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Research/Technical Note

Esterification Between Citric acid and Pumpkin Pips' Organic Molecules – Esters Hydrolysis And Esters Used as Hydrocarbons Additives

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Abstract: Commonly, organicians used various technical like pressurized extraction, solvent extraction to extract essential oils and to isolate thereafter organics molecules in a vegetable organism. This new method consisted in extracting selectively and directly the organic molecules present in a vegetable organism, in this case the organic molecules present in pumpkin pips by esterification with citric acid molecules which were not only esterified by the alcohols and amines functions of the organic molecules present in the vegetable organism but also they esterified, by its alcohol functions, the acid function of the organic molecules present in the same vegetable organism. Operating conditions and procedure were taken into account and these allow us to reach an esterification rate equals to 72.80% at the most. Thus, esters of citric acid and esters of organic molecules' acid present in the pumpkin pips in quantities but also with quality were obtained. The presence of xanthophyll esters, riboflavin esters, citric acid esters, well quality fatty-acid esters and probably amides of citric acid molecules were certain. That led us to do auto-inflammation tests of hydrocarbons (Super-Gasoil-Fueloil) with esters of pumpkin pips' organic molecules as additives. Finally, trying to separate the organic molecules having been esterified or esterifying with the citric acid molecules, hydrolysis reaction tests were carried out with a regeneration rate of citric acid molecules equal to 36.50% compared with the initial citric acid molecules quantities and 50.14% compared with the total ester quantity of the sample. The extraction of pumpkin pips' organic molecules like xanthophyll, riboflavin, well quality fatty-acids and probably other interesting organic molecules according to the bibliography were certain.

Keywords: Citric Acid, Pumpkin Pips, Esterification, Hydrolysis, Xanthophyll, Riboflavin, Fatty-Acids, Auto-Inflammation Test

1. Introduction

The first step of tests was the esterification reaction which consisted not only to esterify the acid function of the citric acid molecules by pumpkin pips' organic molecules but also to esterify the acid functions of the organic molecules by the citric acid molecules' alcohol functions. Thus, esters with citric acid molecules of pumpkin pips' organic molecules were obtained. The quantification of formed esters was made by measuring out with 0.0504N NaOH solution of the rest of citric acid molecules' acid functions not having been esterified. Two measuring out procedures were established: the first was direct which consisted in measuring out of the totality of the solution obtained after esterification followed by decantation; the second was indirect by measuring out separately the three phases obtained after esterification followed by decantation. The results were almost equal. Then, the organoleptic and refractometry characterization followed by bibliographical study of the esters solution obtained allowed us to think that it would be interesting to use it like additive of hydrocarbons used usually (super, gasoil and fueloil). For that, it was

initially necessary to remove the water molecules present in the esters solution. Thus, a procedure of water molecules removal by using gradually adequate organic solvents dichloromethane, acetone and hexane was elaborate. Only after, the auto-inflammation tests of blank hydrocarbons and mixes hydrocarbons- esters with citric acid molecules of pumpkin pips' organic molecules were carried out. Lastly, hydrolysis procedures of the three phases of esters with citric acid molecules were elaborate in order to recover the polar and non-polar organic molecules effectively. Then, these organic molecules were characterized by basic usual chemical and physical methods like refractometry, acid value, saponification value and ester value. The measuring out the olefins quantity in the extract non-polar molecules (fatty-acid) by hydrofluoric acid solution 2.6E-3[mol.l^{-1}] has been done. The determination of the hydrolysis rate was done by direct measuring out of the three phases hydrolysed separately with 0.0504N NaOH solution. Thus, we obtained the regeneration rates of citric acid molecules in each phase compared with the initial citric acid molecules quantities and compared with the total ester quantity of the sample.

2. Citric Acid and Pumpkin Pips General Points

2.1. Citric acid

2.1.1. Citric Acid Acidity

Citric acid $C_6H_80_7$ is a tricarboxylic acid α- hydrolyzed. It contains three acids with pKa such as $pKa_1 = 3.14$, $pKa_2 = 4.77$ and $pKa_3 = 6.39$ and a α-alcohol function with pKa = 14.4 [1, 2, 3] (Figure 1). By its reactivity, the citric acid was the object of several studies and was used in several fields like the cosmetics, the food one, the chemistry and others [4, 5]. Noticed that the acid form is AH with pKa (AH). It was shown that if the pH ≤ [pKa (AH) – 2], the quantity of basic A^- associated to the acid/base couple AH/A^- is negligible in comparison with the AH quantity. And if the pH ≥ [pKa (AH) + 2], the quantity of acid AH associated to the acid/base couple AH/A^- is negligible in comparison with the A^- quantity [6]. For [pKa (AH) – 2] ≤ pH ≤ [pKa (AH) + 2], the basic A^- and the acid AH forms coexist but if [pKa (AH) – 2] ≤ pH ≤ pKa (AH) the acid form AH dominate and if pKa (AH) ≤ pH ≤ [pKa (AH) + 2] the basic form A^- dominate [6]. Consequently, for the citric acid, the acids and basics forms according to the pKa and pH were showed in the following Table 1:

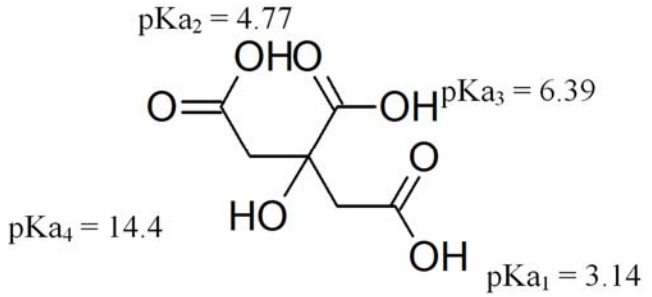


Figure 1. *3-hydroxybutane-1, 2, 4-tricarboxylic acid (Citric Acid).*

Table 1. *Dominant Forms of "Citric Acid" According to the pH.*

pH	Acid/base couple	pKa	Acid/Base reactions	Dominant forms	Dominant molecule/Ions
pH ≤ 3.14	AH_3/AH_2^-	3.14	$AH_3 \rightleftharpoons AH_2^- + H^+$	AH_3	Citric Acid
3.14 ≤ pH ≤ 4.77	AH_2^-/AH^{2-}	4.77	$AH_2^- \rightleftharpoons AH^{2-} + H^+$	AH_2^-	Di-Hydrogenocitrate
4.77 ≤ pH ≤ 6.39	AH^{2-}/A^{3-}	6.39	$AH^{2-} \rightleftharpoons A^{3-} + H^+$	AH^{2-}	Mono-Hydrogenocitrate
6.39 ≤ pH	AH^{2-}/A^{3-}	6.39	$AH^{2-} \rightleftharpoons A^{3-} + H^+$	A^{3-}	Citrate

2.1.2. Citric Acid Physico-Chemical Characteristics

Citric acid is solid with monoclinic as crystal structure, white, odorless and excessively sour flavor (Table 2) [3]. Citric acid exists in hydrates forms, the monohydrate melts towards 343.15 °K and the anhydrous state melting point is 426.15°K. Citric acid is soluble in alcohol, ether, ethyl acetate and DMSO and insoluble in C_6H_6, $CHCl_3$, CS_2, and toluene. Its solubility in ethanol at 298.15°K is 62g/100g. Citric acid is very soluble in water and its solubility increases with the temperature as shown the following table (Table 3) [7].

Table 2. *Citric acid physicochemical properties.*

Physicochemical Properties	CITRIC ACID - $C_6H_80_7$
Appearance	Crystalline white solid
Crystal structure	Monoclinic
Molar mass	192.12 [g.mol^{-1}]
Density	1.665 [g.cm^{-3}] anhydrous 1.542[g.cm^{-3}]monohydrate at 291.15°K
Melting point	426.15°K anhydrous 343.15°K monohydrate
Boiling point	448.15°K
Solubility in ethanol	62g/100g
Solubility in water	59.20% at 293.15°K (Table 3)

Table 3. *Evolution of the citric acid solubility in water (w/w) following to the temperature (°K).*

T°K	283.15	293.15	303.15	313.15	323.15	333.15	343.15	353.15	363.15	373.15
Solubility (% g/100mg)	54.0	59.2	64.3	68.6	70.9	73.5	76.2	78.8	81.4	84.0

2.2. Pumpkin Pips

2.2.1. Pumpkin Pips General Points

The pumpkin pips are white pips washed by distilled water removed from pumpkin. Pumpkin pips were wrapped by a transparent very fine layer [8]. Drawing their origins in Central America, pumpkin are consumed since nearly 10,000 years ago [9]. The two main types are "pepo", which scientists dubbed so-called "small" pumpkins and are often carved into Jack-o-lanterns in the fall, and "maxima", the giant variety grown to enter the "biggest pumpkin" contest at the county fair [10]. The pumpkin pips oil extraction was made by the Austrians since 1735. The pumpkin pips were taken on pumpkins during the winter before being put to dry at low temperature then peeled and finally crushed. Specialty of the Styrie area, it starts to be spread in the world from 1970 [9].

2.2.2. Pumpkin Pip Components

Pumpkin pips are rich in proteins and contains many mineral salts (magnesium, iron, phosphorus, zinc, copper, potassium, calcium), vitamins (A, B1, B2), unsaturated fatty-acid like linoleic acid, alpha-linoleic acid, oleic acid and also saturated fatty-acid like palmitic acid, stearic acid [11-13]. HPLC analysis of the powerful pigments reveals the presence of carotenoids that the main components was said to be lutein and betacarotene [12]. The following table 4 showed the principal components rates [8]. Seeing that pumpkin grains were extracted from pumpkin, it would be interesting to know its components. Pumpkin provide lots of vitamin C, riboflavin, potassium, copper and manganese. Smaller but significant amounts of vitamin E (alpha tocopherol), thiamin, niacin, vitamin B6, folate, iron, magnesium and phosphorus [10]. Also flavonoids such as cryptoxanthine, lutein [10, 12], zeaxanthin and amino acid tryptophan [12].

