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Synthesis of *Capsicum chinense* Citric Acid Esters-Its Methanol Trans-esterification Investigations with hplc Analysis and Its valorization as Gels-Crystals Ca-Salts

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Abstract: The esterification between citric acid molecules and raw material-*Capsicum chinense* organic molecules in excess was carried out at 137°C-410.15°K. The kinetics of the reaction showed that this reaction was second order compared with citric acid and the calculated initial and global speed constant were respectively $1.43\times10^{-1}[L^2\times mol^{-2}\times s^{-1}]$ and $3.84\times10^{-2}[L^2\times mol^{-2}\times s^{-1}]$. The synthesized raw material's-*Capsicum chinense*'s citric acid esters solutions colors was from light yellow-1.5[mn] to highly orange yellow-60[mn] confirming the esterification between citric acid molecules and the main bioactive molecules constituting the *Capsicum chinense* including capsaïcine, quercetin and luteolin whose densities were superior to one and explained the high densities of the *Capsicum chinense* citric acid esters solutions between 0.9825[g/ml] to 1.0636[g/ml]. Then, the alkene concentrations in esters solutions recorded an increase initially and at the middle of the time reaction respectively due to citric acid dehydration and alkenes from the raw material's-*Capsicum chinense*'s bioactive molecules. But, the diminution of these alkene concentrations recorded from 900[s] time reaction confirmed their etherification with carboxylic acid and/or alcohol organic functions catalyzed by citric acid's protonic acid-H^+. Second, the trans-esterification mechanisms of the raw materials' citric acid esters solutions with methanol was explored, inventoried and carried out in order to extract its bioactive molecules and their derivatives synthesized during this trans-esterification reaction where citric acid's protonic acid-H^+ sites functioned as catalyst. Thus, a trans-esterification using reflux-assembly followed by an extraction procedures were established in this manuscript. Indeed, the hplc analysis of the dichloromethane extracts allowed to identify the *Capsicum chinense*'s bioactive molecules and these derivatives. Third, procedures to synthesize crystals and gel calcium salts of raw material's citric acid esters solutions was established. An inventory of these gel-crystals structure was done in this manuscript and it was deduced that the first step of these procedures was the esters solution titration with NaOH-0.05N in order to determine the optimum quantities of calcium hydroxide for the synthesis using reflux assembly at 137°C followed by evaporations procedures. Then, once synthesized gel was suffered under thermic treatment until having solid crystals well-structured, both could be characterized by an established EDTA-0.01N and alkene procedures titrations established in this manuscript. Finally, an established titration procedure with NaOH-0.05N allowed to determine their equivalent citric acid molecules concentrations. It was noticed that their citric acid and calcium weight concentration ratio was respectively 4.354/1.67 (≈2/1) which confirmed the well-structured of the product regarding to the exploration salts synthesis-mechanisms figures done in this manuscript.

Keywords: *Capsicum chinense*, Citric Acid, Esterification, Methanol, Trans-esterification, hplc, Hexane, Dichloromethane

1. Introduction

The first part of this manuscript treated the kinetic study of the esterification between citric acid molecules and spicy-*Capsicum chinense*'s organic molecules in excess and the alkene concentration evolution in this synthesized citric acid esters solutions which was titrated with NaOH-0.05N [1-3] in order to determine the optimum methanol volume

used during its trans-esterification in an reflux assembly. An inventory of the possible trans-esterification reactions was done and allowed to establish an extraction procedure using hexane and dichloromethane whose extracted solution was analyzed by hplc-chromatography analysis according to experimental conditions analysis which could detect capsaïcine, luteolin, quercetin and their derivatives. The second part described the established raw material's-*Capsicum chinense*'s citric acid esters solutions gel-crystals calcium salts synthesis procedures and their established titrations and characterizations procedures with EDTA-0.01N and NaOH-0.05N to quantify respectively their calcium concentrations and their citric acid weight concentration ratio in order to check and confirmed their well-structured. Finally, an HF-0.0026N titration procedure was described and carried out on the synthesized gel calcium salts of *Capsicum chinense*'s citric acid esters solutions according to the established procedure described on paragraph §.4.4. Laboratory materials and chemicals used during these experimentations were beaker-250ml, magnetic stirrer, stirring rod, precision-balance-KERN, test-tube 100ml/50ml, burette 50ml, settling bulb 1l, heater balloon 250ml, flask balloon-250ml, straight condenser, reflux assembly, HF-0.0026N, NaOH-0.05N, EDTA-0,01N, helianthine, bromophenol blue, citric acid, *Capsicum chinense*, dichloromethane, hexane, rotavapor, hplc-UV160A UVvisible recording spectrophotometer shimadzu – column ST5BC 18250 (250*4.6mm).

2. Esterification Between Citric Acid Molecules and Spicy-*Capsicum chinense*'s Organic Molecules

2.1. Esterification Between Citric Acid Molecules and Spicy-Capsicum chinense's Organic Molecules Experimental Conditions

The experimental conditions of this esterification between citric acid molecules and spicy-*Capsicum chinense*'s organic molecules was shown in the following table 1. First of all, blackened spices, inedible spices as well as all spices stalks was removed such as the rest was cut approximately between 2[mm] to 3.5[mm] and putted into a 250[ml] flask-balloon. Then, dissolve the citric acid with the distillated water, put this solution into the flask-balloon which was overhang by straight condenser and placed this reflux-assembly into a balloon heater. Start the heat and the chronometer in the same time; when the reaction time was over, stop the balloon heater and withdraw the flask-balloon in order to decrease rapidly its temperature [1, 2]. Finally, the obtained esters solutions was not only characterized physico-chemically including its volume, density, colors, refractive index with pH but also titrated with NaOH-0.05N to quantify its equivalent rest unreacted citric acid molecules and to deduce the citric acid conversion [1-3].

