Isolation of *Jatropha curcas* Oil Based Linoleic Acid by Using Argentation Column Chromatography

Lye, Yan-Ni; Salih, Nadia; Salimon, Jumat*[+]

School of Chemical Sciences and Food Technology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor Darul Ehsan, MALAYSIA

Al-Mashhadani, Mohammed H.*; Yousif, Emad

Department of Chemistry, College of Science, Al-Nahrain University, Baghdad, IRAQ

ABSTRACT: *Argentation column chromatography is a method used for separating similar molecules such as fatty acids which have slightly different stereochemistry. In this study, silver nitrate-impregnated silica gel was used as the adsorbent to isolate and purify Jatropha curcas oil fatty acids mixtures. The effects of silica gel, silver nitrate, adsorbent quantity, and fatty acid quantity on the purity and recovery percentage, as well as the ratio of linoleic acid to oleic acid, were investigated. The results showed that at the optimal condition of purification, the purity of linoleic acid increased from 38.4% to 78.7%. In the isolation process, linoleic acid adsorbed most strongly on the $AgNO_3/SiO_2$ adsorbent followed by oleic acid due to the greater number of the double bond and high ability to form polar charge-transfer complexes.*

KEYWORDS: *Silver absorbent; Argentation column chromatography; Lenoleic acid.*

INTRODUCTION

Polyunsaturated fatty acids (PUFAs) are fatty acids that contain more than one double bond. These fatty acids such as linoleic acid (C18:2n-6), α-linolenic acid (C18:3n-3), arachidonic acid (C20:4n-6), eicosapentaenoic acid (C20:5n-3) and docosahexaenoic acids (C22:6n-3) are known as essential fatty acids because the human body are not able to synthesize these fatty acids and must obtain them from dietary [1]. Moreover, PUFAs can be used to introduce functional groups such as epoxides into its double bond and also act as intermediates for a variety of products; for example, paints, coatings, additives in lubricants, and stabilizers in the polymer [2-5]. The production of PUFAs from natural sources especially in fish oil and plant oils still remained a topic of interest for their special roles in nutritional and physiological [6]. *Jatropha curcas* oil (JCO) is a non-edible and inexpensive natural source of PUFA which contains high linoleic acid content (C18:2n-6) [7].

The development of PUFAs, especially linoleic acid purification methods has been the subject of intensive research. Several purification methods have been proposed including molecular distillation, enzymatic purification, urea complexation, low-temperature solvent crystallization, membrane filtration, supercritical fluid extraction, and solid phase extraction [8-14]. Chromatography methods such as High-Performance Liquid Chromatography (HPLC),

***** *To whom correspondence should be addressed.*
+ E-mail: jumatsal@gmail.com ; mo_chemical@yahoo.com
1021-9986/2022/5/1634-1642 9/$/5.09

Thin Layer Chromatography (TLC) and column chromatography have long been known to separate fatty acids from mixtures [15-19]. However, HPLC and TLC are used in small-scale isolation.

Column chromatography using silver ion-impregnated silica gel has gained wide acceptance and popularity due to simple ways of separating lipids based on the degree of unsaturation. This argentation column chromatography method is based on the principle that unsaturated organic molecules react reversibly with a transition metal such as silver to form polar charge-transfer complexes. These complexes are formed when the formation of a sigma bond between the occupied 2p π electrons of the double bond and the free 5s and 5p orbitals of the silver ion. A π bond is formed by the overlap of the vacant antibonding 2p π electrons of the double bond and the occupied 4d orbitals of the silver ion [20]. The strength of the complex is determined by the accessibility of the electrons in the filled orbitals and steric inhibition of the orbital [21].

The isolation of *Jatropha curcas* oil-based linoleic acid has never been done especially by using argentation column chromatography. Therefore, there is a need to study the isolation of *Jatropha curcas* oil-based linoleic acid. Nevertheless, γ-linolenic acid (GLA,18:3ω6) has been purified from other seeds oil such as *Anchusa azurea*, *Scrophularia sciophila*, and *Echium fastuosum* involving simultaneous extraction and saponification of the seeds, followed by urea fractionation, urea-concentrate methylation, and argentated silica gel column chromatography. The argentated silica gel chromatography has yielded high purity γ-linolenic acid for *A. azurea* and *S. sciophila* seeds oil with yields of 73 and 64%, respectively. On the other hand, in *E. fastuosum* seed oil the recovery for the combined processes was 60%, with a final purity 86% [22]. Although argentation column chromatography has long been known, it has not been used to purify fatty acid mixtures in larger quantities. The present study involves the development of a simple and easily adaptable method for large quantity fatty acids purification *via* argentation column chromatography without converting it to ester form. The effects of various conditions in isolation of linoleic acid derived from *Jatropha curcas* oil, by isolation in argentation column chromatography, were also investigated. These various conditions are the different percentages of silver ions that are impregnated on the silica gel in the column chromatography, the amount of the adsorbent, and the amount of loaded fatty acid mixtures. In this work, we report the purity of linoleic acid, recovery percentage, and the ratio of linoleic acid to oleic acid in different conditions of argentation column chromatography.

