Fatty Acid Analysis of Lipid Extracted from Rats by Gas Chromatography-Mass Spectrometry Method

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Abstract. The purpose of this study was to identify fatty acid composition in fat extracted from rats using Gas Chromatography Mass Spectrometry (GC-MS) method. The fatty acid composition was determined as the methyl esters of fatty acids. Fat was extracted with chloroform-methanol solvent. Fatty acid methyl esters were prepared by saponification with base and followed by BF_3-catalyzed methylation. Thirty compounds were identified, representing about 100 % of the total extracted lipids as measured by GC peak areas. The major constituents were 9-Octadecenoic acid; Hexadecanoic acid; 9,12-Octadecadienoic acid; Octadecanoic acid; 9-Hexadecenoic acid; Tetradecanoic acid; and Methyl arachidonate. The major constituents were fatty acids with chain lengths from 15 to 21 carbon atoms (mainly C17 and C19).

1. Introduction

Some food products are found to have been mixed with unlawful ingredients, such as rat meat in beef meatballs products [1,2]. Meatballs are one of the typical Indonesian foods where the main component is meat, whether it beef, chicken, or fish. Sometimes, rat meat is chosen to be mixed with beef, as it is easily obtained. The goal is to reduce costs [2]. It is very unfavorable for consumers, especially Muslim consumers.

Analysis of non-halal materials can be carried out by several methods, such as Gas Chromatography – Mass Spectrometry /GC-MS [3], Fourier Transform Infra-Red/ FTIR [4], Polymerase Chain Reaction/PCR [5], electronic noses [6], and Gold Nanoparticle [7].

A fatty acid has a carboxylic acid at one end and a methyl group at the other end. The identification of the fatty acid composition of a meat-based food product can be used to determine the presence of non-halal ingredients. Fatty acid analysis can be done by GC-MS method. The GC-MS method can be used for fatty acid analysis. The purpose of this study was to identify fatty acid composition in fat extracted from rats using Gas Chromatography Mass Spectrometry (GC-MS) method. The fatty acid composition was determined as the methyl esters of fatty acids.

2. Methods

2.1. Lipid extraction

White rats were obtained from laboratory of Pharmacology Universitas Muhammadiyah Purwokerto. Black rats were obtained from local farm in Banyumas regency. Bovine meat was obtained from local market in Purwokerto. Oil samples were extracted using chloroform: methanol by Bligh & Dyer methods.

2.2. Preparation of Fatty Acid Methyl Ester (FAME)

Methyl esterification of samples used in the analyses was performed by BF_3-MeOH method after alkaline hydrolysis. To 50 µL oils were added 1 mL n-hexane and 200 µL 0.2 N $NaOCH_3$ solutions, and the mixture was heated at 60°C for 10 min. To the mixture was added 1.5 mL BF_3-methanol reagent, and heated at 60°C for 10 min. After cooling, 1 mL of saturated NaCl solution was added, followed by a thorough shaking. The resulting hexane layer was used as a sample solution for GC-MS.

2.3. GC-MS method

Analysis of FAME was performed on a GC-MS QP2010 by Shimadzu. Separations were performed using a 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 programmed at 100°C for a hold of 5 min and increased to 240°C at a rate of 4°C/min and hold at the final temperature for 30 min. The GC-MS operation was controlled by LabSolution software. MS spectra were obtained at range width m/z 10-500. FAME peaks were identified by comparing their retention time with respect to standard FAME.

3. Results and discussion

Gas chromatography coupled with mass spectrometry (GC MS) was used to identify and measure the composition of fatty acids present in the lipid extracted from rats and bovine. Fatty acid methyl esters were prepared by saponification with base and followed by BF_3-catalyzed methylation. Thirty compounds were identified, representing about 100 % of the total extracted lipids as measured by GC peak areas. Peak identification of fatty acids in the analyzed samples are carried out by comparing with the retention time and molecular mass of mass spectra of standard, obtained from library (Wiley9.lib) of the GCMS instrument and also confirmed by comparing the Mass Spectrometric Fragmentation Pattern with the standard.

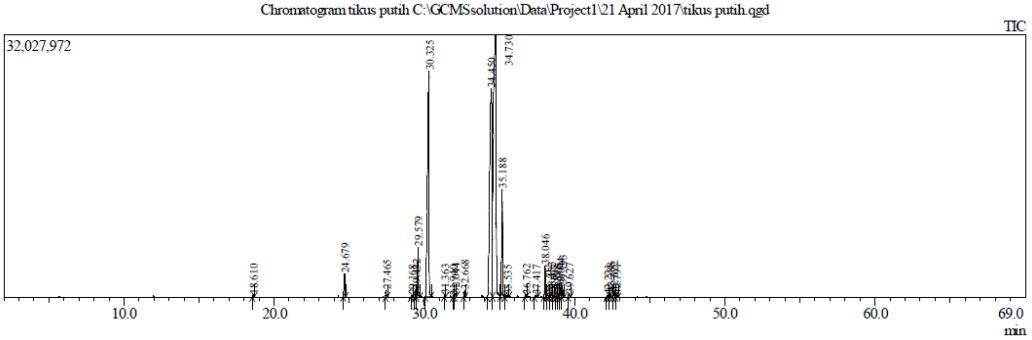

Figure 1. White rat's lipid GC chromatogram

From figure 1 revealed that fatty acid (9-Octadecenoic acid) at 34.730 min retention time had the highest level. The other major constituents were Hexadecanoic acid (retention time/tr 30.325 min); 9,12-Octadecadienoic acid (tr 34.450 mi); Octadecanoic acid (tr 35.188 min); 9-Hexadecenoic acid (tr 29.579 min); and Methyl arachidonate (tr 38.046 min).

