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An Analysis of Rat Meat with FTIR and GC / MS for Halal Authentication

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Abstract

Fourier Transform Infra Red (FTIR) and Gas Chromatography Mass Spectrophotometry (GCMS) method were developed to determine rat fat which is extracted from rat meat. Fat was extracted with methanol and chloroform as a solvent. Derivatization process was conducted to convert fat into a methyl ester form using sodium methoxide and boron trifluoride. GCMS analysis was used to identify the fatty acid composition from rat fat. The FTIR spectral bands correlated with bovine, pork, chicken and rat fat were scanned, interpreted, and identified. Qualitative differences between FTIR spectra were proposed as a basis tools for differentiating between rat fat and other fat. Principal Component Analysis (PCA) at combining wavenumber regions of 1250-1100 cm-1 and 3010-2850 cm-1 was capable of distinguishing rat meat from other meat. GCMS chromatogram showed the fatty acid composition in rat meat which five major compounds were hexadecenoic acid, 9,12-octadecadienoic acid, 9-hexedecenoic acid, tetradecanoic acid and 7-hexadecanoic acid. PCA based on fatty acid composition can distinguish rat meat from other meat.

Key words : Rat meat · FTIR · GCMS ·

1. Introduction

A muslim is majority population in Indonesia, whose must consume halal food products. Thus, it is important to ensure the halals of food products. Adulteration non halal meat in food product become increase and some food products are found to have been adulterated with non halal ingredient, such as rat meat in meatball (1,2).

Meatballs are a popular food in Indonesia where the main component is meat. The meat can be from beef, chicken, or fish. Some food manufacturers replace halal meat with non halal meat such as rat, because it is easier to get and cheaper. The aim to reduce the cost. However, it is unfavorable for consumers and harmful to health because rat can cause some diseases like Salmonellosis, Leptospirosis and Plague (1).

Non halal component in food products can be analyzed with several methods, such as Gas Chromatography-Mass Spectrometry/GCMS (3,4), Fourier Transform Infra Red/ FTIR (5,6,8), Liquid Chromatography/HPLC (9) and Polymerase Chain Reaction/PCR (7). FTIR method is fast and consistent method, even in low analyte concentration (10), non-destructive, sensitive, and does not require complicated sample preparation (11).

However, the FTIR method has limitations, that is it cannot certainly identify the content type of the sample's each fatty acid component (8). Fatty acid analysis can be done by GCMS method. Fatty acid composition of meat can be used for distinguishing rat meat from other meat. The fatty acid composition is determined as methyl ester. The purpose of this research is to identify rat meat in food products by using FTIR and GCMS methods.

2. Materials and Methods

Lipid Extraction :

Black rats were obtained from local farm in Banyumas regency Indonesia. Bovine, pork and chicken meat was obtained from local market in Banyumas Indonesia. Fat was extracted using chloroform: methanol by Bligh & Dyer methods.

GC-MS Analysis :

Fat was hydrolyzed with alkaline to produce fatty acid, and followed with methyl esterification. Transesterification performed by the BF_3-MeOH method to form fatty acid methyl ester. Approximately 50 μL oil samples were added with 1.0 mL n-hexane and 200 μL 0.2 N $NaOCH_3$ solutions and heated at 60°C for 10 min. Then, the mixture was added to 1.5 mL BF_3-methanol reagent, and heated at 60°C for 10 min. After it was cool, 1 mL of saturated NaCl solution was added, and shake. The resulting hexane layer was used as a sample solution for GC-MS. Subsequently, 1 μL of the clear supernatant was taken and injected into a GC-MS (Shimadzu QP2010, Shimadzu Corp., Tokyo, Japan). The column used is a

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SH-Rxi-5Sil MS (5% diphenyl/95% dimethyl polysiloxane) capillary column (30 m x 0.25 mm ID, 0.25 µm film thickness). Helium was used as the carrier gas at flow rates of 1.0 mL/min. The injector temperature was 280°C. The oven temperature was set at 100°C for 5 min, increased to 240°C at a rate of 4°C/min and held at the final temperature for 30 min. The GC-MS operation was controlled by Lab Solution software. MS spectra were obtained in wide range of m/z 10- 500. FAME peaks were identified by comparing their retention time with the FAME standard and similarity index (SI more than 90%).

