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Experimental Investigations of Oleic Acid Separation from Olive Oil and Olive Mill Wastewater: A comparative study

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Abstract

Oleical industry produces along with olive oil, two fractions of wastes: A solid fraction called pomace and a liquid one called olive oil mill wastewater (OMW). The OMW is known for its high concentration in oily residues. These residues remain unused. In this context, this work aims to separate the oily phase (high acidity of 3.397%) which will constitute a raw material for oleic acid production. After undergoing a transesterification reaction at temperatures between -4°C and 20 °C, the oily phase yields methyl esters which are subjected to four crystallizations with urea in order to remove saturated and unsaturated fatty acids. A pure olive oil from the same region is subjected to the same process. This operation was performed to determine the efficiency of our process, thus making it possible for us to compare the quality of both resultant oleic acids. CPG analysis showed that the final product from olive oil contains 97.75% of methyl oleate, while the oleic acid extracted from OMW contains 95.5%. An additional process has been suggested, to isolate the glycerin obtained- as a by-product - during the transesterification this process will furthermore, allow the production of biodegradable soap.

Keywords: Olive mill wastewaters, acidity, oleic acid, transesterification, urea, glycerin.

1. Introduction

Olive oil mill wastewater (OMW) is a liquid by-product obtained during the extraction of olive oil with a rate of 0.5 to 1.5 m3 per ton of olive depending on the technology [1]. The industry of olive oil is no exception to other industries. It also releases OMW and solid wastes called pomace, along with the produced oil .OMW is a black liquid, generally flushed in mid environment without significant treatment, causing great environmental harm due to its acidic pH and its concentration in hardly biodegradable organic matter and polyphenols. While pomace is normally used in composting [2], fuel [3], biogas [4], or animal feed [5]

Nowadays the olive oil extraction industry has remarkably grown, as a consequence of the high nutritive, economical and medical value of the produced oil. A raise of olive oil sells have been noted (3.5 kg/habitant) in 2013[6]. Hence, Olive oil production contributes with a rate of 5% to the country's Domestic agricultural Product (GDP) and with a rate of 15 % to its agro alimentary exportations [7]. With the raising attention to oil production, Morocco encouraged the development of the olive oil production. Leading to an annual production capacity of olive oils that reaches 1.5 million tons (0.6 million tons of olives triturated by 565 modern and semi-modern facilities while 0.16 million tons of olives is annually triturated par 15000 traditional facilities called Maâsra) [8]. Consequently the oil flux production increase, will engender an equivalent increase in the released liquid wastes (OMW), which composition varies depending on many factors such as the variation and maturity of the olive, production period, climatic conditions, cultivation method and the oil extraction technology [9], the **Table 1** below, illustrates an example of OMW composition in Marrakech department [10].

Table 1: Composition of crude olive mill wastewaters from Marrakech; *S.M*: suspended matter, *COD*: chemical oxygen demand, *P.P*: polyphenols.

Parameters	Values
pH	4.2
Colour (A_{395})	16
S.M (g/l)	4
COD (g O_2/l)	50
P.P (g /l)	12
Cl^- (g /l)	11.9
K^+(g/l)	2.5
NH_4^+ (g/l)	0.15

The treatment of OMW has been the aim of several studies. Many processes were developed to treat OMW: Simple physical processes such as dilution, evaporation, sedimentation, filtration and centrifugation [11, 14]. Biological treatment: aerobic digestion [15-16], and anaerobic treatment [17-18]. Thermic treatment [19-21]. As well as physico-chemical and electrochemical processes such as lime treatment [22, 23], coagulation–flocculation–hydrogen peroxide oxidation [24], phenolic treatment [25-27] and Electro-Fenton oxidation [28-30]. The treatments stated previously are performed in order to reduce the organic matter and toxicity to acceptable limits before the OMW's release.