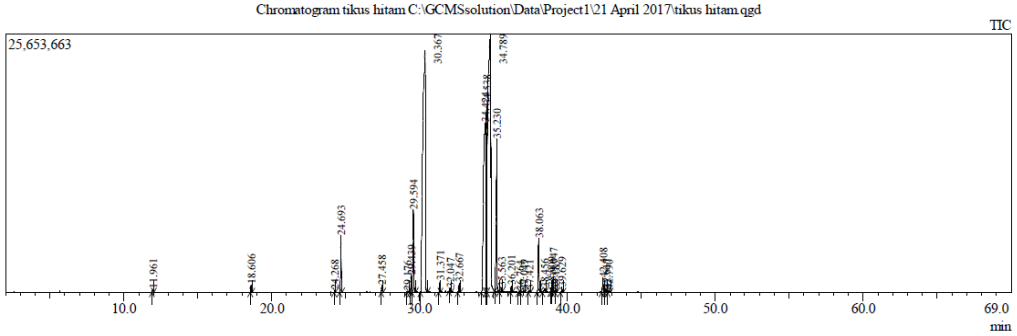

Figure 2. Black rat's lipid GC chromatogram

Gas chromatography (figure 2) revealed that fatty acid (9-Octadecenoic acid) at 34.789 min retention time had the highest level. It shows that 9-Octadecenoic acid was the major fatty acid in the lipid extracted from the rats. The other major constituents were Hexadecanoic acid (retention time/tr 30.367 min); 9,12-Octadecadienoic acid (tr 34.424 mi); 9-Hexadecenoic acid (tr 29.594 min); Tetradecanoic acid (tr 24.64 min); and Methyl arachidonate (tr 38.063 min).

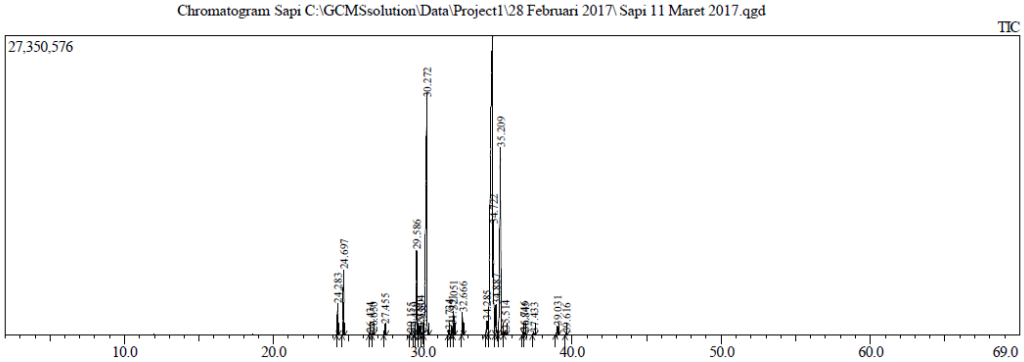

Figure 3. Bovine lipid GC chromatogram

From figure 3, it can be seen that fatty acid 11-Octadecenoic acid at 34.722 min retention time had the highest level. It shows that 11-Octadecenoic acid was the major fatty acid in the lipid extracted from the bovine. The other major fatty acid was 9-Hexadecenoic acid (29.586 min); Octadecanoic acid (35.209 min); 9-Hexadecenoic acid (29.586 min); Tetradecanoic acid (24.697 min); 13-Octadecenoic acid (34.887 min); Cis-9-Tetradecenoic Acid (24.283 min); Cis-10-Heptadecenoic Acid (32.051 min); and 9,12-Octadecadienoic acid (34.285 min).

From table 1, it can be seen the presence of 9-Octadecenoic acid (32.50 – 36.09%) and Methyl arachidonate (1.28 – 1.76%) in lipid extracted from rats, but not in bovine. In contrast, 11-Octadecenoic acid (47.30%) exists only in lipid extracted from bovine. Hexadecanoic acid was found to be approximately equal in all lipid types analyzed. The quantity of 9.12-Octadecadienoic acid in bovine (1.29%) is much lower than in rats (16.11 – 27.46%). Octadecanoic acid, 9-Hexadecenoic acid, and Tetradecanoic acid were found with a higher quantity in lipid extracted from bovine than rats. The fatty acid composition of lipid extracted from rats were unique compared to bovine.