Table 4. *Pumpkin pips components weight per 100g.*

Pumpkin pip components	Weight per 100[g]
Gluicide	1.29 g
Starch	0.74 g
Glucose	1.29 g
Fibre	6.5 g
Protein	29.84 g
Lipid	49.05 g
Pumpkin pip components	Weight per 100[g]
Water	2.03 g
Ash Total	4.37 g
Calcium	52 mg
Iron	8.07 mg
Magnesium	550 mg
Manganese	4.49 mg
Phosphorous	1174 mg
Potassium	788 mg
Selenium	0.0094 mg
Sodium	256 mg
Zinc	7.64 mg
Vitamin B6	0.1 mg
Vitamin C	6.5 mg
Vitamin E	0.65 mg
Vitamin K	0.0045 mg

3. Pumpkin Pips Esterification with Citric Acid Molecules

3.1. Esterification Reaction General Points (with Citric Acid)

Fischer-Speier esterification also called carboxylic acids esterification is a chemical reaction between an alcohol function and a carboxylic acid function catalyzed by the H^+ ions coming from acids AH/A^- [6] such as

$$AH \rightleftharpoons A^- + H^+$$

This reaction is accompanied with water molecule formation (Figure 2) according the general equation [14]

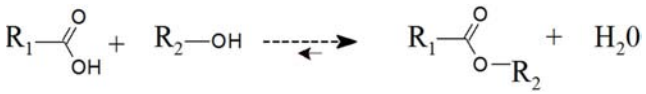


Figure 2. *Esterification reaction general equation.*

Thus, tricarboxylic acids of citric acid could be esterified with three alcohol functions of organic molecules and organic molecules carboxylic acids could be esterified by the alcohol function of citric acid of if the solution pH is respected [6] according to the general equations (Figure 3 – Figure 4):

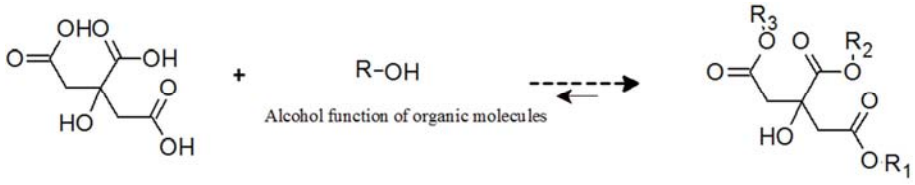


Figure 3. *Tricarboxylic acids of citric acid molecule with three alcohol functions of organic molecules.*

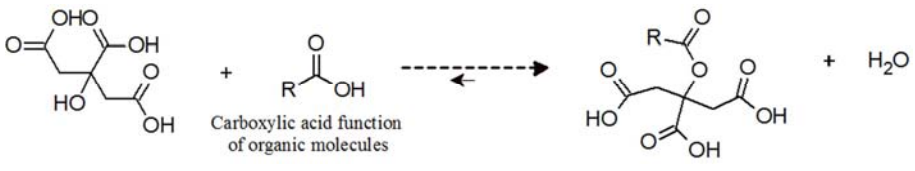


Figure 4. *Carboxylic acid of organic molecule esterified by the citric acid alcohol function.*

However, seeing that pumpkin pips contained amine functions [8] of amino-acids components obtained by hydrolyzed proteins [15], it is possible the amide formation reaction between carboxylic acid function and amine function catalyzed by H^+ ions coming from acids AH/A^-. This reaction is accompanied with water molecule formation according to the general equations (Figure 5 – Figure 6) [16]:

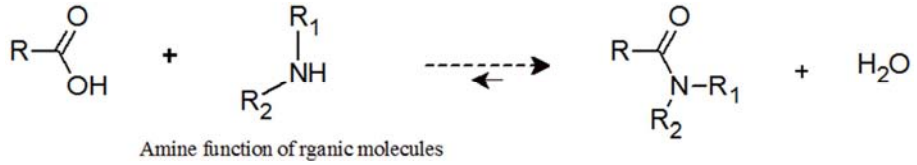


Figure 5. *Amide formation by amine function and carboxylic acid.*

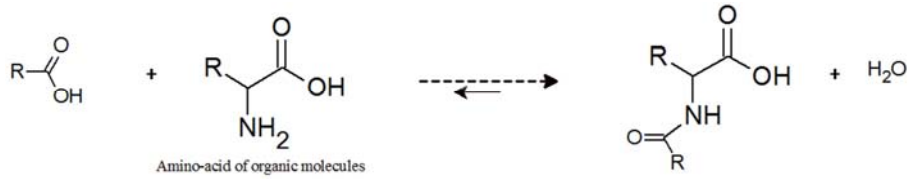


Figure 6. *Amide formation by amino-acid and carboxylic acid.*

Thus, tricarboxylic acids of citric acid could be in reaction with three amino-acids or amines functions of organic molecules if the solution pH is respected [6] to obtain amide molecules according to the general equations (Figure 7 – Figure 8):

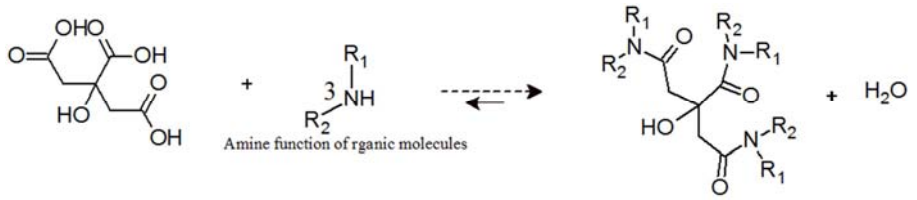


Figure 7. *Amide formation by amine function and tricarboxylic acids of citric acid molecule.*

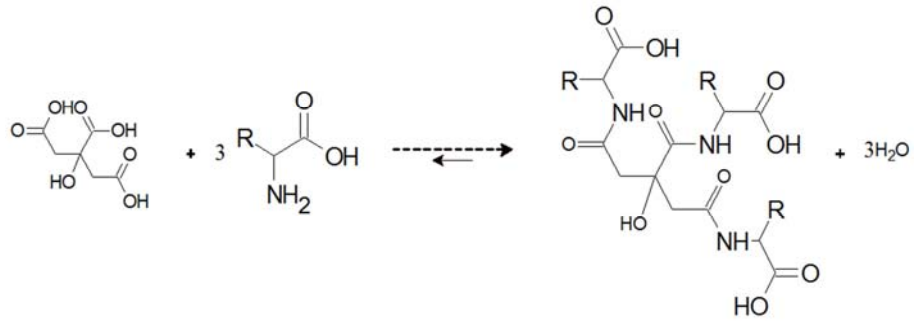


Figure 8. *Amide formation by amino-acid and tricarboxylic acids of citric acid molecules.*

3.2. Citric Acid Molecules Esterification with Pumpkin Pips Molecules Procedure

The Reactional environment pH was estimated by the calculation to determine the ideal and optimal operating conditions for the esterification reactions with tricarboxylic acid of citric acid molecules or esterification of organic molecules carboxylic acid by citric acid alcohol function as seen in the previous paragraph (§ 3.1). Seeing that citric acid is a tri-acid with an alcohol function (Table 1), the pH solution and the estimated pH in the vicinity (V) of each weak acids (Table 5) have been calculated according to the distilled water volume and the weight of citric acid used.

Table 5. *pH solution according to the distilled water volume and the weight of citric acid used.*

m_{Ac}/Volume eau [l]	Calculated pH	pH in V(pKa_1)	pH in V(pKa_2)	pH in V(pKa_3)
5,1[g]/0,810	2.24	2.31	3.13	3.94
5[g]/1,524	2.38	2.45	3.26	4.07
3[g]/1,524	2.5	2.57	3.385	4.195

Equipments, accessories and chemical products used for the esterification of pumpkin pips organic molecules (§ 3.1), the extractions and the separations of obtained molecules thus their characterizations were:

Balloon (2L) – Separatory funnel (2L) – Liebig condenser – Heat balloon – Balance - Magnetic stirrer – Refractometer – Thermometer – Dichloromethane – Hexane – rotary evaporator - NaOH 99% - NaOH 0.05N – CuS04 – Iced cube – pH paper.

Took, wash and weight the pumpkin pips to be esterified. Then, prepare the citric acid solution used for the esterification in the balloon (2L) put the weighted pumpkin pips in. Complete the extraction assembly and finally heat the balloon at the reaction temperature ranging between 130°C and 145°C. After 1H30mn to 2H, stop the heat balloon without stopping the Liebig condenser water refrigeration to reduce the temperature and eliminate the light gas leak risk. Then as soon as possible change the heat balloon to a beaker containing iced cube not only to stop the reactions but also to stop the opposite reaction which is the hydrolysis of the obtained esters. When the balloon temperature was carried out to room temperature, stop the Liebig condenser water refrigeration and finally a homogeneous liquid of persistent slightly yellow color (Figure 9) was obtained in the balloon.

Thus, two esterification extractions were done according to the following characteristics showed in the table 6.

