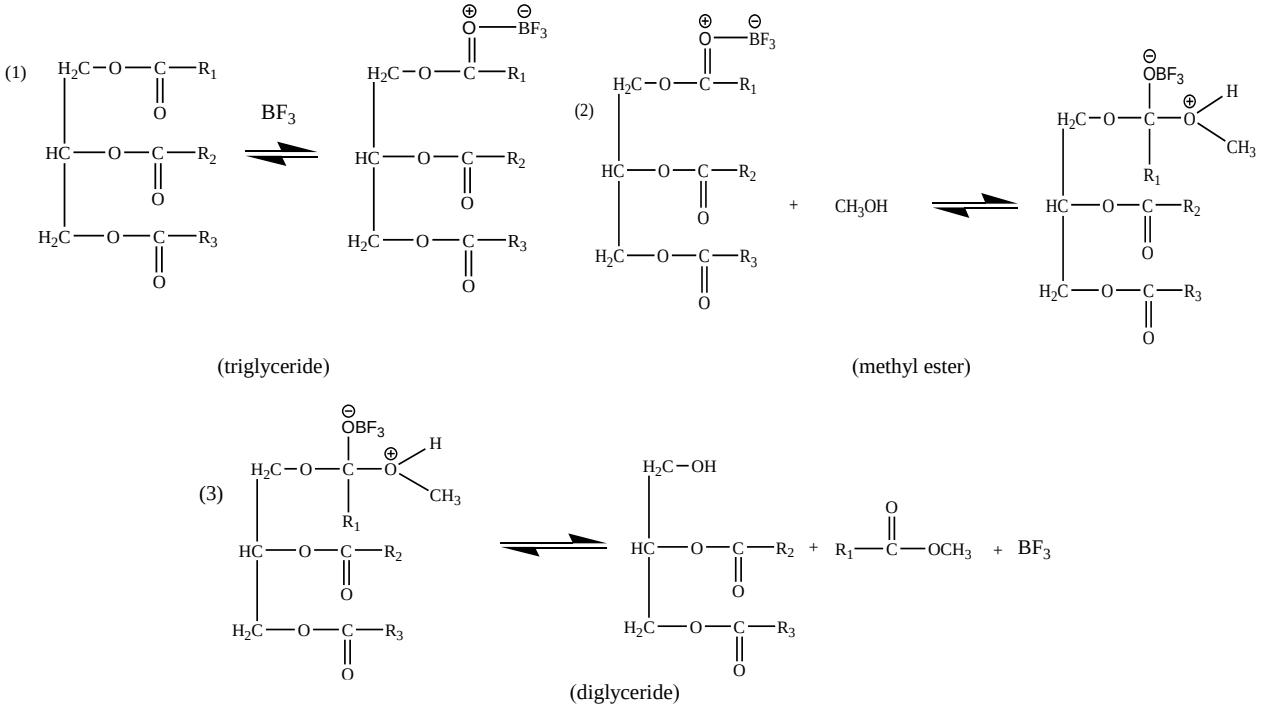

Figure 1. Mechanisms of triglyceride transesterification reaction with an acid catalyst

The product from transesterification of triglycerides is fatty acids that are in the form of methyl esters. These methyl esters are analyzed using GC-MS to determine the fatty acid profile of meat or meatballs. The fatty acid contents in some meats and meatballs are shown in Table 1.

Table 1 shows the types of fatty acids contained in some animal fats in meat or in meatball products. Based on the fatty acid contents, it can be seen that the fatty acids in meat differ from those in meatball products. Even linoleic acid at 8.22% in pork oil and 4.61% in beef fat was not detected in the meatball products. This might be due to the oxidation of unsaturated fatty acids during preparation of meatballs, so that unsaturated fatty acids are lost. We observe further that the missing fatty acids are polyunsaturated fatty acids (PUFA), whereas saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) can still be detected (Table 1).

Figure 2 shows that the polyunsaturated fatty acids (PUFA) component in lard is higher than in beef fat. Beef fat contains many types of saturated fatty acids while lard tends to be more unsaturated fatty acids. Figure 3 also shows that the ratio SFA: MUFA in lard is 0.77 in beef fat it exceeds 3.5. This is similar to beef meatball which has SFA: MUFA ratio 3.6, while pork has a ratio of 1.06, and wild boar meatballs ratio 1.2. In commercial meatball samples the SFA: MUFA ratio was 8.5 (Figure 3). This study showed that the proportions of SFA and MUFA can be used to detect if a product contains pork or not.

Grouping of halal food based on the content of fatty acids is given in Figure 4. Figure 4A shows the grouping based on the types of fatty acids while Figure 4B shows the grouping based saturated and unsaturated fatty acids. It can be concluded that the grouping based on saturated fatty acids and unsaturated fatty acids gave a better result, with beef meatball and beef fat in the same group. In contrast the grouping based on the types of fatty acids shows beef meatball and beef fat as less similar. Nevertheless, the data processing is able to distinguish halal foods from illegitimate foods. In this study it is also seen that the wild boar meatball and pork meatball are the most similar, whereas commercial meatball sample does not contain pork or wild boar or beef ingredients. The commercial meatball also has the highest SFA: MUFA ratio, larger than that for pork meatball, wild boar meatball or beef meatball. Halal analysis of SFA or MUFA or PUFA compositions is one analytical method recomended for supporting other halal analyses developed previously. This analysis is more suitable and better than the color and texture analyses of meatball reported by Tathma *et al.* (2019).

