FOOD
RESEARCH

Determining fatty acids and halal authentication of sausage

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Abstract

Sausages are instant food that requires the halal attention for a Muslim. In general, non-halal issues are usually associated with pig derivatives. One of the pig derivates is lard that can be analyzed using GC-MS via a transesterification catalyzed with a base catalyst like sodium methoxide 2%. This study indicated that pork sausage has different fatty acids compared with beef sausage. The pork sausage contained the dominant fatty acids such as palmitic acid (37.75%), myristic acid (22.24%), oleic acid (25.29%), and lauric acid (8.46%). Whereas, beef sausage has the dominant fatty acids of palmitic acid (42.31%), oleic acid (20.19%), stearic acid (10.92%) and myristic acid (7.66%). The commercial sausages 1, 2, and 3 have similar dominant fatty acids such as palmitic acid, oleic acid, stearic acid, and myristic acid compared with fatty acid types in beef sausage. The discriminant analysis also showed that the beef sausage is separated location from pork sausage and all samples are not containing the pork or lard because they are far away from pork sausage.

1. Introduction

Food is one of the primary needed by humans because it contains some ingredients that the human body needed. The one ingredient is fat or oil in triglyceride form that can be hydrolyzed into glycerol and fatty acids. The hydrolysis process of triglycerides depends on the chain length and molecular position of the fatty acids (Bauer *et al.,* 2005). Based on triglycerides composition, the different animals will result in the differences in triglycerides composition (Rohman *et al*., 2012). This evidence can be used as one way for discriminant analysis between halal food and non-halal food. For a Muslim, food consumption is supposed for good or healthy food and also halal foods (Fadzlillah *et al*., 2011).

In general, non-halal products are the product associated with pig derivatives so a Muslim must be aware and take care to choose the halal product. Either producers or consumers must learn to understand about halal and they ensure the halal certification because it has many advantages in the halal product (Hassan and Awang, 2009). Furthermore, certification halal must be the responsibility of both governments and companies to ensure the products is halal (Khattak *et al*., 2011). Nakyinsige *et al*, (2012) stated that the head of

the meat industry company must know about the rules of sharia in the production of halal meat. This is because the international market for halal food reaches the US $ 580 billion per year (Qureshi *et al*., 2012). On the other hand, there is a big contribution from the halal food industry in the development of society and national economic growth (Bohari *et al*., 2013).

For detection pork or lard in the product, we can analyze the fats content composed of monoglycerides, diglycerides, triglycerides, fatty acids. The biggest component in lard or pork is triglycerides, which is about 95% (Gunstone, 2002; Lobb and Chow, 2007). Triglycerides (TAG) are a reaction formed from one molecule of glycerol and three fatty acids and it can be hydrolyzed to produce glycerol and fatty acids again (Figure 1). The differences of TGA contained in pork compared with other meat animals cause they can be grouped if the foods contain pork. The analytical methods developed to analyze lard or other components of pork. Ahda *et al*. (2016) and also Gutierrez and Perona (2000) analyzed pork based on triglycerides component using HPLC. Furthermore, the FTIR spectroscopy is also used as a pork analysis based on fingerprints of functional group (Guntarti *et al*., 2015; Guntarti and Seshilia, 2017). Besides that, the discriminant analysis also can be grouped based on fatty

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acid types using KLT and GC-MS (Ismiyarto *et al.,* 2006; Almeida *et al.*, 2006; El-Ghorab *et al*., 2009; Setiawati and Edward, 2012). This research is aimed to analyze commercial sausage products based on fatty acid contents using GC-MS. This is a way for giving a solution where each sausage producing can cause the hydrolysis of triglycerides in sausage. So, fatty acid analysis can be a solution in halal authentication because it can ensure the discriminant result is only based on fatty acids components, not both mixture between triglycerides and fatty acid components.
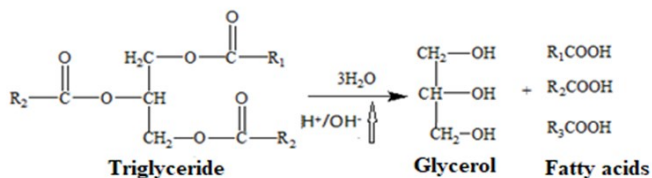

Figure 1. Hydrolysis of triglycerides into fatty acids and glycerol

2. Materials and methods

2.1 Materials

The used materials: n-hexane, Na_2SO_4 anhydride, saturated NaCl, sodium methoxide 2%.

