

Studies on two-step acid-base catalyzed transesterification of refined ostrich oil

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Abstract

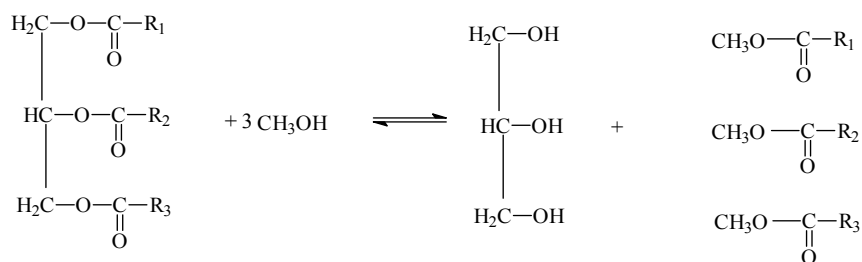
This paper presents studies regarding the conversion of waste (fat ostrich) in the high added value product.. The crude oil was obtained by wet melting of fat from the back and belly obtained from Suraki farmed ostriches.(Rasuceni, Giurgiu district) Refined ostrich oil was obtained in our laboratories, by purification of crude oil through neutralization, washing, drying and fading Conversion of the refined ostrich oil in the fatty acid esters was performed by applying a chemical transesterification. The fatty acid methyl esters was preparation by two-step acid-base catalyzed transesterification of fatty acid triglycerides of refined ostrich oil with methanol, without prior cleavage of the free fatty acids. Determination of fatty acids in samples of refined ostrich oil and of fatty acid methyl esters (FAMEs) was performed by gas chromatography. The evolution of reaction was monitored by thin layer chromatography . The spots were visualised by exposing the plates to iodine vapor.

Keywords: ostrich oil, transesterification, fatty acid, fatty acid methyl esters

Introduction

Fatty acid methyl esters are widely used as intermediates in the production of detergents, emulsifiers, stabilizers, wetting agents, textile processing agents. Smaller amounts of methyl esters of fatty acids are used in a variety of direct and indirect applications: food additives, including dehydrating grapes to produce raisins, synthetic flavoring agents and lubricants in metal to metal intended for use food. Fatty acid methyl esters are also used as intermediates in the production of a variety of food ingredients [1].

Preparation of fatty acid methyl esters is done by the well-known transesterification of fatty acid triglycerides of vegetables oils or animal fats with methanol in alkaline catalysis [2-6], acid catalysis [2,3,7], or two-step acid-base catalyzed transesterification [8-10] without prior cleavage of the free fatty acids, according to the following reaction (Scheme1):



Scheme 1 Transesterification of triglyceride

Transesterification in alkaline catalysis is carried out in the presence of alkaine catalysts (sodium hydroxide, potassium hydroxide, sodium methoxide) (catalyst concentration of 0.5-5% by weight of the raw materials) in methanolic solution at normal pressure and at the boiling point of methanol. The reaction speeds higher than acid catalysis. This method has the disadvantage that it requires raw material to be anhydrous (water content below 0.8% by weight) and have a free acid content, expressed as acid number preferably less than 0.5 less than 0.1.

Transesterification in acid catalysis is carried out in the presence of acid catalyst (catalyst concentration of 0.5-5% by weight of the raw material) (sulfuric acid, hydrochloric acid, *para*-toluenesulfonic acid). This method allows processing animal fats with high free acid and water. It works in methanolic solution, at normal pressure, at the boiling point of methanol. The reaction time is between 2-3 hours.

According to the literature, two step acid-base catalyzed transesterification is carried out as follows: the first step, pre-treatment with acid, when, takes place the esterification of the free acids from the oil and two-step transesterification in alkaline catalysis.

The advantages of the transesterification in two stage are : the yield of methyl esters of fatty acids obtained by esterification and transesterification is 21.5% and 90.6% [8], energy consumption is low and this process can be used for oils having a free acids value greater than 1%.

Purification of fatty acid esters obtained from vegetables oils or animal fats is achieved by chromatographic methods, distillation method, low temperature crystallization, enzymatic methods, supercritical fluid extraction, urea complexation [11].

This paper presents, our results, related to transesterification of fatty acid triglycerides of refined ostrich oil with methanol, in two step acid-base catalyzed transesterification, without prior cleavage of the free fatty acids.

