

Fat Analysis of House Rat (*Rattus tanezumii*) in Meatball Using Gas Chromatography-Mass Spectrometry (GC-MS) Combined with Principal Component Analysis

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ABSTRACT

Counterfeit foods have become a new problem for Indonesians. Special attention has been given, especially to the contamination of non-halal meat in food products. This research was aimed to analyze the fatty acid compositions contained in house rats using the GC-MS method combined with Principal Component Analysis (PCA). The fat extraction was held using an oven at 90°C - 100°C for approximately an hour. The fat was then derived through the derivatization process using NaOCH₃ and BF₃ into a methyl ester that can be easily evaporated. The resulting methyl esters were injected into the chromatography instrument system for GC-MS analysis; the results showed that fatty acids of house rats have SI values > 90. Fatty acids of house rats were composed with methyl myristate (0.19±0.03)%, palmitoleic (2.40±0.29)%, methyl palmitate (27.65±0.32)%, oleate (45.81±3.25)%, and stearate (4.65±0.28)%. The total fat content was 48.21% unsaturated fatty acids, and 31.49% saturated fatty acids. The GC-MS method combined with PCA can post the fat of house rats. Based on PCA's chemometrics, fatty acids from house rats demonstrate chemical-physical properties with fatty acids from chickens.

Keywords: house rat, fatty acids, GC-MS, PCA's chemometrics

INTRODUCTION

Food supervision in Indonesia related to halalness, safety, and health has been ineffective. Most of Indonesia's population are Muslims (Mursyidi, 2013). Current issues are focused on the contamination of haram meat in food products, such as counterfeit meat products made of house rats and field rats (Guntarti and Prativi, 2017). Counterfeit foods are a problem for Muslim communities in Indonesia and Indonesians in general, especially in terms of health (Nakyinsige *et al.*, 2012).

Meats made of rats are classified as non-halal meat. In food production, meats made of rats can be analyzed from various perspectives: religion, economy, and health (Van der Spiegel *et al.*, 2012). Food products circulating in the market, such as meatballs, sausages, and nuggets, which are made of mixed halal and non-halal meats, need to be analyzed with high validity tests (Rahayu *et al.*, 2018). Several methods have been developed to

detect the presence of non-halal components, such as Fourier Transform Infrared (FTIR) (Rahmania *et al.*, 2015) and Differential Scanning Colorimetry (DSC) (Rohman *et al.*, 2012). Other methods that have been proposed to investigate this issue are electronic nose (Indrasti *et al.*, 2010; Nurjuliana *et al.*, 2011) and real-time PCR (Kurniasih *et al.*, 2020). Some researchers also have proposed chromatography-based methods such as High-Performance Liquid Chromatography (Tarola *et al.*, 2012; Von Barga *et al.*, 2013) and Gas Chromatography (Guntarti *et al.*, 2020).

Gas Chromatography-mass Spectrometry (GC-MS) analysis is a fast and accurate method for separating complex mixtures, capable of analyzing small amounts of mixtures (Kumar *et al.*, 2014; Johnsen *et al.*, 2017). Analysis with GC-MS combined with chemometric PCA aims to reduce the number of existing variables without losing the information contained in the original data (Zhao *et al.*, 2014; Grob, 2004).

Guntarti *et al.* (2020) analyzed fatty acids of Wistar rats using the GC-MS method. Fatty acids constituent of Wistar rats, boar, and other animals, are similar to fatty acids of lards based on PCA chemometrics. However, Wistar rats are close to pigs and chickens.

MATERIAL AND METHODS

GC-MS analysis of the research is conducted using Shimadzu GCMS-QP2010 SE (Tokyo, Japan), and equipped with a mass spectrometer detector and AOC-5000 autosampler. Mass spectra determination used WILLEY147 & NIST14 references. Separation was carried out in a Rtx-5ms Restek column (30m x 0.25 mm ID, 0.25 μ m) (Bellefonte, PA, USA), with 100% dimethyl-polysiloxane for its stationary phase. The injector's temperature was 230°C. The column temperature was initially designed at 70°C and increased to 300°C at 10°C/min. The flow rate was 1.15 mL/min. Helium was used as the carrier gas in the mobile phase. The MS detector used was 70 MeV Electron Multiplier Detector (EMD). Other tools used in the research are oven, vortex, and glassware.