From the GC-MS analysis, found that the major constituents of lipid extracted form rats were fatty acids with chain lengths from 15 to 21 carbon atoms (mainly C17 and C19).

Table 1. Fatty acid compositions of lipid extracted from bovine and rats obtained by GC-MS method

Retention time [min]	Fatty acids	Fatty acid Percentage [%]		
		Bovine	White Rats	Black Rats
24.25	**4. Cis-9-Tetradecenoic Acid**	1.32	nd	0.05
24.64	Tetradecanoic acid	3.08	0.85	1.79
27.43	Pentadecanoic Acid	0.43	0.24	0.2
29.12	14-Pentadecynoic acid	0.09	nd	0.04
29.14	7-Hexadecenoic Acid	0.10	0.35	1.31
29.57	9-Hexadecenoic acid	4.14	0.82	1.79
30.32	Hexadecanoic acid	23.31	23.50	25.09
31.32	**5. Cis-10-Heptadecenoic Acid**	1.02	nd	0.10
31.37	Heptadecanoic Acid	0.32	0.07	0.29
31.37	Octadecanoic acid	nd	0.08	0.32
31.95	Hexadecanoic acid, 14-methyl	0.41	-	-
34.48	9,12-Octadecadienoic acid	1.29	27.46	16.11
34.77	9-Octadecenoic acid	td	36.09	32.50
34.72	11-Octadecenoic acid	47.30	nd	nd

Retention time [min]	Fatty acids	Fatty acid Percentage [%]		
		Bovine	White Rats	Black Rats
34.81	13-Octadecenoic acid	1.54	nd	nd
35.22	Octadecanoic acid	12.71	5.05	7.01
36.20	Octadecane, 1,1-dimethoxy	nd	nd	0.18
36.76	10-Nonadecenoic acid	0.14	nd	0.05
37.41	Nonadecanoic acid	nd	0.08	0.08
37.43	9,11-Octadecadienoic acid	0.15	-	-
38.04	Methyl arachidonate	nd	1.28	1.76
38.20	5,8,11-Eicosatrienoic acid	nd	0.08	nd
38.45	7,10,13-Eicosatrienoic acid	nd	0.06	0.14
38.45	6,9,12-Octadecatrienoic acid	nd	0.20	nd
39.04	Cyclopropaneoctanoic acid, 2-hexyl	0.49	0.08	nd
39.18	11-Eicosenoic acid	0.02	nd	0.06
39.61	Eicosanoic acid	0.07	0.13	0.15
42.62	Methyl gamma-linolenoate	nd	0.26	0.13
42.79	Methyl eicosa-5,8,11,14,17-	nd	0.12	0.56

4. Conclusion

The major constituents of lipid extracted from rats were 9-Octadecenoic acid; Hexadecanoic acid; 9,12-Octadecadienoic acid; Octadecanoic acid; 9-Hexadecenoic acid; Tetradecanoic acid; and Methyl arachidonate. The major constituents were fatty acids with chain lengths from 15 to 21 carbon atoms (mainly C17 and C19).

Acknowledgments

We wish to thank to Instrument Laboratory Staff members of the Faculty of Pharmacy, Universitas Muhammadiyah Purwokerto, for the facilities given and their supports, and special thanks are devoted to the Directorate General of Higher Education of Indonesia which has given grants for this research.

References

[1] Widyasari I Y, Sudjadi and Rohman A 2015 Detection of Rat Meat Adulteration in Meat Ball Formulations Employing Real Time PCR *Asian Journal of Animal Sciences* **9** 6 460-465
[2] Rahmania H 2014 *Analisis Daging Tikus dalam Bakso Sapi Menggunakan Metode Spektroskopi Inframerah yang Dikombinasikan dengan Kemometrika (Skripsi)* Yogyakarta: Universitas Gadja Mada
[3] Nurjuliana M, Che Man Y B, Mat Hashim and Mohamed A K S 2011 Rapid identification of pork for halal authentication using the electronic nose and gas chromatography mass spectrometer with headspace analyzer *Meat Sci.* **88** 638–644
[4] Guntarti A, Martono S, Yuswanto A and Rohman A 2015 FTIR Spectroscopy in Combination with Chemometrics for Analysis of Wild Boar Meat in Meatball Formulation *Asian Journal of Biochemistry* **10** 4 165-172
[5] Fibriana F, Widianti T, Retnoningsih A and Susanti 2012 Deteksi Daging Babi Pada Produk Bakso di Pusat Kota Salatiga Menggunakan Teknik Polymerase Chain Reaction *Biosaintifika* **4** 2
[6] Che Man Y B, Rohman A and Mansor T S T 2011 Differentiation of lard from edible oils by means of Fourier transform infrared spectroscopy and chemometrics *Journal of the American Oil Chemists' Society* **74** 187-192
[7] Ali M E, Hashim U, Mustafa S, Che Man Y B and Islam Kh. N 2012 Gold Nanoparticle Sensor for the Visual Detection of Pork Adulteration in Meatball Formulation *Journal of Nanomaterials* Article ID 103607 7