Materials and methods

Ostrich oil was refined in our laboratories, with the following characteristics: density-0,8760-0,9280 g cm⁻³,(40°C), free fatty acids: 0,14-2 mg KOH 1 g⁻¹ oil, peroxid value,1-2,85 mE kg⁻¹, moisture content 0,02-0,1%, iodine value 62-72 g I 100g⁻¹oil, saponification value 185-205 mg KOH 1 g⁻¹ oil.

Fatty acids contained in the refined ostrich oil are:

- 30,6-40,1 % *saturated fatty acids (FS)*: lauric acid C12:0, myristic acid C14:0, palmitic acid C16:0, stearic acid C18:0,arachidic acid C20:0;
- 39,5-58,0 % *monounsaturated fatty acids (MUFA)*: palmitoleic acid C16:1, oleic acid C18:1(n-9) (omega 9 fatty acid), eicosenoic acid C20:1(n-9) (omega 9 fatty acid), erucic aid C22:1 (n-9)(omega 9 fatty acid);
- 8,73-19,73 % *polyunsaturated fatty acids (PUFA)* : Acid alpha-linolenic acid (ALA) C18: 3(n-3) (omega 3 fatty acid), linoleic acid (LA) C18:2 (n-6)(omega 6 fatty acid), gamma-linolenic acid (GLA) C18: 3 (n-6) (omega 6 fatty acid), arachidonic acid (AA) C20:4 (n-6) (omega 9 fatty acid);

Determination of fatty acids in samples of refined ostrich oil and of fatty acid methyl esters (FAME) is performed by gas chromatography, in accordance with EN ISO 15304, using a capillary column with a stationary phase with high polarity, HP88-(88% cyanopropyl) aryl

polysiloxane . Gas chromatography is performed with a gas chromatograph-689N Agilent detector equipped with FID (flame ionization) and autosampler 7683B. Progress of the transesterification was followed by TLG plates on Sigma-Aldrich, eluted with the solvent system : n hexane:ethyl acetate:acetic acid : 90: 10:1(v:v:v). The spots were visualised by exposing the plates to iodine vapor.

Two step acid-base catalyzed transesterification:sulfuric acid-sodium hydroxide (FAME 1)

A mixture of 140 ml (120.9 g) refined ostrich oil and 60 ml methanol abs. was heated with stirring at 60°C, then added the catalyst-sulfuric acid -30% methanolic solution, (1% of the oil). The mixture was heated under stirring for 3 hours at 65°C. After acid catalyzed pretreatment, the reaction mixture, was cooled to 50°C. Then, sodium hydroxide methanolic solution, (1% of the oil) was added and the mixture was heated, under stirring at the same temperature for 3 hours. End of transesterification was revealed by thin layer chromatography, when it noticed missing spot corresponding raw material-refined ostrich oil. Let it separate for 2 hours. The lower layer containing glycerol (51.9 g) was separated. The upper organic layer (123.17 g) containing fatty acid methyl esters (FAMES) are diluted with hexane (150 ml), washed with water (200 ml) to neutral pH, dried over anhydrous sodium sulfate and concentrated. 118 g of oil containing fatty acid methyl esters were obtained. After crystallization from acetone at low temperatures, filtration and concentration there were obtained 75 g of oil containing fatty acid methyl esters.

Two step acid-base catalyzed transesterification:sulfuric acid- sodium methoxide (FAME 2)

A mixture of 110 ml(94,93 g,) refined ostrich oil and 47 ml methanol abs. was heated with stirring at 60°C, then added the catalyst - sulfuric acid -30% methanolic solution, (1% of oil) The mixture was heated under stirring for 3 hours at 65°C. Next, the reaction mixture, is cooled to 50°C. Then, sodium methoxide- methanolic solution (1% of the oil) was added and the mixture is heated, under stirring at the same temperature for 2 hours. End of transesterification was revealed by thin layer chromatography, when it noticed missing spot corresponding raw material-refined ostrich oil. Let it separate for 2 hours. The lower layer containing glycerol (29,79 g) was separated. The upper organic layer (98,35 g) containing fatty acid methyl esters (FAMES) are diluted with hexane (100 ml), washed with water (200 ml) to neutral pH, dried over anhydrous sodium sulfate and concentrated. 95 g of oil containing FAMES were obtained. After crystallization at low temperatures from acetone, filtration and concentration 65 g oil containing fatty acid methyl esters there were obtained.

