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Nina Salamah <nina.salamah@pharm.uad.ac.id>

[IJP] Editor Decision

1 pesan

Editorial Board IJP <mfi@ugm.ac.id> 7 Oktober 2021 09.42
Kepada: Any Guntarti <any_guntarti@yahoo.co.id>, Putri Ayu Lestari <putri1600012036@webmail.uad.ac.id>, Nina Salamah <nina.salamah@pharm.uad.ac.id>, Ibnu Gholib Gandjar <ibngandjar@yahoo.com>

Any Guntarti, Putri Ayu Lestari, Nina Salamah, Ibnu Gholib Gandjar:

We have reached a decision regarding your submission to Indonesian Journal of Pharmacy, "AUTENTICATION OF HOUSE RAT FAT (Rattus tanezumi) WITH GAS CHROMATOGRAPHY MASS SPECTROMETRY (GC-MS) COMBINED CHEMOMETRICS". Please revise the review comment below:

The tittle should be more specific, consider to include the sample analysed. The major discussion of manuscript is about characterization of house rat fat. But authors also conduct analysis of meatball.

The authors should add the background why they analysed the house rat in meatball.
The authors should add the method in analysing meatball samples
Avoid the double presentation of data, as it is found the double presentation of data in table form and figure form table II and figure 2, Table IV and figure 3

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Balas Ke: Editorial Board IJP <mfi@ugm.ac.id>
Kepada: Nina Salamah <nina.salamah@pharm.uad.ac.id>, Any Guntarti <any_guntarti@yahoo.co.id>, Putri Ayu Lestari <putri1600012036@webmail.uad.ac.id>, Ibnu Gholib Gandjar <ibngandjar@yahoo.com>

Dear Mr/Mrs Nina Salamah, Any Guntarti, Putri Ayu Lestari, Ibnu Gholib Gandjar

Thank you for working with us for the paper, "AUTENTICATION OF HOUSE RAT FAT (Rattus tanezumi) WITH GAS CHROMATOGRAPHY MASS SPECTROMETRY (GC-MS) COMBINED CHEMOMETRICS," the editing of your submission is complete. We are pleased inform you that your manuscript has been completed and it was accepted for publication.
In order to improve the clarity of the manuscript, we required the author to seek help for professional proofreading service before we are able to proceed the manuscript to the next step. Please email to us the proofreading version and proofreading certificate of the manuscript.

Regards
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[Kutipan teks disembunyikan]
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Best regards,

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Faculty of Pharmacy
University of Ahmad Dahlan
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AUTENTICATION OF HOUSE RAT FAT (*Rattus tanezumi*) WITH GAS CHROMATOGRAPHY MASS SPECTROMETRY (GC-MS) COMBINED CHEMOMETRICS

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ABSTRACT

Counterfeit food products are starting to become a new problem around the people of Indonesia. Problems that are getting special attention especially the concern of contamination of food products by non-halal meat. This research aim was to analyze the fatty acids contained in house rat. The method that used was GC-MS method combined with Principle Component Analysis (PCA). Method of extraction grease was using oven at 90ºC - 100ºC for approximately one hour. After that, the derivatization process changed the fat into a methyl esters that easily evaporated. The derivatization process were using $NaOCH_3$ and BF_3. Methyl Esther compounds were injected into the chromatography instrument system GC-MS. GC-MS analysis results shows that the composition of fatty acid compounds from house rat fat with SI values > 90. Composition of fatty acids of house rat fats include: Myristate (0,19±0,03)%, Palmitoleat (2,40±0,29)%, Methyl Palmitate (27,65±0,32)%, oleate (45,81±3,25)%, and Stearate (4,65±0,28)%. Total content of house rat fatty acids was as high as 48.21% of unsaturated fatty acid and 31.49% of saturated fatty acids. The GC-MS method combined with PCA can post the fat of house rat. House rat fatty acids based on PCA's chemetrics demonstrate the resemblance of chemical physical properties with chicken fatty acids.