In the present work, the fractional crystallization is found to be a quick, easy, cheap and effective method to recover an expensive product from these huge quantities of effluents, discharged directly into the environment without any significant treatment. The originality of this technique is the separation by a natural settling of an acidic oil (inconsumable) from olive mill wastewater, which requires searching recovery solutions by adaptation and application of a fractional extraction technique of fatty acid from these effluents for their valuation coupled on one hand to produce soap and glycerin and on the other hand to extract pure oleic acid contained in the extracted oil. To determine the efficiency of this new method, an extraction from extra virgin oil from the same region was performed in order compare the yields of the two separations.

2. Materials and methods

After extracting olive oil, either by pressing or centrifugation, the resulting liquid contains both oil and vegetation water. The latteris separated by decantation or centrifugation.

In spite of the treatment, the wastes resulting from the decantation and centrifugation processes still contain important oily residues. These wastes are generally releases in the environment.

The residual oil resulting from OMW separation cannot be edible due to its high acidity (3.39%), which is beyond the admissible value by Codex Alimentarius [31].

In this work, we collected 90 L of OMW from Fes-Boulman region. OMW collected was stored in 5L cans, and then left for 6 months in the laboratory for decantation purposes.

Several separated oil analysis showed that the acidity rate is high and surpasses the standards for human consumption. We will dedicate this work to uncover solutions aiming to upgrade these oils via the manufacturing of biodegradable soap and the oleic acid extraction for pharmaceutical and chemical purposes.

2.1. *Characterization of olive oil and OMW*

The OMW and the virgin oil used were collected during the oleical campaign of 2012/2013, from different traditional trituration plants in Fes-Boulman region. According to the Regional Administration of Agriculture (RAA), the agricultural year of 2012/2013 was exceptional (a raise of 70%) in comparison with its previous one. By dint of the climatic conditions, the production rate was 135 090 tons. The samples were conserved in the laboratory for six months in room temperature.

2.2. *Analyses*

2.2.1. *Oil and soap analysis*

The chemical characterization of recovered oil samples and the soap product was performed according to the method of the International Organization for Standardization (ISO).

2.2.2. *Samples analysis by GC*

The gas chromatography can be applied directly to fatty acids or fatty esters. For the triglycerides, itmust be used after conversion into methyl esters.

During separation, samples were analyzed using a gas chromatography having the following characteristics:
Name: VARIANT 304 CX.
Column length: 50 m.
Stationary phase: silica.
Carrier gas: Helium.
Helium flow rate: 1ml/min
Injection volume split: 0.5 µL.
T column: 210 °C.
Injector temp: 190 °C.
Detector: FID (Flame Ionization Detector).

2.2.3. *Determining 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.

2.2.4. *Determining 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.

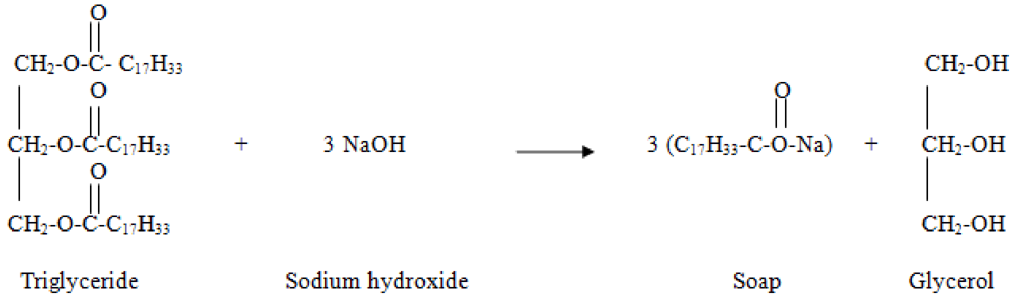
2.2.5. *Determining melting point*

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.

