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Isolation and Ultra-Purification of Oleic Acid Extracted from Olive Oil Using Urea Crystallization

Nisreen Aldaw[1], Mohammad Haroun[2], Mohammad Nasser[3], Yaser Mousa[4]

[1,3]Department of Basic Sciences, Faculty of Pharmacy, Al-Andalus University for Medical Science,Tartous, Syria

[2]Department of Quality Control and Pharmaceutical Chemistry, Faculty of Pharmacy, Al-Andalus University for Medical Science,Tartous, Syria

[4]Department of Chemistry, Faculty of Sciences, Tishreen University, Syria.

*Corresponding Author E-mail: **n.aldaw@au.edu.sy**

ABSTRACT:

Oleic acid is an omega-9 fatty acid that is found naturally in many vegetable sources and animal products The major component in olive oil is the triglyceride esters of oleic acid. This triglyceride is one of the sources of good cholesterol. Oleic acid USP-NF (United States Pharmacopeia-National Formulary) material is a common pharmaceutical excipient that has been widely used in various dosage forms. In this paper we are describing a method for isolation and purification oleic acid in a high purity 99 % from fatty acids extracted from olive oil in coastal region of Syria by selectively using urea addition crystallization and cooling crystallization. A gas chromatography–flame ionization detection (GC–FID) method was used for direct quantitative analysis of oleic acid and related fatty acids in oleic acid USP-NF material.

KEYWORDS: Oleic acid, Isolation, Urea crystallization

1. INTRODUCTION:

Oleic acid is an omega-9 fatty acid that is found naturally in many vegetable sources and animal products The major component in olive oil is the triglyceride esters of oleic acid.This triglyceride is one of the sources of good cholesterol [1,2]. It is rich in antioxidants that help in fighting the effects of free radicals in the body. It also boosts the immune system and helps in fighting diseases by keeping us healthy throughout. It is also used as an antioxidant supplement[3]. Among the products obtained from fats and oils, fatty acids are most extensively produced. These fatty acids are characterized by carbon chain length and the number of double bonds. The characteristic composition of fatty acids is specific for each kind of fat[4,5].

Numerous methods have been established to isolate (or concentrate) and recover specific fatty acids and their derivatives (i.e., esters, free fatty acids, triacylglycerol, etc.) from various naturally occurring sources, but only few are suitable for large-scale production. The more commonly used techniques are based on the fractional distillation [6] and the inclusion with urea [7]. Given its low cost, higher yield, the quality of the obtained product, and the low operating temperatures, the inclusion with urea is considered to be a more attractive technique. However, in order to use such unsaturated fatty acids as the raw material for food and pharmaceutical products they are in need of isolation and purification in a high purity. The use of urea complexes in the preparation of oleic acid of 80-95% purity in good yield from various commercially available inedible animal fat sources was recently reported by Daniel Sweren and Winfred E.Parker [8]. Five acids found in olive oil, listed in Table 1[9],three are unsaturated and two are saturated. Oleic acid and linoleic acid are considerably lower melting and more soluble in organic solvents than the saturated components, and when a solution of the hydrolysate in n-hexane is cooled to - 15°C, about half of the material separates as a crystallize containing the two saturated compounds, stearic acid and palmitic acid [10].

Table 1 Acids of olive oil (typical composition)

Acid	Formula	%	MW	mp(°C)
Oleic	$C_{18}H_{34}O_2$	55-83	282.45	13.6
Linoleic	$C_{18}H_{32}O_2$	3.5-21	280.44	-5
Linolenic	$C_{18}H_{30}O_2$	0-1.5	278.42	Liquid
Stearic	$C_{18}H_{36}O_2$	0.5-5	284.07	69.9
Palmitic	$C_{16}H_{32}O_2$	7.5-20	256.42	62.9

FIG.1 structure of oleic acid and linoleic acid

The unsaturated acid fraction is then recovered from the filtrate andtreated with urea in methanol to form the urea inclusion complex.With the elimination of saturated acids from the olive oil hydrolysate by crystallization from n-hexane, the problem remaining in isolation of oleic acid is to remove the doubly unsaturated linoleic acid.