Table 6. *Citric acid esterification extraction of pumpkin pips organic molecules characteristics.*

REACTIONS	DURATION [mn]	m_{Ac} m[g]	$M_{grains\ courges}$ [g]	Pumpkin pips Number	Veau [l]	pH calculated	pH in V(pKa$_1$)	pH in V(pKa$_2$)	pH in V(pKa$_3$)	pH tournesol paper
REACTION 1	90	5.1000	27.9000	≈ 123	0.810	2.24	2.31	3.13	3.94	-
REACTION 2	120	5.0932	85.4287	376	1.524	2.38	2.45	3.26	4.07	3 à 4

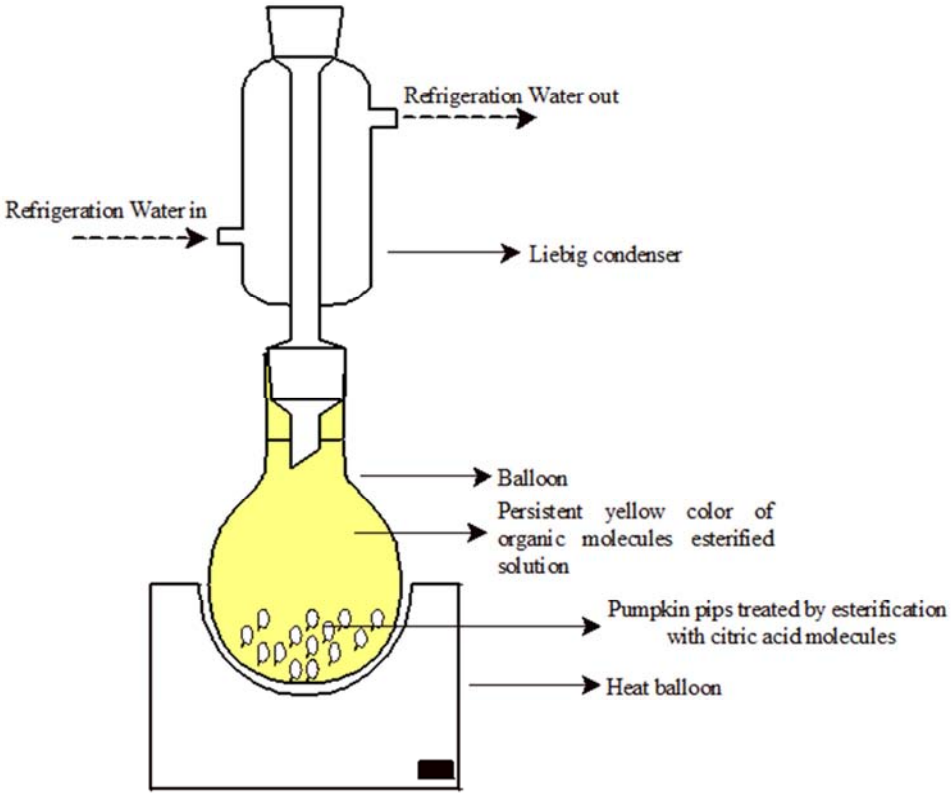


Figure 9. *Pumpkins pips organic molecules extraction by citric acid esterification assembly.*

3.3. Exhaustive Inventory of Pumpkin Pip Organic Molecules Group Probably Esterifying and Esterified Citric Acid Molecules Based on Avocado Stone and Grape Pip Organic Molecules Quoted in Bibliography

According to the bibliography [17,18] which correspond to the organic molecules discovered in pip of avocado and grapes, the organic molecules group probably esterifying and esterified citric acid molecules (§ 3.1) showed in the following table.

Table 7. *Probably* ***p****umpkin pips organic molecules group could esterify and could be esterified respectively the tricarboxylic acid and by the alcohol function of citric acid molecules.*

Organic molecules group **with alcohol function which could esterifying the tricarboxylic acid of citric acid molecules (§ 3.1)**	**Organic molecules with carboxylic acid function could be esterified by the alcohol of citric acid molecules (§ 3.1)**
Alcohols like hexanol (17) *Acyclic oxygenated monoterpenes alcohol* like linalol, geraniol, nerol, citronnellol, …(17) *Cyclic oxygenated monoterpenes alcohol* like myrtenol, terpineol, …(17) *Acyclic oxygenated diterpenes alcohol* like phytol, …(17) *Phenolic compound* like flavonones (apigenine), flavonols (quercetine), flavanones (hesperetine), hydroxyflavanones (dihydroquercetine) (17) *Hydrolysable flavonoids* (17) *Non hydrolysable flavonoids* (17) *Cyclic oxygenated sesquiterpenes alcohol* like elemol, eudesmol, cadinol, viridiflorol, …(17) *Acyclic oxygenated diterpenes alcohol* like phytol, …(17) *Saponins* (17)	*Saturated fatty-acids* like palmitic acid, stearic acid, …(18) *Unsaturated fatty-acids* like oleic acid, linoleic acid (18) *Alkaloid acid* derived from L-tryptophane (17) *True alkaloid acid derived from amino-acids* like *L*-phenylalanine et le L-tryptophane *presenting at least one heterocycle* (17) *Saponins* (17)

3.4. Measuring Out of Citric Acid Molecules non-Esterified

3.4.1. The Rest of Citric Acid Molecules Extraction Procedure

Before the measuring out of citric acid molecules non-esterified, it's necessary to extract them from the final solution. Thus, transvase the esterified organic molecules in the separatory funnel and added dichloromethane solvent which extracted the rest of citric acid molecules [19] followed by soft agitation of the balloon. Let allow to settle for a few minutes. Then, take and measure each volume of phase and refrigerate each phase recently extracted in three different containers. The aqueous Phase 2 was treated by non-polar solvent hexane with softly agitation. Thus, half of hexane solution with its soluble organic molecules was distilled in a rotary evaporator. Finally, Biuret test and refractive index test were carried out with aqueous Phase 2 (Hexane) and Phase 1 (Dichloromethane). Their study would be treated in the following paragraph.

3.4.2. Results and Discussions

(i). Three Distinct Homogeneous Phases After Dichloromethane Solvent Treatment

Three distinct homogeneous phases were obtained: The lowest phase (Phase 1) which contains the rest of citric acid molecules non reactant soluble in dichloromethane solvent [19]. On the top, there was the aqueous phase (Phase 2) with water which kept the persistent slightly yellow color. In the middle of Phase 1 and Phase 2, there was the third phase (Phase 3) characterized by fine layers of initially white-yellow strips more or less soluble in the aqueous phase (Phase 2) but attracted progressively in the vicinity of the dichloromethane polar solvent. These results confirmed the esterification of pumpkin pips organic molecules, a part was water soluble ester (in Phase 2) obtained not only by esterification of the one carboxylic citric acid with pKa1 (3.14) with alcohol function of organic molecules extracted (Figure 4 - Table 7 – [18]) but also by esterification of pumpkin pips fatty-acid by the citric acid molecules alcohol function (Figure 3). Contrary to these, the Phase 3 esters were obtained with not only esterification of the organic molecules by fatty-acid of citric acid molecules but also the esterification of at least the two carboxylic acids (pKa1=3.14, pKa2=4.77) belonging to the same citric acid molecules which was, consequently, less soluble in water but more attracted by the polar solvent dichloromethane (Figure 4 – Figure 3). This doesn't exclude the esterification of the carboxylic acid with pKa3=6.39 (Figure 4 – [6]).

Figure 10. *Dichloromethane solvent treatment: three homogeneous phase Phase 1 – Phase 2 – Phase 3.*

The aqueous Phase 2 was treated by non-polar solvent hexane with softly agitation. After a few minutes of decantation, there were the organic phase (in top) composed by hexane and organic molecules soluble in this solvent like citric acid carboxylic acids (only pKa1 = 3.14 or all pKa) esterified by fatty-acid and the rest were intact. Then, the aqueous phase (in bottom) preserved its slightly yellow color (Figure 11).

Figure 11. *First treatment of Phase 2 with hexane solvent.*

After some hexane treatments, the aqueous yellow color was clarified but preserved at heart its slightly yellow color. Nevertheless, a fine yellow layer appeared on the upper surface of hexane (Figure 12).

Figure 12. *Fine yellow layer above the solvent after some hexane solvent treatment.*

According to the bibliographies, only two natural organic molecules are potentially responsible for yellow naturalness: riboflavin (Figure 13) and xanthophyll (Figure 14). Riboflavin is the only water-soluble yellow vitamin and its color is responsible for the yellow dyeing of solid preparations or aqueous solution of vitamin B.

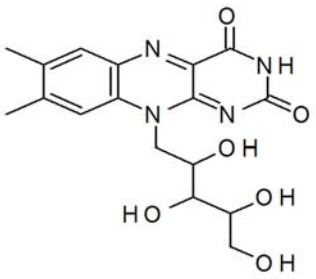


Figure 13. *Riboflavin.*

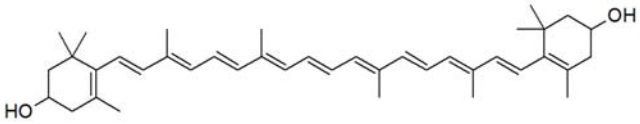


Figure 14. *Xanthophyll.*

Let's noted that both riboflavin and xanthophyll molecules contained more than two alcohol functions. Thus, they were organic molecules which esterify citric acid carboxylic acids (Figure 3). According to the structure of these molecules responsible of yellow natural pigments, yellow molecules above hexane were xanthophyll-citric acid esters (Figure 15) whereas the water-soluble yellow molecules were riboflavin-citric acid esters (Figure 16).