2.3. *Saponification*

The Saponification process is based on the hydrolysis of the ester linkages between fatty acids and glycerol. The process requires the presence of a strong base (NaOH (sodium hydroxide)):

2.3.1.



$$\mathrm{(CH_2\text{-}O\text{-}CO\text{-}C_{17}H_{33})(CH\text{-}O\text{-}CO\text{-}C_{17}H_{33})(CH_2\text{-}O\text{-}CO\text{-}C_{17}H_{33}) + 3\,NaOH \longrightarrow 3\,(C_{17}H_{33}\text{-}CO\text{-}O\text{-}Na) + CH_2OH\text{-}CHOH\text{-}CH_2OH}$$

Implementation of the saponification reaction

150 ml of oils obtained from OMW, 150ml of ethanol, 30%of sodium hydroxide and some pumice were mixed.

The mix is heated with reflux for 6 hours till the solution becomes limpid. The 6 hours are considered enough for the saponification reaction to end. The mix is left to rest for few minutes and then separated to obtain two phases: An aqueous phase, rich of glycerin and a heavier one which is soap.

2.3.2. *Salting out*

NaCl is much more soluble in water than soap, the salt is dissolved in soap water, which contains Na^+ ions. Result: The excess of sodium hydroxide in the solution is neutralized with sodium chloride, and the soap precipitates. Thus, helping to improve the biphasic separation.

2.3.3. *Separation by filtration*

The mix is left to naturally decant over night for a period of 12 hours. We proceed then by filtration on a Büchner vacuum filter on using a filter cloth (40 µm) to filter the mix.

The solid phase (soap) is washed with distilled water and dried in sunbeam.

2.4. *Extraction of oleic acid*

Oleic acid is a fatty unsaturated acid. It is very essential in chemical fields. The oleic acid is used as a surfactant to modify the surface of magnetite particles [32]. It is also considered to be a raw material for bio products production which are rarely available in their natural state [33].

Several techniques were applied to extract oleic acid out of food wastes. The more commonly used techniques are based on the fractional distillation [34] and the inclusion with urea [35]. 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.

In this work, we chose to adopt the fractional crystallization with urea method that has been described by Lucie FRÉMONT, Marie-Thérèse GOZZELINO [36]. This method allowed us to recover oleic acid with both high purity and quality.

The originality of this work lies in the adaptation and application of a fractional extraction technique of fatty acids from the oils obtained from Moroccan OMW. The resultant products will be then upgraded by soap and glycerin production on the one hand and oleic acid extraction on the other hand.

A parallel study was carried out on an extra virgin oil to make a comparison between oleic acid resulting from OMW and that extracted directly from olive oil.

2.4.1. *Transesterification*

The aim of this technique is to transform the triglycerids, forming the oil, into methyl esters. To do so, 200 ml of the oil sample, and 600 ml of methanol were mixed and catalyzed by 100 ml of sodium methoxide. The mixture is heated with reflux for 3 hours. The 3 hours period is considered sufficient for the perfect homogenization of the mixture.

The mixture is then separated in a bulb decanter for 4 hours, till two phases appear: Upper phase rich in methyl esters and a lower phase rich in glycerol.

After recovering the glycerol, the upper phase is separated and thus washed with hydrochloric acid to neutralize the excess of sodium hydroxide

The upper phase resulting from the first separation is then washed again with 100 ml of distilled water which leads two phases formation: an upper phase rich in pure methyl esters and a lower one rich in water and methanol.

After separating the esters from the upper phase, the lower is then introduced with hexane into a bulb decanter. In order to maximize the recovery of methyl ester we proceeded by a 3 staged separation. The hexane was then added successively with a rate of 25 ml. Every addition was followed by a decantation. Methyl ester concentrated solution is mixed with the one obtained previously the mixture is then concentrated by evaporation using a rotatory evaporator. The esters are then purified, and weighed with an electronic scale.

2.4.2. *Crystallization with urea*

This stage aims to crystalize methyl oleate, obtained from fatty methyl acid, with four successive crystallizations and a purity of 95.5 % approximatively.

As for the first separation, 100g of methyl esters, 100g of urea and 1L of methanol are mixed. In order to solubilize the mixture in alcohol a water bath is being used.

