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Effect of Fat Extraction Methods on Fatty Acid and Infrared Profiles of Chicken Fat Using GC-MS and FTIR

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Chicken fat was obtained from rendering and chicken meat processing. The objective of present work was to study fatty acid and infrared characteristics of chicken fat based on several extraction methods. The sample preparation using Soxhlet, Folch, Bligh & Dyer and Hara & Radin methods, analysis of fatty acid using GC-MS and infrared characteristics of chicken fats using FTIR. Analysis of fatty acid showed that both SFA and USFA in Folch were not significantly different with those in Soxhlet and Hara and Radin at 5 % level, however significantly different with those in Bligh and Dyer. In addition, PCA showed fatty acids in Folch can be separated from others. FTIR spectra in general showed no significant differences in C-H stretching at 2922-2852 cm^{-1}, C=O stretching at 1744-1376 cm^{-1} and the fingerprint absorption at 1000-900 cm^{-1}. In addition, there were two peaks that indicate C-O-C group with force constant of C-O was about 1940 Nm^{-1}.

Keywords: Chicken fat, Extraction, Fatty acid composition, PC analysis.

INTRODUCTION

Poultry commodities, especially chicken, have excellent market prospects due to their characteristics of poultry products such as meat and eggs, which are favoured by the Indonesian people. The higher the population and the income level lead to the higher the demand for meat and chicken fats and their processed products. The chest part of chicken is a place of pile of lipid and rich in cholesterol, especially under the skin [1]. Triyantini *et al.* [2] reported that thigh part has higher fat content compared to chest. In addition, the physical quality of the chest meat is relatively better than the thigh. Chicken fat is obtained from the cutting process of broiler chicken with the standard procedure where all the cutting process must meet the requirements of safe, healthy, hygienic and halal.

One method that can be developed in analyzing the purity of food products containing animal fats is study the fatty acid composition contained in it using the gas chromatography-mass spectrometry (GCMS) by esterifying the fatty acid into its ester derivative or fatty acid esterification [3]. Another analysis that can be done is from spectra pattern using Fourier transform infrared (FTIR) spectrophotometry. FTIR is highly potential to be utilized as a rapid fat detection tool with consistent results even with very low content. This method also does not require complicated sample preparation where both solid and liquid samples can be directly analyzed to produce spectra. The use of FTIR and GCMS in analyzing the finger print and fatty acid composition of vegetable and animal fats have been done by several researchers. Jaswir *et al.* [4] have conducted lard analysis using FTIR, while David and Sandra [5] reported animal fats analysis of chicken, beef and lard using GC-MS. Hermanto *et al.* [6] analyzed the characteristics of fatty acids in chickens, beef fat and lard using FTIR and GCMS.

In this research, Soxhlet, Folch, Hara & Radin and Bligh & Dyer methods are used in sample preparation. According to Macedo *et al.* [7] and Habeck *et al.* [8], different extraction methods will provided data on the number and types of different fats. The objective is to study the fatty acid composition and PCA analysis as well as the infrared characteristics of chicken fats based on four extraction methods.

EXPERIMENTAL

Sample preparation: Chicken fats were prepared by rendering the adipose tissue of chicken, obtained from the meat market in Pasar Bogor, West Java, Indonesia. There were four extraction methods which were used namely Soxhlet (SO), Folch (FO), Bligh & Dyer (B&D) and Hara & Radin (H&R) methods which were based on AOAC [9], Folch *et al.* [10], Bligh & Dyer [11] and Hara & Radin [12] methods.