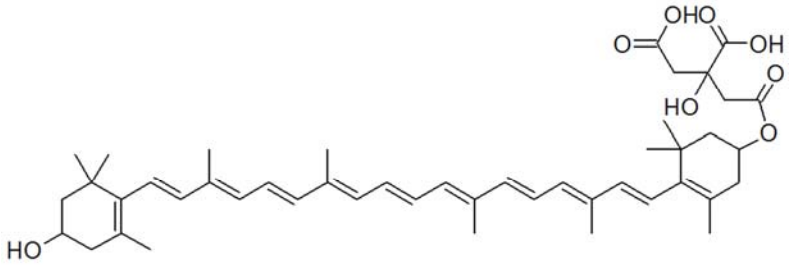


Figure 15. *Citric acid carboxylic acid (pKa1=3.14) esterified by pumpkin pips' xanthophyll.*

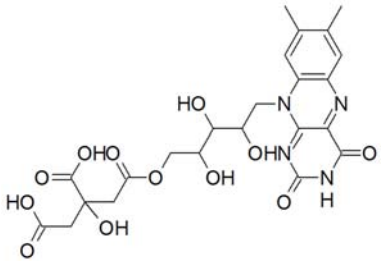


Figure 16. *Citric acid carboxylic acid (pKa1=3.14) esterified by pumpkin pips' riboflavin.*

Those didn't exclude the esterification of other organic molecules with citric acid molecules. Consequently, the difference three phases noticed after dichloromethane treatment (figure 10 - §3.4.2) could be explain by the reactivity and the participation of the three citric acid's carboxylic acids in esterification reactions (Figure 17) with organic molecules probably present in pumpkin pips (Table 7).

Thus, the Phase 1 which was soluble in dichloromethane was composed of not only the rest of citric acid molecules non-reactant but also citric acid's carboxylic acids (pKa1 and pKa2 and pKa3) molecules esterified by organic molecules (table 7 – Figure 3 – Figure 4 – Figure 5 – Figure 6 – Figure 7 – Figure 8). Whereas, the Phase 2 was composed of esterified soluble organic molecules, in the otherwise, it must be composed of citric acid's carboxylic acid (only pKa1) esterified by organic molecules (Table 7 – Figure 15 – Figure 16) or organic molecules' carboxylic acid esterified by the citric acid's alcohol function (Figure4). Finally, the Phase 3 (between the Phase 1 and Phase 2) must be composed of citric acid's carboxylic acid (pKa1 and pKa2) esterified by two different pumpkin pips' organic molecules such as the one was non-polar soluble in water and the other was polar soluble in dichloromethane as showed in the following figure (Figure14).

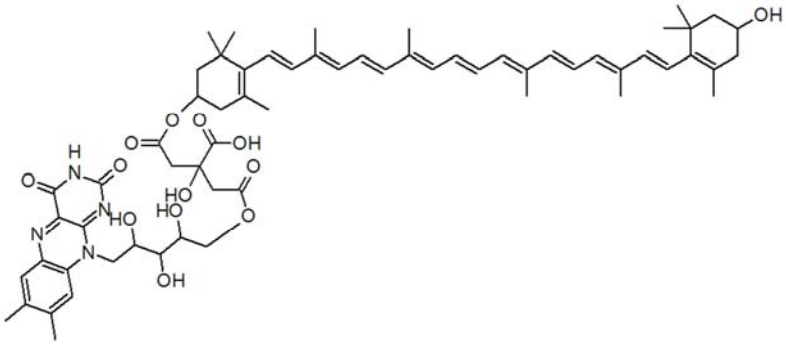


Figure 17. *Citric acids' carboxylic acid (pKa1 and pKa2) esterified by non-polar water soluble (xanthophyll) and polar dichloromethane soluble (riboflavin) forming a molecule located in phase 3.*

(ii). Phases Characterizations by Biuret Test and Refractive Index

To concentrate the organic phase obtained by treatment (of aqueous Phase 2 - §3.4.1), took it to be distilled on the rotary evaporator at 313.15[°K]. An organic solution which smelled not only the perfume characteristic of pumpkin but also the perfume of light gazes with alcohol function esterifying citric acid's carboxylic acids like hexanol (Table 7 – Figure 3) or directly light gazes obtained by fatty-acid cracking during the citric acid esterification [22] were obtained. The rate of Phase 1' organic molecules esterified or not soluble in hexane solvent was 20.80[%].

(ii). Biuret test of Aqueous Phase 2 and Its Organic Molecules Extracted by Hexane Solvent

Take 1[g] of NaOH (99%), 0.2[g] of $CuSO_4$ and 4.1[ml] of aqueous Phase 2 with persistent yellow color in a test tube. The solution color was initially blue and turn progressively to purple. Also, 2.2[ml] of organic molecules extracted by hexane solvent were tested. The solution color was directly purple. These results confirmed that not only the distillation and purification on the rotary evaporator was efficient but also the Phase 2 contained, certainly, nitrogenized compounds like organic molecules with amine function such as amino-acids, alkaloids, and maybe non-hydrolyzed proteins (Table 7) esterified by citric acid molecules (§3.1). Also, the presence of amides obtained by reactions between citric acid molecules and amine function (§3.1 – Figure 5 – Figure 6 – Figure 7 – Figure 8) weren't excluded. The presence of riboflavin molecule esterifying citric acids' carboxylic acid molecules was confirmed (Figure 15).

(ii). Refractive Indexes of Extracted Solutions

The refractive index of hexane Phase 2 extracted solution and dichloromethane extracted solution for the two reactions (Table 6) were shown in the following solution:

Table 8. *Refractive indexes of extracted solutions.*

REACTIONS/I_R	Hexane Phase 2 extracted solution	Dichloromethane Phase 1 extracted solution
REACTION 1	1.6465	1.6275
REACTION 2	1.6295	1.6299

In the following table 9 were presented the refractive indexes of probably organic molecules in the extracted solution according to the previous results and discussions.

Table 9. *Refractive indexes of probably organic molecules in the extracted solution according to the tests refractive indexes (table 8).*

Organic molecules	Categories	Precursors	Refractive indexes	Bibliographies
Riboflavin	Product		1.4278	sciences-physiques.ac-montpellier.fr/ABCDORGA/Famille/Produit/Medicam.htm [23]
Oleic acid	Product		1.4582	sciences-physiques.ac-montpellier.fr/ABCDORGA/Famille/Produit/acidolei.html [24]
Linoleic acid	Product		1.4758	sciences-physiques.ac-montpellier.fr/ABCDORGA/Famille/.../ACIDES%20GRAS.html [25]
Citric acid	Reagent	-	1.493 – 1.509	http://www.chemicalbook.com/ChemicalProductProperty_EN_CB9854361.htm [26]
Polyester	Similable product to polyester obtained by citric acid auto-esterification	Citric acid	1.567	(en) Marvin J. Weber et al. (préf. Marvin J. Weber), Handbook of Optical Materials, CRC Press, 24 September 2002, 536p. ISBN 0-8493-3512-4 et 978-0849335129 [27]
Phenylalanine	Amino-acid	-	1.58	https://www.scbt.com ›... › Inhibitors, Activators, Substrates › Protein › CPA [28]
Xanthophyll	Product	-	1.583	www.chemspider.com/Chemical-Structure.21111876.html [29]
Lycorine	True Alkaloids	Phenylalanine	1.733	www.molbase.com/en/476-28-8-moldata-1486454.html [30]

Organic molecules	Categories	Precursors	Refractive indexes	Bibliographies
L-Tryptophane (100%)	Amino-acid	-	1.84	https://www.researchgate.net/publication/259650812 [31]
Harmine	True Alkaloids	L-Tryptophane	1.647	www.chembk.com/en/chem/Harmine [32]
Psylocine	True Alkaloids	L-Tryptophane	1.646	www.chemspider.com/Chemical-Structure.4807.html [33]
Tryptamine	True Alkaloids	L-Tryptophane	1.668	www.chembk.com/en/chem/Tryptamine [34]
Serotonine	Amine	L-Tryptophane	1.71	www.chembk.com/en/chem/Serotonine [35]
Zolmitryptan	True Alkaloids	L-Tryptophane	1.619	www.chemnet.com › CAS [36]
Ajmalicine	True Alkaloids	L-Tryptophane	1.656	www.chemnet.com › CAS [37]
Tabersonine	True Alkaloids	L-Tryptophane	1.651	www.chembk.com/en/chem/Tabersonine [38]
Apigenin	Phenolic compound	-	1.732	www.chembk.com/en/chem/apigenin [39]
Quercetine	Phenolic compound	-	1.823	www.chemspider.com/Chemical-Structure.4444051.html [40]
Pentagalloylglucose Tanin	Phenolic compound	-	1.87	www.chembk.com/en/chem/pentagalloylglucose [41]

Comparing the table 8 and table 9 results, the presence of not only alkaloids and their corresponding amino-acid precursors but also phenolic compounds and amides as well as amines esterified or esterifying with citric acid molecules were checked. Indeed, bibliography said that pumpkins contained amino-acid tryptophan and flavonoids [10] such as lutein [10–11] and zeaxanthin [10]. The low value of tested (reactions) refractive indexes compared to those in table 9 of bibliography could be explained by the non-negligible presence of citric acid polyester formed by its auto-esterification. Indeed, the bibliography affirmed that the presence and the increase in mass proportion of polyethylene in a L-tryptophan solution decreased the value of the refractive index of solution up to 23[%] [42].

3.4.3. The Rest of Citric Acid Molecules Non-Esterified Measuring Out

(i). Direct Measuring Out Procedure

After carrying out the esterification of citric acid molecules with Pumpkin pips' molecules according to the procedure described in paragraph 3.2 (§3.2), agitate the solution in the balloon a few seconds to be homogeneous, take 5[ml] of this solution in a beaker. Dilute this sample with 15[ml] of distilled water, then add two or three drops of helianthine. The solution turn immediately to red. Place the 0.0504N NaOH solution in an oilcan and the beaker on the magnetic stirrer. The measuring out can begin by falling into the beaker drip the oilcan 0.0504N NaOH solution and mixing the beaker solution with the magnetic stirrer. When the beaker solution turn to orange yellow, closed the oilcan and record the equivalent 0.0504N NaOH volume which correspond to the equivalent point. Then, calculate the rest of non-reactant citric acid molecules as shown in the following (Table 10) the results for the reaction 2 (Table 6).