After a night long of cooling at 4°C, the solution is vacuum filtered using sintered glass. To finally obtain two phases: solid phase C1 and a liquid phase F1

The filtrate F1 is mixed with 200g of urea. The mixture is maintained at 4°C to recover crystals C2. These crystals will be rinsed and mixed with 1.5l of methanol and then vacuum filtered at room temperature.

The filtrate F3 resulting from the second crystallization is mixed with 120g of urea and then held at a temperature of 4°C. The mixture is left to rest till a crystallized phase appears C4.

This final phase contains, in addition to methyl ester, the urea that should be eliminated with a solution of hydrochloric acid and then separated with a means of natural decantation. Esters are then extracted by hexane. The excess acid is eliminated by pure water and dried afterwards with sodium sulfate

After removing the solvent with the rotatory evaporator, esters are then weighed

2.5. *Glycerin extraction*

Glycerol (propane-1, 2, 3-triol) is a co-product which has three hydroxyl groups functionalisable used infood [37, 38], and cosmetics [39, 40]. There are two type of glycerol, a synthetic obtained by the petrochemical process which propene is converted to glycerol, and natural oneformed in the two processes of saponification and transesterification.

The aqueous solution produced by the saponification and transesterification, was neutralized with concentrated hydrochloric acid. After evaporation and distillation, an extra pure glycerin was recovered.

3. Results and discussion

Oil was recovered of OMW after a natural decantation for six months. 90 L handed 20 L, a yield of 23 %. This value is slightly higher than that given by (FestasRos de Ursinos) [41] who found that the residual oil concentration contained in the OMW is very variable depending on the extraction method used.it varies between 0.02 and 1%. Which means that these effluents still contain high amounts of oils, despite the treatment. These oil characteristics are presented in **Table 2** below, along with characteristics of virgin oil extracted from the same region:

Table 2:Analysis of virgin oil and oil of OMW samples

Parameters	**Virgin oil**	**Oil of OMWW**	**Norm CODEX**	**Methods**
Acidity (%)	0.46	3.397	0,3-1	ISO660 (Determination of acid value and acidity)
Iodine index (g/100g)	86.14	82.17	75-94	ISO3961 (Determination of iodine value)
Peroxide index (meq O_2/kg)	9.61	11.26	≤20 - ≤15	ISO3960 (Determination of peroxide value)
Saponification index (mg KOH/g)	187	189	184 – 196	ISO 3657 (Determination of saponification value)
Refractive index ($n^d{}_{20}$)	1.4701	1.4678	1.4677 – 1.4705	ISO 6320 (Determination of refractive index)

Examination of the characteristics of the recovered oil allowed illustrating that the acidity surpasses the standard of Codex Alimentarius for oils. This is due to the instability of the oils and the bad storage conditions and the low peroxide index. The peroxide index explains the resistance to the oxidation through the storage.

The olive oil samples met the required values of acidity, and the others index for the “extra virgin olive oil” quality category.

3.1. *Soap and saponification yield*

The soap obtained after the saponification has a beige color and a smooth clean aspect with a consistent and abundant soapsuds.

A comparison between the resulting soap characteristics and the pomace soap characteristics [42], showed that the two categories of values are comparable, which they are presented in **Table 3**.

From 138 g of oil we could produce approximately 141.67 g soap
Knowing that
M (oil)=884 g/mol et M (soap)=304 g/mol
et n (oil)=n (soap)/3
m (oil)/M (oil) =m(soap)/3*M(soap)
With m (oil) =138g and m (soap) exp =141.67g
m (soap) theoretical =3*304*138/884=142.37 g