EXPERIMENTAL SECTION

Materials. *Jatropha Curcas* seeds were collected from the experimental plots of Universiti Kebangsaan Malaysia. Silica gel (Aldrich, 200-400 mesh) and silver nitrate (System) were used in this study. All other chemicals and solvents were reagent grade and were used as received.

Preparation of FFA. Crude JCO was extracted from the seed by Soxhlet extraction method with n-hexane as the solvent. Free fatty acids (FFAs) were obtained by the hydrolysis of crude JCO [23]. Crude JCO (25 g) and 150 mL potassium hydroxide in 90% aqueous ethanol (1.5 N) were placed in a two-necked round bottom flask. The mixture was heated at 60ºC and continued for 2 h. After heating, the reaction mixture was placed in a separatory funnel and allowed to cool to room temperature. Distilled water (100 mL) and aqueous hydrochloric acid (6 N, 50 mL) were added to the reaction mixture for the protonation of the carboxylate ion. Hexane was added and used as the solvent for the FFAs from the hydrolysis reaction. The lower layer in the separatory funnel was removed and then washed with 2 x 50 mL of hexane. All of the FFAs containing hexane solutions were collected and washed out of the aqueous residue with distilled water. The FFAs containing hexane solution was dried over anhydrous sodium sulfate. After filtering by filter paper Whatman No.4, the solvent was evaporated in a vacuum rotary evaporator at 40ºC.

Preparation of argentation silica gel ($AgNO_3/SiO_2$). The solution of $AgNO_3$ (1.04 – 8 g) in deionized water (120 mL) was added to SiO_2 (200 – 400 mesh) (6 – 36 g) and stirred at room temperature (30 ºC) for 15 min. After that, the silver nitrate-impregnated silica gel was activated by 24 hours of heating at 120ºC to furnish a free-flowing white solid. This material was cooled and kept in the dark in a desiccator until needed [24].

Isolation of fatty acids by argentation column chromatography. The procedure was followed as reported study with modification [25]. A glass column (50 cm x 3 cm) was prepared and the exit of the chromatography column was plugged with glass wool to retain solids. The slurry of $AgNO_3/SiO_2$ in hexane was poured into the column

that previously had been filled with some hexane. A slight flow of hexane was allowed to avoid the air bubble trapped in the packing. The hexane was dropped out until it was 1 cm above the stationary phase. A solution of fatty acids (4 – 20 g) in 100 mL hexane was added on top of the $AgNO_3/SiO_2$ and the mixture was allowed to stand for 30 min. Then, the solution was filtered to give a hexane solution. The remaining $AgNO_3/SiO_2$ was washed with 2 x 50 mL hexane and collected together with the former hexane solution. After that, $AgNO_3/SiO_2$ has washed again with 4 x 50 mL diethyl ether to strip the PUFAs which were bonded with silver ions in $AgNO_3/SiO_2$. After filtering by filter paper Whatman No.5, hexane was evaporated in a vacuum rotary evaporator at 40°C while diethyl ether is at 30°C. All of the fractionations were carried out at room temperature. The compositions of hexane and diethyl ether fractions were analyzed using GC-FID. All the experiments were repeated at least three times and the average value was recorded.

Instrument. Gas chromatography with a flame ionization detector (Shimadzu, 17A series) equipped with column BPX 70 (30m x 0.25 mm x 0.25 μm film thickness; SGE) was used to determine the composition of the fatty acid mixtures. The injector was maintained at 280 °C. Operating conditions were as follows: helium as the carrier gas was at a flow rate of 1mL/min, injection volume 1 mL, and a split ratio of 60:1. The oven temperature was maintained at 120 °C and increased to 245 °C and hold for 15 min at a rate of 3 °C per minute for 56.6 min of analysis. Fatty acids were converted to fatty acid methyl esters (FAMEs) according to PORIM Test Method by esterification with methanol in the presence of hydrochloric acid. A reagent mixture of 10 mL methanol and 2.5 mL concentrated hydrochloric acid (37%) was used. 2 g of the fatty acid sample was placed in a small (50 mL) two-neck round-bottom flask, equipped with a standard taper joint (19/38) and short condenser. A 7.5 mL methanol was added to 1.5 mL of the previous reagent followed by 1.5 mL of toluene. The mixture was then heated at 65 °C for 1.5 hours. The heated mixture was subsequently transferred into a separatory funnel. 15 mL of hexane and 10 mL of distilled water were added to the mixture. The mixture was left to stand until two distinct layers emerged. The upper layer was decanted and dried using anhydrous sodium sulfate Na_2SO_4 overnight. The FAME was then injected into a gas chromatography. The FAME peaks were classified and quantified by comparing their peak areas and retention times with that of the pure standard FAME.