The primary sample used was fats taken from house rats that were obtained from PASTY (Yogyakarta Animal and Ornamental Plant Market), Dongkelan, Bantul, Yogyakarta. Supporting samples consist of fats taken from lard, chicken, and goat, purchased from the local market; other additional solutions used were n-hexane (Merck 104367), methanol (Merck 106009), solid NaOH (Merck 106498), BF₃ solution (Merck 801663), saturated NaCl (Merck 106404) and anhydrous Na₂SO₄ (Merck 106649)(Guntarti *et al.*, 2020).

Sample identification

Prior to sample preparation, sample identification was first conducted at the Animal Systematics Laboratory (SH), Faculty of Biology, Gadjah Mada University.

Fat extraction with oven heating

Fats of house rats, lard, chicken fat, goat fat, and those taken from meatballs bought on the market were cut into small pieces. Those fats were subjected to rendering in an oven at 90-100°C for 1-2h. The obtained fats were then filtered with a flannel cloth. The fat fraction was then taken, added with anhydrous Na₂SO₄, and centrifuged at 3000 rpm for 20min. The oily layer was decanted, filtered with Whatman paper, and then placed with anhydrous Na₂SO₄. After that, the solution was stored in the refrigerator at -20°C in a closed tube.

Therefore, they were ready to be used for esterification (Guntarti *et al.*, 2020).

Esterification

A total of 50 μ L of oil or fat of house rats was added with 1.0 mL of n-hexane and 200 μ L of 0.2 N NaOCH₃ solution, and heated in a water bath at 90°C-100°C for 10 minutes. The 0.2 N methanolic NaOH solution was obtained by mixing 800 mg of solid NaOH in 100 mL of methanol. The mixture was cooled, added with 1.5 mL of BF₃ solution, vortexed, and heated in a water bath at a temperature of 90°C-100°C for 10 minutes. The mixture was re-cooled, and 1.5 mL saturated NaCl was added to it. The supernatant containing methyl ester (the derivative of fatty acids) was transferred into the vial, and 1 μ L of the supernatant was injected into the GC-MS system for further analysis (Rahayu *et al.*, 2018b)

Data analysis

In the form of methyl esters, fatty acids were used for the Gas Chromatography-Mass Spectrometry (GC-MS) analysis. The content of methyl esters in each sample (fats of house rats, pigs, chickens, goats, and meatballs bought from the market) was subjected to chemometric PCA analysis using Minitab 19 software (Danzer and Currie, 2015).

RESULTS AND DISCUSSION

Samples of house rats were identified as species of *Rattus tanezumi*, which belong to the genus of *Rattus* Fischer. Sample identification was performed at the laboratory of Biology, Gadjah Mada University, Yogyakarta.

Fat extraction

Extraction by rendering produces different yields. The differences can be seen in the amount of percentage yield, fat used, and fat content. The fat/oil obtained was added with Na₂SO₄ to remove the water content in the fat extract (Rohman and Che Man, 2012). Identification of yield and extracted fats of house rats, pigs, chickens, goats, and meatballs (Table I).

The oil obtained from each animal was subjected to a derivatization process. Some solutions, such as n-hexane, methanol, and solid NaOH, separated fatty acids from the triglycerides. Furthermore, BF₃ solution was used as an acidic catalyst, and saturated NaCl was used to precipitate protein salts, separate the glycerol, and clarify the layer (Figure 1).

Table I. Identification results of fat extracted from house rats, pigs, chickens, goats, and meatballs

Fat	Fat weight (gr)	Oil weight (gr)	Oil Color	Yield (%)
House rat	8.15	0.35	White	4.34
Pig	49.90	4.13	White	8.28
Chicken	49.94	4.60	Yellow	9.21
Goat	50.26	4.74	White	9.42
Meatball A	48.45	7.07	White	14.59
Meatball B	49.73	8.28	Yellow	16.64
Meatball C	50.31	9.35	Yellow	18.58