Table 1. *Experimental conditions of esterification between citric acid molecules and spicy-Capsicum chinense's organic molecules.*

	Reaction time [mn]	*Capsicum chinense* weight [g]	Citric acid weight [g]	Citric acid [moles]	Water volume [ml]	Expected calculated pH	Evaluated Molar ratio (*Capsicum chinense* bioactive molecules/citric acid)
Capsicum chinense	1.5	16.6925	0.1013	5.2727E-4	64.5	2.85	101.0763
	3	16.6925	0.1076	5.6007E-4		2.83	
	15	16.6992	0.1035	5.3873E-4		2.84	
	30	16.7105	0.1035	5.3873E-4		2.84	
	60	16.7065	0.1045	5.4393E-4		2.84	

Noticed that the evaluated total bioactive moles of the used *Capsicum chinense*, which could reacted with the total moles of citric acid, were highly in excess; 101.0763 twice relative to citric acid moles. And the weight ratio between the *Capsicum chinense* and citric acid was 160.5292 [4-8].

2.2. Esterification Between Citric Acid Molecules and Spicy-Capsicum chinense's Organic Molecules Results

The following table 2 showed the physico-chemically characteristics of the obtained citric acid-spicy *Capsicum chinense* esters solutions. Noticed that the citric acid-spicy *Capsicum chinense* esters solution colors was from light yellow (1.5mn) to highly orange yellow (60mn) characteristics colors of the main bioactive molecules components of spicy-*Capsicum chinense* including capsaïcine (white color – [9]), quercetin (yellow color – [10]) and luteolin (yellow color – [11-13]) and explained the high density of the obtained citric acid's spicy-*Capsicum chinense* esters solutions [9-13].

Table 2. *Results of esterification between citric acid molecules and spicy-Capsicum chinense's organic molecules.*

	Reaction time [mn]	Citric acid spicy-*Capsicum chinense* esters solutions volume [ml]	Density of citric acid's spicy-Capsicum chinense esters solutions [g/ml]	Citric acid's spicy-*Capsicum chinense* esters solutions colors	Citric acid's spicy-*Capsicum chinense* esters solutions refractive index	Recorded Citric acid's spicy-*Capsicum chinense* esters solutions pH	Moles of NaOH-0.05N used for titration [moles]	Moles of equivalent citric acid titrated [moles]	Citric acid conversion [%]
Capsicum chinense	1.5	62	0.9825	Light yellow	1.325	3.91	7.75E-4	4.4621E-4	15.3738
	3	64.5	0.9825	Orange yellow	1.325	3.55	6.45E-4	4.2850E-4	23.4919
	15	67	1.0636	Orange yellow	1.321	3.77	5.025E-4	2.8164E-4	47.7216
	30	61	1.0074	Orange yellow	1.324	4.01	4.575E-4	2.5529E-4	52.6129
	60	66	0.996	Orange yellow	1.323	4.25	4.95E-4	2.5248E-4	53.5817

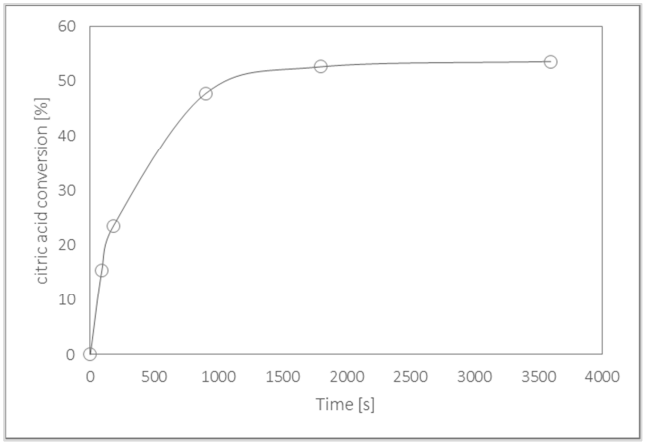

Figure 1. *Equivalent citric acid conversion evolution with reaction time.*

Noticed also that 1.51 was the refractive index of the capsaïcine [14] and dihydrocapsaïcine [15] which were the main components of the spicy [4]. But, the recorded refractive indexes on the table 2 were slightly inferior certainly because of the dissolution in distillated water (64.5[ml] – Table 1) which decrease its refractive index as showed in bibliography [16] and confirmed by these results on the table 2 such as when the citric acid spicy-*Capsicum chinense* esters solutions volume increased from 61[ml] to 67[ml], its recorded refractive index decreased from 1.325 to 1.321 (Table 2). In all cases, the synthesized citric acid spicy-*Capsicum chinense* esters solutions tasted very hot characteristics of capsaïcine and dihydrocapsaïcine which hold the highest percent of spicy components and the highest scoville heat units respectively 69% - 16,000,000 and 22% - 16,000,000 [4].