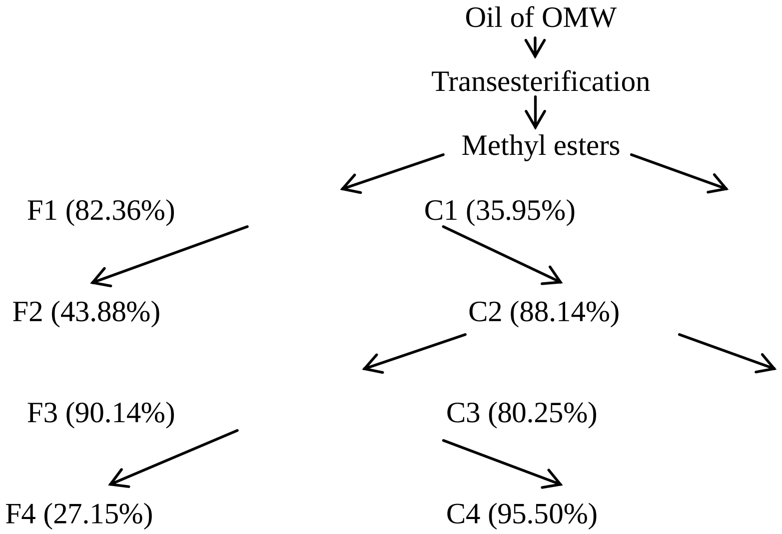
$$Y = (m\ (soap)\ exp/m\ (soap)\ th)*100$$
$$Y = (141.67/142.37)*100 = \mathbf{99.50\%}$$

The aim of the yield calculation is to determine the operating conditions, in order to approach as close as possible to 100%, 0.5% yield losses can have various causes: parasitic reactions, losses at the various stages of the synthesis (filtration, drying and crystallization).

Table3: Characteristics of the resulting soap.

Parameters	Values	Literature [42]	Methods
pH	8,7		
Fatty acid	65.30%	64.60%	ISO 685 (Determination of total alkali content and total fatty matter content)
Moisture	5.60%	5.00%	ISO 672 (Determination of moisture and volatile matter content)
Combined alkali	8.85%	8.85%	ISO 456 (Determination of free caustic alkali)
Chloride	3.70%	3.90%	ISO 457 (Determination of chloride content -- Titrimetric method)
Free alcali	0.10%	0%	ISO 684 (Determination of total free alkali)
Glycerol	0.70%	0.70%	ISO 1066 (Determination of glycerol content -- Titrimetric method)

3.2. *Extraction of oleic acid from OMW*



F: filtrate, C: crystals

Figure 1: Methyl oleate crystallization steps from methyl esters of OMW

The majority of saturated fatty acids are eliminated in the first separation at a temperature of 4°C, in the same condition and by adding the urea to the filtrate F1, the rest will be eliminated in the second crystallization , which gives rise to a phase C2 having in addition to the oleic acid an amount of saturated fatty acid.

This phase will be diluted with methanol at 20 °C to prevent the formation of inclusions while the elimination of fatty acids and the recovery oleic acid in the filtrate F3 with 90.1357% purity.

A final crystallization gave us by the addition of urea at 4°C, a final phase rich in oleic acid (95.50%) (**Fig. 1**).

After a series of successive fractional crystallizations, we were able to obtain a crystalized phase C4, riche in pure methyl oleate (95.50%). The advancement of the composition of fatty acids during the four crystallizations is shown in the **Table 4**.

Table 4: Massfatty acid compositionof thecrystallizationphase of OMW esters.

Fattyacids	Structure	Composition	F1	C1	F2	C2	F3	C3	F4	C4
Palmiticacid	C16:0	9.3429	2.1019	38.8984	0.2920	4.2546	1.7856	12.9456	1.0024	2.5164
Palmitoleicacid	C16:1	0.6096	1.6539	0.0357	2.8123	2.2454	2.5475	4.5789	6.6221	1.9015
Stearicacid	C18:0	2.9059	0.0476	13.9547	0.2225	0.1614	-	-	-	-
Oleicacid	C18:1	74.018	82.3594	35.9476	43.881	88.1354	90.1357	80.2536	27.1524	95.495
Linoleicacid	C18:2	10.9884	12.2856	4.8458	46.5568	3.6213	5.4456	2.2219	64.6214	1.5654
Linolenicacid	C18:3	2.1352	1.5516	6.3178	6.2354	1.5819	0.0856	-	0.6017	0.4231

After the first separation, saturated acids C16:0 and C18:0 were removed with high percentages (38.89% and 13.95%). Then, after performing the second crystallization, a portion of both these saturated acids and unsaturated acids were recovered in the filtrate F2, Oleic acid included.