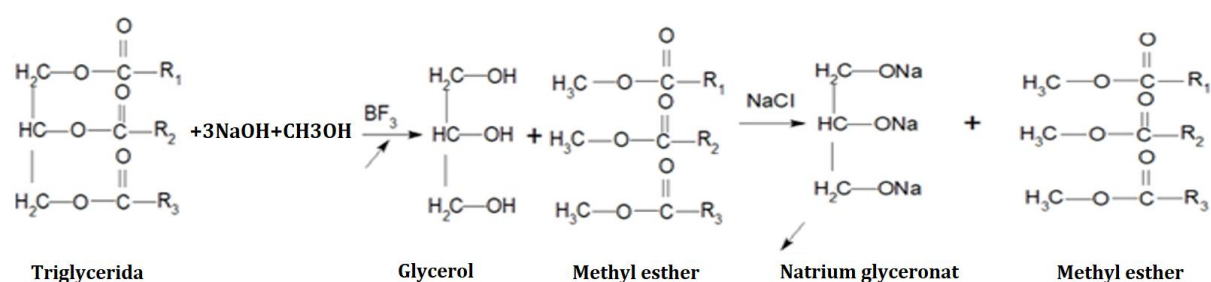


Figure 1. Formation of FAME and its reactions

Table II. Analysis of separated fatty acids taken from house rats with GC-MS

No.	tR (min)	% Area ± SD (n=3)	CV	SI	MW	Compound
1	19.19	0.19±0.03	15.80	96	242	Myristic (C14:0)
2	20.63	2.40±0.29	12.10	96	268	Palmitoleic (C16:1)
3	20.91	27.65±0.32	1.15	97	270	Palmitic (C16:0)
4	26.00	45.81±3.25	7.10	96	296	Oleic (C18:1)
5	26.24	4.65±0.28	6.03	97	298	Stearic (C18:0)

SD=standard deviation; CV= coefficient of variation; SI= similarity index; MW= molecular weight.

Fatty Acid Methyl Ester (FAME) of house rat

Fatty acid analysis of house rats was done using GC-MS. GC-MS is a method of separating organic compounds using two methods of compounds analysis. 1) Gas Chromatography (GC) to analyze the compound's types qualitatively, and 2) Mass Spectra (MS) to obtain relative molecular mass information from the sample (Grob, 2004) (Table II). The separation results of fatty acids taken from house rats were analyzed (Table II). Type identification of fatty acids using mass spectrometry (MS) resulted in SI (similarity index), which is a comparison of their mass spectra with data in the GC-MS library (WILLEY147 & NIST14). The resulting SI is >90; this indicates a similarity in chemical structure to fatty acids of field rats (Guntarti *et al.*, 2020).

Fatty acids of house rats were composed of three types of saturated fats (myristic, palmitoleic, palmitic) and two types of unsaturated fats (oleic and stearic). The highest content of saturated fat was found as palmitic acid (27.65%), while the highest content of unsaturated fat was found as oleic acid (45.81%) (Table II). According to study findings by Guntarti (2020), the GC-MS analysis results of Wistar rats contained six types of methyl esters; oleic acid was the highest content (40.48%), followed by linoleic (30.14%). Wistar rats are specifically bred for animal testing, while house rats are common rats that usually live in households. Wistar rats, as research-specific rats, have certain criteria to meet before being used as samples.

Table III. Percentage of methyl ester found in fats of house rats, pigs, chickens, and goats based on GC-MS acquisition

Methyl ester	Percentage (% area) of methyl ester			
	House rat	Pig	Chicken	Goat
Myristic (C14:0)	0.19	0.16	0.12	0.12
Pentadecanoic (C15:0)	NA	NA	NA	0.11
Palmitoleic (C16:1)	2.40	0.82	2.33	0.36
Palmitic (C16:0)	27.65	0.53	22.84	27.65
Oleic (C18:1)	45.81	36.09	71.38	3.99
Stearic (C18:0)	4.65	11.63	1.90	45.39
Arachidic (C20:0)	NA	0.11	NA	NA

NA: Not Available

Table IV. PCA Analysis Report of SD house rats, and sample products of other animals and their Eigen analysis