The initial conversion of the citric acid was very important, these values increased from zero percent to 23.49[%] only during 3[mn]. These results confirmed not only the low quantities of citric acid used compared to the spicy-*Capsicum chinense's* bioactive molecules (Table 1) promoting their esterification. From the reaction time 3[mn], noticed that the slope of the conversion curve decreased as a function of time which indicated that on these experimental conditions (Table 1) a part of the citric acid's carboxylic acid functions spend their role as source of protonic acid H^+ catalyst for the esterification reactions and in long-term protonic acid H^+ catalyst for the probably formation of ether by addition-reaction between alkenes functions from raw materials or citric acid dehydrations [17,18] and oxygen from alcohol function or carboxylic acid function or ketone function as described on the following figure 2, figure 3 and figure 4.

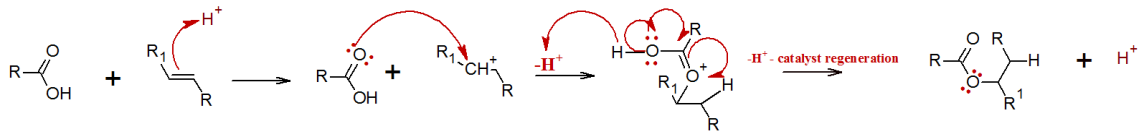

Figure 2. *Ether formation by addition between alkene function and oxygen from carboxylic acid function catalyzed by citric acid's protonic acid-H^+.*

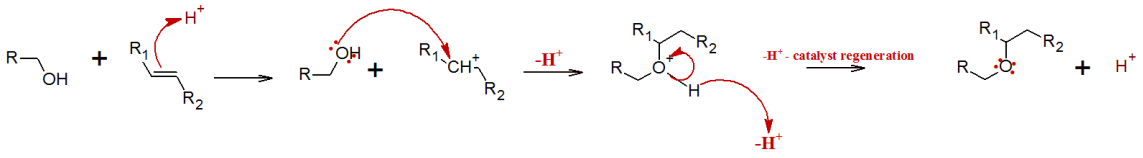

Figure 3. *Ether formation by addition between alkene function and oxygen from alcohol function catalyzed by citric acid's protonic acid-H^+.*

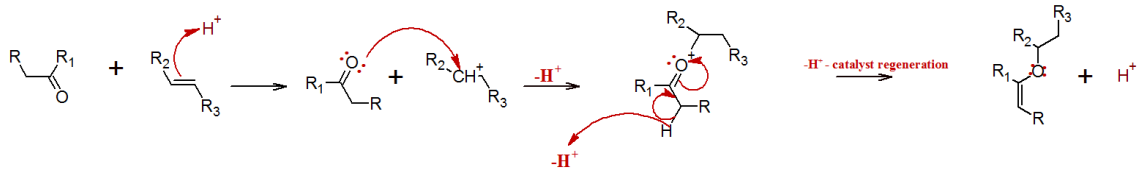

Figure 4. *Ether formation by addition between alkene function and oxygen from ketone function catalyzed by citric acid's protonic acid-H^+.*

Indeed, following the alkene concentration in the citric acid spicy-*Capsicum chinense* esters solutions with time reaction by hydrofluoric HF-0.0026N titration [19, 20] the table 3 and the figure 5 were obtained. These results confirmed and showed that first, the alkenes formed initially were reached by citric acid dehydration [18, 21]; second the alkenes formed after in the middle, from 900[s] to 1800[s], were composed by alkenes formed by the previous mechanism added with *Capsicum chinense's* (Capsaicinoïdes, luteolin, quercetin) bioactive molecules alkenes indeed its total quantities in the order of 10^{-3} (Table 3) were superior to the total initial citric acid moles in the order of 10^{-4} (Table 1); and third from 900[s] reaction time a diminution of alkene concentration were recorded and supported not only the transformation of citric acid's dehydrated alkenes to yellow citric acid monomers [17, 18, 21] but also the transformation of some alkenes to ethers according to the mechanisms described on the previous figures (Figure 2 to Figure 4). The approximate minimum bioactive *Capsicum chinense's* (Capsaicinoïdes with alkene function, luteolin, quercetin) bioactive molecules quantities in the synthesized citric acid spicy-*Capsicum chinense* esters solutions in the table 3 were calculated by doing the difference

between the maximum value of alkene total titrated (In this case equals to 2.34E-02 [moles] at 900[s]-15 [mn] reaction time) and the initial average value of initial citric acid moles.

Table 3. *Citric acid spicy-Capsicum chinense esters' alkene function evolution with reaction time.*

	Reaction time [mn]	**Citric acid spicy-*Capsicum chinense* esters solutions volume [ml]**	**Alkene Total moles in solutions [moles]**	**Alkene concentrations [mol/L]**	**Average value of initial citric acid moles [moles]**	**Approximate minimum bioactive *Capsicum chinense*'s (Capsaicinoïdes, luteolin, quercetin) bioactive molecules quantities [moles]**
Capsicum chinense	1.5	62	3.39E-04	5.46E-03		-
	3	64.5	1.84E-04	2.86E-03		-
	15	67	1.57E-03	2.34E-02	5.4175E-4	
	30	61	1.27E-03	2.08E-02		1.0283E-3
	60	66	6.86E-04	1.04E-02		