Commented [H2]: The meatball analysis result should be mention in the abstract

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

INTRODUCTION

Supervision of food in Indonesia related to halal, safety, and health has not been effective. That is because the majority of Indonesia's population is Muslim (Mursyidi, 2013). The problem that is getting a lot of attention today is the concern about contamination of food products by haram meat, one of which is the meat of house rats, field rats (Guntarti, A. and Prativi, S.R., 2017). The issue of food counterfeiting is not only a problem for Muslim communities in Indonesia, also can be a problem for the people of Indonesia in general, especially in terms of health (Nakyinsige et al., 2012).

Rat meat is classified as non-halal meat. In food production, rat meat can be seen from various aspects, including: religion, economy, and health (Van der Spiegel et al., 2012). The existence of halal meat mixed with non-halal meat in market food products such as meatballs, sausages, naget, needs to be analyzed with a method that has high validity (Rahayu et al., 2018). Several methods have been developed to detect the presence of non-halal components. The methods that have been developed include Fourier Transfor Infrared (FTIR)

(Rahmania et al., 2015), Differential Scanning Colorimetry (DSC) (Rohman et al., 2012), electronic nose (Indrasti et al., 2010; Nurjuliana et al. , 2011), real time PCR (Kholif et al., 2020), High Performent Liquid Chromatography (Tarola et al., 2012; Von Bargen et al., 2013), Gas Chromatography (Guntarti et al., 2020).

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 existing variables to be fewer without losing the information contained in the original data (Zhao et al., 2014; Grob, R.L., 2004).

Guntarti et.al., 2020 conducted an analysis of fatty acids in Wistar rats using the GC-MS method. The constituent fatty acids of Wistar rats and various other animal fatty acids, and boar fatty acids based on PCA chemometrics are close to lard fatty acids, while Wistar rats are close to pigs and chickens.

Commented [H3]: The authors also analysed meatball, Add the background in analyzing house rat in meatball

RESEARCH METHODS

Equipment: GC-MS distributor from Ditek Jaya, Merck Shimadzu, Japan, type GCMS-QP2010 SE with a mass spectrometer detector with an autosampler machine. Separation was carried out in a DBI-MS Restech column 30m x 0.25 mm ID, 0.25 µm, with Polymethyl xiloxan stationary phase, injector temperature 230^{o}C, column temperature 70^{o}C and increased to 300^{o}C in increments of 10^{o}C / min, flow rate 1 , 15 mL / minute. Helium gas mobile phase. The MS detector used was 70 MeV Electron Multifier Detector (EMD), oven, vortex, glass ware.

Material: The main sample used was house rat fat obtained from PASTY (Yogyakarta Animal and Ornamental Plant Market), Bantul KM 1, Dongkelan, Yogyakarta. Supporting samples consist of: lard, chicken fat, and goat fat purchased from the market in Yogyakarta, n-hexane, methanol, solid NaOH, BF3 solution, saturated NaCl, anhydrous Na2SO4 (Guntarti el.al., 2020).

The course of research

Sample Determination

Prior to sample preparation, sample determination was first carried out at the Animal Cytematics Laboratory (SH), Faculty of Biology, Gadjah Mada University.

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Fat Extraction Process With Oven Heating

House rat fat, lard, chicken fat, goat fat, and fat from meatballs on the market are cut into small pieces. Fat is subjected to the rendering procedure in an oven at 90ºC-100ºC for 1-2

hours. The fat obtained is filtered with a flannel cloth. The fat fraction is taken and anhydrous Na_2SO_4 is added which is then centrifuged at 3000 rpm for 20 minutes. The oil layer was decanted, filtered with Whatman paper and then placed with anhydrous Na_2SO_4. The solution is stored in the refrigerator at -20 ° C in a closed effendrof. Furthermore, the solution can be used for the esterification process (Guntarti et al., 2020).