C2 crystals contain small amount of saturated acids. These acids will be crystallized during the third separation, where an important amount of methanol was added, held at room temperature to form inclusions with these acids. In both C3 and F3 phases, it has been noted that there is an important distribution of oleic acid.

The fourth crystallization of the F3 filtrate yielded a crystallized phase C4 with acid oleic purity of 95.50%.

The **Fig. 2** presents the variation of the methyl oleate composition during the crystallization stages.

The initial mixture (separated oil) contains 74.02% of methyl oleate, after the first cristallization we are left with a percentage of 82.36%, the crystals C2 resulting from the second separation have a purity of 88.14%.

F3 and C4 contain 90.14 %, 95.50% respectively. Lucie FREMONT et al were able to extract an oleic acid with purity of 99.5 % but by starting from an olive oil and cooling to -60 ° C of the final phase C4 mixed with an acetone solution.

The purity of the resultant oleic acid is 95.4951% which is admissible given the fact that our raw material is OMW instead of virgin olives.

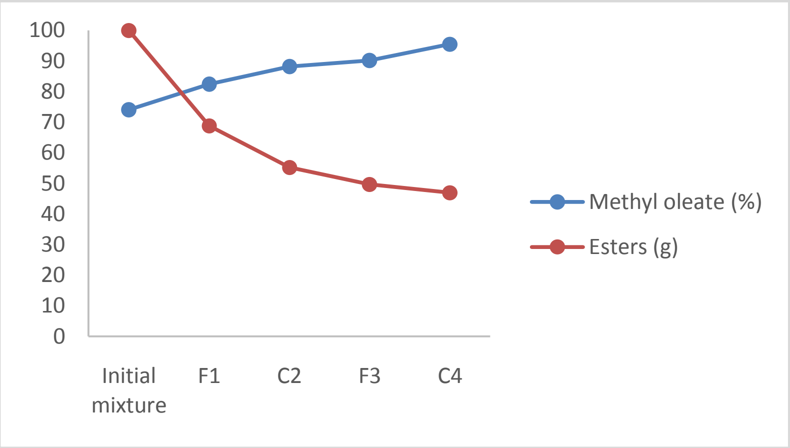
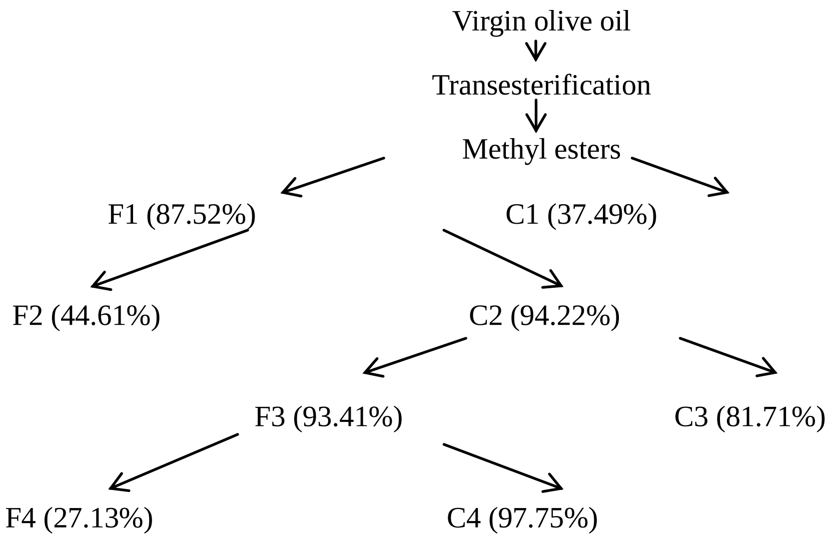


Figure 2: Mass evolutionof methyl oleateand performance weight during the crystallization steps of OMWW

Transesterification of 200 ml oil gave approximately 180.65 g of methyl esters which 100 g are used for crystallization. After the four separation steps, were able to get a final phase C4 with mass of 46.98 g.