Two step acid-base catalyzed transesterification:para-toluenesulfonic acid-sodium hydroxide (FAME 3)

A mixture of 140 ml (120.9 g) refined ostrich oil and 60 ml methanol abs. was heated with stirring at 60°C, then added the catalyst-para-toluenesulfonic acid monohydrat (1,209 g) (1% of the oil). The mixture was heated under stirring for 3 hours at 65°C. Next, the mixture is cooled to 50°C. Then, sodium hydroxide methanolic solution (1% of the oil) was added and the mixture is heated, under stirring at the same temperature for 3 hours. End of transesterification was revealed by thin layer chromatography, when it noticed missing spot corresponding raw material–ostrich oil refined. Let it separate for 2 hours. The lower layer containing glycerol (51.9 g) was separated. The upper organic layer (117,3 g) containing methyl esters of fatty acids are diluted with hexane (150 ml), washed with water (200 ml) to neutral pH, dried over anhydrous sodium sulfate and concentrated. 118 g of oil containing FAMES were obtained. After crystallization from acetone at low temperatures, filtration and concentration 72,5 g of oil containing fatty acid methyl esters there were obtained.

Two step acid-base catalyzed transesterification: para-toluenesulfonic acid-sodium methoxide (FAME 4)

A mixture of 140 ml (120,9 g,) refined ostrich oil and 40 ml methanol abs. was heated with stirring at 60°C, then added the catalyst-*para*-toluenesulfonic acid monohydrate (1% of oil). The mixture was heated under stirring for 3 hours at 65°C. Next, the mixture is cooled to 50°C. Then, sodium methoxide-methanolic solution (1% of the oil) was added and the mixture is heated, under stirring at the same temperature for 2 hours. End of transesterification was revealed by thin layer chromatography, when it noticed missing spot corresponding raw material-refined ostrich oil. Let it separate for 2 hours. The lower layer containing glycerol (50 g) was separated. The upper organic layer (115 g) containing methyl esters of fatty acids are diluted with hexane (100 ml), washed with water (200 ml) to neutral pH, dried over anhydrous sodium sulfate and concentrated. 95 g of oil containing methyl esters of fatty acids were obtained. After crystallization at low temperatures from acetone, filtration and concentration 70 g oil containing fatty acid methyl esters there were obtained.

Results and discussions

Experimental studies for conversion of waste (fat ostrich) in product with high added value are presented.

Refined ostrich oil was obtained in our laboratories, by purification of crude oil through neutralization, washing, drying and fading. The crude oil was obtained by wet melting of fat from the back and belly obtained from Suraki farmed ostriches. (Rasuceni, Giurgiu district)

Characteristics of the refined ostrich oil are: semisolid mass, unctuous (20°C), pale yellow color, odorless, relative density: 0.8760 to 0.9280 (40°C), free fatty acids: 0,14-2 mg KOH 1 g⁻¹ oil, peroxide value, 1-2,85 mE kg⁻¹, moisture content 0,02-0,1%, iodine value 62-72 g I 100 g⁻¹ oil, saponification value 185-205 mg KOH 1 g⁻¹ oil.

A number of experimental studies were done: two step acid-base catalyzed transesterification of refined ostrich oil under optimum conditions of methanol to oil ratio 1:2,33 (v/v), temperature 60°C and 50°C for acid and base transesterification respectively, and catalyst concentration of 1 % (w/w) for acid (sulfuric acid or *para*-toluenesulfonic acid) and 1 % (w/w) for base (sodium hydroxide or sodium methoxide). Progress of the transesterification was followed by TLG plates on Sigma-Aldrich, eluted with the solvent system : n hexane:ethyl acetate:acetic acid:90:10:1(v:v:v).

The spots were visualised by exposing the plates to iodine vapor.

Transesterification proceeds in two steps:

1) acid pretreatment step

The mixture of methyl alcohol and refined ostrich oil in the proportion of 3:7 (v/v) was introduced into the reaction vessel and heated to 60°C with stirring, then added the catalyst:

a) a mixture of conc. sulfuric acid (1 % w/w) with methanol (30 % v/v) (who was separately heated at the same temperature); the mixture was heated for 3 or 4 hours at 65°C;

b) *para*toluenesulfonic acid (1 % w/w); the mixture was heated for 3 or 4 hours at 65°C.