3. Conclusions

Halal food analysis through fatty acids determination showed that SFA: MUFA ratios of lard, pork meatball, and wild boar meatball were 0.77, 1.06, and 1.2 times, respectively. In contrast, the SFA: MUFA ratios of beef fat and beef meatball were higher than for lard, with beef fat about 3.5 fold and beef meatball about 3.6 fold. The PCA also distinguished halal food from forbidden food to separate clusters, based on fatty acid profiles.

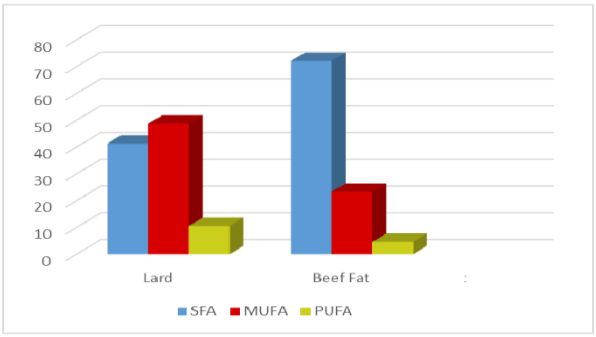

Figure 2. The proportions of SFA, MUFA and PUFA of lard and beef fat

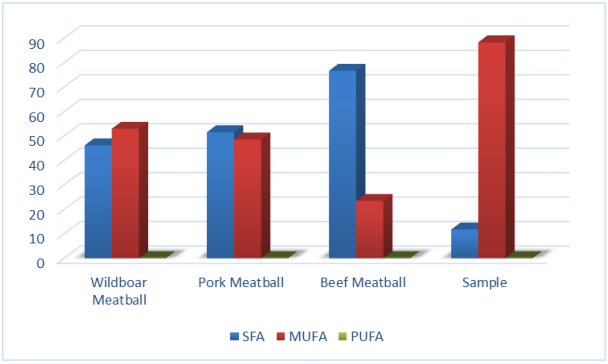

Figure 3. The proportions of SFA, MUFA and PUFA in meatballs made of different meats

Table 1. Fatty acid compositions of meatball products and some animal fats

Time retention	Molecule structure	Fatty acid types	*Relative Percentages (%)					
			Wild boar meatball	Pork meatball	Beef meatball	Lard	Beef fat	Meatball sample
29.068	$C_{12}H_{24}O_2$	Lauric Acid	2.28	3.27	Nd	2.68	16.09	Nd
33.804	$C_{14}H_{28}O_2$	Myristic Acid	2.98	3.06	9.96	2.96	9.03	Nd
38.122	$C_{16}H_{32}O_2$	Palmitic Acid	25.27	34.22	48.72	22.00	30.05	11.18
41.542	$C_{18}H_{32}O_2$	Linoleic Acid	Nd	Nd	Nd	8.22	4.61	Nd
41.634	$C_{19}H_{36}O_2$	Oleic Acid	52.89	48.52	23.40	48.68	23.31	88.26
42.077	$C_{19}H_{38}O_2$	Stearic Acid	16.58	10.92	Nd	13.64	16.91	0.55
44.650	$C_{19}H_{34}O_2$	Arachidonic Acid	Nd	Nd	Nd	2.13	Nd	Nd

Nd: Not Detected, Transesterification process using BF_3 catalyst

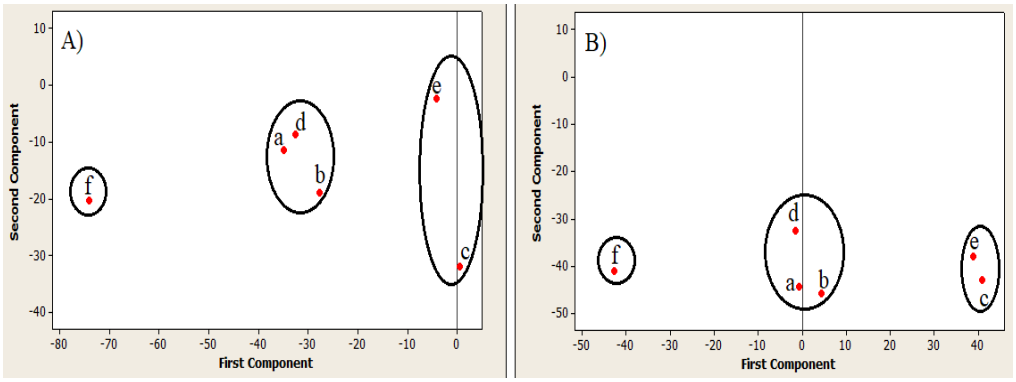

Figure 4. The results of principal component analysis (PCA): A) Based on fatty acid content; B) Based on saturated and unsaturated fatty acids (SFA, MUFA and PUFA). a) Wild boar meatball, b) Pork meatball, c) Beef meatball, d) Lard, e) Beef fat, and f) commercial sample

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