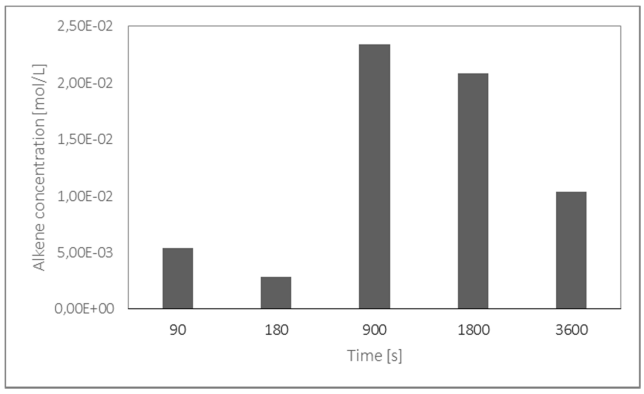

Figure 5. *Citric acid spicy-Capsicum chinense esters' alkene function evolution with reaction time.*

2.3. Esterification Between Citric Acid Molecules and Spicy-Capsicum chinense's Organic Molecules Kinetics

Seeing that not only this esterification occurred approximately at constant temperature and volume (Table 1, Table 2) but also the evaluated molar ratio between *Capsicum chinense*'s bioactive molecules and citric acid was equal to 101.0763; it could be deduced that the concentration of the *Capsicum chinense*'s bioactive molecules was largely in excess and could be taken as practically constant during the esterification reaction with citric acid molecules [2] according to degeneration theory in kinetics. Thus, in this condition the order of this esterification reaction was equal to the citric acid order – "a" such as:

$$v = k \times [citric\ acid]^a \times [raw\ matrials\ reactive\ molecules]^b \quad (1)$$

The observed speed constant became k_{obs} was $k_{obs} = k \times [raw\ materials\ reactive\ molecules]^b$ (2)

Thus,

$$v = -\frac{d[citric\ acid]}{dt} = k_{obs} \times [citric\ acid]^a \quad (3)$$

Studying the citric acid concentrations curve evolution with time conducted to the determination of citric acid partial order.

1) Firstly, assuming that the reaction was the first order, the equation (3) became

$$v = k_{\text{obs}} \times [citric\ acid]^1 = -\frac{d[citric\ acid]}{dt} \quad (4)$$

$$\leftrightarrow -\frac{d[citric\ acid]}{[citric\ acid]^1} = k_{\text{obs}} \times dt \quad (5)$$

Resolving this equation conducted to

$$(k_{\text{obs}} \times \text{t}) + \text{K} = -\text{Ln}[\text{citric acid}] \quad (6)$$

such as K is a constant. At initial time t=0

$$[citric\ acid] = [citric\ acid]_{initial}, \text{ so K} = -\text{Ln}([citric\ acid]_{initial}) \quad (7)$$

The equation (6) became,

$$(k_{obs} \times \text{t}) = \text{Ln}(\frac{[citric\ acid]_{initial}}{[citric\ acid]_{\,t}}) \quad (8)$$

In the other words, if this reaction is first order compared with citric acid-$C_6H_8O_7$, the previous equation (8) must be a straight line according to time t and its slope gave the observed speed constant k_{obs}

2) Secondly, assuming that the reaction was the first order, the equation (3) became

$$v = k_{\text{obs}} \times [citric\ acid]^2 = -\frac{d[citric\ acid]}{dt} \quad (9)$$

$$\leftrightarrow -\frac{d[citric\ acid]}{[citric\ acid]^2} = k_{\text{obs}} \times dt \quad (10)$$

Resolving this equation conducted to

$$(k_{\text{obs}} \times \text{t}) + \text{K} = \frac{1}{[citric\ acid]} \quad (11)$$

such as K is a constant

At initial time t=0, $[citric\ acid] = [citric\ acid]_{initial}$, so

$$\text{K} = \frac{1}{[citric\ acid]_{initial}} \quad (12)$$

The equation (11), became

$$(k_{\text{obs}} \times \text{t}) = (\frac{1}{[citric\ acid]_{\,t}} - \frac{1}{[citric\ acid]_{initial}}) \quad (13)$$

In the other words, if this reaction is second order compared with citric acid-$C_6H_8O_7$, the previous equation (13) must be a straight line according to time t; and its slope gave the observed speed constant k_{obs} The following table 4 gave the evolution of citric acid concentrations and the results of the previous equations (8) and (13) according to time.

Table 4. *Kinetics experimental results of Esterification between Citric Acid Molecules and Spicy-Capsicum chinense's Organic molecules kinetics.*

Time [s]	$[C_6H_8O_7]_{initial}$	$[C_6H_8O_7]_t$	$\mathbf{Ln}\left(\frac{[C_6H_8O_7]_{initial}}{[C_6H_8O_7]}\right)$	$\left(\frac{1}{[C_6H_8O_7]_t}-\frac{1}{[C_6H_8O_7]_{initial}}\right)$
60	8.17E-03	7.20E-03	1.27E-01	16.6202
90	8.68E-03	6.64E-03	2.68E-01	35.3614
900	8.35E-03	4.20E-03	6.87E-01	118.1678
1,800	8.31E-03	4.19E-03	6.86E-01	118.6392
3,600	8.43E-03	3.83E-03	7.90E-01	142.8222

Drawing the curves of $\mathrm{Ln}\left(\frac{[C_6H_8O_7]_{initial}}{[C_6H_8O_7]_t}\right)$ and $\left(\frac{1}{[C_6H_8O_7]_t}-\frac{1}{[C_6H_8O_7]_{initial}}\right)$ evolutions compared with the time reactions for the three initial times and for all-global times; the following figures figure 6-a with figure 6-b and figure 7-a with figure 7-b were obtained respectively for the first order case and second order case.