The evolution of reaction was monitored by thin layer chromatography. ($R_{f\text{ oil}} = 0,519$, $R_{f\text{ FAME}} = 0,649$). After 3 or 4 hours approximately 20 % of refined oil was transformed into fatty acid methyl esters. It was observed, also, the disappearance of the corresponding spots of the free fatty acids, mono and diglycerides. ($R_f = 0,299$; 0,260; 0,182). After acid transesterification step, the mixture was cooled to 50°C.

2) *base catalysed transesterification*

a) Over the mixture obtained from the first step, heated to 50°C, was added sodium hydroxide (1% of the oil) methanolic solution, and was heated under stirring at the same temperature for 1.5-3 hours.

b) Over the mixture obtained from the first step, heated to 50°C, was added sodium methoxide (1% of the oil) methanolic solution, and was heated under stirring at the same temperature for 1-2 hours.

End of transesterification reaction was determined by thin layer chromatography. ($R_{FAME} = 0,649$).

Conditions for preparation and the yield of fatty acid methyl esters are presented in Table 1.

Table 1 Condition for fatty acid methyl esters preparation from refined ostrich oil

| Samples | The first step pre-treatment with acid, | Two-step transesterification in alkaline catalysis. | yield of FAME (%) based on the starting oil |
|---------|---|--|---|
| FAME 1 | catalyst-sulfuric acid 1% temperature- 60°C time – 3 h | catalyst-sodium hydroxide 1% temperature- 50°C time – 2 h | 98 |
| FAME 2 | catalyst-sulfuric acid 1% temperature- 60°C time – 4 h | catalyst- sodium methoxide 1% temperature- 50°C time – 1 h | 97 |
| FAME 3 | catalyst- <i>para</i> -toluenesulfonic 1% temperature- 60°C time – 3 h | catalyst- sodium hydroxide 1% temperature- 50°C time – 2 h | 90 |
| FAME 4 | catalyst- <i>para</i> -toluenesulfonic 1% temperature- 60°C time – 4 h | catalyst- sodium methoxide 1% temperature- 50°C time – 1 h | 85 |

The characteristics of the product "fatty acid methyl esters" are: oily liquid appearance, colorless to pale yellow, characteristic odor, relative density: 0.8400 to 0.8931 g cm⁻³ (20°C); refraction index: 1.4041 to 1.4951 (20°C).

Determination of fatty acids in samples of refined ostrich oil and of fatty acid methyl esters (FAMES) is performed by gas chromatography, in accordance with EN ISO 15304, using a capillary column with a stationary phase with high polarity, HP88-(88% cyanopropyl) aryl polysiloxane. Gas chromatography is performed with a gas chromatograph-689N Agilent detector equipped with FID (flame ionization) and autosampler 7683B. (Table 2) (Fig.1-Fig. 4)

Table 2 Composition of ostrich oils and of FAMES (%)