Urea alone crystallizes in a tightly packed tetragonal structure with channels of 5.67 A° diameter. However, in the presence of long straight-chain molecules, it crystallizes forming a hexagonal structure with inner channels of 8–12 A° diameter [11]. From examination of the oleic acid model together with the 16.2-cm cylinder, it is evident that when the carbon atoms are arranged in the particular manner shown in Fig. 2 the oleic acid molecule can be accommodated in this channel. Careful study of the drawing in Fig. 3 will show that an attempt to insert the linoleic acid model into the same 16.2-cm cylinder meets with failure; the carboxy half of the molecule is accommodated, including the 9,10-double bond, but the 12,13-double bond imposes a stoppage and leaves a five-carbon tail projecting.

Thus, when a solution of the two acids in hot methanol is treated with urea and let cool, crystals of the oleic acid complex separate and the linoleic acid is retained in the mother liquid[12].

FIG.2 Model of linoleic acid molecule does not have adequate space in the 16.2-cm cylinder.	**FIG. 3 Oleic acid model in aplastic cylinder, 16.2 cm**

Specifically, the present study provides a method for isolating and purifying oleic acid as unsaturated fatty acids, in a high purity of at least 99% by subjecting fatty acids derived from oils, particularly, a vegetable oil containing oleic acid at a high concentration, such as olive oil, as the raw material to two-step urea-addition crystallization using methanol and urea and then crystallizing the concentrated unsaturated fatty acid from an organic solvent under cooling at temperature of —5°C. to ~—10°C. Without stirring.

cooling at temperature of —5°C. to —10° C. Without stirring.

2. MATERIAL AND METHOD:

2.1. Chemicals:

All chemical reagents were analytical grade. Urea and methanol were the purest available reagent grades. The standard of oleic methyl ester (C17:0 ME) and were purchased from Merck.

2.2.Samples:

Costal Syrian olive oil was purchased directly from producer, determine iodine number on the basis of (ISO3961 Determination of iodine value) and acidity number on the basis of (ISO660 Determination of acid value and acidity) iodine number was78, acidity number 0.56 and free fattyacid 0.62% as table 2.

Table2: parameters of olive oil sample

Methods	Olive oil	Parameters
ISO660 (Determination of acid value and acidity)	0.56	Acidity (%)
ISO3961 (Determination of iodine value)	78	Iodine inde(g/100g)
ISO3960 (Determination of peroxide value)	9.6	Peroxide index (meq O2/kg)
ISO 3657 (Determination of saponification value)	185	Saponification index (mgKOH/g)

2.3. Conversion of Triglycerides into Fatty Acids:

The conversion of triglycerides into fatty acids Was conducted on the basis of AOAC method. First, NaOH (48g) and Na2 EDTA (0.5 g)Were dissolved in the mixed solution of Water (160 ml) and ethanol (160 ml) at 60° C., and then triglycerides (100 g) Was added to induce saponification for 30 minutes. Then, hexane (700 ml) and Water (80 ml) Were injected into the mixture, stirred for one hour and then allowed to stand. The unsaponificated material of the upper layer Was removed and then, the pH value Was adjusted to 1 by adding concentrated hydrochloric acid to the solution of the lower layer and then the fatty acid layer of the upper layer Was recovered and then evaporated With a vacuum rotary evaporator to remove hexane.

2.4.Isolation and Purification of Oleic Acid in a High Purity:

150 g of urea was added to 400 ml of methanol and then completely dissolved at elevated temperature of 75°C. Then, 100 g of the fatty acids (composition: palmitic acid 12 wt %, palmitooleic acid 2 wt %, stearic acid 4 wt %, oleic acid 70 wt %, linoleic acid 12 wt %) derived from olive oil as converted according to The conversion of triglycerides into fatty acids Was conducted on the basis of AOAC method. Was added to the resulting urea solution in portions over 7 times and cooled to room temperature at the cooling rate of 0.3°C./min. The resulting reaction mixture was filtered under reduced pressure, and the filtrate Was evaporated using a vacuum rotary evaporator to remove the residual methanol thereby obtaining the solid product. In order to remove any trace amount of urea and methanol present in the solid product, 400ml of Water and a small amount of hydrochloric acid were added to the solid product and the mixture was stirred. Then, the upper layer of unsaturated fatty acids was recovered. Subsequently, 200 g of urea was again added to 600 ml of methanol and then completely dissolved at elevated temperature of 70°C. Then, the unsaturated fatty acid obtained above was added to the resulting urea solution in portions over 6 times and cooled to room temperature at the cooling rate of 0.2°C. /min. The reaction mixture was then filtered under reduced pressure to recover the solid particles to which water (200ml) and hexane (200m1) were added and then a small amount of hydrochloric acid was added to cause the phase separation of urea and concentrated oleic acid. The upper layer of oleic acid having a high purity was recovered. The separated upper hexane layer was washed two to three times with water, evaporated using a rotary evaporator to remove hexane thereby obtaining 63g of high-purified oleic acid.

2.4.1. purification of oleic acid:

63 g of high purified oleic acid obtained above Was completely dissolved in 70 ml of hexane and then crystallized by cooling to -5°C. to -10° C. Without stirring. The resulting crystals Were filtered and then evaporated to remove hexane thereby obtaining 58 g of high-purified oleic acid in a yield of 92% and a purity of 99%.

2.4.2.Determination of density:

For measuring the density, our sample is weighed with a balance, and introduced it into a graduated cylinder filled with 100 ml of water. The elevation value of water volume in the graduated cylinder has allowed us to calculate the value of the density.

2.4.3.Determination of boiling point:

To characterize our resultant products we determine their boiling point, by placing an amount of product (oleic acid, glycerol) in a test tube and a thermometer in place, and then heated with a hotplate until the appearance of the first bubble of vapor by reading the temperature displayed by the thermometer is the boiling temperature of our product at atmospheric pressure.

2.4.4. Determination of melting point:

The Thiele tube is used to determine the melting point of our products, the sample is placed in a capillary connected with a thermometer and immersed in the tube and after heating, the product began to melt where we note the melting temperature.

3.RESULT AND DISCUSSION:

Wherein the fatty acids derived from olive oil are subjected to two-stepurea-addition crystallization using methanol and urea andthen crystallized from an organic solvent under cooling attemperature of —5°C. to —10° C. Without stirring to isolateand purify oleic acid. The Characteristics of separated oleic acid were in strict accordance with the standard specifications of oil acid as table 3.

Table3: Characteristics of separated oleic acid

Density	0.892
Boiling point (°C)	360
Melting point (°C)	13.6

3.1. Samples analysis by GC-FID:

The gas chromatography can be applied directly to fatty acids or fatty esters. For the triglycerides, it must be used after conversion into methyl esters. Sample oleic acid (0.1 g) was dissolved in 2 mL of hexane and shaken. The sample was then trans-esterified with 0.2 mL of 2 N methanolic potassium hydroxide solution and vigorously shaken. The upper layer of the solution was analysed by GC–FID [13]. GC was performed on During separation, samples were analyzed using a gas chromatography having the following characteristics: Name: Shimadsu 2010 DB-WAX Column length: 30 m. Stationary phase: silica. Carrier gas: Helium. Helium flow rate: 1ml/min Injection volume split: 0.5 µ l T column: 180°C. Injector temp: 250°C. Detector: FID (Flame Ionization Detector) **Fig 4** chromatogram of oleic acid extracted from olive oil in coastal region of Syria using urea crystallization.

No interfering peaks were found at the retention time of oleic acid and related fatty acids while injecting the diluent hexane into the system. The retention times of oleic acid and related fatty acids were confirmed by comparing their retention times with those obtained from each co-injected individual fatty acid **Fig 5**.

FIG4 chromatogram of oleic acid extracted from olive oil using urea crystallization

FIG5: Typical fatty acids methyl esters chromatogram of olive oil sample

4. CONCLUSION:

According to the present study, oleic acid can be isolated and purified in a high purity of at least 99% by subjecting fatty acids derived from vegetable oils containing oleic acid high concentration, such as olive oil, as the raw material to two-step urea-addition crystallization using methanol and urea and then crystallizing the concentrated unsaturated fatty acid from an organic solvent under cooling temperature of —5°C. to —10°C.

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