Esterification Process

A total of 50 µL of oil or fat of house rats was added with 1.0 mL of n-hexane and 200 µL of 0.2 N $NaOCH_3$ solution, and heated in a water bath at 90ºC-100ºC 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 and added with 1.5 mL of BF3 solution vortexed and heated in a water bath at a temperature of 90ºC-100ºC for 10 minutes. The mixture was cooled and 1.5 mL saturated NaCl was added. The supernatant containing methyl ester fatty acid derivatives was transferred into the vial and injected as much as 1 µL into the GC-MS system (Rahayu et al., 2018b)

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Data analysis

The data from the Gas Chromatography Mass Spectrometry (GC-MS) analysis are in the form of fatty acids in the form of methyl esters. The content of fatty acid methyl esters of each of the fat of house rats, pigs, chickens, goats, and meatball samples in the market were grouped using chemometric PCA with minitab 19 (Danzer and Currie, 2015).

RESULTS AND DISCUSSION

The results of the identification of house rat in the laboratory of Biology, Gadjah Mada University, Yogyakarta, including the Rattus Fischer Genus with the species: Rattus tanezumi.

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Fat Extraction

The extraction process by rendering produces different yields. The difference in yield% is caused by the extraction process, the part of the fat used, and differences in the amount of fat content of each animal. The fat / oil obtained was added with Na_2SO_4 to remove the water content in the fat extract (Rohman, A. and Che Man, Y.B., 2012). Table I presents the yield and fat color of house rats, pigs, chickens, goats, and meatball samples on the market.

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Tabel I. Result and identification of fat color on house rat, pig, chicken, goat, and meatball

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

The oil obtained from each animal is subjected to a derivatization process. N-hexane, methanol and solid NaOH aims to separate fatty acids from triglycerides. Furthermore, BF_3 is used as an acidic catalyst and uses Saturated NaCl to precipitate protein salts and to separate the glycerol and clarify the layer. Derivatization reaction is presented in Figure 1.

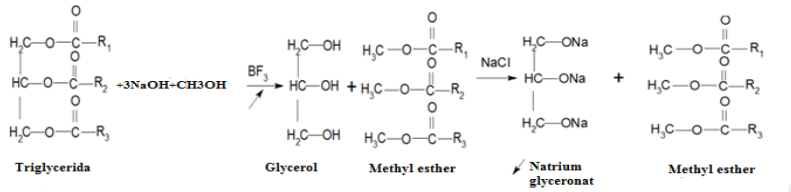

Figure 1. FAME form reaction

Fatty Acid Methyl Esther (FAME) of House Rat

Fatty acid analysis of house rat by GC-MS. GC-MS is a method of separating organic compounds using two methods of analysis of compounds, namely Gas Chromatography (GC) to qualitatively analyze the types of compounds and Mass Spectra (MS) to obtain relative molecular mass information from the sample compounds (Grob, 2004). The results of the analysis of house rat fat using the GC-MS method in the form of its methyl ester can be seen in Table II.

Tabel II. Analysis of the separation of fatty acids in house rat with GC-MS

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

SD=standard deviation; CV= coefficient of variation; SI= Similarity Index; MW= molecule weight.

Table II shows the results of the separation of fatty acid analysis on the fat of house rat. Identification of the types of fatty acids was carried out by a mass spectrometer (MS) with a reading at the peak and SI (similarity index) is a comparison value with the spectra in the GC-MS library software WILLYEY147 & NIST47 states the SI value> 90, this indicates a similarity in chemical structure to fatty acids field rat (Guntarti et al., 2018).

The coefficient of variation (CV) in% area varies widely. CV price shows homogeneity of data. The difference in CV value is caused by the fat extraction process, the derivatization process and, the conversion of liquid methyl ester into vapor in the gas chromatography process. Figure 2 shows the histogram of fatty acid acquisition of house rat.