Eigen analysis of the Correlation Matrix								
Eigenvalue	4.1633	2.0310	1.1190	0.3341	0.2753	0.0541	0.0170	0.0063
Proportion	0.520	0.254	0.140	0.042	0.034	0.007	0.002	0.001
Cumulative	0.520	0.774	0.914	0.956	0.990	0.997	0.999	1.000
Variable	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
Myristic	0.385	0.049	0.501	-0.261	0.480	0.402	-0.316	0.197
Pentadecanoic	0.480	-0.026	-0.065	-0.030	-0.135	-0.645	-0.569	-0.077
Palmitoleic	-0.185	0.514	0.496	0.206	0.244	-0.517	0.263	0.130
Palmitic	0.271	0.512	-0.304	0.321	-0.259	0.238	-0.064	0.584
Margaric	0.428	-0.003	0.285	-0.440	-0.525	-0.036	0.513	0.032
Oleic	-0.375	0.253	-0.282	-0.760	0.069	-0.152	-0.140	0.301
Stearic	0.381	-0.213	-0.432	-0.015	0.570	-0.216	0.465	0.186
Arachidic	-0.211	-0.602	0.241	0.117	-0.138	-0.166	-0.057	0.687

Comparison of fatty acids of house rat, pig, chicken, and goat

Derivatization results of extracted animal fats taken from pigs, chickens, and goats are similar to derived fats of house rats. Their results were analyzed using GC-MS in methyl esters (Table III).

Linoleic acids could not be found in fats of house rats, while quite high content (30.14%) was found in fats of Wistar rats. Linoleic acid has an important function for cell and brain development (Hausman *et al.*, 2018). Fats of rats have 48.21% unsaturated fatty acid constituents (palmitoleic, and oleic) and 31.49% saturated fatty acid constituents (myristic, palmitic, and stearic). Wistar rats contain 70.62% total unsaturated fatty acids (oleic and linoleic), while only 21.78% saturated fatty acids are contained (Guntarti *et al.*, 2020). Compared to that of Wistar rats, fats of house rats have a significant difference in unsaturated fatty acid content. House rats are smaller than Wistar rats. Unsaturated fatty acids

can help increase good cholesterol (HDL), reduce bad cholesterol (LDL), and help maintain heart health (Lusas *et al.*, 2012).

Chickens have the highest unsaturated fatty acids (oleic acid) (71.38%), followed by house rats (45.81%), pigs (36.09%), and goats (3.99%). The highest saturated fat content (stearic acid) was found from goats (45.39%), pigs (11.63%), house rats (4.65%), and chickens (1.90%). In addition, fats taken from goats have pentadecanoic acid (0.11%), which cannot be detected in other animals (Table III). Fats of pigs (lards) contain arachidic acid, which is unnoticeable in fats of other animals.

Principal Component Analysis (PCA)

Principal component analysis, commonly referred to as PCA, is an analytical method for building multivariate linear models on complex data. PCA simplifies the data by reducing the number of variables to a smaller number of orthogonal variables. (Milller and Miller, 2010).

Eigenvalue analysis results using Minitab 19 based on PCA for house rats, chickens, goats, pigs, and three meatball product samples were provided (Table IV).

The PCA analysis that was conducted using Minitab 19 software obtained 8 PCs. The selection of the number of PCs in PCA can be determined by observing the eigenvalues obtained from the result. The number of PCs relevant to explaining the preliminary information from data was PC with an eigenvalue > 1. Below this limit, PCs were considered irrelevant (Table IV). PC1 with an eigenvalue of 4.1633 could describe 52.0% of the original data. Meanwhile, PC2 with an eigenvalue of 2.0310 could describe 25.4% of the total original data variables. PC3 with an eigenvalue of 1.1190 was able to describe 14.0% of the total original data variables. Therefore, only by using 3PCs, 91.4% of original variables can be represented, and the results were relevant enough to describe the original characteristics (Miller and Miller, 2010).

