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Alternative Techniques for Animal Fat Authentication Based on Microscopic Crystal Pattern and Fatty Acid Composition Using Gas Chromatography-Mass Spectrometry (GC-MS)

Diana Candra Dewi[1,2], Chanif Mahdi[1], Hermin Sulistyarti[1,3*], Aulanni`am Aulanni`am[1]

[1] Department of Chemistry, Faculty of Science, Brawijaya University, Malang, Indonesia
[2] Chemistry Study Program, Faculty of Science and Technology, Maulana Malik Ibrahim Islamic State University, Malang, Indonesia
[3] Research Group of Low Cost and Automated Method and Instrumentation (LCAMIA), Department of Chemistry, Faculty of Science, Brawijaya University, Malang 65145, Indonesia

*Corresponding author email: hermin@ub.ac.id

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ABSTRACT

Fat authentication is strongly required for the reason of religious food ethics, human health, and preferences. This research reported two simple techniques approach for recognizing fat based on the character of microscopic and physicochemical properties of fat extracted using different polarity solvents. The formation of microcrystals as well as fatty acid composition were investigated by involving variables of four different types of animal fat and three different solvents. The isolate fats were directly crystallized under incubation at room temperature and observed using an optical microscope while fatty acid composition was determined by hydrolyzing and trans-esterifying fat samples before analysis using GC-MS. The microscopic structures of the obtained crystals are needle-based with a radial orientation in spherulitic pattern which characteristic to each type of fat. The lard fat crystal was authentically distinguished from its shape of a partially radial fan, whilst the shape of fat crystals from beef, goat, and lamb are needle-shaped with a full radial orientation. Fatty acids obtained from all types of fats and types of solvents show similar types but different abundances, the main saturated fatty acids are palmitate, stearate myristic and the main unsaturated fatty acids are oleic, palmitoleic, and linoleic. Gas chromatography-mass spectrometry (GC-MS) analysis exhibited that lard composed higher ΣUSFA compared to ΣSFA but other fats (tallow, goat, and lamb) showed converse behavior.

Keywords: fat, crystal structure, microscopic, fatty acids, GC-MS

INTRODUCTION

Lard (pork fat) is purposefully added to food products in certain nations where it is one of the most affordable edible fats and oils due to its low cost of manufacturing. Because it is less expensive than other consumable oil, lard is specifically included to food goods to save production costs [1]. Certain religions, like Judaism and Islam, forbid the use of lard in any kind of food products. For Muslims, one factor that determines halal food is free from lard. Thus, it is forbidden from a religious perspective for any food products to contain lard. So, it is obvious that there is a high demand for actions to assist Muslims in fulfilling their religious obligation to eat on halal foods which free from lard [2]. Since there are more Muslims in the globe than ever before, the authorized individual should lead in developing halal analysis technologies.

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Numerous analytical methods, encompassing both chemical and physical approaches, have been devised to distinguish lard from other fats. Gas chromatography coupled to time of flights mass spectrometry combined PCA has been used to examine differences in fatty acid methyl esters to distinguish lard from fat of other species. In order to determine the species origin of fat from different animals, comparison of fatty acid data obtained by FAMEs analysis with gas chromatography-mass spectrometry (GC-MS) combined with PCA was found to be insufficient. This is because both lard and chicken fat, as well as beef and mutton fat, shared common fatty acid profiles. According to high performance liquid chromatography (HPLC) research, the triacylglycerols (TAGs) profile of lard was found to be very comparable to chicken fat but significantly different from that of either beef or mutton tallow [3]. HPLC has also been used to study the composition of TAGs, which is used to distinguish between lard and other animal fats (such as those from beef, mutton, and poultry). On further categorize lard and other materials, principal component analysis was applied on the TAGs composition. According to the PCA score plot, the TAGs composition of lard and chicken fat are identical [4].

Lard, like other animal fats and vegetable oils, primarily consists of triacylglycerols (TAGs), diacylglycerols (DAGs), free fatty acids, and various trace components such as phospholipids, sterols, tocopherols, carotenoids, and fat-soluble vitamins. The characteristics of fats, both at a macroscopic and microscopic level, are heavily influenced by the crystalline network of fats and the polymorphic forms of solid TAGs. Factors such as the fat's source, extraction method, composition of TAGs and fatty acids, as well as crystallization conditions including temperature and rate of cooling, and storage duration, all play significant roles in determining the fat's physical and chemical properties [5]. Each type of fat possesses its distinct chemical compositions and physical characteristics. The disparities in these properties between lard and other animal fats could potentially serve as a straightforward and rapid means of detection. Certain of the aforementioned approaches are quite costly and call for certain preparatory processes.

Fat samples exhibit observable physical properties like melting point, refractive index, and specific gravity, while indicators such as iodine number can reveal the degree of fat saturation, alongside acid number, saponification number, and fatty acid composition which signify chemical properties. Fatty acid composition is particularly crucial for discerning the traits of a fat sample and is typically analyzed via GC-MS. This method offers high precision as it swiftly identifies macro and micro components, yielding excellent chromatographic outcomes [6].

Information about fat crystals is obtained from characteristics such as their size, shape, and distribution, as well as the overall rheological properties of the fat. Various studies have qualitatively examined the microstructure of fat crystals, describing attributes like crystal cluster size and shape, as well as the rheological behavior of fat crystal tissue. Microscopy techniques are commonly employed to visualize the microstructure of fat crystals, providing insights into their morphology. The relationship between physical properties and fat microstructure can also be quantitatively explored, generating data for further analysis. Additionally, both large and small deformation rheology have been utilized to investigate the microstructure of fat crystals [7].

Microstructure of hard fat from margarines had examined using polarized light microscopy and revealed that the texture of the margarine is significantly influenced by the production of big spherulites. The mechanical properties of fat are largely determined by its microstructure. Large spherulites' development has a significant impact on margarine texture,

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according to morphologic investigations of the microstructure of margarines made using a polarized light microscope. Large spherulites in margarine are thought to reduce the amounts of crystals and the strength of mutual attraction as the temperature rises [8]. Cryogenic transmission electron microscopy (Cryo-TEM) and polarized light microscopy (PLM) were used to characterize crystal size, demonstrating the considerable influence of matrix concentration on micro/nanoscale structure. Higher cooling rates during crystallization cause smaller nano- and meso-structure components to develop [9]. The macroscopic, textural, and mechanical properties of the composite system can be significantly impacted by variations in the monoglycerides network's shape [10].