Esterification of fatty acids and analysis of fatty acid composition using GC-MS: The extracted chicken fat was placed into a 20-60 mg tube, then added 1 mL NaOH of 0.5 N in methanol and heated on a water bath for 20 min. After that, added 2 mL BF_3 20 % and reheated for 15 min then cooled. Added 1 mL hexane then shaked strongly for 1 min and added 2 mL saturated NaCl and shaked again for 1 min. After 2 layers were formed, removed the top layer (supernatant) and inserted into vials that have 0.1 g of anhydrous Na_2SO_4 and shaked strongly for 1 min then let stand for 20 min. The esterification results (fatty acid methyl ester, FAME) were then inserted into the empty vial to be analyzed by GCMS [13]. A 1 µL fame was then injected into the GC column by the autosampler method and set the injector temperature at 260 °C. Initial temperature of oven was set at 50 °C for 0.5 min, then raised to 195 °C with increasing rate of 8 °C/min, then raised again to 225 °C with rate of 1 °C/min and held for 22 min. Helium gas flow was set at rate of 1.8614 mL/min wher, the MS detector used was electron multifier detector (EMD) 70 eV. The mass spectral results were processed by GCMS post run analysis software using chemstation.

Infrared characteristics of chicken fat using FTIR spectrometer: The liquid extracted chicken fat after extraction process was put on the plate of Thermo Scientific Nicolet iS5 FT-IR spectrometer. Each time measuring the sample, the plate was cleaned using pure ethanol solvent pro-analysis then air spectrum was used as a background and recorded in graph of absorbance or transmittance value. The FTIR spectra were analyzed at wavenumbers of 4000-600 cm^{-1} using OMNIC software. The results were in the form of infrared spectra graph where the x-axis was wavenumbers and the y-axis was absorbance or transmittance intensity.

Data analysis: Data analysis using Anova and Duncan's posthoc test if $p < 0.05$. In order to differentiate and to classify fatty acids of chicken fat using Soxhlet, Folch, Bligh & Dyer and Hara & Radin extraction methods, the principal component analysis was performed with the aid of open chrom community edition (Diels).

RESULTS AND DISCUSSION

Fatty acid composition of chicken fat using GCMS: Based on chromatogram of chicken fat using GCMS as shown in Table-1 which is the retention time corresponding to the fatty acid composition, the percentages of saturated fatty acid (SFA) and unsaturated (UFA) in Soxhlet, Folch, Bligh & Dyer and Hara & Radin methods are 31.16 ± 0.10 and 68.84 ± 0.09, 32.66 ± 0.92 and 67.20 ± 1.03, 29.58 ± 0.40 and 70.15 ± 0.38 and 31.68 ± 0.82 and 68.27 ± 0.86, respectively. There are five dominant fatty acids present in all methods namely palmitic acid, palmitoleic acid, stearic acid, oleic acid and linoleic acid. This is consistent with Rohman and Triyana [14] who reported that these fatty acids were predominant in chicken fat with percentage of saturated and unsaturated fatty acids of about 36.3 and 62.3 %, respectively. In addition, statistical analysis shows both saturated and unsaturated fatty acids in Folch method are not significantly different with those in Soxhlet and Hara & Radin methods at 5 % level, however significantly different with those in Bligh & Dyer method. Poly unsaturated fatty acids (PUFA) such as linoleic and linolenic acid are no significantly different for all methods, while arachidonic acid is only present in Bligh & Dyer method. Rohman and Triyana [14] stated that fatty acids more than 20 carbons can be detected in small amounts. In addition, Hilda [15] found 0.50 % of arachidonic acid in chicken fat and Guntarti *et al.* [16] also explained that this was likely due to the arachidonic acid was lost by heating or derivatization during the extraction process. Smink *et al.* [17] reported that chicken fat has high linoleic acid (omega-6 fatty acid) with percentages of 7.9-22.8 %. This can be seen that linoleic acid in GCMS results has percentage of about 15 % and it is one of dominant fatty acids in chicken fat.