3.3. *Extraction of oleic acid from extra virgin oil*

Following the same step mentioned in the previous section 3.2, we have followed the evolution and distribution of saturated and unsaturated fatty acids between crystals and filtrate (**Fig. 3**):



F: filtrate, C: crystals

Figure 3: Methyl oleatecrystallizationsteps from methyl esters ofolive oil

Table 5 presents the evolution of methyl oleate concentration during the four separations, starting with an olive oil which contains 78 127% oleic acid to have a pure final product (97.75%).

Table 5: Mass fatty acid composition of the crystallization phaseofolive oilesters

Fattyacids	**Structure**	**Composition**	**F1**	**C1**	**F2**	**C2**	**F3**	**C3**	**F4**	**C4**
Palmiticacid	C16:0	10.601	2.825	41.4827	0.4029	2.8739	0.7713	13.7924	-	-
Palmitoleic acid	C16:1	0.945	1.2741	-	3.114	0.5191	0.7911	1.8223	4.4507	1.1276
Stearicacid	C18:0	3.147	0.0283	14.8917	-	-	-	-	-	-
Oleicacid	C18:1	78.127	87.5241	37.4951	44.6167	94.2206	93.416	81.7169	27.137	97.753
Linoleicacid	C18:2	6.659	7.3942	1.3349	48.1263	2.1115	5.0216	2.6684	60.471	1.1193
Linolenicacid	C18:3	0.521	0.9543	4.7956	3.7401	0.2749	-	-	7.9415	

Oleic acid, recovered from virgin olive oil, has a purity greater than 97 %, with a mass loss of about 50 g. The starting weight of methyl esters was 100 g we obtained at the end final weight C4 50.19 g (**Fig. 4**).

3.4. *Separation yields*

In order to evaluate the efficiency of our process, we calculated the yield of different stages of the crystallization, based on the relation below

$$R= ((\text{ester mass}*\text{purity}) / (\text{initial mass}*\text{initial purity}))*100$$

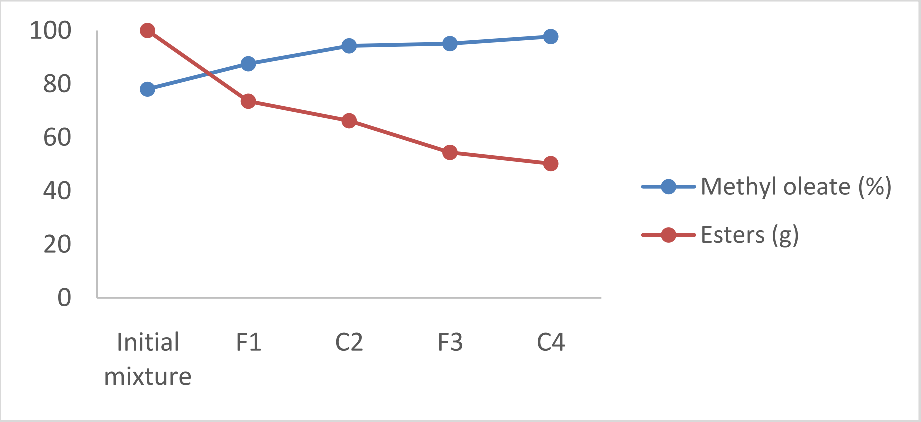


Figure 4: Mass evolution of methyl oleate and performance weight during the crystallization steps of virgin oil

In the final stage of separation, we obtained 46.98 of esters with a purity of 95.49%. This means that the yield of our process is very satisfying, since we reached 60.61% (**Fig. 5**).