Zampouni et al (2022) also reported that depending on the percentage of monoglycerides, distinct oil- monoglycerides mixes had different crystal morphologies when storing oleo-gels at different temperatures. It's also important to note that the inclusion of poly-glycerides as a structure resulted in additional changes to the morphology of the crystals and the crystal networks [11]. Park et.al (2019) reported, the physicochemical parameters of commercial lard and tallow were compared with horse fat. It was discovered that horse fat had greater acid values than those in lard and tallow, but the saponification values were not significantly different. Lard and horse fats had equal iodine values, but beef tallow having the lowest value. There were two distinct endothermic and exothermic peaks on the melting and crystallization curves of horse fat. The amount of saturated fatty acids in tallow is greater than lard and horse fat while the unsaturated content is smaller [12]. Fatty acids (FAs) as the main constituent of fat or triglycerides, and its profiles can be used to assess the validity or purity of animal fats because the content of FAs can differ across their origin sources [13].

The research mentioned above revealed that there were only few discussions in current microscopic techniques on the relationship between the structure of fat microcrystals and the kind of animal or source of fat. The GC-MS method has been employed to ascertain the composition; nevertheless, reports about crystal pattern and the fatty acid content of different kinds of fat extracted using various solvents have not been published. Thus, this study was conducted to develop microscopic analysis as a simple substitute method to distinguish between pork fat and fats from beef, goat, and sheep. Because the polarity of the solvent affects the solubility of fats, a further test involved analyzing the fatty acids recovered from three solvents with distinct polarity was conducted using GC-MS. The solvents chosen for this extraction process are n-hexane, diethyl ether, and chloroform which are categorized as non and semi polar solvents commonly used to extract fat based on their polarity. The differences in physical and chemical properties of lard and other animal fats were expected to have the potential as the basis for developing a fast and easy method of fat authentication.

EXPERIMENT

Materials and instruments

The materials used in this study included lard, beef tallow, lamb fat, and goat fat, diethyl ether (E-Merck), n-hexane (E-Merck), chloroform (E-Merck), ethanol 97% (E-Merck), anhydrous Na_2SO_4 (E-Merck), $Na_2S_2O_3$ (E-Merck), glacial acetic acid (E-Merck), Whatman paper No. 1, Hanus reagent (E-Merck), potassium iodide (E-Merck), HCl (E-Merck), KOH (E-Merck), deionized water (One), starch solution, phenolphthalein indicator (E-Merck).

The tools used in this research include a set of glassware, thermometer, scales, spatula, knife/cutter, a set of rotary evaporators, refrigerator, Olympus CX22LED Microscope with Optilab Digital, GCMS-QP2010S SHIMADZU.

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Sample preparation (Fat Extraction)

Each fat from adipose tissue of pork, beef, lamb, and goat was cut into small pieces and put into four Durant bottle beakers. Then, each of them was added with different solvent of n-hexane, diethyl ether, and chloroform under ratio of animal fat and organic solvent (w/v) of 1: 2 (200g:400mL) and allowed shaking for 24 hours, and filtered using Whatman paper. Extract of 200 mL of each solvent was concentrated using a rotary evaporator at a temperature of 40 ^{o}C to remove residual solvent. The remaining extracts were crystallized for microscopic analysis.

Microscopic analysis

Crystallization of each fat was done by placing 5 mL fat extract into a test tube and closed tightly, then allowed to stand at room temperature for 24 hours. The obtained fat crystal was swapped onto a microscope slide and covered with glass cover. Digital optical micrographs of each fat crystals were taken using an OLYMPUS CX22LED optical microscope (Olympus, Japan) using a 400× objective lens and OPTILAB digital camera.

Determination of melting point

Each of molten fats was transferred into a capillarity glass tube and put into a glass beaker containing ice cubes. Let it stand for 16 hours in the refrigerator at temperature of 4 °C. The capillary glass containing the solid fat was transferred into a water bath setting at 30 °C with increased rate of 1 ^{o}C/3 min. Melting point was determined when all solid fat all in the capillary tube was fully melted and appeared as a transparent liquid. Observation was conducted when the temperature of solid start melting (softening point) until all the sample became completely clear and liquid-like.

Determination of iodine number

Fat sample as much as 5 g was placed into an Erlenmeyer flask and added with 10 mL of chloroform to dissolve completely and 25 mL of Hanus reagent. The mixture was shaken and stored in a dark place for 30 mins, then mixed with 10 mL of 15% KI solution. Then, this solution was titrated by standard sodium thiosulphate solution using starch as an indicator with vigorous shaking to extract the iodine from the chloroform layer until discharge of blue color. The procedure was conducted in triplicates and applied to all types of fats.

Determination of saponification number

Fat extract of 5 g was placed into a 250 ml Erlenmeyer flask, followed by the addition of 50 mL of 0.5 M alcoholic KOH. The mixture was then heated until complete saponification, as indicated by the absence of visible oil droplets in the solution. After cooling, 3 drops of phenolphthalein indicator were introduced, and the solution was titrated with 0.5 M HCl until the pink color disappeared. This process was repeated three times and conducted for all types of fat extracts.

Determination of fatty acid using GC-MS

The process was commenced with transesterification of the fat samples. Methyl esterification of the samples utilized in the analyses was achieved through the BF_3-MeOH method following alkaline hydrolysis. In this method, 20 mg of fat extract was combined with 2 mL of 0.5 mol/L NaOH-methanol solution and heated at 100 °C for 7 minutes. After cooling, 3 mL of 14% BF_3 in methanol was added, and the mixture was sealed and heated at 100 °C for 5 minutes. Upon cooling, 2 mL of hexane and 7 mL of saturated NaCl solution were added,

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followed by thorough shaking. The resulting top layer of hexane was separated via centrifugation and purified by the addition of anhydrous Na_2SO_4 to remove any water phase [14]. The esterified fat extract was then transferred into a vial for GC-MS analysis by injecting 1 µL of the ester under following specified GC-MS settings: column Rts-Wax (Shimadzu), column size: 30 m x 0.25 mm x 0.25 m, oven column temperature: 70 ºC, injector temperature: 300 ºC, carrier gas: helium carrier gas speed: 28 mL/min, operational method: initial temperature 70 ºC for 5 mins, increased temperature 5 ºC/min to 300 ºC, increased temperature 10 ºC/min to 240 ºC, and maintained temperature at 240 ºC for 5 mins.