RESULTS AND DISCUSSION

JCO in this study was containing Saturated Fatty Acids (SFA), Mono Unsaturated Fatty Acids (MUFA), and Poly Unsaturated Fatty Acids (PUFA). The major fatty acids composition in JCO were oleic acid (C18:1n-9) 41.2% and followed by linoleic acid (C18:2n-6) 38.4%, palmitic acid (C16:0) 13.3%, stearic acid (C18:0) 6.5% and the minor fatty acid was palmitoleic acid (C16:1) 0.6%. This minor fatty acid sometimes was not able to detect in GC analysis because the quantity was too little. The result was in agreement with other studies regarding the Malaysia *Jatropha curcas* oil [26].

Argentation silica gel column chromatography is sorted in adsorption chromatography. This study was used for the isolation of linoleic acid from JCO. The stationary phase is silver ion-impregnated silica gel ($AgNO_3/SiO_2$) as an adsorbing surface while the moving phase is hexane and diethyl ether. These two solvents were used in this study due to their different polarity property. When the solutes are polar and tightly adsorbed to a surface, it is necessary to use a highly polar solvent to move them [17]. The more polar solvent in this study is diethyl ether. Furthermore, the number, position, and geometric configuration of double bonds determine the order of elution of unsaturated fatty acids [27].

When the mixtures of the JCO fatty acids were added to the argentation column chromatography, polar charge-transfer complexes were formed due to the interaction between double bonds and silver ions. The unsaturated compound acted as an electron donor while the silver ion was an electron acceptor. The stability of the complexes increased with an increasing number of double bonds, *cis* double bond isomers were held more strongly than *trans* isomers, and stability decreased with increasing chain length. Therefore, complexes of linoleic acid were retained on the $AgNO_3/SiO_2$ more stable than oleic acid. Hexane was used as the first solvent to dissolve SFA and some oleic acid and moved out from the column. This phenomenon is called elution. The second solvent used as diethyl ether, which was more polar than hexane and tended to compete with the solute (linoleic acid) for adsorption sites and therefore moved them along. Thus, this stripping step is called the displacement phenomenon [17]. The extent and the strength of the complexation

Table 1: Fatty acid composition (%) in the diethyl ether fraction from extractions of JCO fatty acids with decreasing the amount of SiO_2.

				%$AgNO_3/SiO_2$[a]		
Fatty acids		Feed	10%	20%	30%	40%
C16:0		13.3 ± 0.3	12.8 ± 0.5	0	0	0
C16:1		0.6 ± 0.1	0	0	0	0
C18:0		6.5 ± 0.2	6.2 ± 0.3	0	0	0
C18:1		41.2 ± 0.6	43.3 ± 0.6	33.6 ± 0.5	22.0 ± 0.6	21.3 ± 0.6
C18:2		38.4 ± 0.8	37.7 ± 0.9	66.4 ± 1.0	78.0 ± 1.0	78.7 ± 1.5
C18:2/C18:1 ratio		0.9	0.9	2.0	3.5	3.7
Recovery (%)		100	84.5	50.6	27.2	15.2

[a] *4.0 g of $AgNO_3$ in each adsorbent with different amounts of SiO_2.*

controls the mobility of the solute, and so does the polarity of the mobile phase.

Effect of SiO_2 amount.

The first parameter examined was decreasing the amount of SiO_2 in argentation column chromatography. Four different compositions (10%, 20%, 30%, and 40%, wt/wt) of $AgNO_3/SiO_2$ were prepared by adding the same amount of $AgNO_3$ but decreasing the amount of SiO_2. The $AgNO_3$/SiO2 adsorbents were prepared by adding 4 g of $AgNO_3$ to 36 g of SiO_2 (10%), 16 g of SiO_2 (20%), 9.33 g of SiO_2 (30%), and 6 g of SiO_2 (40%). Results of the purification of 4g of fatty acids mixture was shown in Table 1. For the 10% $AgNO_3/SiO_2$ adsorbent, almost all the fatty acids were recovered in the diethyl ether fraction. When only 10% of $AgNO_3$ was impregnated on the surface of SiO_2, the topology of the adsorbent had a very high SiO_2 surface area but low $AgNO_3$ surface density. Therefore, 10% $AgNO_3/SiO_2$ was sufficient to adsorb all fatty acids due to its larger surface area compared to 40% $AgNO_3/SiO_2$. This is because the larger surface was attributed to the quantity of SiO_2 which is a polar adsorbent. These adsorbents adsorbed polar solutes more tightly than less polar solutes [17]. Fatty acids contained a polar COOH group and a tendency to adsorb on the SiO_2. This phenomenon prompted that SiO_2 itself did not affect a significant fractionation of the fatty acid mixture. The result was in agreement with the effect of SiO_2 in the fractionation of methyl coyote by argentation column chromatography.