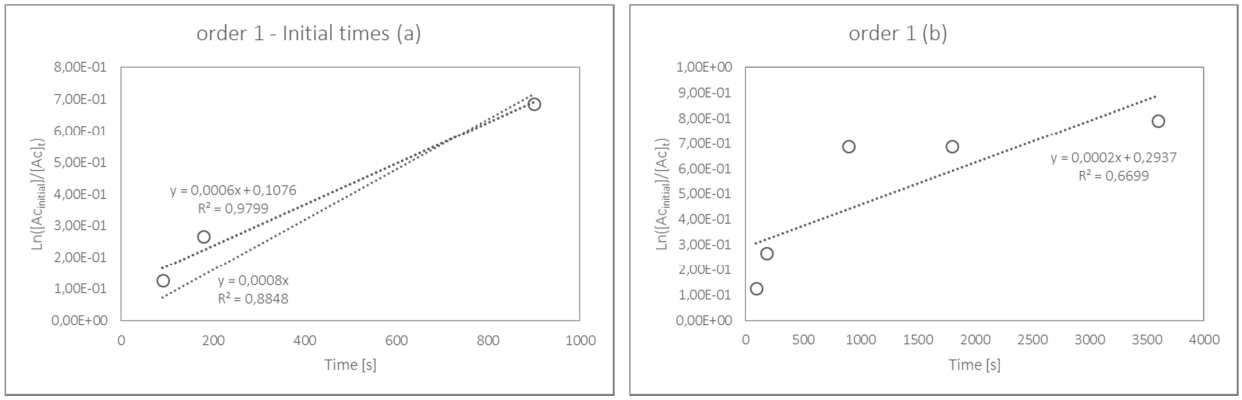

Figure 6. *Order 1 case evolution drawn with initial time (a) and drawn with full-global times (b).*

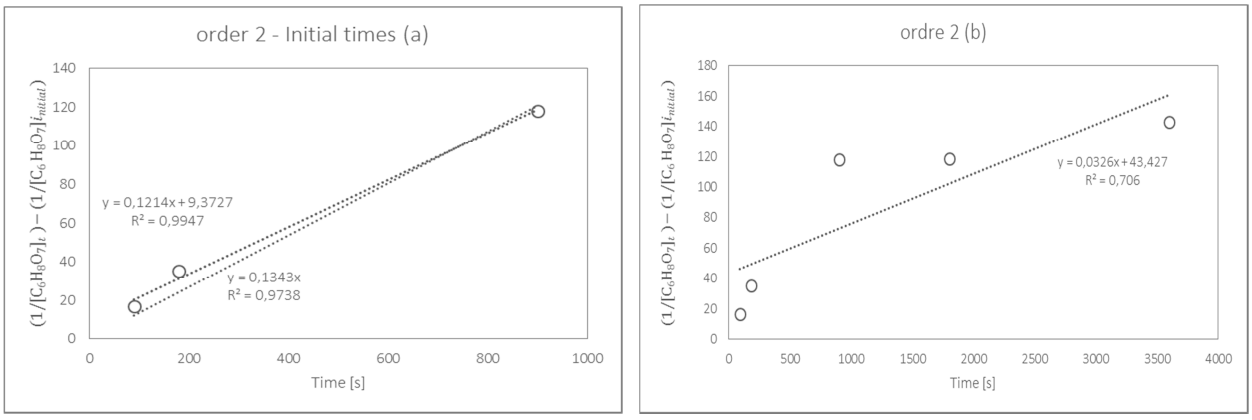

Figure 7. *Order 2 case evolution drawn with initial time (a) and drawn with full-global times (b).*

Seeing that the (R^2) of these initial times figures 6(a) and 7(a) were in the vicinity of 0.90 but its (R^2= 0.706) for the global times figure 7(b) was superior than for the figure 6(b) (R^2= 0.6699); seeing also the conversion evolution curve (Figure 1) such as the concentration of the *Capsicum chinense*'s bioactive molecules was largely in excess and could be taken as practically constant during their esterification reaction with citric acid molecules compared with citric acid molecules (Table 1) and whereas noticing that the interception with the origin $\mathcal{O}(0,0)$ on figure 7(a) (R^2 = 0.9738) was higher than those for the figure 6(a) (R2 = 0.8848); it was more adequate to affirm that, according these results and the experimental conditions (Table 1), this esterification reaction between citric acid molecules and *Capsicum chinense*'s bioactive molecules was second order compared with the citric acid concentration. This second order against to citric acid explained and confirmed not only the important initial conversion (figure 1) of citric acid molecules to reactions products as esters, monomers of citric acid but also the adsorption, desorption and move of reactants, products and citric acids' protonic acid H^+ catalysts molecules on *Capsicum chinense*'s surfaces/structure aromatics and polynuclear alkenes by hydrogen bond with water [2, 17-18]. Indeed, in this experimental condition (Table 1), where the raw materials *Capsicum chinense*'s bioactive molecules was highly in excess than the citric acid molecules, it was noticed a 55.55[%] diminution of alkene concentration in citric acid spicy-*Capsicum chinense* esters solutions with the conversion

from 15[mn] to 60[mn] confirming not only the transformation of some alkenes to ethers according to the mechanisms described on the previous figures (Figure 2 to Figure 4) but also the citric acids' protonic acid H^+ catalysts activities in diminution due to this etherification.