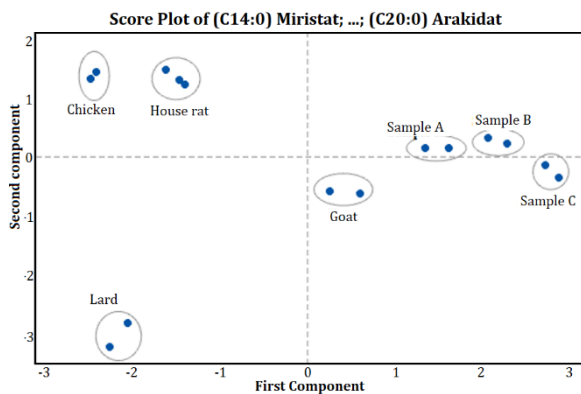


Figure 2. PCA analysis of fatty acid profiles of house rats, pigs, chickens, goats, and three samples of meatball products from the market

The different group on the score plot profiles of fatty acids (Figure 3), the PCA chemometric results showed that the fatty acid profile of house rats was in the same quadrant as that of chickens. This is because there are similarities in the content of fatty acids. Meanwhile, three fat samples of meatball products bought from the local market have similar fatty acid constituents to that of goats. This is in line to study findings (Guntarti *et al.*, 2020) that the fatty acid profile of goats is similar to that of cows.

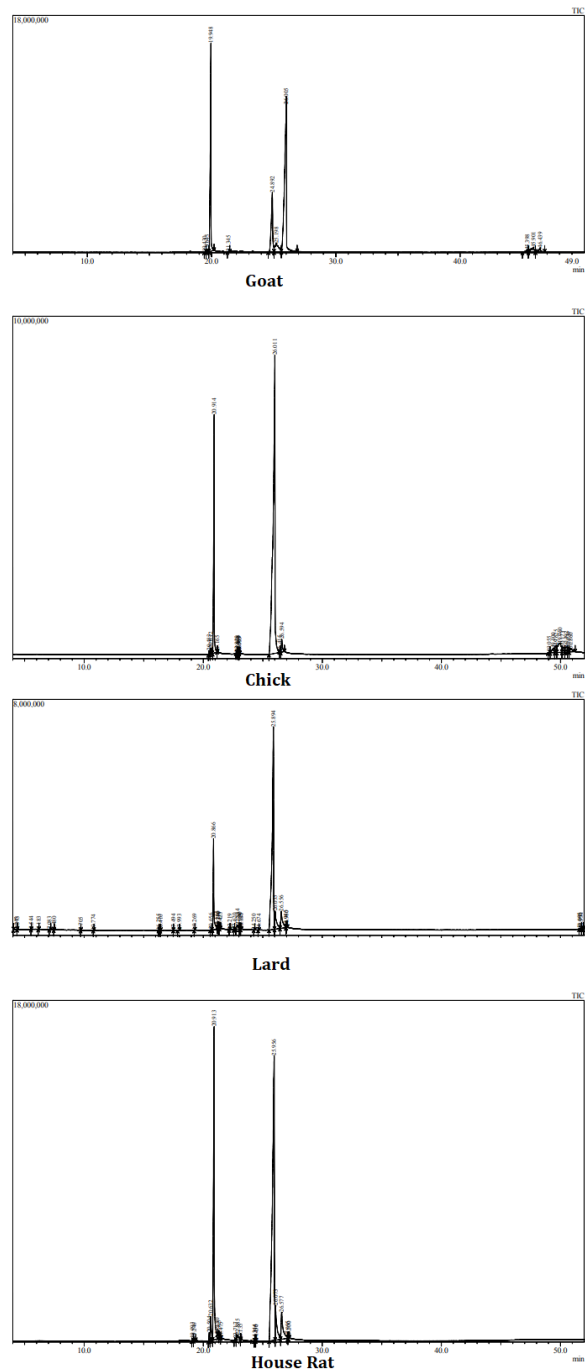


Figure 3. Chromatograms of various animal types (rat, pig, chicken, and goat) with GC-MS

CONCLUSION

GC-MS method can be used to analyze fat compositions of house rats, along with fats of other animals. Fat composition of house rats is the most similar to that of field rats. However, the percentage of methyl esters resulting from MS

acquisition needs further analysis to differentiate meatball samples. Chemometric Principle Component Analysis (PCA) can classify fats of house rats from other animal fats, and profile the fat of meatball samples; samples of meatball products that were bought on the market have different fat profiles to those of house rats.

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