Table 1 indicated that the isolation process started from 20% $AgNO_3/SiO_2$ due to higher $AgNO_3$ surface density in 20% $AgNO_3/SiO_2$ as compared with 10% $AgNO_3/SiO_2$ adsorbent. Certainly, more polar charge-transfer complexes were formed in 20% $AgNO_3/SiO_2$. Furthermore, linoleic acid with two double bonds was held stronger than other fatty acids. When 20%, 30%, and 40% $AgNO_3/SiO_2$ were used as adsorbents, all of the SFA such as palmitic acid and stearic acid were removed in the hexane fraction. Although palmitoleic acid had one double bond, it was also removed due to its shorter chain length compared to oleic acid and linoleic acid.

The effects of SiO_2 amount on the purity of linoleic acid, recovery percentage, and the ratio of linoleic acid to oleic acid were shown in Fig. 1. At a fixed amount of $AgNO_3$, the linoleic acid purity increased tremendously with decreasing SiO_2 amounts until 30% of $AgNO_3/SiO_2$ while the corresponding recovery percentage was also decreased tremendously until 40% of $AgNO_3/SiO_2$. For example, the composition of linoleic acid could be increased from 37.7± 0.9% to 10% $AgNO_3/SiO_2$ to 78.7± 1.5% in 40% $AgNO_3/SiO_2$ with the recovery of 84.5% to 15.2%. In terms of selectivity, a high ratio of C18:2 to C18:1 represented the higher selectivity of linoleic acid as compared with oleic acid in the argentation column chromatography. Linoleic acid showed the highest selectivity in this series of experiments–with a value of 3.7. As in Fig. 1, the selectivity of linoleic acid increased tremendously from 10% to 30% $AgNO_3/SiO_2$ but no significant change from 30% to 40% $AgNO_3/SiO_2$. When higher the percentage of $AgNO_3/SiO_2$ was used, the amount of SiO_2 was lesser. The surface density of $AgNO_3$ on SiO_2 was increased until the SiO_2 surface was completely covered and may be plugged by more than one layer of $AgNO_3$ (*Ghebreyessus et al.* 2006). Therefore, the complexes were only formed with the upper layer of silver ions regardless of the percentage of $AgNO_3/SiO_2$.

Table 2: Fatty acid composition (%) in the diethyl ether fraction from extractions of JCO fatty acids with Increasing the amount of $AgNO_3$.

			%$AgNO_3/SiO_2$[a]		
Fatty acids	Feed	10%	20%	30%	40%
C16:0	13.3 ± 0.3	2.3 ± 0.1	0	0	0
C16:1	0.6 ± 0.1	0	0	0	0
C18:0	6.5 ± 0.2	1.1 ± 0.1	0	0	0
C18:1	41.2 ± 0.6	30.4 ± 0.6	21.4 ± 0.6	22.0 ± 0.5	25.1± 0.5
C18:2	38.4 ± 0.8	66.2 ± 1.4	78.6 ± 1.3	78.0 ± 1.4	74.9 ± 1.3
C18:2/C18:1 ratio	0.9	2.2	3.7	3.5	3.0
Recovery (%)	100	23.0	20.8	27.2	25.8

[a] *9.33 g of SiO_2 in each adsorbent with different amounts of $AgNO_3$.*

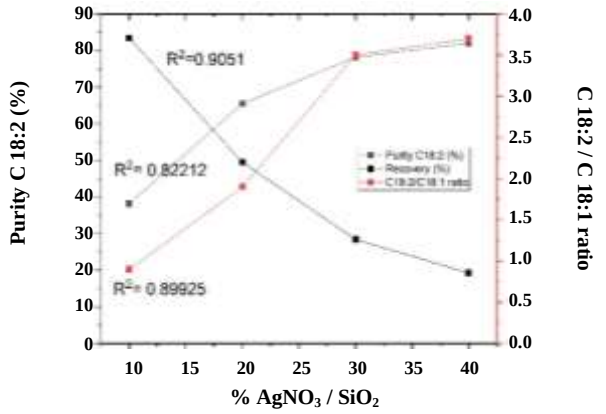

Fig. 1: The effect of SiO_2 amount on the purity of linoleic acid, recovery percentage, and the ratio of linoleic acid to oleic acid ($AgNO_3$ = 4.0 g and FFA = 4.0 g).