Thus, the previous equation (13) $\left(\frac{1}{[C_6H_8O_7]_t} - \frac{1}{[C_6H_8O_7]_{initial}}\right) = (k_{obs} \times t)$ was experimentally verified and the initial and global observed speed constant

$$k_{obs} = k \times [Capsicum\ chinense\ bioactive\ molecules]^{\beta} \quad (14)$$

for this esterification reaction between citric acid molecules and *Capsicum chinense's* bioactive molecules was equals respectively to the slope of the figure 7(a) and 7(b) that is to say respectively 0.1214[L×mol^{-1}×s^{-1}] (initial) and 0.0326[L×mol^{-1}×s^{-1}] (global).

Seeing that the rough calculated initial *Capsicum chinense's* bioactive molecules concentration value was 0.8490[mol.l^{-1}], thus the approximate values of the initial and the global speed constant were respectively 1.43E-1 [L^2×mol^{-2}×s^{-1}] and 3.84E-2 [L^2×mol^{-2}×s^{-1}].

3. Trans-Esterification of Citric Acid Esters Solutions - Citric Acid *Capsicum chinense* Esters

The objective was to trans-esterify the synthesized citric acid esters solutions [1] with methanol in order to analyze by chromatography the obtained products composed basically of raw materials' bioactive molecules and its derivatives with eventually regeneration of citric acid molecules. Indeed, the global inventory of this trans-esterification between raw materials' bioactive molecules of citric acid esters solutions with methanol permitted to obtain methanol-ether of raw materials' bioactive molecules with regeneration of citric acid (Figure 8) and/or alcohol of raw materials' bioactive molecules with methanol esters of citric acid (Figure 9) and/or methanol-esters of raw materials' bioactive molecules with regeneration of citric acid (Figure 10) and/or carboxylic acid of raw materials' bioactive molecules with methanol esters of citric acid (Figure 11).

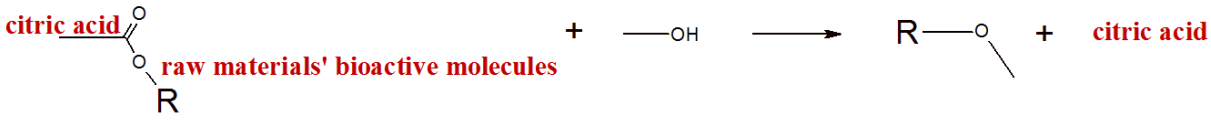

Figure 8. *First case of trans-esterification of raw materials' bioactive molecules citric acid esters with methanol.*

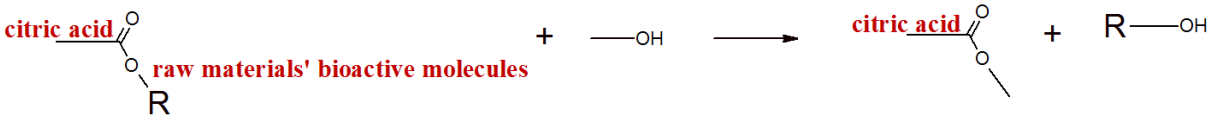

Figure 9. *Second case of trans-esterification of raw materials' bioactive molecules citric acid esters with methanol.*

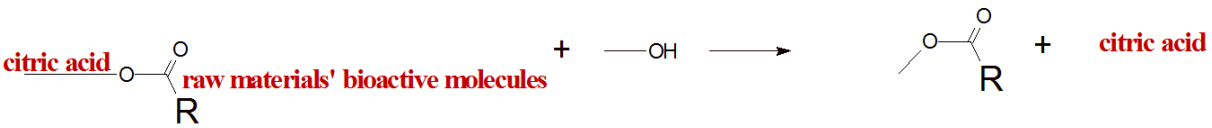

Figure 10. *Third case of trans-esterification of raw materials' bioactive molecules citric acid esters with methanol.*

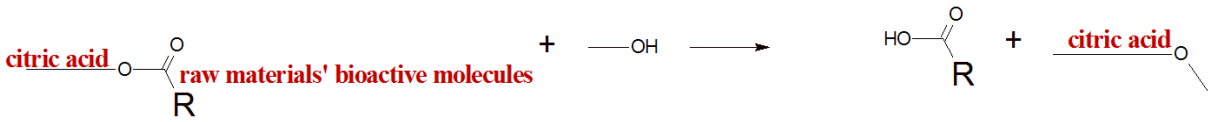

Figure 11. *Fourth case of trans-esterification of raw materials' bioactive molecules citric acid esters with methanol.*

In addition, even if the capsaïcin's quantities on spicy was in the range of micrograms per gram [22], during its esterification with citric acid molecules catalyzed by citric acid protonic acid-H^+ it was possible that alkene organic function was transformed to an citric acid esters [23] which could be trans-esterified by methanol according the following mechanism in the figure 12 and figure 13 for this case of capsaïcine and in general according to the previous mechanism in figure 10 and figure 11.