| | Oil 1 | FAME 1 | Oil 2 | FAME 2 | Oil 3 | FAME 3 | FAME 4 |
|--|--------------|---------------|--------------|---------------|--------------|---------------|---------------|
| C12:0 | 0,04 | 0,04 | 0,05 | 0,05 | 0,06 | 0,05 | 0,06 |
| C14:0 | 0,66 | 0,64 | 0,81 | 0,79 | 0,90 | 0,86 | 0,88 |
| C16:0 | 27,49 | 27,37 | 30,32 | 26,62 | 31,11 | 30,80 | 30,76 |
| C16:1c | 7,06 | 6,96 | 10,49 | 10,51 | 9,18 | 8,97 | 9,03 |
| C18:0 | 5,88 | 5,95 | 5,07 | 4,99 | 5,53 | 5,65 | 5,55 |
| C18:1t | 0,26 | 0,28 | 0,35 | 0,39 | 0,35 | - | - |
| C18:1c | 35,31 | 35,53 | 36,64 | 37,29 | 33,76 | 34,64 | 34,30 |
| C18:2c | 18,10 | 18,00 | 11,12 | 11,26 | 13,11 | 13,07 | 13,09 |
| C20:0 | 0,05 | 0,05 | 0,04 | 0,03 | 0,04 | - | - |
| C18:3α | 1,41 | 1,39 | 0,89 | 0,89 | 1,59 | 1,55 | 1,56 |
| C18:3γ | 0,055 | 0,05 | 0,06 | 0,06 | 0,05 | 0,05 | 0,05 |
| C20:1c | 0,24 | 0,25 | 0,24 | 0,24 | 0,23 | 0,25 | 0,25 |
| C20:4 | 0,23 | 0,22 | 0,15 | 0,15 | 0,15 | 0,15 | 0,15 |
| Σ C18:1 trans | 0,26 | 0,28 | 0,35 | 0,39 | 0,35 | - | - |
| Σ C18:2 trans | 0,17 | 0,09 | 0,23 | 0,24 | 0,26 | 0,29 | 0,62 |
| Σ C18:3 trans | 0,1 | 0,1 | 0,16 | 0,15 | 0,13 | 0,12 | 0,12 |
| Σ FA trans | 0,53 | 0,47 | 0,73 | 0,78 | 0,74 | 0,41 | 0,75 |
| Σ n-3 FA | 1,41 | 1,39 | 0,89 | 0,89 | 1,59 | 1,55 | 1,56 |
| Σ n-6 FA | 18,39 | 18,27 | 11,33 | 11,41 | 13,31 | 13,27 | 13,29 |
| Σ n-9 FA | 35,55 | 35,78 | 36,98 | 37,53 | 33,39 | 34,89 | 34,55 |

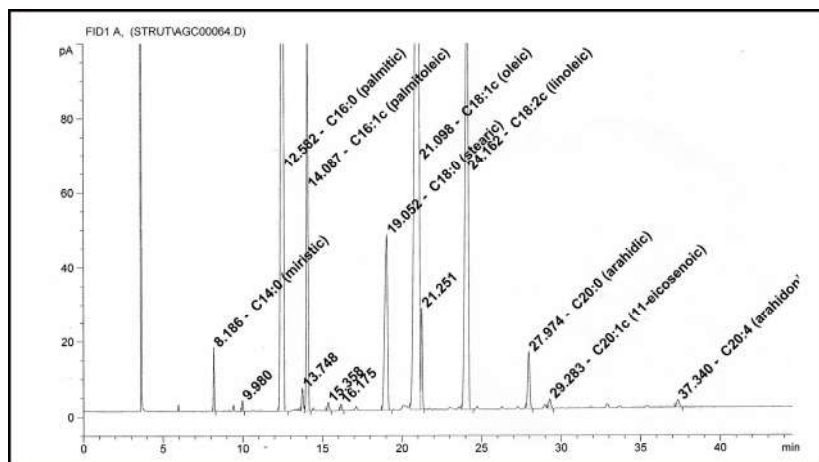


Fig.1. GC analysis of the product of transesterification of refined ostrich oil in two step acid-base catalyzed transesterification (*sulfuric acid-sodium hydroxide*)-FAME 1

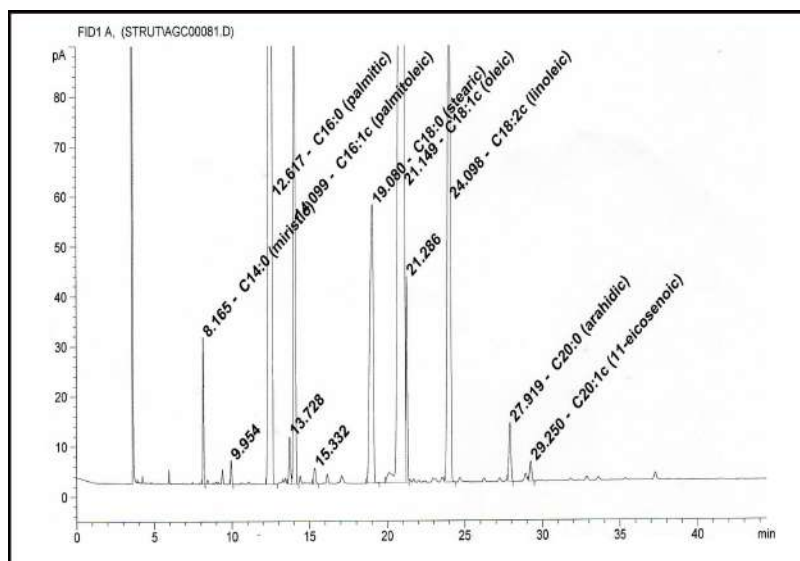


Fig. 2. GC analysis of the product of transesterification of refined ostrich oil in two step acid-base catalyzed transesterification (*sulfuric acid-sodium methoxide*)-FAME 2