Fig. 1 indicated that a lower amount of SiO_2 with a constant amount of $AgNO_3$ was preferred for the isolation of linoleic acid in argentation column chromatography.

Effect of $AgNO_3$ amount. In this series of experiments, extractions were performed with $AgNO_3/SiO_2$ containing the same amount of SiO_2 but increasing the amount of $AgNO_3$ from 10% to 40%. Fatty acids compositions in the diethyl ether fractions were shown in Table 2. When 10% $AgNO_3/SiO_2$ was used, almost all the fatty acids were extracted in the diethyl ether fraction with the percentage of linoleic acid being the highest among all fatty acids in this extraction. Linoleic acid has a high ability to form stronger reversible interactions with Ag^+ ions because the greater the number of double bonds, the stronger complexation will form (Li et al. 2009). The fatty acid compositions for both 10% $AgNO_3/SiO_2$ in Tables 1 and 2 were different due to the different amounts of adsorbent ($AgNO_3/SiO_2$) used. In Table 1, the total amount of 10% $AgNO_3/SiO_2$ was 40 g but only 10.34 g in Table 2. Thus, the surface area of SiO_2 for 10% $AgNO_3/SiO_2$ in Table 1 was higher than in Table 2. Therefore, more fatty acids remained in the adsorbent. It is worth noting that when the fatty acids mixtures were extracted by 20%, 30%, and 40% $AgNO_3/SiO_2$ adsorbents, palmitic acid, palmitoleic acid, and stearic acid were completely separated from the fatty acid mixtures. However, some oleic acid was still retained on the adsorbent because the double bond in the oleic acid was competing with linoleic acid.

The effect of $AgNO_3$ amount on the purity of linoleic acid, recovery percentage, and the ratio of linoleic acid to oleic acid was shown in Fig. 2. At a fixed amount of SiO_2 at 9.33 g, the highest purity of linoleic acid was the extraction from 20% $AgNO_3/SiO_2$. The purity increased gradually from 10% $AgNO_3/SiO_2$ to 20% $AgNO_3/SiO_2$. As seen in Fig. 2, there was no significant change from 20% to 30% $AgNO_3/SiO_2$ in the purity of linoleic acid, however, it started to decrease gradually from 30% to 40% $AgNO_3/SiO_2$. The highest recovery percentage of linoleic acid was 27.2%. All recovery percentages were very low in this series of experiments due to the small total amount of adsorbent ($AgNO_3/SiO_2$). In terms of linoleic acid selectivity, the highest ratio showed at 20% of $AgNO_3/SiO_2$ and decreased gradually afterward because the surface density of $AgNO_3$ was saturated on SiO_2 and affected the formation of polar charge-transfer complexes. Fig. 2 indicated that the amount of $AgNO_3$ on SiO_2 was depending on the total amount of adsorbent for the purification of linoleic acid in argentation column chromatography.

Table 3: Fatty acid composition (%) in the diethyl ether fraction from extractions of JCO fatty acids with an increasing amount of 20% $AgNO_3/SiO_2$.

Fatty acids	Feed	Mass of 20% AgNO3/SiO2 (AgNO3 + SiO2)[a]		
		10 g	13.33 g	20 g
C16:0	13.3 ± 0.3	0	0	0
C16:1	0.6 ± 0.1	0	0	0
C18:0	6.5 ± 0.2	0	0	0
C18:1	41.2 ± 0.6	25.0 ± 0.5	24.2 ± 0.5	33.6 ± 0.8
C18:2	38.4 ± 0.8	75.0 ± 1.3	75.8 ± 1.2	66.4 ± 1.4
C18:2/C18:1 ratio	0.9	3.0	3.1	2.0
Recovery (%)	100	22.8	37.0	50.6

[a] *4 g of fatty acids mixture was used in each quantity of adsorbent.*

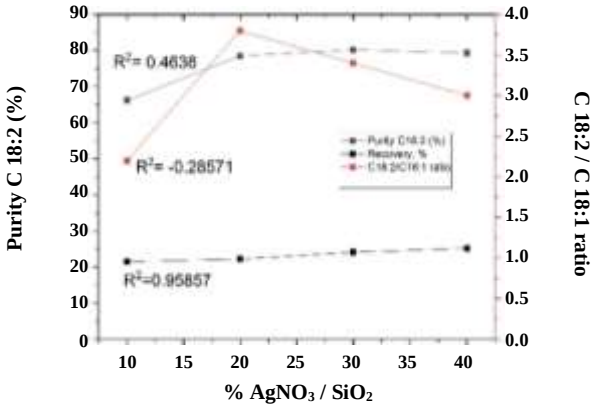

Fig. 2: The effect of $AgNO_3$ amount on the purity of linoleic acid, recovery percentage, and the ratio of linoleic acid to oleic acid (SiO_2 = 9.33 g and FFA = 4.0 g).