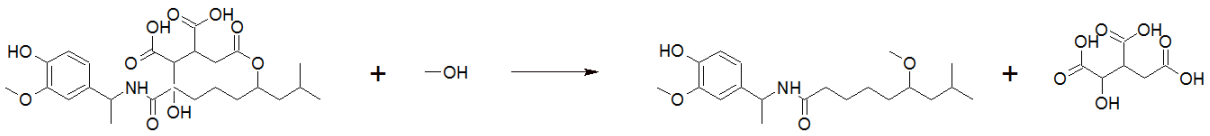

Figure 12. *First possible trans-esterification of alkene- capsaïcin's' citric acid esters with methanol.*

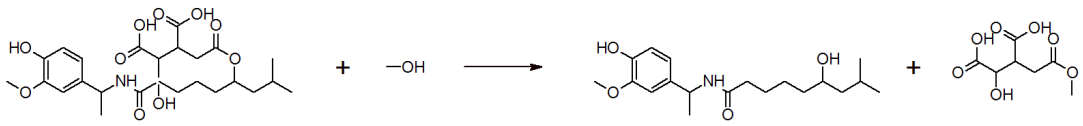

Figure 13. *Second possible trans-esterification of alkene- capsaïcin's' citric acid esters with methanol.*

Thus, during esterification with citric acid molecules catalyzed by citric acid protonic acid-H^+ it was possible an addition reaction between ketone organic function and citric acid molecule according the mechanism on the figure 14.

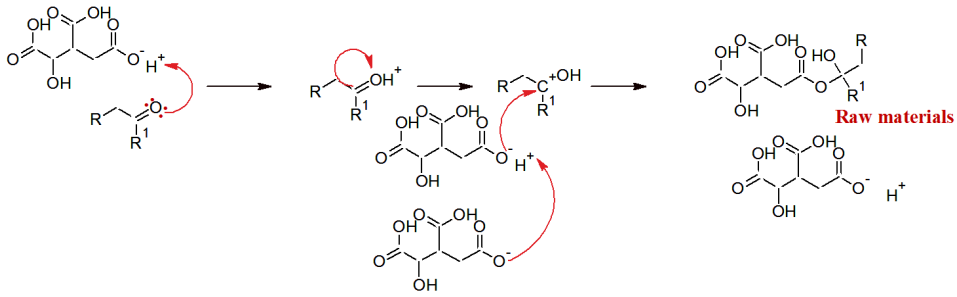

Figure 14. *Esterification between ketone's raw material and citric acid catalyzed by citric acid protonic acid-H^+.*

The trans-esterification of this previous product on figure 14 gave the following products on the figure 15 and the figure 16 according to the previous general mechanisms on the figure 8 and the figure 9.

Figure 15. *First case of product by trans-esterification between methanol and ketone's raw material- citric acid esters.*

Figure 16. *Second case of product by trans-esterification between methanol and ketone's raw material- citric acid esters.*

Then, during raw materials' bioactive molecules esterification with citric acid molecules catalyzed by citric acid protonic acid-H^+ it was possible an addition reaction between amine organic function and citric acid molecule according these mechanisms on figure 17 and figure 18.

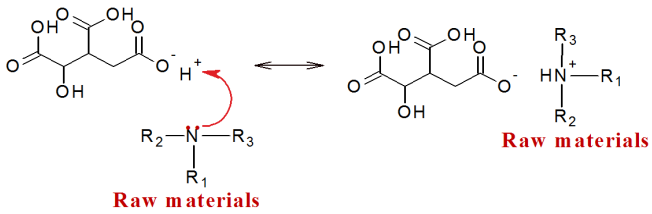

Figure 17. *First case of addition reaction between citric acid and raw materials' amine function catalyzed by citric acid protonic acid-H^+.*

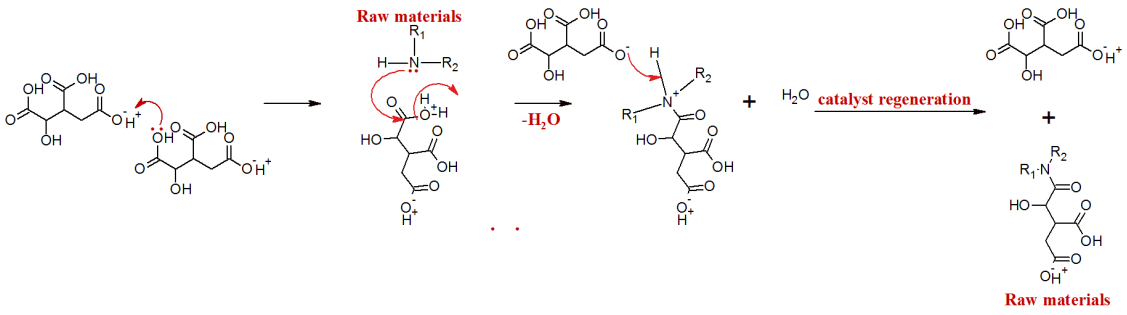

Figure 18. *Second case of addition reaction between citric acid and raw materials' amine function catalyzed by citric acid protonic acid-H^+.*

The trans-esterification of these previous products on figure 18 gave the following products on the figure 19 to the figure 20 according to the previous general mechanisms on the figure 8 and the figure 9.