Table 10. *The rest of citric acid molecules quantity measuring out by direct procedure.*

REACTION 2	
Sample volume [ml]	5
NaOH concentration [mol.l^{-1}]	0.0504
The rest of citric acid quantity [moles]	7.21398E-3
Initial citric acid quantity [moles]	0.0265
Converted citric acid quantity [moles]	0.0193
Conversion rate - χ [%]	72.79

(ii). Indirect Measuring Out Procedure by Three Phases Measuring Out Separately

After carrying out the extraction of the rest of citric acid molecules according to the procedure in paragraph 3.4.1 (§3.4.1) and the dichloromethane solvent treatment leading to the three distinct homogeneous phases according to the procedure in paragraph 3.4.2 (§3.4.2), take samples volumes between 1[ml] to 5.1[ml] for each phases in a beaker to be measuring out with NaOH solution for quantifying the rest of citric acid molecules (in each phase). Dilute each sample with 15[ml] of distilled water, then add two or three drops of helianthine. The solution turn immediately to red. Place the 0.0504N NaOH solution in an oilcan and the beaker on the magnetic stirrer. The measuring out can begin by falling into the beaker drip the oilcan 0.0504N NaOH solution and mixing the beaker solution with the magnetic stirrer. When the beaker solution turn to orange yellow, closed the oilcan and record the equivalent 0.0504N NaOH volume which correspond to the equivalent point. Then, calculate the rest of non-reactant citric acid molecules as shown in the following (Table 11) the results for the reaction 1 and reaction 2 (Table 6).

Table 11. *The rest of citric acid molecules quantity measuring out by indirect procedure phase by phase.*

PHASES - REACTIONS		REACTION 1		REACTION 2	
		Initial citric acid quantity [moles]	0.0265455	Initial citric acid quantity [moles]	0.0265101
PHASE 1	Rest of citric acid [moles]	2.7403E-3		4.7559E-3	
	Rate [%]	10.32		17.94	
PHASE 2	Rest of citric acid [moles]	8.1828E-3		2.4553E-3	
	Rate [%]	30.83		9.26	
PHASE 3	Rest of citric acid [moles]	1.2547E-4		2.6700E-7	
	Rate [%]	0.47		1.007E-3	
TOTAL	Rest of citric acid [moles]	0.0110486		7.211467E-3	
	Rate [%]	41.62		27.20	
Conversion rate of citric acid - χ [%]		58.38		72.80	

(iii). Results and Discussions

First of all, it was confirmed that direct measuring out was efficient than the indirect measuring out since they gave the same result equal to 72.79[%] in citric acid molecule conversion rate (Table10 – Table11). Then, during the dichloromethane solvent treatment, the balloon agitation had played a significant role on the rest of citric acid molecules distribution. Thus, the reaction1 agitation was more softly than the reaction2 one leading not only the reduction of the rest of citric acid rate in Phase 1 for the reaction1 but also the increase of its quantity in Phase 2 and other polar organic molecules which should be in Phase 1. It wasn't the case for the reaction2. Secondly, according to the following table (Table 12) showing the effect of some parameters, the pH which reflects the ion H^+ concentration played a significant role on the increase in conversion. Looking at the effects of other parameters like duration, pumpkin pips weight, pumpkin pips quantity relate to the pH, overall the reaction1 was much more profitable than the reaction2. However, theoretically when pH increase, the carboxylic acid with pKa = 6.39 should be more active and should increase the conversion rate without considering steric congestion (Figure 3). In all the cases, It was confirmed that the pumpkin pips were real micro-reactors in which were carried out the citric acid esterification, catalyzed by citric acid's protons H^+, to esters and amides diffused through its walls.

Table 12. *Parameters effects to citric acid molecules conversion rate.*

PARAMETERS/REACTIONS	REACTION 1	REACTION 2	REACTION1/REACTION2
pH	2.24	2.38	1/1.06
Duration (D-mn)	90	120	1/1.33
Pumpkin pips weight (M-g)	27.9	85.4287	1/3.06
Pumpkin pips quantity (N)	≈ 123	376	1/3.06
Conversion rate (χ -%)	58.38	72.79	1/1.25
χ / pH	26.0625	30.5840	1/1.17
χ / D	0.6487	0.6066	1.06/1
χ / (pH×D)	0.2895	0.2549	1.14/1
χ / (D×M)	0.0232	0.0071	3.27/1
χ / (pH×D×M)	0.0104	0.003	3.47/1
χ / N	0.4746	0.1936	2.45/1
χ / (pH×N)	0.2119	0.0813	2.61/1
χ / M	2.09	0.8521	2.45/1
χ / (pH× M)	0.93	0.3580	2.60/1

4. Hydrolysation of Pumpkin Pips' Organic Molecules Esterified with Citric Acid Molecules

4.1. Hydrolysis Reaction General Point

Hydrolysis reaction of esters (Figure 18) [43] or amides (Figure 19) [44] are their transformations to respectively acids and alcohols or acids and amines organic functions under water molecules actions. This is a slow reaction but can be catalyzed by H^+ ions or OH^- ions according to the pH operating conditions. In this manuscript, H^+ (H^+_{Ac}) ions from the rest of non-reactant citric acids' tricarboxylic acid were used to catalyze this hydrolysis reaction.

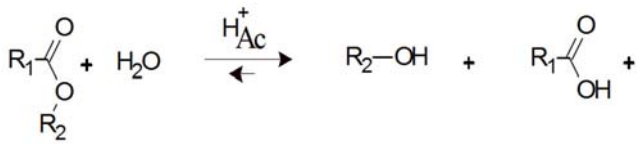


Figure 18. *Hydrolysis reaction of esters.*

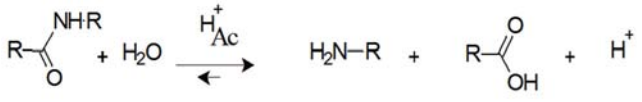


Figure 19. *Hydrolysis reaction of amides.*

4.2. Hydrolysation of Pumpkin Pip Organic Molecules Esterified with Citric Acid procedure

Hydrolysis of the three phases obtained previously (§ 3.4.2) were carried out separately to be efficient both in quantity and quality of organic molecules which must be obtained (§ 3.4.2). In short, the opposite reactions of reactions presented by Figure 2 to Figure 8 (§ 3.1) were attended.

4.2.1. Hydrolysation of Pumpkin Pip Organic Molecules Esterified with Citric Acid in Phase 2 and Phase 3 Procedure

Phase 2 (aqueous) and Phase 3 (medium) hydrolysis were carried out in a 250[ml] balloon. Put 75[ml] of the phase to be hydrolyzed in the balloon. Then, add 125[ml] of distilled water. The solution's pH was in the vicinity of 3 (pH paper). Thus, H^+ (H^+_{Ac}) ions from the rest of non-reactant citric acids' tricarboxylic acid with pKa1 (3.14) and pKa2 (4.77) were able to catalyze the hydrolysation reactions (Table 1). Complete the extraction assembly (Figure 9) and finally heat the balloon at the reaction temperature ranging between 125°C and 150°C. After 1H, stop the heat balloon without stopping the Liebig condenser water refrigeration to reduce the temperature and eliminate the light gas leak risk. Then as soon as possible change the heat balloon to a beaker containing iced cube not only to stop the hydrolysis reactions but also to stop the opposite reaction which is the esterification of the obtained organic molecules (§ 3.1). When the balloon temperature was

carried out to room temperature, stop the Liebig condenser water refrigeration and finally a homogeneous liquid of persistent very slightly yellow color with organic molecules (Table7 - § 3.4.2) were obtained in the balloon.

4.2.2. Hydrolysation of Pumpkin Pip Organic Molecules Esterified with Citric Acid in Phase 1 (Dichloromethane) Procedure

Phase 1 (Dichloromethane) hydrolysis were carried out in a 250[ml] balloon. Put 15[ml] of the phase to be hydrolyzed in the balloon. Then, add 25[ml] of distilled water. The solution's pH was taken more than 3 (pH paper). Thus, H^+ (H^+_{Ac}) ions from the rest of non-reactant citric acids' tricarboxylic acid with pKa1 (3.14) and pKa2 (4.77) were able to catalyze the hydrolysation reactions (Table 1). Complete the extraction assembly (Figure 9) and finally heat the balloon at the reaction temperature ranging between 40°C and 90°C-maxi. After 1H, stop the heat balloon without stopping the Liebig condenser water refrigeration to reduce the temperature and eliminate the light gas leak risk. Then as soon as possible change the heat balloon to a beaker containing iced cube not only to stop the hydrolysis reactions but also to stop the opposite reaction which is the esterification of the obtained organic molecules (§ 3.1). When the balloon temperature was carried out to room temperature, stop the Liebig condenser water refrigeration and finally a liquid with two phases slightly yellow colored with organic molecules (Table7 - § 3.4.2) were obtained in the balloon.