Effect of adsorbent quantity

Three batches of experiments with different quantities (10 g, 13.33 g, and 20 g) of the adsorbent (20% $AgNO_3/SiO_2$) were used to extract 4 g of JCO fatty acids mixture in 100 mL of hexane (Table 3). The percentage of linoleic acid increased as the total amount of adsorbent decreased due to the smaller surface area of SiO_2. There was no significant change for 10 g and 13.33 g adsorbents. SFA and palmitoleic acid were completely separated from the mixture of the fatty acid. When the total amount of 20% $AgNO_3/SiO_2$ adsorbent increased from 10 g to 20 g, silver ion and SiO_2 also increased in the same ratio. The surface density of $AgNO_3$ on the 20% adsorbent was the same for three batches of the experiment, but the surface area of SiO_2 increased as the total amount of adsorbent increased. Consequently, more oleic acid was adsorbed on the 20 g 20% $AgNO_3/SiO_2$.

The effect of adsorbent quantity on the purity of linoleic acid, recovery percentage, and the ratio of linoleic acid to oleic acid was shown in Fig. 3. The purity of linoleic acid decreased as the total amount of adsorbent increased while the corresponding recovery percentage in diethyl ether fraction increased from 22.8% to 50.6%. Although the recovery percentage was the highest, the ratio of linoleic acid to oleic acid was only 1.9 which was the lowest among the tests. This is due to the amounts of the adsorbent used having reached the optimal condition and no further increase in separation. Fig. 3 indicated that a lower total amount of adsorbent showed better isolation results than linoleic acid in argentation column chromatography.

Effect of JCO fatty acid concentration

Extractions of linoleic acid were conducted with 4 g, 10 g, and 20 g of JCO fatty acids mixture respectively in 100 mL hexane (Table 4). The standard $AgNO_3/SiO_2$ adsorbent used in this experiment was 20% $AgNO_3/SiO_2$ with 4 g of $AgNO_3$ on 16 g of SiO_2. It was found that the amount of linoleic acid adsorbed on the $AgNO_3/SiO_2$ increased as the concentration of the fatty acid mixture increased. Table 4 indicated that linoleic and some oleic acids were separated from SFA and palmitoleic acid for more concentrated fatty acid mixtures.

The purity of linoleic acid was increased as the amounts of fatty acids increased (Fig. 4). the same percentage of $AgNO_3/SiO_2$ adsorbents provided the same surface density of $AgNO_3$ on SiO_2. In this circumstance, silver ions were fixed in each of the experiments and could interact with linoleic acid as much as possible. Moreover, linoleic acid

Table 4: Fatty acid composition (%) in the diethyl ether fraction from extractions of JCO fatty acids in different amount of fatty acids.

Fatty acids	Feed	Fatty acids used in 100 mL hexane[a]		
		4 g	10 g	20 g
C16:0	13.3 ± 0.3	0	0	0
C16:1	0.6 ± 0.1	0	0	0
C18:0	6.5 ± 0.2	0	0	0
C18:1	41.2 ± 0.6	33.6 ± 0.8	24.5 ± 0.8	24.7 ± 0.9
C18:2	38.4 ± 0.8	66.4 ± 1.3	75.5 ± 1.5	75.3 ± 1.4
C18:2/C18:1 ratio	0.9	2.0	3.1	3.0
Recovery (%)	100	50.6	19.4	10.3

[a] *20 g of 20% $AgNO_3/SiO_2$ was used in each quantity of fatty acids mixture.*

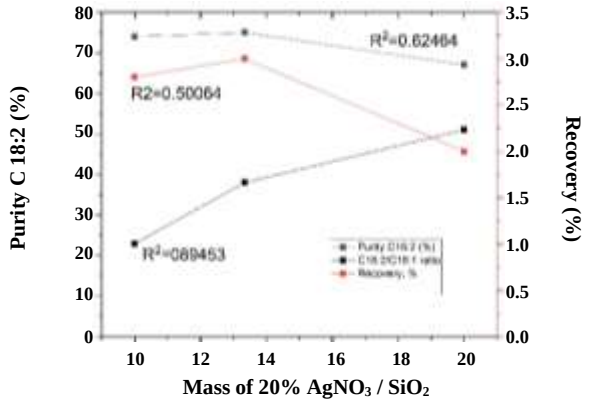

Fig. 3: The effect of adsorbent quantity on the purity of linoleic acid, recovery percentage, and the ratio of linoleic acid to oleic acid (20% of $AgNO_3/SiO_2$ and 4 g of FFA).