RESULT AND DISCUSSION

Fat authentication in this study was done via two methods, microscopic crystal pattern and GC-MS fatty acid composition. Extraction of fat animal using different polarity solvents were investigated in order to find out the differences microscopic, physical, and physico-chemical properties of four types of animal fats, including lard, tallow, goat fat, and lamb fat. The microscopic, physical, and chemical properties were conducted to determine the type of organic solvent which can significantly distinguish the source of fats. The parameters studied were microscopic properties (crystal pattern), physical properties (refractive index, specific gravity, and melting point), and chemical properties (iodine number and saponification value). The physico-chemical properties of fat extracts were studied using GC-MS based on the type and composition of fatty acids in the fat extract to find out the influence of solvents to the characteristics of lard compared to other animal fats for fat authentication.

The microstructure of fat crystal

The microstructure of fat crystal examined under light microscope performed bright needle-like crystals created aggregates from a nucleation center. Crystal aggregates have a distinctive rod-like structure, composed of several needle-shaped crystals interacting with one another as they grew from a nucleation point in a single direction [15]. Figure 1 indicates that the morphology of the crystals of fat at different animal under varied solvent resulted different shape. The composition of high and low melting in the mixture affects the crystal morphology and produces different structures when observed under a microscope. In all fat samples, microscopic observations showed all needle-shaped crystals with various patterns.

Lard extracted with diethyl ether and chloroform form needle crystals with a spherulite pattern, which is rounded with an imperfect core in the middle, meaning that some crystals are formed partially rounded like a fan. Thus, the shape of lard crystals is needle-based with a radial orientation partially fan-like. Lard extracted with hexane form needle crystal but less obvious pattern. The shape of the tallow crystal is needle-shaped with a full radial orientation which tends to be large and blunt, while the shape of the goat and lamb fat crystal is needle-based with a full radial orientation evenly. Tallow, goat fat, and lamb fat solidified at room temperature and the fat crystals observed in the microscope are needle-shaped with radial patterns. These dense network-like structures might be responsible for their ability to retain the composition of the formed polycrystalline structure [16]. Microstructure pattern of the crystals obtained from fat extract using different type of solvents, such as hexane, diethyl ether, and chloroform did not yield a noticeably different microscopic structure.

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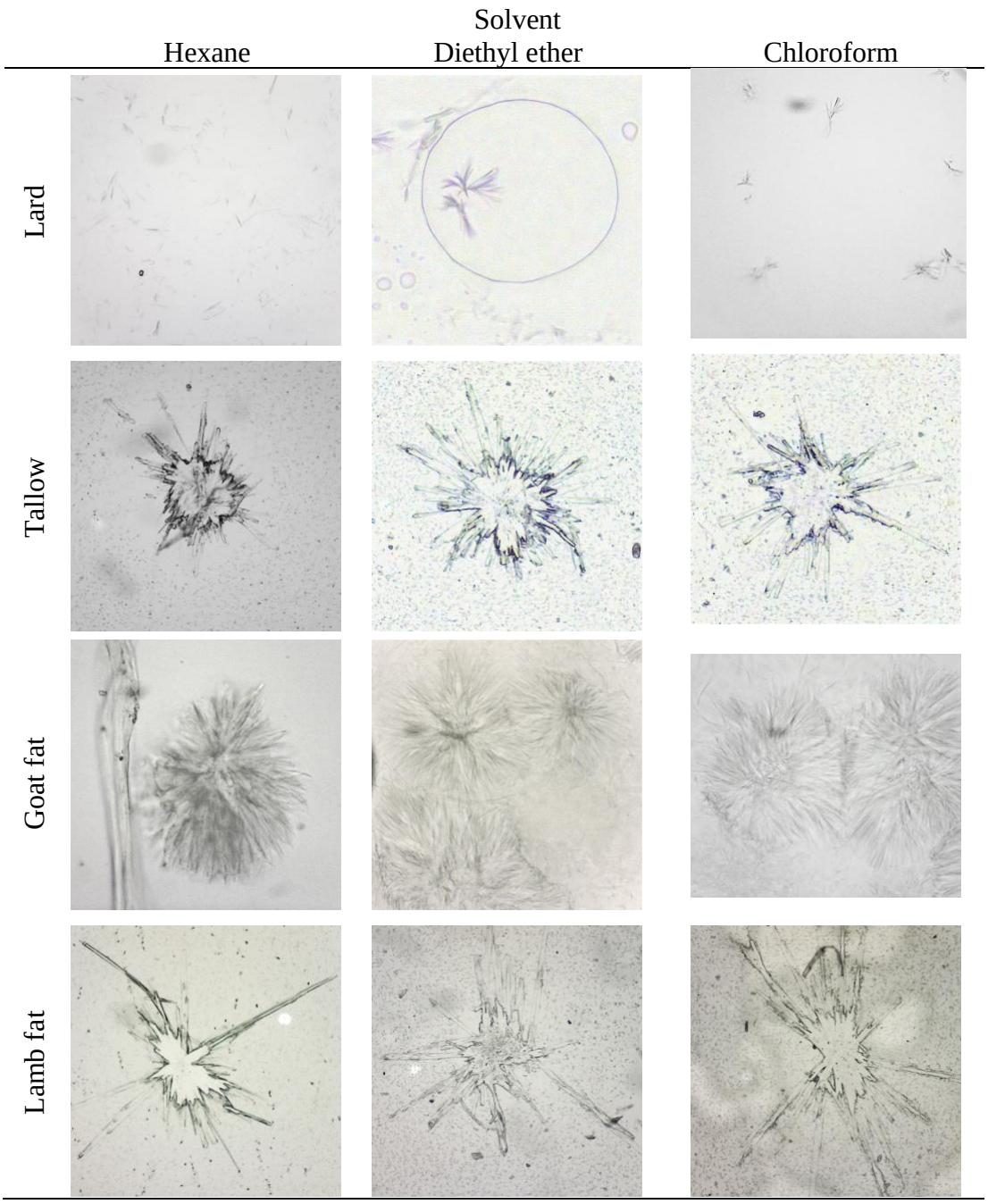

Figure 1. Microstructure of fat crystals observed by optical microscope

Melting point test

The melting point was observed at the temperature at which a solid fat changed into a liquid at a pressure of one atmosphere. The melting point of each fat under three different solvents of hexane, diethyl ether, and chloroform is depicted at Table 1.