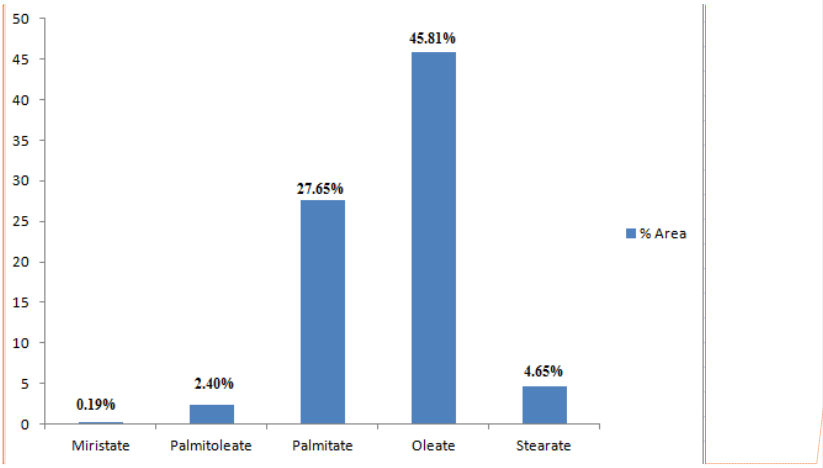

Figure 2. The result of separation of fatty acids in the house rat fat with GC-MS

Figure 2, there are 5 types of fatty acids in house rat. There are 3 types of saturated fatty acids with the highest content of palmitic acid (27.65%), There are 2 types of unsaturated fatty acids, which have the highest content of oleic acid (45.81%). Research by Guntarti (2020), the results of analysis by GC-MS Wistar rats contained 6 types of methyl esters, the highest being oleic (40.48%), then linoleic (30.14%). Wistar rats are rats for animal testing, while house rats are rats that usually live indoors. Wistar rats as research-specific rat, have certain criteria. Figure 3 comparison of methyl ester content in Wistar rats and house rat.

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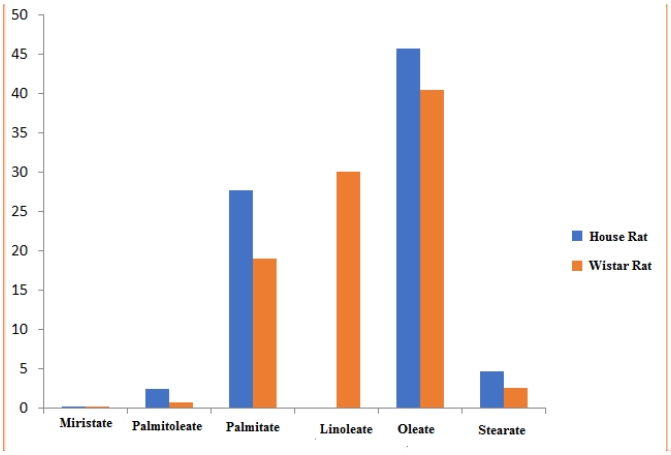

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Figure 3. Comparison of methyl esters content in house rat and wistar rat

The data presented In Figure 3 showed that~~,~~ house rat do not contain linoleic fatty acids, while Wistar rats are quite high (30.14%). Linoleic acid has an important function for cell and brain development (Hausman et al., 2018). Rats have many types of unsaturated fatty acids with a total percentage of 48.21% (palmitoleate, linoleate, and oleate) and types of saturated fatty acids with a total percentage of 31.49% (myristate, palmitate, and stearate). In Wistar rats, total unsaturated fatty acids are 70.62% (oleic and linoleic), while saturated fatty acids are only 21.78 (Guntarti et al., 2020). Significant difference in unsaturated fatty acid content. The house rat is smaller than the Wistar rat. Unsaturated fatty acids can help increase good cholesterol (HDL), reduce bad cholesterol (LDL), and helps maintain heart health (Lusas et al, 2012).

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

Pig, chicken, and goat animal fats in the extraction process, derivatization as in the fat of house rat. The results of analysis by GC-MS in the form of methyl ester are presented in the table presented in Figure 4 presenting the chromatograms on fat of house rats, pigs, chickens, and goats. Table III presents the methyl esters.