***Fat analysis using FTIR* :**

Fat from each meat was dropped on the ATR crystal, which was placed in a controlled temperature (20°C) as much as 1 drop. Then, the fat was scanned for 32 times at the wave number of 4000-650 cm^{-1} with a resolution of 4 cm^{-1} and was recorded in the form of absorbance. FTIR spectra were analyzed using chemometrics in the form of PCA using Horizon MB software.

3. Results and Discussion

Fatty acid composition was identified and measured with gas chromatography with mass spectrometry (GC MS) which present in the lipid extracted from rats and other meat. Saponification with alkaline and followed by BF^3-catalyzed methylation were used to form fatty acid methyl ester. Peak identification of fatty acids methyl ester in the analyzed samples was conducted by comparing the retention time and molecular mass of mass spectra of standard mass spectra, which were obtained from library (Wiley9.lib) of the GCMS instrument and also confirmed by comparing the mass spectrometric fragmentation pattern with the standard.

Composition of fatty acid can be used to differentiate rat fat from other species. GCMS chromatogram at figure 1 revealed that hexadecanoic acid has the highest level fatty acid from rat fat. The other major constituents are; 9,12-octadecadienoic acid (tr 34.424 min); 9-hexadecenoic

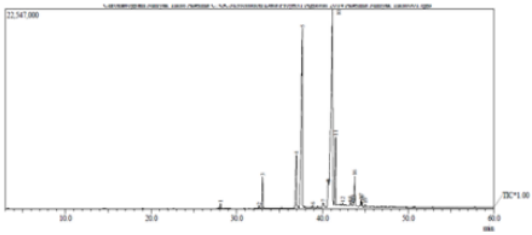

Figure 1. Rat's fat GC chromatogram

Table 1: Fatty Acid Compositions of Lipid Extracted from Bovine, Rat, Chicken and Pork Obtained By GC-MS Method

Fatty Acid	Fatty Acid Percentage (%)			
	Rat	Bovine	Chicken	Pork
Dodecanoic Acid	0	0	0.07	0.2
pentadecanoic acid	0.2	0.61	0.06	0.09
tetradecanoic acid	1.79	3.18	0.56	1.82
9-octadecenoic acid)	0	24.52	46.57	37.62
cis-9-tetradecenoic acid	0.05	1.32	0	0
7-hexadecanoic acid	1.31	0.1	0.28	0.42
hexadecanoic acid	25.09	23.75	24.62	24.19
9-hexedecenoic acid	1.79	4.64	3.7	2.27
octadecanoic acid	0.32	12.75	7.74	13.15
heptadecanoic acid	0.29	1.17	0.1	0.44
cis-10-heptadecenoic acid	0.1	1.02	0.04	0.25
9,12-octadecadienoic	16.11	1.57	15.59	17.36
5,8,11,14-eicostate	0	0	0	0.22
6,9,12-octadecadienoic	0	0	0	0.13
eicosenoic acid	0.15	0.07	0.06	0.18
methyl eicosanoic acid	0.56	0	0	0
10-nonadecenoic acid	0	0.24	0	0
11,13-eicosadienoic	0.06	0	0	0.45
11-octadecenoic acid	0	47.43	0	0

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acid (tr 29.594 min); tetradecanoic acid (tr 24.64 min); and 7-hexadecanoic acid (tr 29.140 min). From Table 1 it can be seen that rat has a larger percentage of saturated fatty acids than beef, chicken and pork. The differences in the degree of unsaturated fatty acid in animal fats could be due to the individual fatty acid distribution pattern (12). The presence of methyleicosanoic acid is found in lipid extracted from rats, but it is not found in bovine. In contrast, 11- octadecenoic acid (47.30%) exists only in lipid extracted from bovine. Hexadecanoic acid is found to be approximately equal in all analyzed lipid types. The quantity of 9.12-Octadecadienoic acid in bovine (1.57%) is much lower than in rats (16.11 %). The fatty acid composition of lipid extracted from rats were unique compared to bovine, chicken and pork. From the GC-MS analysis, it is found that the major constituents of lipid extracted form rats were fatty acids with chain lengths of 15 to 21 carbon atoms (mainly C17 and C19). PCA score plot in Figure 2 showed that fatty acid composition can distinguish rat fat from other fat. Rat fat is located at different quadran from other fat.

