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 BF3-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](image1)

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](image2)
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 min); 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](image)

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-Octadecanoic acid (32.50 – 36.09%) and Methyl arachidonate (1.28 – 1.76%) in lipid extracted from rats, but not in bovine. In contrast, 11-Octadecanoic 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).

| 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       |
| 37.31                | 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                | Cyclopropanocticanoic 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-linolenate           | 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).

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