4.3. Direct Measuring Out of Citric Acid Molecules Regenerated by Hydrolyze Procedure

The citric acid molecules regenerated by hydrolyze procedure were done by direct measuring out with NaOH – 0.0504 [mol.l^{-1}]. Take 5[ml] and 2[ml] of the hydrolyzed solution in a beaker respectively for Phase 2-3 and Phase 1. Dilute sample with 15[ml] of distilled water, then add two or three drops of helianthine. The solution turn immediately to red. Place the 0.0504N NaOH solution in an oilcan and the beaker on the magnetic stirrer. The measuring out can begin by falling into the beaker drip the oilcan 0.0504N NaOH solution and mixing the beaker solution with the magnetic stirrer. When the beaker solution turn to orange yellow, closed the oilcan and record the equivalent 0.0504N NaOH volume which correspond to the equivalent point. Then, the regenerated citric acid molecules rate (χ) compared with respectively initial citric acid molecules (χ (% Citric acid)) and initial esters or amides of citric acid molecules (χ (% Esters)) considering the rate of non-dissociated (HA) and dissociated (A^-/H^+) acid forms [45] have been calculated as shown in the following (Table 13) the results for the three phases.

4.4. Results and Discussions

Dichloromethane is a polar solvent and extracted esters or amides of citric acid molecules and amines (Table 7) whose refractive index were checked as discussed in the paragraph § 3.4.2 – table 8. It could be said that this extraction-method by citric acid esterification was qualitatively very interesting with an extraction rate of important organic molecules (Table 7) among other things riboflavin and xanthophyll (§ 3.4.2) at least equal to 1.30[%] to 15.22[%]. Then, seeing that fatty-acids were in the form of esters of citric acid (Figure 3) mainly soluble in the aqueous phase 2, their rate can go to 24.48[%] (33.62[%]) whose refractive index were discussed and verified according to the paragraph § 3.4.2 – table 8 and table 13. Lastly, the regeneration of citric acid molecules was very interesting with a hydrolysis rate of esters more than 50[%]. Consequently, at large this extraction method by citric acid esterification was also quantitatively interesting.

Table 13. *Regenerated citric acid molecules rate compared with initial citric acid and initial esters of citric acid.*

PHASES	REACTION 2				
	χ (% Citric acid) [%]	**χ (% Esters) [%]**	**χ-Cumulated (%Citric acid)[%]**	**χ-Cumulated (% Esters) [%]**	***I_R of the solution***
PHASE 1 (CH_2Cl_2) Esters – Amides	0.94	1.30	0.94	1.30	-
PHASE 2 (aqueous)	24.48	33.62	25.42	34.92	1.6345
PHASE 3 (middle)	11.08	15.22	36.50	50.14	1.6349

5. Pumpkin Pips' Organic Molecules Extractions

5.1. Extraction of Pumpkin pips' Organic Molecules Procedure

Mix the hydrolyzed phases obtained previously. Notice that the mixed aqueous phase obtained smelled characteristic pumpkin odor and light petroleum gas odor (Table 7). Take 200[ml] of the hydrolyzed phases solution into a separatory funnel and add 100[ml] of dichloromethane polar solvent. Agitate smoothly the solution obtained a few minutes in order to recover in the polar solvent dichloromethane the rest of citric acid molecules [19] but also hydrolyzed polar organic molecules. Then, let allow to settle during 5[mn] to 10[mn] until obtaining two distinct phases: organic phase in lower part and aqueous phase above. Transvase the organic phase in another separator funnel and add 75[ml] of distilled water. Agitate smoothly the obtained solution a few minutes, distilled water dissolved the little rest of citric acid molecules and fatty-acids whereas the organic phase became increasingly rich in polar organic molecules. Let allow to settle during 5[mn] to 10[mn] until obtaining two distinct phases: organic phase rich in polar organic molecules in lower part and aqueous phase above. Recover the organic phase in a container for conservation. Then, recover the aqueous phase rich in fatty-acids in a separatory funnel and add 100[ml] of

non-polar solvent hexane. Agitate smoothly the obtained solution a few minutes in order to recover fatty-acid molecules obtained after hydrolysis reaction. Let allow to settle during 5[mn] to 10[mn] until obtaining two distinct phases: organic phase above rich in fatty-acids and aqueous phase below containing water and low rate of citric acid molecules. Thus, a rather notable drops of fatty-acid molecules completely soluble in the non-polar solvent hexane grouping at the bottom of the container were recovered (photo 4).

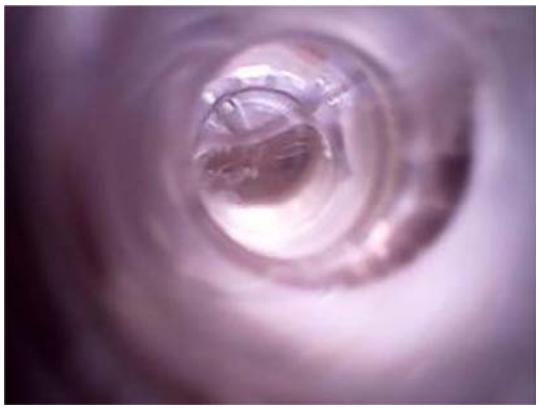


Figure 20. *Pumpkin pips' fatty-acid molecules extracted.*

5.2. Pumpkin Pips' Organic Molecules Extracted by Esterification with Citric Acid Molecules Characteristics

5.2.1. Biuret test and Refractive Index Results for the 1 and 2 Reactions' Three Solutions Phases

Table 14. *Results of three phases' Biuret test and refractive index.*

PHASES	REACTION 1	REACTION 2	BIURET TEST
	I_R	I_R	
PHASE 1 (CH_2Cl_2) Esters – Amides	1.6475	-	Positive
PHASE 2 (aqueous)	1.6310	1.6345	Positive
PHASE 3 (middle)	-	1.6349	Positive

The results in table 14 confirmed the presence of amides, esters and amines extracted with the citric acid esterification discussed previously in paragraph § 3.4.2.

5.2.2. Alkene Content of Pumpkin Pips' Fatty-Acid Molecules Extracted by Hexane Solvent Measuring out by Hydrofluoric Acid

The alkene content of pumpkin pips' fatty-acid molecules extracted by hexane solvent (§ 5.1 – Figure 20) were measuring out by hydrofluoric acid according to the procedure described in bibliography [46-47]. While referring to the iodine index of organic molecules which is the weight quantity [g] of iodine molecules fixed on the alkene functions in 100[g] of fatty-acid molecules [47], the HF index like the weight quantity [g] of hydrofluoric acid molecules fixed on the 100[g] of fatty-acid seeing that in theory were proposed to be defined, it's enough to make react an halogen in solution on a fat to determine its non-saturation degree [46-48-49]. Thus,

$$HF\ index = \frac{(2{,}6E-3 \times V_{HF} \times 20)}{Sample\ weight} \times 100$$

Table 15. *Alkene content of pumpkin pips' fatty-acid molecules extracted by hexane solvent measuring out by hydrofluoric acid.*

ALKENE CONTENTS ($C^{=}$) OF PUMPKIN PIPS' FATTY-ACID MOLECULES EXTRACTED BY HEXANE SOLVENT	
HF concentration [mol.l^{-1}]	2.6E-3
Sample weight [g]	0.1346
HF volume at the equivalent point [l] [46]	7.5625E-3
Moles of HF [moles]	1.96625E-5
HF at the equivalent point weight [g]	3.9325E-4
HF index [g_{HF}/100g $_{of\ sample}$]	0.2922
[Moles of $C^{=}$/100g $_{of\ sample}$]	0.0146

The alkene content of the sample (0.1346 [g]) was 1.96625E-5 [moles], that is to say 0.0146 [Moles of alkenes] in 100 [g] of pumpkin pip's fatty acid extract by hexane (§ 5.1).

5.2.3. Acid Value or Acidity of Pumpkin pips' Fatty-Acids Extracted with Hexane

Acid value or acidity is the mass of potassium hydroxide (KOH) in milligrams that is required to neutralize one gram of chemical substance (fatty-acids) [49-50] as shown in the table 16.

Thus,

$$Acid\ value = \frac{m_{KOH}}{Sample\ weight} \times 1000$$

Table 16. *Acid value of the fatty-acids extracted by hexane.*

ACID VALUE OF THE PUMPKIN PIPS' FATTY-ACIDS EXTRACTED BY HEXANE	
KOH concentration [mol.l^{-1}]	0.2
Sample weight [g]	1.0676
KOH volume at the equivalent point [l]	5.05E-3
Equivalent KOH quantity [moles]	1.01E-3
KOH weight equivalent [g]	0.05656
Acid value [mg_{KOH}/1g $_{of\ sample}$]	53

5.2.4. Saponification Value of Pumpkin Pips' Fatty-Acids Extracted with Hexane

Saponification value also referred to as "sap" represents the number of milligrams of potassium hydroxide required to saponify 1[g] of fat under the conditions specified [49-51] as shown in the table 17.

Thus,

$$Saponification\ value = \frac{[N_{HCl} \times (V_{HCl\ at\ blank\ test} - V_{HCl\ test}) \times 56.1]}{Sample\ weight} \times 1000$$

Table 17. *Saponification value of the fatty-acids extracted by hexane.*

SAPONIFICATION VALUE OF THE PUMPKIN PIPS' FATTY-ACIDS EXTRACTED BY HEXANE	
HCl concentration [mol.l^{-1}]	0.4972
Sample weight [g]	0.3127
HCl volume at the equivalent point for the blank test [l]	5.55E-3
HCl volume for the test with sample [l]	3.90E-3
KOH weight equivalent [g]	0.04594
Saponification value [mg_{KOH}/1$g_{of\ sample}$]	147

Bibliography [11] presented a bio pumpkin pips oil with saponification value 185 which isn't far from 147 and confirmed that probably pumpkin pips' oil fatty-acid have been extracted. However, the low value could be explained by the presence not only of short chain but also long chain fatty-acids [51] extracted by esterifying citric acid molecules (§ 3.1 - § 3.2).