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Table 1. The results of fat melting point test with solvent variations

	Various solvent		
	Hexane (°C)	Diethyl ether (°C)	Chloroform (°C)
Lard	33-37	31-37	32-37
Beef fat	40-44	38-44	39-44
Goat fat	43-46	43-47	42-47
Lamb fat	42-46	42-47	41-46

Table 1 illustrates that lard stands out from other fats sourced from various animals due to its notably lower melting point, ranging from 33-37°C. In contrast, fats from beef, goat, and lamb exhibit similar melting points of 40-44°C, 43-46°C, and 42-46°C, respectively. Additionally, different solvents used in the study showed no significant variance in melting points for each type of fat. Melting point serves as an indicator of the relative strength of intermolecular bonds; stronger bonds require more energy to break, thus leading to higher melting points. In fats, the strength of intermolecular forces is influenced by the composition of saturated and unsaturated fatty acids. Saturated fatty acids, with their linear structure, promote strong intermolecular interactions, resulting in higher melting points as they readily stack together. Conversely, unsaturated fatty acids, with double bonds (DB) typically in cis configuration, hinder intermolecular interactions, leading to weaker bonds and lower melting points compared to saturated fatty acids.

The melting points of saturated fatty acids adhere to the principle of the boiling point, where higher molecular weight corresponds to higher melting points. For instance, the melting points of lauric (C12), palmitic (C16), stearic (C18), and arachidic acid (C20) increase progressively from 45°C to 76°C. Conversely, the melting points of unsaturated fatty acids are primarily influenced by the number of double bonds present. An increase in double bonds, or higher unsaturation, weakens the bonds between molecules of unsaturated fatty acids. This is evident in oleic (C18-1DD), linoleic (C18-2DD), and linolenic (C18-3DD) acids, where the melting point dramatically decreases from 13°C to -11°C with an increase in the number of double bonds. The significantly low melting point of lard suggests that its fatty acid composition likely contains the highest proportion of unsaturated fatty acids compared to other fats studied.

Iodine value

The iodine value (or number) is an important characteristic of fats as it indicates the proportion of unsaturated fatty acids present. Iodine value is defined as the number of mg of iodine required to saturate the fatty acids present in 100 mg of the oil or fat. This test aims to state the degree of unsaturated fat. In fatty acids, unsaturation occurs mainly as double bonds which are reactive towards the iodine. Thus, fat rich in saturated fatty acids have low iodine value, while fats rich in unsaturated fatty acids have high iodine value. The iodine value obtained from iodometric method is depicted in Table 2.

Table 2 shows that the greatest iodine number was found in lard (73.30), followed by goat (60.74), lamb (61.58), and beef (45.55) fats, and the different solvents did not affect

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significantly to the value. This proves that lard contained the most unsaturated fatty acids among the other fats. The fact supported the melting point results, in which the highest unsaturated fatty acids content of lard gave results to the lowest melting point. The more unsaturated fatty acids composition in fats are more likely in liquid phase at room temperature; thus, visually, lard at room temperature is in liquid form indicating that it contains more unsaturated fatty acids, while beef, lamb, and goat fats which compose of more saturated fatty acids are in solid phase [17]. Iodine value can be used to distinguish fat from different type of animals studied except for goat and lamb which have similar value to each other.

Table 2. Iodine value of fat with solvent variation

	Solvent Variations		
	Hexane	Diethyl ether	Chloroform
Lard	73.30±0.15	72.77±0.23	72.74±0.19
Beef fat	45.55±0.08	44.75±0.19	44.10±0.11
Goat Fat	61.58±0.12	61.47±0.07	61.03±0.16
Lamb fat	60.74±0.05	60.37±0.11	59.55±0.24

Saponification value

The saponification value test is conducted to estimate the average molecular weight or chain length of all the fatty acids present in the sample in the form of triglycerides. It is determined by the amount of potassium hydroxide (KOH) required to neutralize the fatty acids obtained through complete hydrolysis (saponification) of 1 gram of fat. A higher saponification value indicates a shorter average length of fatty acids, smaller mean molecular weight of triglycerides, and vice versa. Table 3 presents the saponification values obtained from fats extracted using three different solvents across four types of animals studied.

Table 3 Saponification value of fats with solvent variation

	Solvent Variations		
	Hexane	Diethyl ether	Chloroform
Lard	259.06±1.26	258.74±0.76	264.33±1.06
Beef fat	233.89±0.27	236.16±0.26	235.90±3.10
Goat Fat	212.47±0.54	205.55±0.53	223.81±0.75
Lamb fat	250.07±1.14	257.17±0.26	261.28±0.95

Table 3 demonstrates that pork fat gives the highest saponification values, followed by lamb, beef, and goat. The highest saponification values of pork fat indicate that the average molecular weight of fatty acids from lard is smaller than that of other animal fats. The used of semipolar solvent (chloroform) slightly increased the saponification value of all fats except of beef fat. This might be due to the content of fatty acids, monounsaturated fatty acids predominated in

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nonpolar lipids, whereas saturated and polyunsaturated acids were more prominent in polar lipids. From the resulted values, lard feasibly be identified among other fats of beef, goat, and lamb from the highest saponification values of 259 under hexane solvent.