2.2 The extraction of triglycerides in pork, beef and sausage.

Samples of meat (pork, beef) sausages were sliced into small pieces, put in a glass beaker, put in an oven at a temperature of 50°C until it was melted. Furthermore, the above fat liquid was extracted with n-hexane and then added Na_2SO_4 anhydride to bind the water. The fat liquid was evaporated before the transesterification process.

2.3 Transesterification process of triglycerides of sausages samples

A total of 20 mg of samples fat was added using 50 mL of sodium methoxide solution (NaOH: methanol 2% w/v) and then heated at 60°C for 60 mins. After that, the solution was kept at room temperature and then added the saturated NaCl. The supernatant can be separated using centrifugation method and up layer injected on the GC-MS instrument.

2.4 Data processing

Chemometrics analysis of principal component analysis (PCA) using Minitab 16 software. The variables used for discriminant analysis are fatty acid types and relative fatty acid content, in each sausage will be determined.

3. Results and discussion

Analysis of fatty acid components in sausage is very important from the difference of carbon atoms length, fatty acids saturation degree, and double bonds position in the chain of fatty acids (Dupuy, 1996). Fatty acids can be classified to become saturated fatty acids, monounsaturated fatty acids, and also polyunsaturated fatty acids (Coltro, 2005). Figure 2 showed a difference in chromatogram profiles of all sausage samples. 100% pork sausage (red color) appeared to have a different chromatogram profile with 100% beef sausage (blue color). Commercial sausage samples (yellow color) have similarities with 100% beef sausage (blue color). The fatty acids in 100% pork sausage are more than the fatty acids contained in 100% beef sausage and commercial sausages.

The composition of the sausage samples with values that vary in each fatty acid. Palmitic acid contained in all the sample sausage has the highest percentage. In comparison, 100% pork sausage containing palmitic acid is lower than 100% beef sausage and commercial sausage. Oleic acid which is contained in the fifth sample is also very varied. 100% pork sausage contained the dominant fatty acids such as palmitic acid (37.75%), myristic acid (22.24%), oleic acid (25.29%), and lauric acid (8.46%). While 100% of beef sausage contained the predominant fatty acid such as palmitate acid (42.31%), oleic acid (20:19%), stearic acid (10.92%) and myristic (7.66%). Commercial sausage 1 contained dominant fatty acids such as palmitic acid (45.99%), oleic acid (27.55%), stearic acid (12.81%) and myristic acid (4.71%). Commercial sausage 2 contained dominant fatty acids such as palmitic acid (47.29%), oleic acid (38.08%), stearic acid (6.10%), and palmitoleic acid (2.26%). Commercial sausage dominant 3 contained dominant fatty acids such as palmitic acid (46.79%), oleic acid (27.65%), stearic acid (11.71%) and myristic acid (5.37%). The fatty acids contained in beef sausage, pork sausage, and commercial sausages are tabulated in Table 1.