5.2.5. Ester Value of Pumpkin pips' Fatty-Acids Extracted with Hexane

Ester value of fatty-acid extracted with hexane results from its acid value and saponification value as shown in the table 18.

Table 18. *Ester value of the fatty-acids extracted by hexane.*

ESTER VALUE OF THE PUMPKIN PIPS' FATTY-ACIDS EXTRACTED BY HEXANE	
SAPONIFICATION VALUE (I_S)	147
ACIDE VALUE (I_A)	53
ESTER VALUE = ($I_S - I_A$)	94

6. Pumpkin Pips' Organic Molecules Esterified with Citric Acid Molecules Increase in Value to Hydrocarbons (Super-Gasoil-Fueloil) Additive

Gasoline additives are organic molecules which increase gasoline's octane rating. They can be oxygenates organic molecules like alcohols, ethers, esters [52], black citric acid polymers [53], nitrogenized composed with strong oxygen content (NSO) [54], antioxidants as stabilizers like BHT, 2,6-DTBP, p-phenylenediamine, ethylene diamine, antiknock agents. It was seen previously that pumpkin pips' organic molecules esterified with citric acid molecules solution contained probably not only oxygenated nitrogenized compounds (§3.3 -§3.4) but also light short chain fatty-acid with carbon quantity between five and eight in chains (§3.4.2 - §5.1 - §5.2.4) like "pentane plus" which were very top quality liquids [55]. Thus, the first step was to eliminate the water molecules in this solution before the auto-inflammation test of hydrocarbons – dehydrated pumpkin pips' organic molecules esterified with citric acid solution mixes.

6.1. Elimination of Water Molecules in Pumpkin Pips' Organic Molecules Esterified with Citric Acid Molecules Solution with Organic Solvents Procedure

Put pumpkin pips' organic molecules esterified with citric acid molecules solution (citric acid's ester, amides of citric acid or amines) to be treated in a separatory funnel. Add dichloromethane (polar solvent) whose volume was two or three times the volume of the solution to be treated. Mix the obtained solution and add acetone at least equal to its volume. Mix this solution and let elutriate at least 15 minutes until obtaining two quite distinct organic phases: the heaviest below phase, dichloromethane, insoluble in water and rich in polar oxygenated molecules like citric acid's esters, citric acid's amides, and amines. The above phase was acetone containing not only polar oxygenated organic molecules but also water molecules which were heavier than acetone solvent molecules, soluble in its and thus located in the border of dichloromethane and acetone solvents. Then, add gradually acetone in excess (approximatively half of the acetone's volume used at the beginning) without disturbing nor mixing the two organic phases obtained recently. Let elutriate during 15 minutes until obtaining two quite distinct organic phases, then recover firstly the lower phase dichloromethane rich in polar oxygenated molecules like citric acid's esters, citric acid's amides, and amines. Take care to turn off the separatory funnel's faucet a little before the frontier of two phases to make sure that it didn't contain any water molecules. Secondly, recover the rest rich in water, acetone with citric acid's esters of organic molecules in another separatory funnel where must added gradually hexane which volume was equal to 2/3 to the obtained above phase solution. Hexane which was non-polar solvent insoluble in water dissolved acetone and the rest of citric acid's esters of organic molecules. Let elutriate during at least 15 minutes and recover the lower phase within taking care to turn off the separatory funnel's faucet a little after the two phases' frontier to make sure that the totality of the water molecules present in the solution were recovered. Then, recover the above phase rich in acetone, hexane and the rest of citric acid's esters of organic molecules-amides and mix it with the dichloromethane phase recovered before. Third, test that this obtained solution didn't contain any more water molecules by adding hexane solvent. Let elutriate during 15 minutes until obtaining two quite distinct phases with a clearly fine visible and flexible frontier justifying the non-presence of water molecules. In this case, a solution with two quite distinct phases (Figure 21) were obtained: an organic phase above yellow colored rich in acetone, hexane and citric acid's esters, citric acid's amides, and amines such as riboflavin, xanthophyll showed previously in paragraph §3.4.2 and the other organic phase below transparent rich in dichloromethane and citric acid's esters, citric acid's amides, and amines of pumpkin pips' organic molecules. Finally, pass the obtained solution under filtration to remove possible organic solid matters or mineral solid. The refractive index of the two phases were determined on a refractometer and showed in the following table 19.

Figure 21. *Without water molecules citric acid's esters, citric acids amides and amines of Pumpkin pips' organic molecules.*

Table 19. *Citric acid's esters, citric acids amides and amines of Pumpkin pips' organic molecules characteristics.*

I_R / ORGANIC PHASES	ABOVE PHASE	BELOW PHASE
PHASES COLORS	YELLOW	TRANSPARENT
I_R (Refractive Index)	1.6295	1.6299
COMPOSITIONS	citric acid's esters, citric acid's amides, and amines of pumpkin pips' organic molecules citric acid's esters of Riboflavin citric acid's esters of xanthophyll	citric acid's esters, citric acid's amides, and amines of pumpkin pips' organic molecules

6.2. Assets and Added Value Provided by Pumpkin Pips' Organic Molecules Esterified with Citric Acid Molecules Solution

According to its definition, combustion is a chain reaction mechanism with formation of intermediate active species, radicals, which have one very short lifetime (similar to μs) [56]. Generally, combustion is composed of three steps that depend on the properties of the combustible substance: firstly the initiation step characterized by radicals species formation from initiation reactions, secondly propagation reactions where radicals formed in the first step react with reactant molecules or other radical to form one or other radicals, finally the termination reactions [57-58]. Notice that the first step is an endothermic reaction and need activation energy according to the hydrocarbons characteristics [56-57]. Referring to the pumpkin pips' organic molecules esterified with citric acid molecules solution, it contained probably not only oxygenated nitrogenized composed (§3.3 -§3.4) but also light short chain fatty-acid with carbon quantity between five and eight in chains (§3.4.2 - §5.1 - §5.2.4) like "pentane plus" which were very top quality liquids [55] needing low energy to form radical species and potentially favoring not only the initiation steps but also the propagation steps (§6). To confirm experimentally these theories [56-57-58] and observations [52-53], auto-inflammation tests [53] (blank tests) of hydrocarbons (super-gasoline-fuel oil) compared to mixing solution prepared with hydrocarbons (super-gasoline-fueloil) and pumpkin pips' organic esterified with citric acid molecules solution like additive were carried out.

6.3. Auto-Inflammation Test Procedure of Hydrocarbons (Super-Gasoil-Fueloil) - Pumpkin Pips' Organic Molecules Esterified with Citric Acid Molecules Mixes Solution

Trying to respect the hydrocarbons standards concerning the esters content; 10% in volume for the Super98 [59] – 5% in volume for the road gasoil [60] – 7% in volume for the non-road gasoil [61]; the polycyclic aromatic hydrocarbons and oxygenated compounds, 10% in volume for Super98 [59] – 8% in volume for the non-road gasoil [61] – 11% in mass for the road gasoil; the azote content 3400[mg/kg] for the fuel oil [62] and seeing that pumpkin pips' organic molecules esterified with citric acid molecules mixes solution contained not only fatty-acid, light short chain fatty-acid with carbon quantity between five and eight in chains (§3.4.2 - §5.1 - §5.2.4) molecules esterified by citric acid molecules but also pumpkin pips' organic molecules esterified with citric acid molecules solution contained probably oxygenated nitrogenized compounds (§3.3 -§3.4); take 5[ml] of hydrocarbons (super-gasoline-fueloil) samples mixes with 0.4[ml] of pumpkin pips' organic molecules esterified with citric acid molecules solution without water molecules. Put each sample prepared in a porcelain crucible then carried in an oven programmed to increase gradually its temperature speed at 276.15[°K/mn]. When unusual speed variations compared to the speed programmed were noted on the temperature screen, the sample must be checked. Thus, when a persistent flame (for the gas oil mixture) and/or a persistent smoke were noted both accompanied with coke deposit formation, the temperature displayed on the temperature screen was retained like the auto-inflammation temperature of the mixes sample solution composed with hydrocarbons (super or gasoil or fueloil) - pumpkin pips' organic molecules esterified with citric acid molecules.

6.4. Results and Discussions

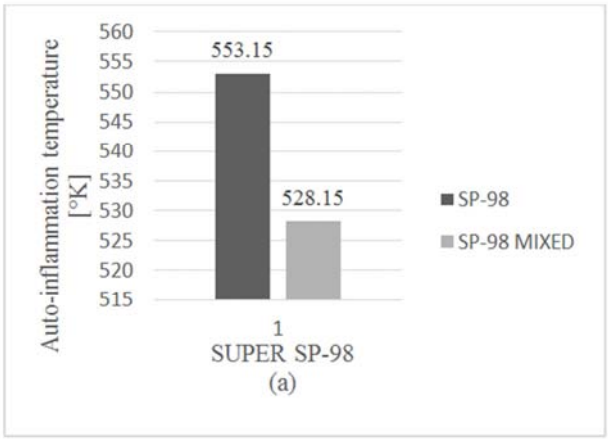
The auto-inflammation test results of the hydrocarbons (super or gasoil or fueloil) blank samples and the mixes hydrocarbons (super or gasoil or fueloil) - pumpkin pips' organic molecules esterified with citric acid molecules samples were presented in the following table 20.