Fatty acids composition

The composition of fatty acids of the extracted fats were done by converting the fatty acids to their derivatized methyl esters prior to GC-MS analysis. The chromatograms of fatty acid composition from each type of fat animals extracted under three different solvents of hexane, diethyl ether, and chloroform are depicted in Figure 2. As shown in Figure 2, the total chromatogram peaks obtained from all solvents showed nine predominant fatty acids of caproic, lauric, myristic, palmitoleic, palmitic, oleic, linoleic, stearic, and arachidic) which appear at retention time (t_R) in the range of around 25-39 mins. Fatty acids extracted from diethyl ether showed all the eight fatty acids (Figure 2b), while the other two solvents (hexane and chloroform) showed seven out of the eleven fatty acids, in which arachidic acids is absent from chromatogram (Figure 2a and 2b). The chromatogram's profile revealed traits specific to each kind of sample in terms of peak appearance and intensity. This discovery chromatograms made from animal fat's fatty acids can be suggested as a technique for differentiating between halal and non-halal foods [18].

Each kind of fat extracted with three different solvents has distinct properties in the chromatogram profile which is characteristics for each type of animal fats in term of the appearance and the intensity of peaks. These discoveries can be suggested as a mean for fat authentication of halal and non halal identification. From chromatogram of fatty acids obtained from hexane extracted fat (Figure 2a), fatty acids from different type of animal fats showed different profiles: 4 peaks for lard (palmitoleic, palmitic, oleic, and stearic acids); 6 peaks for tallow (myristic, palmitoleic, palmitic, oleic, linoleic, and stearic acids); 7 peaks for lamb fat (caproic, myristic, palmitoleic, palmitic, oleic, linoleic, and stearic acids); 4 peaks for goat fat (palmitic, oleic, linoleic, and stearic acids). Chromatograms profiles from diethyl ether fat extract (Figure 2b) is like those obtained from hexane fat extract (Figure 2a) except of that from lamb fat in which the chromatogram shows 5 peaks of caproic, palmitic, oleic, stearic, and arachidic acids. The chromatogram profile from chloroform is also like that of hexane extract, with exception of that from lard which shows 6 peaks of myristic, palmitoleic, palmitic, oleic, linoleic, and stearic acids and that from lamb fat which demonstrates 6 peaks of caproic, lauric, palmitic, oleic, stearic, and arachidic acids (Figure 2c).

The type and abundance of fatty acids obtained from the chromatograms are presented in Table 4. Component analysis from GC-MS (Table 4) showed that there is similarity type of fatty acids but different in abundance observed under different type of solvents. Besides, there are certain fatty acids that can also be used for identification. Lignoceric acid is only found in goat fat with the abundance of 0.84 % (hexane), 0.83% (diethyl ether), and 0.93% (chloroform) acceptable for identification of goat fat. Arachidic acid is found in lamb fat with relatively high abundance (13.30%) and used as marker for identification of lamb fat. Caproic acid can be used as an additional character for goat and lamb fats. Tallow can be recognized from the presence of myristic acids with the abundance of 3.59% under hexane solvent. No specific fatty acid observed for lard; however, lard can be identified from the highest abundance of oleic acid (76.58%) under hexane solvent.

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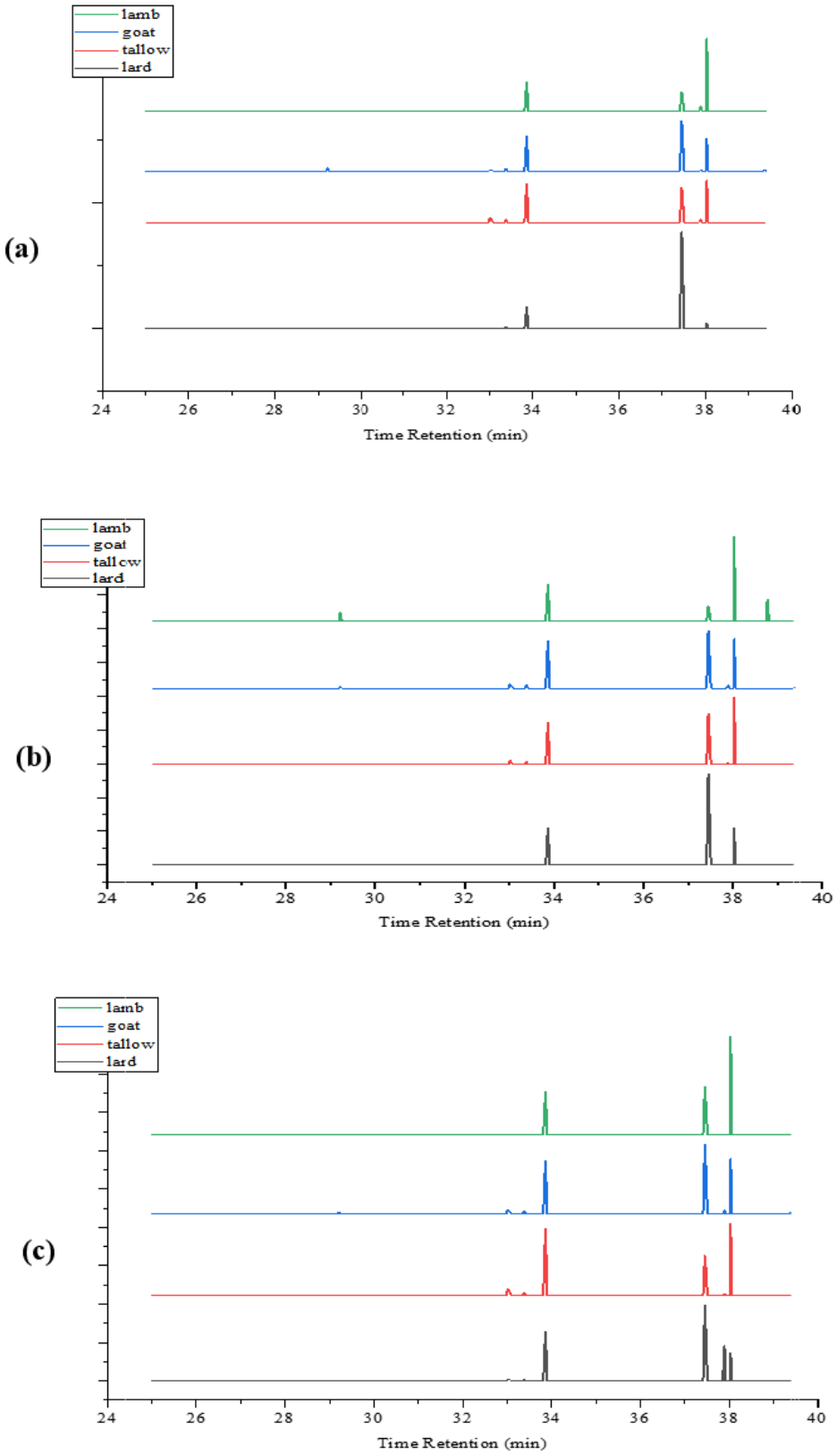