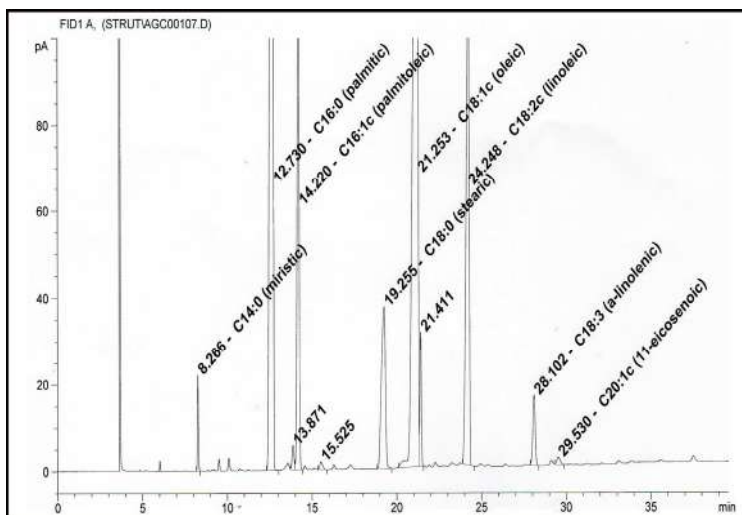


Fig. 3. GC analysis of the product of transesterification of refined ostrich oil in two step acid-base catalyzed transesterification (*para*-toluenesulfonic acid monohydrat -sodium hydroxide)- FAME 3

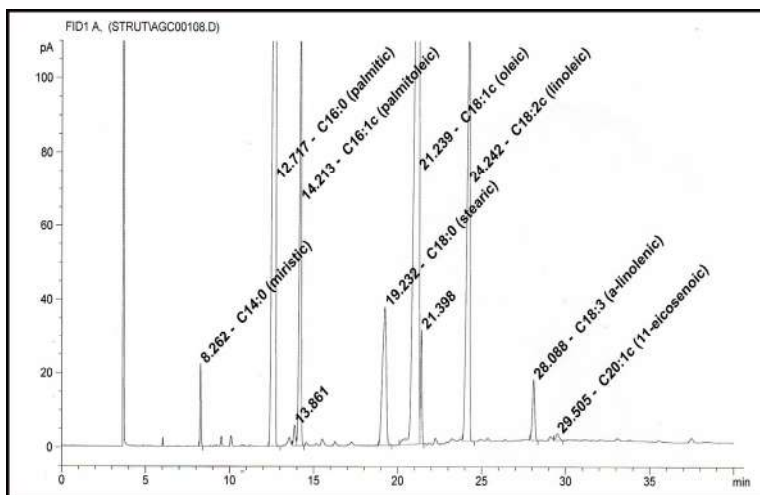


Fig. 4. GC analysis of the product of transesterification of refined ostrich oil in two step acid-base catalyzed transesterification (*para*-toluenesulfonic acid monohydrat- sodium methoxide)-FAME 4

Conclusions

Studies regarding the conversion of waste (fat ostrich) in high added value product were carried out in order to preparation fatty acid methyl esters. The conversion of the crude product in the fatty acid esters was performed by applying a chemical transesterification.

Fatty acid methyl esters were obtained by two step acid-base catalyzed transesterification:

- the first step, pretreatment with acid, when takes place the esterification of the free acids from the refined ostrich oil;
- two-step transesterification in alkaline catalysis.

The advantages of the transesterification in two stage are : the yield of methyl esters of fatty acids obtained is 80-90% and this process can be used for oils having a free acids value greater than 1%. Transesterification was carried out in two steps in acid-base catalysis, without isolating the product obtained in the first step-acid pretreatment of the refined ostrich oil. The second stage of the transesterification was carried out in the same reaction vessel, by adding the basic catalyst to the reaction mixture resulting from the first stage of the transesterification. It was observed that the presence of free fatty acids in refined oil, has not led to the formation of soap, as it happens in basic catalyst.

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