Fig. 1 illustrates the score plot of PCA of four extraction methods describing the projection of samples defined by the first (PC1) and second (PC2) components. Based on the score plots, it is known that Folch can be separated from others in which Folch has negative side in PC1 and positive side in PC2. In addition, fatty acids in Soxhlet method has similar fatty

TABLE-1
FATTY ACID COMPOSITION OF CHICKEN FAT BY SEVERAL EXTRACTION METHODS

Fatty acid		Peak area (%)			
		Soxhlet	Folch	Bligh & Dyer	Hara & Radin
Lauric acid	C12:0	0.06 ± 0.00^{a}	0.05 ± 0.00^{a}	0.06 ± 0.01^{a}	0.06 ± 0.00^{a}
Myristic acid	C14:0	0.79 ± 0.04^{b}	0.76 ± 0.02^{ab}	0.7 ± 0.03^{a}	0.78 ± 0.01^{b}
Myristoleic acid	C14:1	0.18 ± 0.04^{a}	0.15 ± 0.01^{a}	0.15 ± 0.01^{a}	0.15 ± 0.01^{a}
Pentadecanoic acid	C15:0	0.09 ± 0.01^{a}	0.09 ± 0.01^{a}	0.08 ± 0.00^{a}	0.09 ± 0.01^{a}
Palmitic acid	C16:0	24.35 ± 0.53^{b}	25.12 ± 0.52^{b}	22.91 ± 0.38^{a}	24.17 ± 0.43^{ab}
Palmitoleic acid	C16:1	6.38 ± 0.86^{a}	5.78 ± 0.09^{a}	5.84 ± 0.01^{a}	5.95 ± 0.30^{a}
Margaric acid	C17:0	0.11 ± 0.01^{a}	0.13 ± 0.01^{c}	0.11 ± 0.00^{ab}	0.12 ± 0.00^{bc}
cis-10 Heptadecanoic acid	C17:1	0.11 ± 0.01^{a}	0.12 ± 0.01^{a}	0.11 ± 0.00^{a}	0.11 ± 0.00^{a}
Stearic acid	C18:0	5.78 ± 0.47^{a}	6.53 ± 0.37^{a}	5.87 ± 0.02^{a}	6.47 ± 0.40^{a}
Oleic acid	C18:1	45.85 ± 0.09^{a}	48.09 ± 0.54^{b}	46.84 ± 0.40^{ab}	46.58 ± 0.79^{a}
Linoleic acid	C18:2	15.04 ± 0.88^{b}	12.07 ± 1.49^{a}	15.79 ± 0.04^{b}	14.27 ± 1.12^{ab}
Linolenic acid	C18:3	0.83 ± 0.08^{b}	0.55 ± 0.01^{a}	0.89 ± 0.02^{b}	0.69 ± 0.16^{ab}
cis-11,13 Eicosenoic acid	C20:1	0.48 ± 0.11^{a}	0.45 ± 0.00^{a}	0.55 ± 0.01^{a}	0.53 ± 0.06^{a}
Arachidonic acid	C20:4	nd	Nd	0.13 ± 0.01	nd
Saturated fatty acid		31.16 ± 0.10$^{a.b}$	32.66 ± 0.92^{b}	29.58 ± 0.40^{a}	31.68 ± 0.82^{b}
Unaturated fatty acid		68.84 ± 0.09$^{a.b}$	67.20 ± 1.03^{a}	70.15 ± 0.38^{b}	68.27 ± 0.86$^{a.b}$

The value of each fatty acid is a mean ± standard deviation of two replications. Each row with different letters is significantly different ($P < 0.05$). nd is not detected.

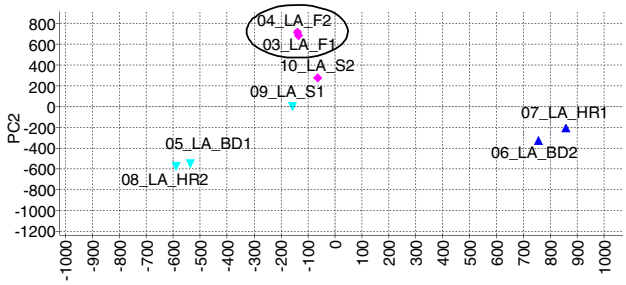

Fig. 1. Score plot of PCA model using fatty acid composition of chicken fat in Soxhlet, Folch, Bligh & Dyer and Hara & Radin

acid composition with Folch method, among others, as shown by the close distance of Soxhlet to Folch.

In order to know the number of PCs suggested by software to be used in PCA model, the residual analysis was constructed. Based on the predicted residual error sum of square values (Fig. 2), it can be stated that 5 PCs is necessary for PCA model, because at this PC number, PRESS value reach minimal [18].