Figure 2. Gas Chromatogram of fatty acid extracted with hexane (a), diethyl ether (b), and chloroform (c).

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Table 4. Fatty acids composition of fat extract from different solvent

Fatty acid composition of fat extracted with hexane (%)				
Fatty acid	lard	tallow	goat	lamb
Butyric(C4:0)	n.d	n.d	n.d	0.102
Caproic (C6:0)	n.d	n.d	2.485	0.132
Lauric (C12:0)	n.d	n.d	n.d	n.d
Myristic (C14:0)	0.765	3.582	0.901	n.d
Palmitic (C16:0)	17.025	31.117	27.601	23.018
Stearic (C18:0)	4.297	33.045	25.655	57.581
Lignoceric (C24:0)	n.d	n.d	0.839	n.d
ΣSFA	**22.087**	**67.744**	**57.480**	**80.833**
Palmitoleic (C16:1)	1.338	2.241	1.926	n.d
Oleic (C18:1)	76.575	27.664	39.818	15.163
Linoleic (C18:2)	n.d	2.351	0.776	4.004
ΣUSFA	**77.913**	**32.256**	**42.520**	**19.167**
Fatty acid composition of fat extracted with diethyl ether (%)				
Fatty acid	lard	tallow	goat	lamb
Caproic (C6:0)	n.d	n.d	1.096	5.260
Myristic (C14 :0)	0.602	2.522	2.513	n.d
Palmitic (C16:0)	22.232	24.730	28.446	22.081
Stearic (C18:0)	21.921	39.876	29.354	50.212
Arachidic (C20:0)	0.121	n.d	n.d	13.295
Lignoceric (C24:0)	n.d	n.d	0.832	n.d
ΣSFA	**44.876**	**67.128**	**62.241**	**90.848**
Palmitoleic (C16:1)	0.532	1.672	1.927	n.d
Oleic (C18:1)	54.080	30.091	34.038	9.152
Linoleic (C18:2)	0.512	1.109	1.794	n.d
ΣUSFA	**55.124**	**32.872**	**37.759**	**9.152**
Fatty acid composition of fat extracted with chloroform (%)				
Fatty acid	lard	tallow	goat	lamb
Butyric(C4:0)	n.d	n.d	n.d	0.204
Caproic (C6:0)	n.d	n.d	0.803	0.344
Lauric (C12:0)	n.d	n.d	n.d	0.312
Myristic (C14 :0)	1.032	3.862	2.119	n.d
Palmitic (C16:0)	26.101	35.115	27.958	22.324
Stearic (C18:0)	14.737	37.553	28.689	51.393
Arachidic (C20:0)	n.d	n.d	n.d	0.313
Lignoceric (C24:0)	n.d	n.d	0.927	n.d
ΣSFA	**41.870**	**76.530**	**60.496**	**74.890**
Palmitoleic (C16:1)	0.692	1.726	1.759	n.d
Oleic (C18:1)	39.349	20.896	36.088	25.110
Linoleic (C18:2)	18.089	0.848	1.657	n.d
ΣUSFA	**58.130**	**23.47**	**39.504**	**25.110**

As seen in Table 4, fatty acids from fat of different type of animals showed different ΣUSFA and ΣSFA. Total of unsaturated fatty acids (ΣUSFA) of lard extracted with different

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solvent respectively 77,91% (n-hexane), 55,12% (diethyl ether), and 58,13% (chloroform). On the other hand, the total of saturated fatty acids (ΣSFA) of tallow extracted with different solvent respectively 67,74% (n-hexane), 67,13% (diethyl ether), and 76,53% (chloroform). Lard was dominated by unsaturated fatty acids; however, tallow, goat fat, and lamb fat were dominated by saturated fatty acids. This fact is matched with iodine value and melting point, in which lard showed higher iodine value with lower melting point compared to those of tallow, goat fat, and lamb fat. The high quantity of unsaturated fatty acids is directly correlated with the high iodine value, but it is negatively correlated with the low melting point. Figure 3 presents that different type of solvents affect the composition of ΣUSFA and ΣSFA [19], in which under hexane solvent, lard fatty acids was dominated by USFA with the highest percentage (77.91%), followed by lamb (42.52%), tallow (32.57%), and goat fat (19.17%). The proportion of ΣUSFA is lesser under diethyl ether for all type of fats sample giving percentage with the same order, i.e. 55,12% (lard), followed by lamb (37.76%), tallow (32.87%), and goat fat (9.15%). The ΣUSFA from lard and goat fat extracted using chloroform showed similar results to those obtained by diethyl ether, giving results with respective percentage of 58.13% (lard), 39.50% (goat), 23.47% (tallow), and 25.11% (lamb). On the other hand, total saturated fatty acids (ΣSFA) of fat extracted with different solvent inversely related to the ΣUSFA.

Based on the composition of ΣUSFA and ΣSFA, it can be concluded that lard and lamb can be recognized from the composition ratio of ΣUSFA: ΣSFA (78:22) and (20:80) under hexane solvent. Goat fat can also be distinguished from other fats under chloroform solvent with ratio ΣUSFA: ΣSFA of 9:91. The composition ratio of ΣUSFA: ΣSFA from tallow was close to that of goat; thus, these two fats cannot be easily distinguished from each other.