After comparing the two extractions yields, since we have had a separation efficiency of about 60.61 % for OMW and 62.80% for virgin olive oil. We can confirm that this technique is very effective for extraction of fatty acids from oily residues of OMW.

In order to separate the oleic acid, we proceed by a saponification followed by adding of hydrochloric acid, until the appearance of a precipitate of oleic acid. The characteristics of this oleic acid are presented in the **Table 6**.

For both samples, the resulting oleic acids have the same appearance; a yellow color, insoluble in water, and soluble in some solvent such as: ethanol, ether and chloroform. This product is used to prepare esters, alcohols, and organometallic salt. With the quality and purity of this compound, we can produce biodiesel by esterification with alcohol. The oxidative cleavage of the acid produces two unsaturated carboxylic acid: azelaic acid (or acid nonanedioic) used in the cosmetic and pharmaceutical field (treatment of skin diseases including acne), and pelargonic acid (or nonanoic acid) frequently used as fragrance for perfumes, antibacterial, tensioactifs and others [43, 44].

The glycerol resulting from the saponification reaction and the transesterification process is a viscous liquid, transparent with a sugary flavor. The glycerin characteristics are shown in the **Table 7**.

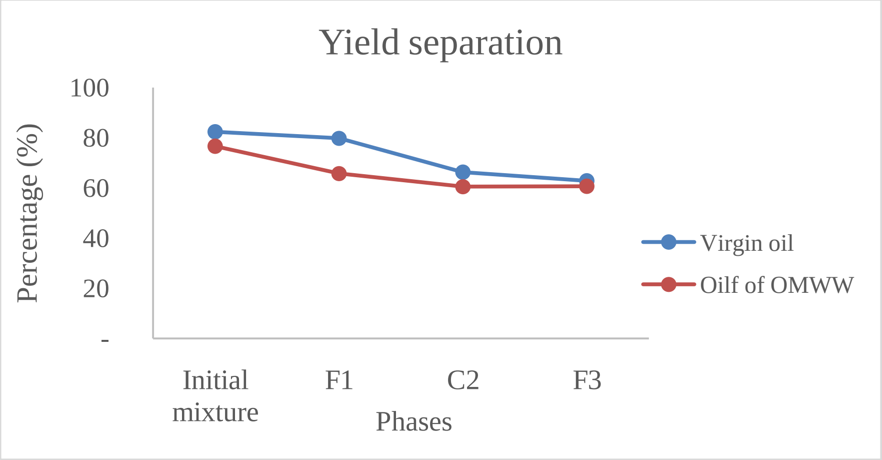


Figure 5: Yield of four crystallizations.

Table 6: Characteristics of separated oleic acid.

Parameters	Literature [36]	Virgin oil	Oil of OMWW
Density	-	0.893	0.898
Boiling point (°C)	-	360	360
Melting point (°C)	14 <<15	14.1	13.7

Table7: Characteristics of the resulting glycerol.

Parameters	Literature [35]	Values
Density	1.26	1.263
Boiling point(°C)	290	290
Melting point(°C)	17-18	17.5

Glycerin is soluble in water and in alcohols, very stable under normal conditions of use, non-toxic and has no negative impact on the environment.

From 138 g of olive oil we could produce 9.14 g of glycerol by saponification, and from 184 g were able to get 11.31 g by transesterification.

Conclusions

This study, suggests a new technique for the production of oleic acid from Moroccan olive mill wastewaters. The propounded technique is based on the inclusion with urea, it is a simple method with a low cost. In comparison with the extraction of oleic acid from raw olives, we concluded that this process is applicable and the resulting oleic acid has nearly the same characteristics as the one resulting from raw olives. This proves that Moroccan OMW contains oily residue.

The oily phase is separated from the OMW, has very poor quality and nutritive value due to its strongly acidic aspect. Thus, it can be used to manufacture biodegradable, high quality, soap and glycerin.

This new upgrading technique tends to purify, as much as possible, our food wastes to ensure a better environmental protection and to contribute to the improvement of the profitability of the oleical field, through the recovery of a multiple valuable products.

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