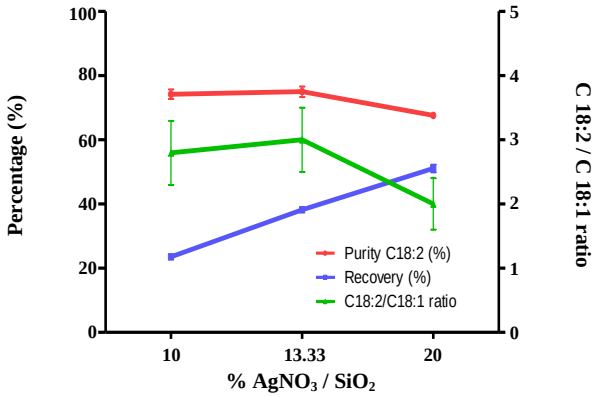

Fig. 4: The effect of fatty acids quantity on the purity of linoleic acid, recovery percentage, and the ratio of linoleic acid to oleic acid. 20% of $AgNO_3/SiO_2$ = 20 g.

could replace less unsaturated fatty acids (oleic acid) that have already coordinated with Ag^+ ions. Therefore, it was enhancing the isolation of linoleic acid. In Fig. 4, the purity of linoleic acid did not show significant changes for 10 g and 20 g. Furthermore, the ratio of linoleic acid to oleic acid also increased as the amount of fatty acids mixture increased. For example, the linoleic acid selectivity increased from 1.8 with 4 g of fatty acids mixture to 3 with 20 g of fatty acids mixture used. However, the recovery percentage of linoleic acid decreased from 50.6% to 10.3% as the amount of fatty acids mixture increased. It is suggested that the fatty acids retained on the adsorbent and not stripped off from it were due to the large amounts of fatty acids adsorbed on $AgNO_3/SiO_2$.

CONCLUSIONS

The argentation column chromatography method was shown as a suitable method for separating PUFA especially linoleic acid from saturated fatty acids. At the optimal conditions of purification, the purity of linoleic acid can be increased up to 78.7 ± 1.3%, and reduced oleic acid down to 21.4 ± 0.6%. However, this method was not significant for separating mono- and unsaturated fatty acids completely with the same number of carbon atoms as oleic acid and linoleic acid.

Acknowledgments

We would like to thank Universiti Kebangsaan Malaysia for financial support for research grants UKM-GUP-NBT-08-27-113 and UKM-OUP-NBT-29-150/2011 and to the supporting staff of the School of Chemical Sciences and Food Technology that has contributed greatly in our research.

Received : Mar. 3, 2021 ; Accepted : Jun. 14, 2021

REFERENCES

[1] Dobson G., Christie W.W., Nikolova-Damyanova B., Silver Ion Chromatography of Lipids and Fatty Acids, *Journal of Chromatography B*, **671**: 197-222 (1995).

[2] Don V.N., Bunmi S.M., John C., Peter D.N., Aduli E.O.M-A., Supplementation with Plant-Derived Oils Rich in Omega-3 Polyunsaturated Fatty Acids for Lamb Production, *Veterinary and Animal Science*, **6**: 29-40 (2018).

[3] Du G., Tekin A., Hammond E. G., Woo L.K., Catalytic Epoxidation of Methyl Linoleate, *Journal of the American Oil Chemists' Society*, **81**: 477-480 (2004).

[4] Francisco M-A., Aránzazu F-G., Surfaces and Interfaces for Renewable Energy, *Coatings*, **9(12)**: 838-842 (2019).

[5] Gámez-Meza N., Noriega-Rodríguez J. A., Medina-Juárez L.A., Ortega-García J., Monroy-Rivera J., Toro-Vázquez F.J., García H.S., Angulo-Guerrero O., Concentration of Eicosapentaenoic Acid and Docosahexaenoic Acid from Fish Oil by Hydrolysis and Urea Complexation, *Food Research International*, **36**: 721-727 (2003).

[6] Ghebreyessus K.Y., Schiltz H., Angelici R.J., Partial Separation of Polyunsaturated Fatty Acid Esters from FAMEs Mixtures by Adsorption on Silver Nitrate-Impregnated Silica Gel, *Journal of the American Oil Chemists' Society*, **83**: 645-652 (2006).

[7] Guil-Guerrero J.L., Belarbi E.H., Purification Process for Cod Liver Oil Polyunsaturated Fatty Acids, *Journal of the American Oil Chemists' Society*, **78**: 477-484 (2001).

[8] Guil-Guerrero J.L., Campra-Madrid P., Belarbi E., γ-Linolenic Acid Purification from Seed Oil Sources by Argentated Silica Gel Chromatography Column, *Process Biochemistry*, **36**: 341-354 (2000).