In Table 1, 100% pork sausage is detected lauric acid on while it's undetected in 100% beef sausage and commercial sausages. This fact has influenced high temperatures that cause fat damage through hydrolysis reaction and oxidation reaction during the process of derivatization. The process of fat oxidation will occur in the unstable hydroperoxide of long-chain fatty acids become short-chain fatty acids. Hence, the lauric composition in pork sausage is very high. The speed of the oxidation process also depended on the type of fat and storage conditions. There are some undetected fatty acids in the samples at the time of the analysis, so the value of the total fatty acids is lower than the supposed
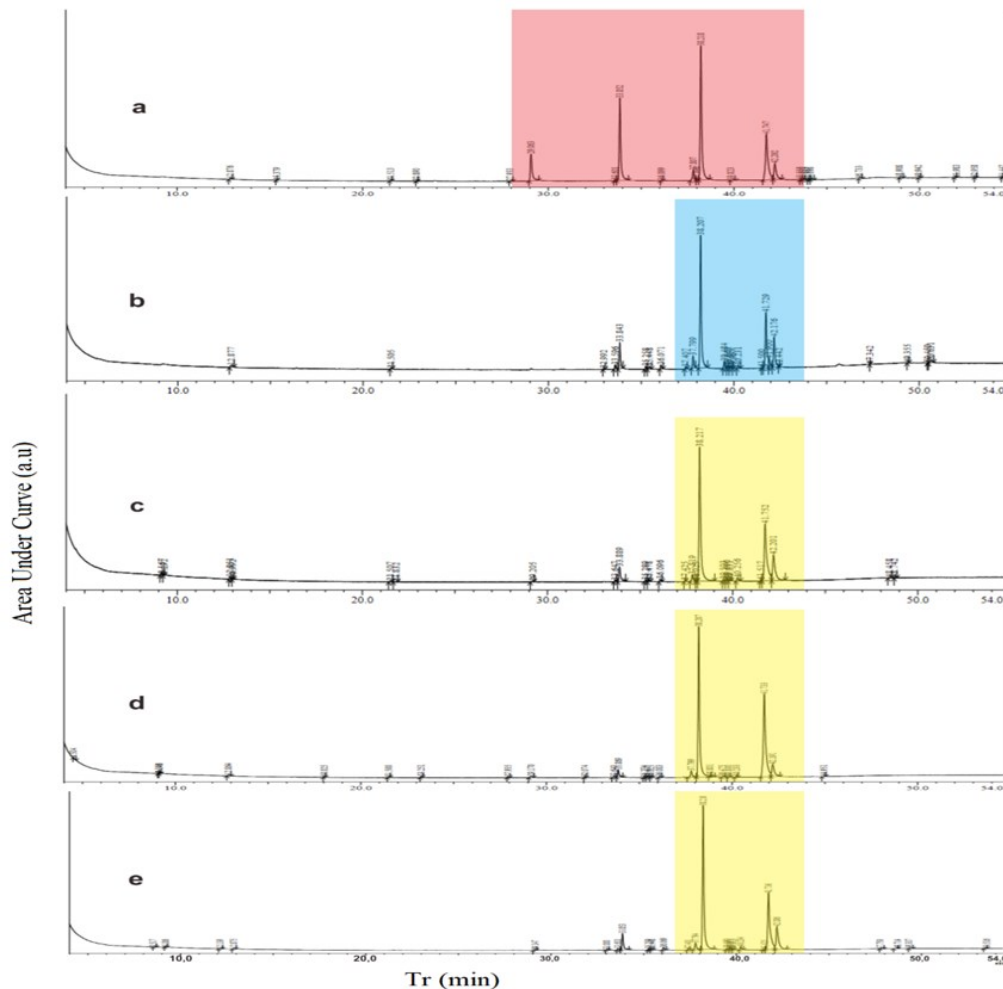
Figure 2. Chromatogram profiles of sausage samples a) 100% Pork Sausage, b) 100% Beef sausage, c) Commercial sausage 1, d) Commercial Sausage 2, e) Commercial sausage 3.

Table 1. Composition of fatty acid methyl ester (FAME) in sausage samples

Retention time	Chemical formula	Fatty acid name	FAME relative (%)					SI	BM
			100% pork sausage	100% beef sausage	Commercial sausage 1	Commercial sausage 2	Commercial sausage 3		
29,063	C12:0	Lauric acid	8.46	-	-	-	-	94	200
33,852	C14:0	Myristic acid	22.24	7.66	4.71	2.2	5.37	96	228
36,071	C15:0	Pentadekanoat acid	0.1	1.06	0.68	0.23	0.7	84	242
37,807	C16:1	Palmitoleic acid	3.2	3.71	2.19	2.26	2.53	96	254
38,218	C16:0	Palmitic acid	37.75	42.31	45.99	47.29	46.79	97	256
40.234	C17:0	Heptadecanoic acid	0.15	0.97	0.9	0.3	0.95	87	270
41.736	C18:1	Oleic acid	25.29	20.19	27.55	38.08	27.65	95	282
42,202	C18:0	Stearic acid	6.56	10.92	12.81	6.1	11.71	96	284

value. 100% pork sausage contained the highest myristic acid compared with other sausage fat which is 22.24%, while the beef fat contained 7.66% myristic acid, all the three samples of commercial sausage contained 4,71%; 2,20%; and 5,37% myristic acid respectively. This shows that commercial sausage resembled 100% beef sausage shown in Table 1.

Differences are also seen in palmitic acid, which is 37.75% contained in 100% pork sausage, while 100% beef sausage has a higher value which is 42.31%, Same

thing went for the three commercial sausages with a value of 45.99%; 47.29%; and 46.79% respectively, the stearic acid content in 100% beef sausage is 10.92% higher than 100% pork sausage with a content of 6.56%, a different fatty acid composition among samples of 100% beef sausage and 100% pork sausages based on the results of GC-MS analysis in which the saturated fatty acid content of 100% beef sausage is greater than 100% pork sausage. The identification of the three commercial sausages above has a similar fatty acid content with 100% beef sausage. This is consistent with

the results seen in the grouping of PCA as seen in the commercial sausages plot included in the same quadrant with beef sausage.