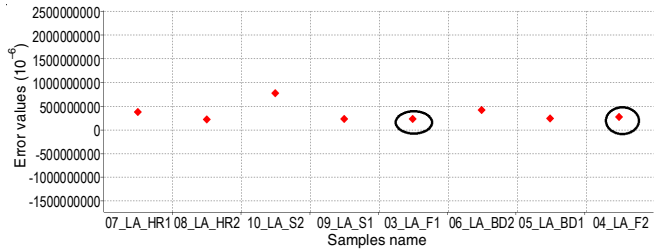

Fig. 2. Residual error of PCA model for determination of optimum principle components used in PCA model

Infrared characteristics of chicken fat using FTIR: The FTIR analysis is based on the characteristics of the functional groups in the extracted chicken fat using four extraction methods. FTIR spectra data of each sample is obtained from the scanning of chicken fat using Thermofisher Scientific FTIR Nicolet iS5 at wavenumbers of 4000-600 cm^{-1} which can be seen in Fig. 3. Based on these data, it can be seen that FTIR spectra of chicken fat generally showed no significant difference in C-H stretching at wavenumbers of 2922-2852 cm^{-1}. The high peak of chicken fat absorption in these areas shows the presence of unsaturated fatty acid content, especially linoleic acid, where the unsaturated fatty acids contribute to the high absorbance value of the C-H stretching vibration region of the *cis* double bond [19]. The absorption of C=O stretching groups of aldehydes at 1744-1376 cm^{-1} and the fingerprint absorption pattern at 1000-900 cm^{-1} also do not show any significant differences or an overlaping. In addition, there are two peaks that indicate same functional group and vibration mode appear at 1116 and 1159 cm^{-1} which show stretching vibration of C-O-C group [20].

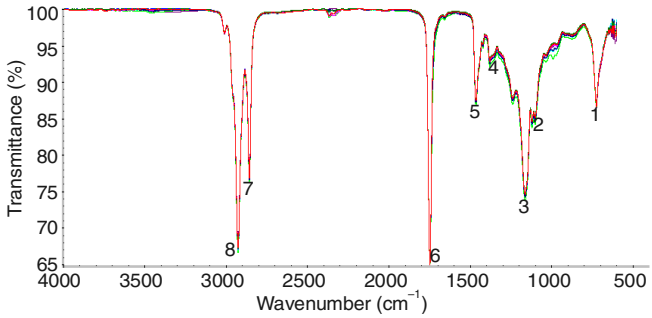

Fig. 3. FTIR spectra of chicken fat

Other infrared characteristic that can be obtained from those data is the bonding force or interaction which can be characterized by the force constant (k). Vibration mode of stretching C-O-C is found in chicken fat samples using FTIR. Stretching vibration is the movement of regular atoms along the bond axis between two atoms so the distance between atoms can increase or decrease. This vibration can be determined the magnitude of the force constant by assuming anharmonic oscillation. This constant can be calculated from eqn. 1.

$$f = \frac{1}{2\pi}\sqrt{\frac{k}{\mu}} \text{ atau } k = 4\pi^2 f^2 \mu \quad (1)$$

where $\mu = \frac{Mm}{M+2m}$, with M and m are C mass (1.944×10^{-26} kg) dan O mass (2.655×10^{-26} kg), respectively and f is the frequency (s^{-1}) that can be obtained from eqn. 2:

$$f = \frac{c}{\lambda} = c\bar{\omega}_e \quad (2)$$

where c is the speed of light (3×10^{10} cm s^{-1}) and $\bar{\omega}_e$ is vibrational constant (cm^{-1}). This is related to anharmonic constant (x_e) based on eqns. 3 and 4:

$$\Delta\varepsilon = \bar{\omega}_e(1 - 2x_e)\ cm^{-1} \quad (3)$$

$$\Delta\varepsilon = 2\bar{\omega}_e(1 - 3x_e)\ cm^{-1} \quad (4)$$

where $\Delta\varepsilon$ is wavenumber (cm^{-1}) of the obtained peak of C-O-C.