[9] Soheila S., Karim Z., Mohammad M.S., The Effects of pH and Chelating Agent on Synthesis and Characterization of Ni Mo/γ- Alumina Nanocatalyst for Heavy Oil Hydrodesulfurization, *Iran. J. Chem. Chem. Eng. (IJCCE)*, **40(1)**: 21-34 (2021).

[10] Bertrand M., Özcan M. M., Fatty Acid Composition and Tocopherol Contents of some Sesame Seed Oils, *Iran. J. Chem. Chem. Eng. (IJCCE)*, **37(5)**: 151-155 (2018).

[11] Ahmed W.A., Salih N., Salimon J., Derawi D., Yarmo M.A., Al-Mashhadani M.H., Yousif E., Lubrication Properties of Dodecanedioate Esters-Based Bio-Lubricant, *Letters in Applied NanoBioScience*, **9(2)**: 1117-1125 (2020).

[12] Li M., Pham P.J., Wang T., Pittman C.U., Li T., Selective Extraction and Enrichment of Polyunsaturated Fatty Acid Methyl Esters from Fish Oil by Novel π-Complexing Sorbents, *Separation and Purification Technology*, **66**: 1-8 (2009).

[13] Juliette L., Vincent L., Claire N., Jean-Jacques R., Sylvain C., Fatty Acid-Based Radically Polymerizable Monomers: from Novel Poly(meth)acrylates to CuttingEdge Properties, *Biomacromolecules, American Chemical Society*, **20(1)**: 4-26 (2019).

[14] Martins P.F., Ito V. M., Batistella C.B., Maciel M.R.W., Free Fatty Acid Separation from Vegetable Oil Deodorizer Distillate Using Molecular Distillation Process, *Separation and Purification Technology*, **48**: 78-84 (2006).

[15] Miyagi A., Nabetani H., Subramanian R., Purification of Crude Fatty Acids Using PDMS-Based Composite Membrane, *Separation and Purification Technology*, **77**: 80-86 (2011).

[16] Morris L.J., Separations of Lipids by Silver Ion Chromatography, *Journal of Lipid Research*, **7**: 717-730 (1966).

[17] Ozlem C., Separation techniques: Chromatography. *North Clin Istanb.*, **3(2)**: 156–160 (2016).

[18] Russel J.M., Werne J.P., The Use of Solid Phase Extraction Columns in Fatty Acid Purification, *Organic Geochemistry*, **38**: 48-51 (2007).

[19] Salimon J., Abdullah R., Physiochemical Properties of Malaysian *Jatropha curcas* Seed Oil, *Sains Malaysiana*, **37(4)**: 379-382 (2008).

[20] Usman A., Adamu U., Sani U., Adamu S.G., Quantitative Structure-Properties Relationship of Lubricating Oil Additives and Molecular Dynamic Simulations Studies of Diamond-Like-Carbon (DLC), *Iran. J. Chem. Chem. Eng. (IJCCE)*, **39(4)**: 281-295 (2020).

[21] Ghasem Z., Ali T.M., Siamak M., Alireza R., Babak J., Evaluation of a New Agent for Wettability Alteration During Enhanced Oil Recovery, *Iran. J. Chem. Chem. Eng. (IJCCE)*, **39(5)**: 333-341 (2020).

[22] Shimada Y., Sugihara A., Tominaga Y., Enzymatic Purification of Polyunsaturated Fatty Acids, *Journal of Bioscience and Bioengineering*, **91(6)**: 529-538 (2001).

[23] Siew W.L., Tang T.S., Tan Y.A., "PORIM Test Methods", Vol.1. Bandar Baru Bangi: Palm Oil Research Institute of Malaysia (1995).

[24] Wagner H., Luther R., Mang T., Lubricant Base Fluids Based on Renewable Raw Materials Their Catalytic Manufacture and Modification, *Applied Catalysis A: Gener*al, **221**: 429-442 (2001).

[25] Wanasundara U. N., Shahidi F., Concentration of Omega 3-Polyunsaturated Fatty Acids of Seal Blubber Oil by Urea Complexation: Optimization of Reaction Conditions, *Food Chemistry*, **65**: 41-49 (1999).

[26] Wilson R., Sargent J. R., Chain Separation of Monounsaturated Fatty Acid Methyl Esters by Argentation Thin-Layer Chromatography, *Journal of Chromatography A*, **905**: 251-257 (2001).

[27] Yamamura R., Shimomura Y., Industrial High-Performance Liquid Chromatography Purification of Docosahexaenoic Acid Ethyl Ester and Docosapentaenoic Acid Ethyl Ester from Single Cell Oil, *Journal of the American Oil Chemists' Society*, **74**: 1435-1440 (1997).