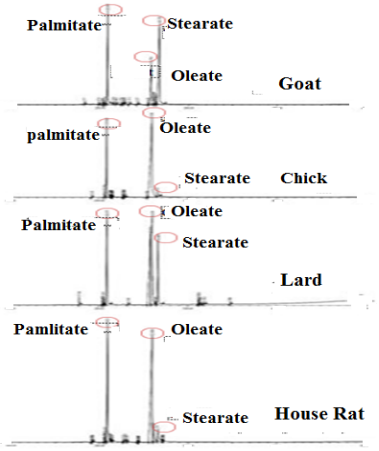

Figure 4. Chromatograms of various animal types (rat, pig, chicken, and goat) with GC-MS

Table IV. Acquisition of the methyl ester percentage in house rat, pig, chicken, and goat fat with GC-MS

Methyl ester	Percentage (% area) of methyl ester			
	House rat	Pig	Chicken	Goat
Miristat (C14:0)	0.19	0.16	0.12	0.12
Pentadekanoat (C15:0)	Na	Na	Na	0.11
Palmitoleat (C16:1)	2.40	0.82	2.33	0.36
Palmitat (C16:0)	27.65	0.53	22.84	27.65
Oleat (C18:1)	45.81	36.09	71.38	3.99
Stearat (C18:0)	4.65	11.63	1.90	45.39
Arakidat (C20:0)	Na	0.11	Na	Na

Na: Not available

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Figure 4 and Table IV above show that chicken has the highest unsaturated fatty acids (oleic) (71.38%), then house rat (45.81%), pigs (36.09%), and goats (3.99%). The highest saturated fat content (stearic acid) was goat (45.39%), pig (11.63%), house rat (4.65%), and chicken (1.90%). In addition, Goats have pentadecanoic acid (0.11%), other animals are not detected. Pigs have arachidates, other animals are not detected.

***Principal Component Analysis* (PCA)**

Principal component analysis, or 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. (Millller and Miller, 2010). Table V presents the eigenvalue with minitab 19 on PCA for house rats, chickens, goats, pigs, and 3 meatball product samples.

Table V. The Report of PCA analysis of SD House Rat, Other Animal and Sample Product at Markets of its Eigen analysis

Eigenalysis 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
Comulative	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
Myristate	0.385	0.049	0.501	-0.216	0.480	0.402	-0.316	0.197
Pentadecanoate	0.480	-0.026	-0.065	-0.030	-0.135	-0.645	-0.569	-0.077
Palmitoleate	-0.185	0.514	0.496	0.206	0.244	-0.517	0.263	0.130
Palmitate	0.271	0.512	-0.304	0.321	-0.259	0.238	-0.064	0.584
Margarate	0.428	-0.003	0.285	-0.440	-0.525	-0.036	0.513	0.032
Linoleate	-0.375	0.253	-0.282	-0.760	0.069	-0.152	-0.140	0.301
Oleate	-0.385	-0.213	-0.432	-0.015	0.570	-0.216	0.465	0.186
Stearate	-0.211	-0.602	0.241	0.117	-0.138	-0.166	-0.057	0.687

The PCA analysis that conducted using minitab 19 software obtained 8 PCs. The selection of the number of PCs in PCA can be determined by observing the eigenvalue that obtained from the result. The number of PCs which were relevant to explain the preliminary information from data was PC with an eigenvalue > 1. Below this limit, PCs were considered irrelevant. Table V shows that the PCA analysis produced 8 PCs. PC1 with an eigenvalue of 4.1633 was able to describe 52.0% of the total original data variable. PC2 with an eigenvalue of 2.0310 was able to describe 25.4% of the total original variable. PC3 with an eigenvalue of 1.1190 was able to describe 14.0% of the total original variables. Therefore, only by using 3PCs, one can already describe 91.4% of all original data variables and were relevant enough to explain the characteristics of the initial variables (Miller and Miller, 2010). Figure 5 shows the result of the fatty acid plot score: *SD* rats, boars, goats, cows, as well as meatball (sample A, B and C).