In addition, the value of Similarity Index (SI) may indicate the accuracy of the data based on the similarity of fragmentation pattern and basic peak (base chromatogram) which referred to the literature of standardized instruments. The greater its SI value, the more accurate the identification of compounds on the MS instrument. Each of the identified compounds can then obtained information about the relative molecular mass using MS spectra. Then, the data of fatty acids content were analyzed using principal component analysis for discriminant and grouped the commercial sausage products and this method more effective and to be easier to conclude that the commercial sausage product is not containing lard or pork. Based on Figure 3, pork sausage and beef sausage are separate in different quadrants. This is a piece of evidence that fatty acid content in both beef sausage and pork sausage is different. The commercial product 2 made is not containing pork or lard or beef. In contrast, both commercial products 1 and 3 showed that both are located in the same quadrant with beef sausage. It can be concluded that both made from beef because they have similar fatty acid contents as beef sausage.
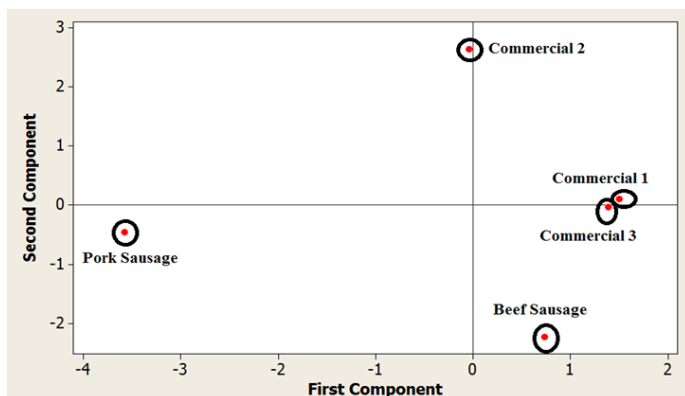

Figure 3. PCA Score Plot of fat from Pork sausage, Beef sausage, Commercial 1, Commercial 2, and Commercial 3, by using fatty acids as a variable.

The results of PCA analysis using Minitab resulted in 8 PCs are presented in Figure 4. Each PC displays eigenvalue, proportion, and cumulative values. Eigenvalue variations can explain the data on each PC and show how much influence a variable on the formation of the characteristics of a matrix (Miller and Miller, 2005). In Figure 4, PC1 with eigenvalue 4.3992 is able to describe 55.5% of the total original data variables while PC2 with eigenvalue 3.0274 is able to describe 37.80% of the total original variables. Thus, 2 PCs described the illustration data for discriminant analysis of sausage samples of 92.80%.

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Eigenanalysis of the Correlation Matrix

Eigenvalue  4,3992  3,0274  0,5490  0,0244  0,0000  0,0000  0,0000  -0,0000
Proportion  0,550   0,378   0,069   0,003   0,000   0,000   0,000   -0,000
Cumulative  0,550   0,928   0,997   1,000   1,000   1,000   1,000    1,000


Variable         PC1     PC2     PC3     PC4     PC5     PC6     PC7     PC8
Lauric         -0,456  -0,085  -0,339  -0,249   0,560  -0,454   0,286   0,083
Myristic       -0,426  -0,221  -0,303  -0,363  -0,556  -0,089  -0,391  -0,276
Pentadecanoate  0,352  -0,375   0,233   0,185   0,030  -0,670  -0,111  -0,431
Palmitoleate   -0,177  -0,463   0,620  -0,295  -0,018  -0,021  -0,092   0,524
Pamlitic        0,395   0,316   0,023  -0,641   0,332  -0,016  -0,468  -0,047
Heptadecanoic   0,408  -0,287  -0,155  -0,482  -0,229   0,131   0,646  -0,094
Oleic           0,021   0,572   0,120  -0,091  -0,457  -0,549   0,197   0,317
Stearic         0,365  -0,282  -0,562   0,184  -0,066  -0,134  -0,264   0,588
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Figure 4. The report of PCA analysis of sausage and some parameters of its Eigenanalysis

4. Conclusion

The beef and pork sausages have different fatty acids contents, where pork sausage has palmitic acid (37.75%), myristic acid (22.24%), oleic acid (25.29%), and lauric acid (8.46%). Whereas, beef sausage contains palmitic acid (42.31%), oleic acid (20.19%), stearic acid (10.92%) and myristic acid (7.66%). The sausage commercials contain fatty acid dominant such as palmitic, oleic, stearic, and myristic where they have similarity fatty acids content like fatty acids of beef. The PCA analysis showed all of the commercial sausages did not contain the pork or lard because they located far away from pork sausage based on its fatty acid content.

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