From Table-2, it can bee seen that peaks of C-O-C groups occured at 1116.81-1117.07 cm^{-1} and 1158.95-1159.06 cm^{-1}. Based on these data and eqns. 3 and 4, the anharmonic constant (x_e) of 1940.990-1942.567 Nm^{-1} and vibrational constant ($\bar{\omega}_e$)

TABLE-2
FUNCTIONAL GROUPS AND VIBRATION MODES OF CHICKEN FAT BASED ON FTIR SPECTRA

Wavenumber (cm^{-1})				Functional group and vibration mode	Ref.
Soxhlet	Folch	Bligh & Dyer	Hara & Radin		
721.33	721.50	721.33	721.36	=C-H, bending	[20]
1116.99	1116.81	1117.07	1116.91	C-O-C, stretching	[20]
1159.06	1159.06	1158.95	1158.99	C-O-C, stretching	[20]
1376.25	1376.13	1376.19	1376.16	C-H-(CH_3), bending	[16]
1461.37	1461.05	1461.20	1461.16	=C-H- (CH_2), bending	[16]
1744.09	1743.93	1744.07	1744.04	C=O, stretching	[20]
2852.78	2852.79	2852.77	2852.76	-C-H (CH_2), stretching	[16]
2922.05	2922.07	2922.05	2922.02	-C-H (CH_2), stretching	[16]

TABLE-3
ANHARMONIC CONSTANT VALUE (Nm^{-1}) WITH ASYMMETRY STRETCHING VIBRATION OF C–O FROM C–O–C

Extraction method	$\Delta\varepsilon_1$ (cm^{-1})	$\Delta\varepsilon_2$ (cm^{-1})	x_e	$\bar{\omega}_e$ (cm^{-1})	f (10^{13} Hz)	k (Nm^{-1})
Soxhlet	1116.99	1159.06	0.2452	2191.91	6.5757	1941.947
Folch	1116.81	1159.06	0.2452	2191.37	6.5741	1940.990
Bligh & Dyer	1117.07	1158.95	0.2452	2192.26	6.5768	1942.567
Hara & Radin	1116.91	1158.99	0.2452	2191.74	6.5752	1941.646

of 2191.37-2192.26 cm^{-1} are obtained. This is in accordance with Brooks [21] who reported that $\bar{\omega}_e$ value of C-O was about 2170 cm^{-1}. Finally, the force constant (k) of C-O from C-O-C group can be calculated using these data, where the frequencies (f) are in the range of 6.5741-6.5768 × 10^{13} Hz using eqn. 2 and resulting in the range of 1940.990-1942.567 Nm^{-1} (Table-3). Brooks [21] reported that force constant of C-O is 1860 Nm^{-1}.

Conclusion

The percentages of saturated fatty acid and unsaturated in Soxhlet, Folch, Bligh & Dyer and Hara & Radin methods were 31.16 ± 0.10 and 68.84 ± 0.09, 32.66 ± 0.92 and 67.20 ± 1.03, 29.58 ± 0.40 and 70.15 ± 0.38 and 31.68 ± 0.82 and 68.27 ± 0.86, respectively. In addition, saturated and unsaturated fatty acids in Folch method were not significantly different with those in Soxhlet and Hara & Radin methods at 5 % level, however significantly different with those in Bligh & Dyer method. PCA analysis showed fatty acids in Folch can be separated from others in which Folch has negative side in PC1 and positive side in PC2. In addition, fatty acids in Soxhlet method has similar fatty acid composition with Folch method, among others, as shown by the close distance of Soxhlet to Folch. FTIR spectra of chicken fat in general showed no significant differences in C-H stretching at 2922-2852 cm^{-1}, C=O stretching at 1744-1376 cm^{-1} and the fingerprint absorption at 1000-900 cm^{-1}. In addition, there are two peaks that indicate same functional group and vibration mode which show stretching vibration of C-O-C group with the force constant of C-O from C-O-C was about 1940 Nm^{-1}.

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