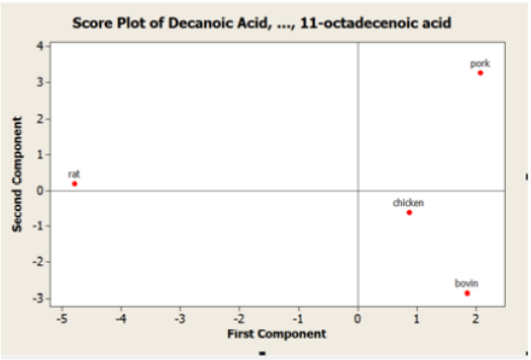

Figure 2. PCA Score Plot from rat, chicken, pork and bovine fat based on fatty acid composition

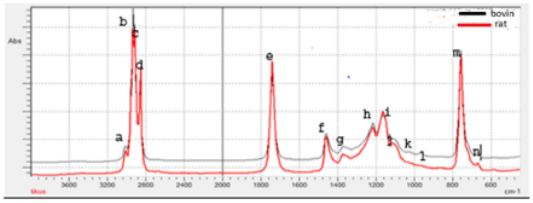

Figure 3. FTIR spectrum rat and bovin fat

FTIR analysis was conducted based on the differences between the functional groups of fat from rat, chicken, pork and bovine meat, which were measured at wave number 4000-650 cm^{-1}. Figure 3 shows that the difference of FTIR spectrum between rat and bovine fat is a typical

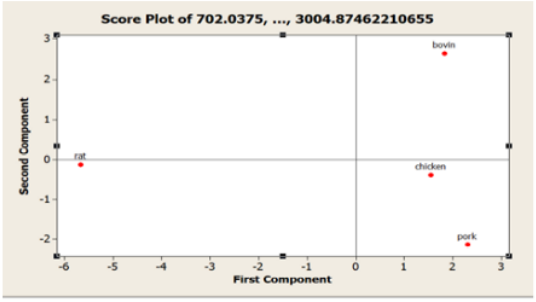

Figure 4. PCA Score Plot From Rat, Chicken, Pork And Bovine Fat at wavenumber 1250-1100 cm^{-1} and 3010-2850 cm^{-1}

peak at wave number 3010-2950 cm^{-1}. The absorption pattern at wave number 3008.95 cm^{-1} (peak a) for rat fat shows relatively higher peaks compared to bovine fat. The high peak of rat fat absorbance in the area shows the presence of unsaturated fatty acid content which contributes to the high absorbance value, namely the C-H stretching vibration area of the cis double bond.

Fingerprint area (1500-700 cm^{-1}) that is at wavenumber of 1118.71 cm^{-1} (i) shows the typical spectrum of rat fat that is the C-O stretching vibration area. The third point of difference is located in wavenumber 1026.13 cm^{-1} (k) and 972.12 cm^{-1} (l) in which this area does not show any absorption in the rat fat spectrum.

Figure 4 showed that PCA can be accomplished to classify between rat, pork, chicken and bovine fat. Rat fat stand far from others fat, chicken and pork stand at same quadrant which it showed they have similarity.

4. Conclusion

This study investigated application of GC-MS and FTIR to identify rat meat based on fat and fatty acid profile. The high constituents of lipid extracted from rats are 9-Octadecenoic acid; hexadecanoic acid; 9,12-octadecadienoic acid; octadecanoic acid; 9-hexadecenoic acid; tetradecanoic acid; and 7 hexadecanoic acid. The major constituents are fatty acids with chain lengths of 15 to 21 carbon atoms (mainly C17 and C19) and unsaturated fatty acid higher than saturated fatty acid. GCMS method can be used to authenticate rat meat with fatty acids content.

Application of multivariate statistical analysis such as PCA would be required to determine source of the origin. Hence, this study showed that fatty acid data allowed separation rat fat from other animal fats.

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The difference of rat and beef fat is located in wavenumber 1026.13 cm^{-1} (k) and 972.12 cm^{-1} (l) in which this area does not show any absorption in the rat fat spectrum. FTIR spectroscopy at wavenumber region 1250-1100 cm^{-1} and 3010-2850 cm^{-1} combined with chemometrics techniques can be used to determine rat meat.

Acknowledgements

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Conflict of Interest

The authors declare no conflict of interest.

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