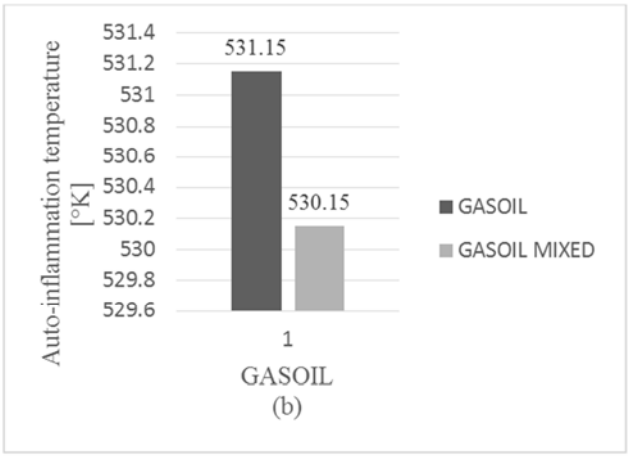
Table 20. *Auto-inflammation tests results.*

AUTO-INFLAMMATION TEMPERATURE [°K]			
TESTS/HYDROCRBONS	**SUPER SP-98**	**GASOIL**	**FUELOIL**
BLANK TESTS	553.15	531.15	633.15
MIXES HYDROCARBONS- PUMPKIN PIPS' ORGANIC MOLECULES ESTERIFIED WITH CITRIC ACID MOLECULES SAMPLES	528.15	530.15	528.15

Notice that the presence of pumpkin pips' organic molecules esterified with citric acid molecules solution decrease considerably the auto-inflammation temperature of blank hydrocarbons essence sp-98 – gasoil – fueloil according to the following figures 22(a)-22(b)-22(c). This was probably due to these molecules especially xanthophyll, riboflavin

(§3.4.2) which were answerable to the yellow coloration of the pumpkin pips' organic molecules esterified with citric acid molecules solution and light short chain fatty-acid with carbon quantity between five and eight in chains esterifying citric acid molecules (§3.4.2 - §5.1 - §5.2.4). Thus, these molecules acted well as initiation molecules additives whose transformation in the initiation step of combustion (§6.2) could be presented in the Figure 23 - Figure 24 – Figure 25 referring to the bibliography [57]. And, it was also probable that citric acid molecules, acid functions and oxygenated molecules participated to the combustion and contributed to the diminution of mixed samples' auto-inflammation temperature [53-57].





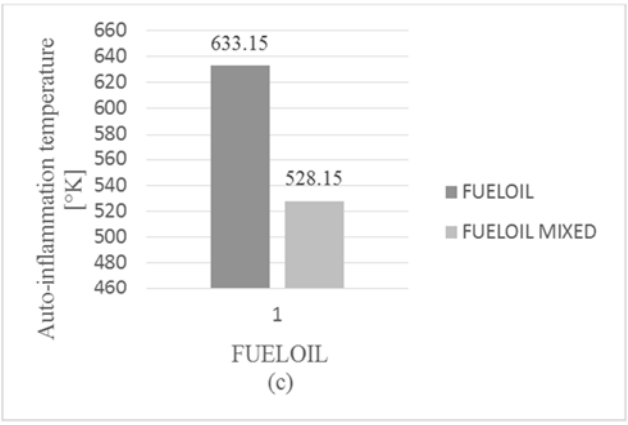


Figure 22. *Auto-inflammation temperature comparison of hydrocarbons and hydrocarbons mixed with pumpkin pips' organic molecules esterified with citric acid molecules solution: (a)-Super sp-98 (b)-Gasoil (c)-Fueloil.*

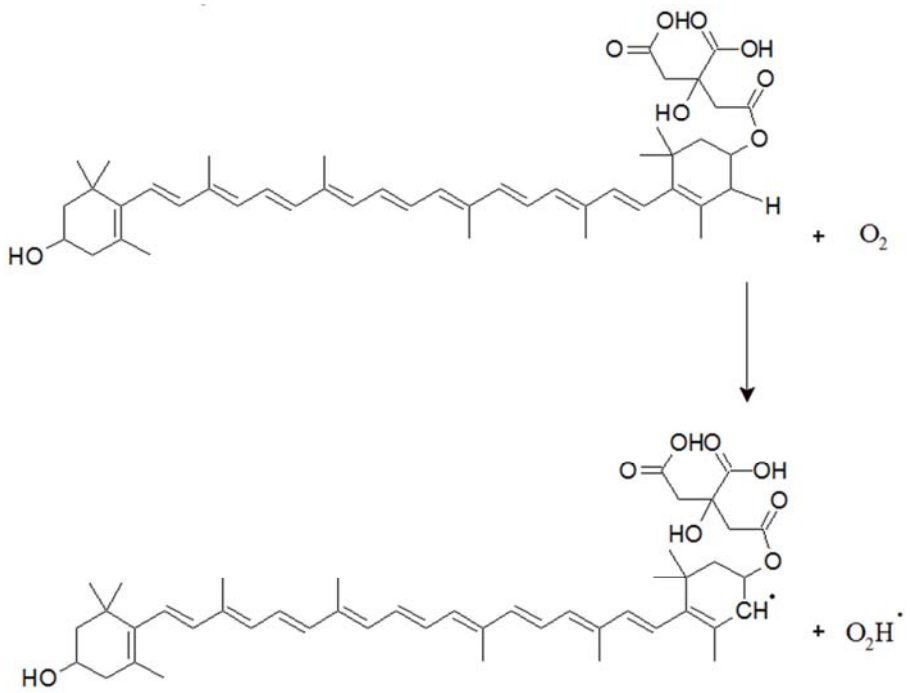


Figure 23. *Probably initiation step mechanism of xanthophyll molecule.*

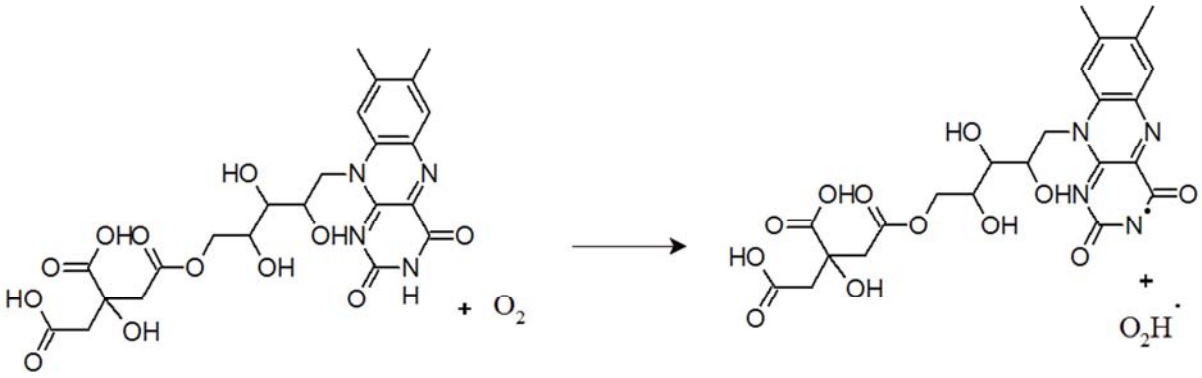


Figure 24. *Probably initiation step mechanism of riboflavin molecule.*

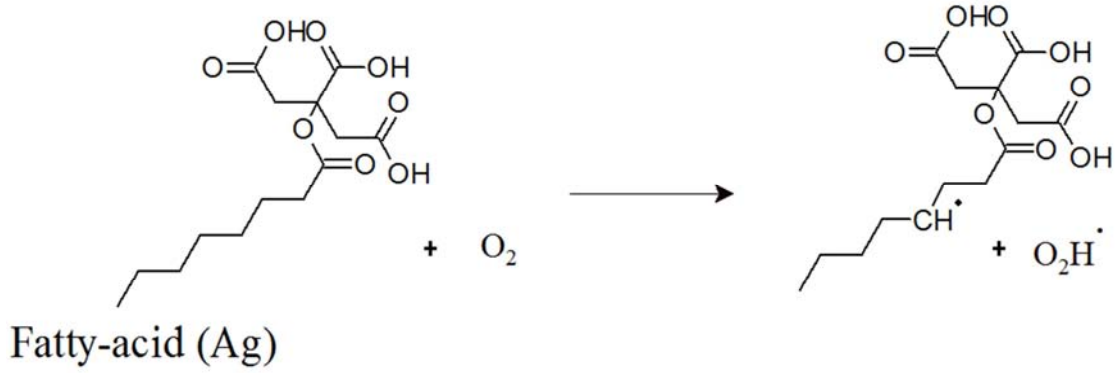


Figure 25. *Probably initiation step mechanism of fatty-acid molecule.*

7. Conclusion

Esterification with citric acid molecules was a technique which goes well to extract organic molecules in a vegetable organism. In this case, obtaining an important conversion into esterification wasn't explained quite simply by the pH and the environment acidity but also by the pumpkin pips which are real mini-reactors where esterification reactions of its organic molecules are favoured and through its walls will be able to move citric acid molecules and esters molecules produced. Two important molecules responsible for the yellow colouring of pumpkins, xanthophyll and riboflavin but also high quality fatty-acids and probably other organic molecules among other things responsible for the pumpkins odor characteristic have been extracted from pumpkin pips. The use of esters with citric acid as additive of hydrocarbons (super, gasoil, fueloil) after water removal according to a procedure using organic solvents (acetone, dichloromethane, and hexane) was very interesting [63]. Indeed, they make it possible to decrease up to 16.58% the auto-inflammation temperature of the mixture esters with citric acid-fueloil (8-100). Much more coke formation were noticed in this case; this can being due to the presence of citric acid molecules (in esters form). Indeed, it was shown by previous studies [53] that the presence of citric acid molecules (in black polymer of acid citric-PN form) not only decreased the auto-inflammation temperature but also contributed to the total coke formation particularly soluble coke.

Acknowledgements

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