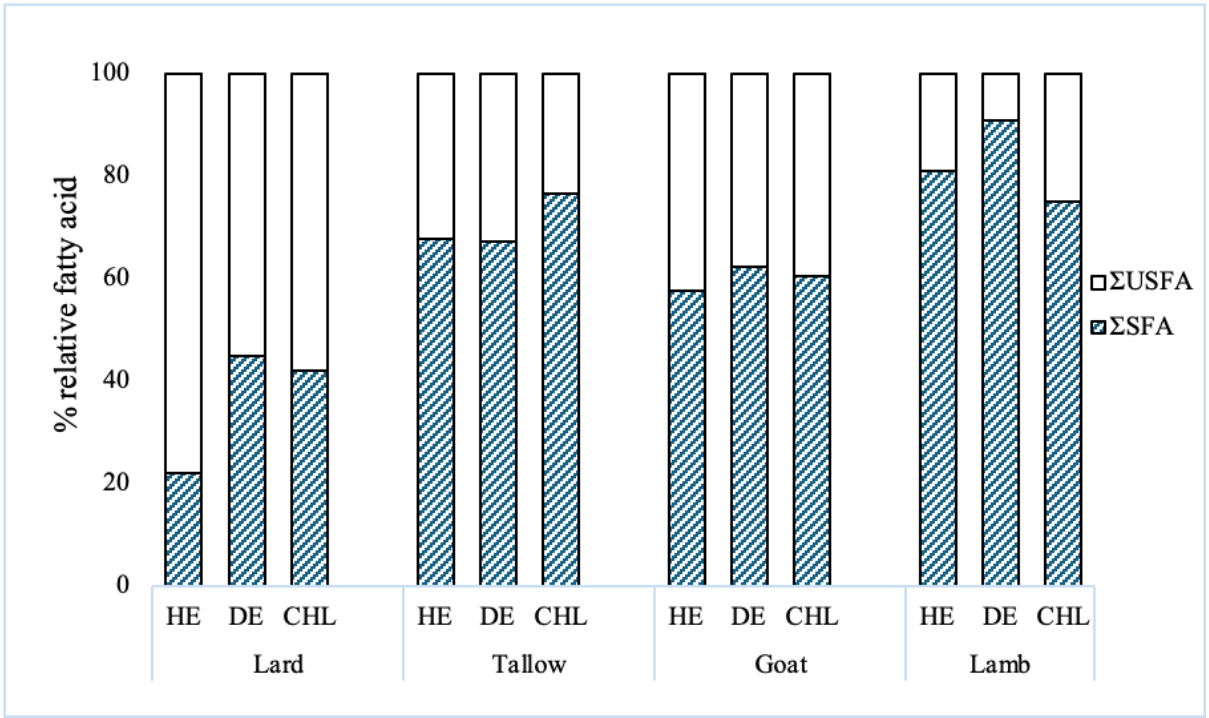

Figure 3. Total SFA and USFA of several fat extracted with different solvent

The fatty acids composition seems has relationship to the crystal pattern. Crystal of lard was shaped partially radial fan-like as it is dominated by unsaturated fatty acid and may contain

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unsaturated chains TAGs. Lard extracted with diethyl ether and chloroform form crystals formed partially rounded like a fan as their balance composition of ΣUSFA: ΣSFA are 55:45 (diethyl ether) and 58:42 (chloroform). The higher unsaturated fatty acids (ΣUSFA: ΣSFA 78:22), lard extracted with hexane formed less obvious pattern needle crystal. Crystal of other fats (tallow, goat fat, and lamb fat) which rich of saturated fatty acids gave results to needle-shaped crystal with a full radial orientation with the needle tends to be large and blunt for tallow. Saturated fatty acids predominated in these fats, which may be indicative of TAGs with saturated chains [20,21]. The bulk of fats, approximately 98% are comprised of triacylglycerols (TAGs), with minor components comprising other polar lipids. Fat extracts typically consist of mixtures of TAGs with varying compositions of fatty acids, including both high-melting and low-melting lipid classes. The unsaturated fatty acids have lower melting points than the saturated fatty acids. Upon crystallization and observation under a microscope, the crystal morphology and microstructure can be discerned. The crystal morphology is predominantly influenced by the high-melting components, as they precipitate more readily at the same temperature compared to the low-melting components [22]. Thus, fat with high SFA performs perfect crystal structure as observed.

In this study, the liquid fat was slowly cooled, allowing the lipid molecules sufficient time to organize themselves into lamellae and form three-dimensional structures through crystallization. The pace of cooling, temperature of crystallization, agitation rate, and composition of the lipid phase are among the variables influencing the arrangement of molecules into a crystalline state. The transition from liquid to crystalline phase was a significant factor in regulating crystallization. The nucleation rate primarily determines the number and size of crystals formed, their polymorphic form, and the eventual distribution of crystalline solids. It was observed that when the nuclei are formed, rapid crystal growth occurs. Fat extracts that are rich in saturated fats such as beef, lamb and goat fat showed more easily forming crystal nuclei followed by crystal growth to form perfectly circular spherulites. On the other hand, fat extracts that are rich in unsaturated fats such as lard will take longer to form crystal nuclei followed by slow crystal growth speed forming partially circular spherulites or one side like a fan. Fat extracts contain numerous triacylglycerol (TAG) and non-TAG components can undergo various crystallization mechanisms. Minor components may pose challenges in nucleation. Spherulitic growth is common in TAGs, but the exact mechanisms of crystal growth remain poorly understood due to the absence of a comprehensive theory. Heterogeneous nucleation may play a role in promoting the crystallization process. The crystallization form characterized by a distinct spherulitic pattern with well-fitted spherulites, resulting in sharp edges and straight lines. Fat crystals often exhibit spherulitic growth, their shape and size can vary significantly, depending on the crystallization conditions and compound composition [23,24]. This model relies on a geometric framework that simulates the real process of crystallization, including nucleation and crystal growth. The heterogeneity of fatty acid components in triacylglycerols (TAGs) leads to complex behavior, especially in mixed-acid TAGs with varying lengths of fatty acids, and TAGs containing both saturated and unsaturated fatty acids. The incompatibility of TAGs due to differences in chain length and degree of saturation results in immiscibility in the solid state.