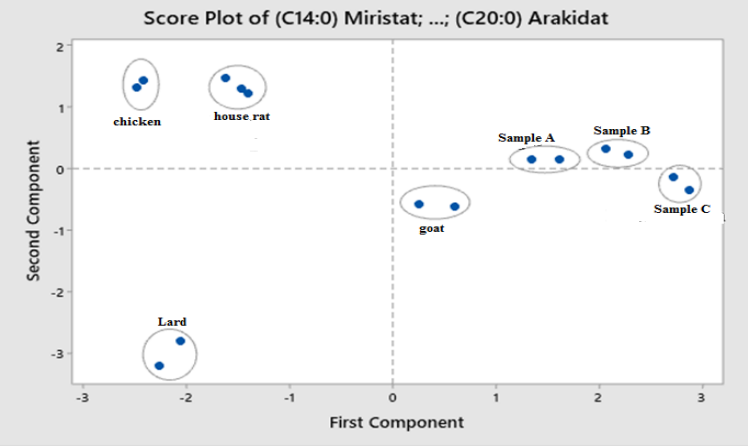

Figure 5. PCA analysis of fatty acid profiles of house rat, pig, chicken, goat, and 3 meatball sample products from the market

The score plote in Figure 5 shows the different profiles of types of fatty acids. The PCA chemometric results showed that the fatty acids of house rats and the fatty acids of chicken

were in the same quadrant. This is because there are similarities in the fatty acid content. Meanwhile, 3 meatball product samples on the market are close to each other with goats. In the study of Guntarti et al., 2020, the fatty acid profile of goat is similar to that of cow.

CONCLUSION.

Fatty acids in house rat contain 6 types of fatty acids, namely: Methyl Oleate (52.348%), Methyl Palmitate (20.65%), Methyl Oleate (5.21%), Methyl Stearate (3.66%), Methyl Palmitoleate (2.60%), and Methyl Miristate (0.19 %). Chemometric Principle Component Analysis (PCA) can classify house rat fat with other animal fats and fat from meatball products on the market.

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

Authors:

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The manuscript was edited for proper English terms, grammar, punctuation, and overall style. Author's intended meaning was not altered during the process. All amendments were tracked with Microsoft Word "Track Changes" feature.

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Fat Analysis of House Rat (*Rattus tanezumi*) 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 90ºC - 100ºC for approximately an hour. The fat was then derived through the derivatization process using $NaOCH_3$ and BF_3 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 Bargen *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; Haiyan *et al.,* 2007).

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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, 0.25µm) (Bellefonte, PA, USA), with 100% dimethylpolysiloxane 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 Multifier 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 Na_2SO_4, and centrifuged at 3000 rpm for 20min. The oily layer was decanted, filtered with Whatman paper, and then placed with anhydrous Na_2SO_4. 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 µL of oil or fat of house rats was added with 1.0 mL of n-hexane and 200 µL of 0.2 N $NaOCH_3$ solution, and heated in a water bath at 90ºC-100ºC 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 90ºC-100ºC 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 µL 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 Na_2SO_4 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 can be seen in 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, BF_3 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

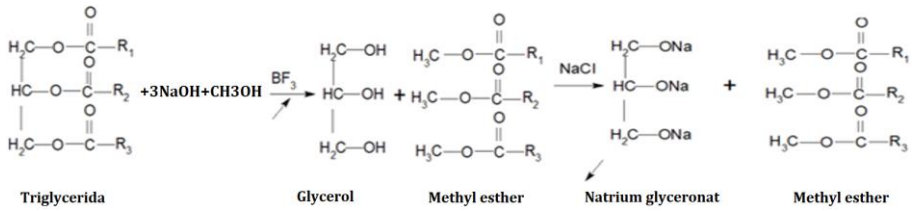

Figure 1. Formation of FAME and its reactions

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 (Haiyan *et al.,* 2007) (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.

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).

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

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 4 shows plot scoring results of fatty acids taken from *SD* rats, boars, goats, cows, and meatballs (samples A, B, and C).

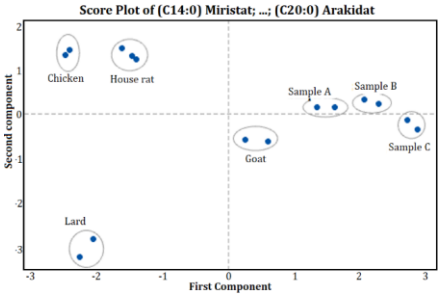

Figure 2. PCA analysis of fatty acid profiles of house rats, pigs, chickens, goats, and three samples of meatball products from the market

The score plot in Figure 3 shows different group profiles of fatty acids. 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.

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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