CONCLUSION

Microscopic analysis of fat microstructure allowed to to explain the origin of fat. The morphology of the microstructure of goat and lamb tallow crystals is considerably different from that of the lard crystals, which are needle-based with an equally distributed radial

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orientation and a somewhat fan-like microstructure. Organic solvents used to extract animal fats give different physical properties such as melting point and chemical properties (iodine number and saponification number) but are not specific. Iodine value is higher in lard, while the melting point was lower rather than tallow, goat fat and lamb fat. The results of the GCMS are consistent with microscopic analysis, which shows that fat extracts from lamb, goat, and tallow were dominated by saturated fats whereas fat extracts from lard were primarily unsaturated fats. Total of unsaturated fatty acids (ΣUSFA) of lard fats were greater than those saturated fatty acids (ΣSFA), on the other hand, total amount of saturated fatty acids (ΣSFA) of tallow, goat and lamb fats were greater than those unsaturated fatty acids (ΣUSFA). Saturated fat-rich fat extracts, such that found in goat, lamb, and beef fat, are more likely to produce crystal nuclei and then crystal development into precisely round spherulites. On the other hand, fat extracts high in unsaturated fats, like lard, will form crystal nuclei more slowly, which will result in partly circular spherulites or fans with one side. The findings of microscopic crystal pattern and fatty acids composition from GC-MS can be suggested as a useful means for fat authentication as well as identification of halal and non halal food.

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CONFLICT OF INTEREST

All authors declare that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

DCD conducted the experiment and write draft of the manuscript, HS and CM wrote and revised the manuscript, and AA conducted proof reading. All authors agreed to the final version of this manuscript.

REFERENCES

[1] Mohamad, F., Sairin, M. A., Nizar, N. N. A., Aziz, S. A., Hashim, D. M., *9th International Conference on Sensing Technology (ICST)*, **2015**, 739–744.
[2] Masyitah Binti Nizara, Nina Naquiah Ahmad Nizar, Muhammad Syarafuddin Abdul Shukor, Ruzanna Zainal Siti Roha Ab Mutalib and Siti Aimi Sarah Zainal Abidin, *Halal Sphere*, **2023**, 3, 2, 79-90.
[3] Marikkar, N., Alinovi, M., and Chiavaro, E., *Ital. J. Food Sci.*, **2021**, 33 (1), 106–115.
[4] Rohman, A., Triyana, K., Sismindari and Erwanto, Y., *Int. Food Res. J.*, **2012,** 19 (2), 475-479.
[5] Sato, K. and Ueno, S., *Curr. Opin. Colloid Interface Sci.*, **2011**, 16, 384–390.
[6] Abidin, S. Z, Patel, D. and Saha, B., *Can J. Chem. Eng.*, **2013**, 91, 1896-1930.
[7] Shi Y., Liang B., and Hartel, R. W., *J. Am. Oil. Chem. Soc.*, **2005**, 82, 6, 399-408.
[8] Liu, Y., Meng, Z., Zhang, F., Shan, L., & Wang, X., *Eur. Food Res. Technol.*, **2010**, 230, 5, 759–767.
[9] Maleky F., Acevedo N. C., and Marangoni A. G., *Eur. J. Lipid Sci. Technol*. **2012**, 114, 7, 748-759.
[10] Chen, C. H., and Terentjev, E. M., *Langmuir*, **2009**, 25, 12, 6717–6724.
[11] Zampouni K., Soniadis A., Moschakis T., Biliaderis C.G, Lazaridou A., and Katsanidis

The journal homepage www.jpacr.ub.ac.id
p-ISSN : 2302 – 4690 | e-ISSN : 2541 – 0733

E., *LWT*, **2022**, 154 (2022) 113172.
[12] Park, Y. H., Cho, M. J., and Kim, H. J., *Korean J. Food Technol.*, **2019**, 51 (1), 1-6.
[13] Hauff, S. and Vetter, W., *J. Agric. Food Chem*., **2010**, 58, 2, 707–712.
[14] Moigradean, D., Poiana, M. A., Alda, L. M., and Gogoasa, I., *J. Agroaliment Processes Technol.,* **2013**, 19(4), 459-463.
[15] Ojijo, N. K., Neeman, I., Eger, S., and Shimoni, E., *J. Sci. Food Agric.*, **2004**, 84(12), 1585–1593.
[16] Bin Sintang, M. D., Rimaux, T., Van de Walle, D., Dewettinck, K., & Patel, A. R., *Eur. J. Lipid Sci. Technol.,* **2017**, 119 (3), 1500517.
[17] Huang, Y., Li, F., Bao, G., Li, M., and Wang, H., *Environ. Sci. Pollut. Res.*, **2022**, 29, 2432–2447.
[18] Dewi, D. C., Mahdi, C., Sulistyarti, H., and Aulani`am, A., *J. Pure App. Chem. Res.*, **2023**, 12 (02), 65-79.
[19] Al-Juhaimi, F., Uslu, N., Babiker, E. E., Ghafoor, K., Ahmed, M., and Özcan, M. M., *J. Oleo Sci.*, **2019**, 68, 11, 1099-1104.
[20] Himawan C., Starov V.M., and Stapley A.G.F., *Adv. Colloid Interface Sci.,* **2006**, 122, 3–33.
[21] Katharina, S., Sigurd, S. and Martin, M., *J. Am. Oil. Chem. Soc.,* **2014**, 91, 7, 1217-1224.
[22] Palla, C., de Vicente, J., Carrín, M. E., Ruiz, M. J. G., *Food Res. Int.*, **2019**, 125, 108613.
[23] Bouzidi, L. and Narine, S.S., *Chem. Phys. Lipids*, **2012**, 165 (1), 105–119.
[24] Bouzidi, L. and Narine, S.S., *Chem. Phys. Lipids*, **2012**, 165 (1), 77–88.

The journal homepage www.jpacr.ub.ac.id
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