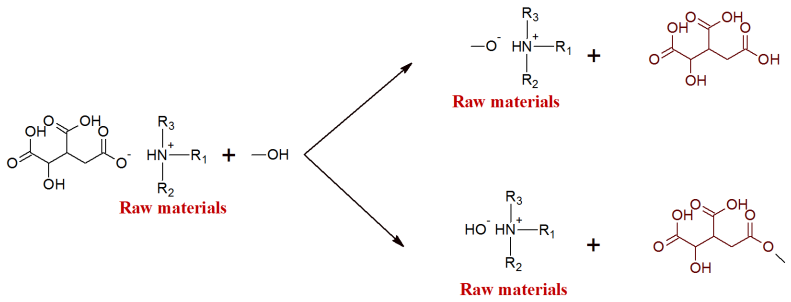

Figure 19. *First case of products by trans-esterification between methanol and citric acid-amine salt product on figure 17.*

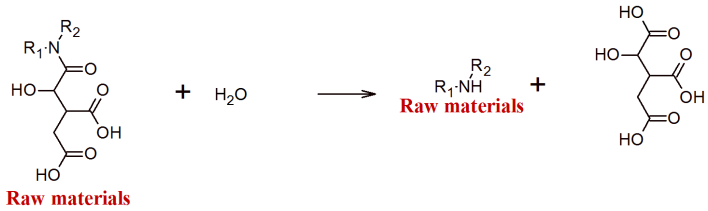

Figure 20. *Second case of products hydrolysis during trans-esterification- between methanol and amino-acid product on figure 18.*

3.1. Trans-Esterification of Citric Acid Esters Solutions with Methanol Procedure – Case of Spicy Capsicum Chinense Esters Solutions

First, the raw materials' citric acid esters solution was prepared and characterized by NaOH-0.05N titration in order to determine the moles-quantities of NaOH titrants then its citric acid (unreacted and reacted) concentration (§2.2) [1]. In the same time, the inventory of the raw materials' bioactive molecules able to react with citric acid (in this case *Capsicum Chinense's* bioactive molecules*)* could be done to evaluate their weight and molar percentages then their minimum total moles. These raw materials' citric acid esters solutions values information permitted to determine the optimal methanol in excess quantities necessary for its trans-esterification. Also, the previous raw materials' bioactive molecules inventory permitted to anticipate the possible products organic functions characteristics according to the figure 8 to the figure 11. The following table 5 showed the case of spicy *Capsicum chinense esters solutions* methanol trans-esterification to evaluate the optimal methanol quantities.

Once determined, the quantities of the esters solutions to be trans-esterified (in this case the *Capsicum chinense's* citric acid esters volume = 200[ml]) and the quantities of the methanol (in this case equals to 41.28[ml]) were putted into a 250[ml] flask-balloon which was overhang by straight condenser and placed this reflux-assembly into a balloon heater. Start the heat and the chronometer in the same time; the temperature was in the vicinity of 141[°C] during the trans-esterification. When the reaction time was over, stop the balloon heater and withdraw the flask-balloon in order to decrease rapidly its temperature then to freeze reactions. At the end, the synthesized raw materials' (Capsicum chinense) methanol-monoglyceride bioactive molecules solution composed by a majority with methanol-monoglyceride molecules-products, including the derivatives molecules of raw materials' bioactive molecules R-, citric acid and their derivatives (on figure 8 to figure 20), was subjected to the following procedure in paragraph §3.2. to extract solution able for chromatography analysis. In the case of the *Capsicum chinense*-raw material, its bioactive molecules R- were capsaicinoïdes, luteolin and quercetin.

Table 5. *Evaluation of the optimum methanol quantities for the spicy Capsicum chinense esters solutions methanol trans-esterification.*

Inventoried *Capsicum chinense's* bioactive molecules	**Estimated molar percentage [%]**	**Citric acid concentration of *Capsicum chinense's* citric acid esters solution [mol/L]**	**Volume of *Capsicum chinense's* citric acid esters to be trans-esterified [ml]**	**Reactant citric acid moles of *Capsicum chinense's* citric acid esters to be trans-esterified [mol]**	**Evaluated minimum bioactive molecules esterified quantities [mol]**	**Evaluated total minimum bioactive molecules esterified quantities [mol]**	**Evaluated optimum ethanol volume for trans-esterification of *Capsicum chinense's* citric acid esters solution [ml]**
capsaïcine	68.93				1.50E-01		
dihydrocapsaïcine	21.83				4.74E-02		
nordihydrocapsaïcine	7.28				1.58E-02		
homodihydrocapsaïcine	0.95	5.19E-3	200	6.51E-3	2.06E-03	2.71E-1	41.28
homocapsaïcine	0.96				2.07E-03		
luteolin	3.07E-3				5.00E-06		
quercetin	5.11E-2				6.66E-05		

3.2. Extraction Procedure of Raw Materials' (Capsicum chinense's) Methanol-Monoglyceride Bioactive Molecules

Noticed that according to the nature of the organic functions which composed the raw materials' (in this case *Capsicum chinense)* bioactive molecules R-, the methanol-monoglyceride bioactive molecules (figure 8 to figure 20) were soluble in distillated water and/or in polar solvent and/or in non-polar solvent though the methanol-citric acid monoglyceride and the regenerated citric acid molecules formed (figure 8 to figure 20) were certainly highly soluble in distillated water like citric acid molecules. Thus, in this extraction procedure dichloromethane (polar solvent), hexane (non-polar solvent) and distillated water were used.

Firstly, the first step washing the raw materials' (*Capsicum chinense)* methanol-monoglyceride bioactive molecules with icy-distillated water was done according to the distillated water quantities used during the raw materials' bioactive molecules-citric acid esters synthesis (in this case of *Capsicum chinense,* distillated water volume = 64.5[ml] – Table 1) such as the quantities-moles of icy-distillated water was in excess against the used quantities-moles methanol (§3.1. – Table 5). The objective of this first step was to dissolve in the icy-distillated water the previous citric acid and its methanol-monoglyceride derivatives with eventually the methanol-monoglyceride bioactive molecules, if R- was soluble in water, without their hydrolysis. In this case of *Capsicum chinense* this first step wasn't necessary. Thus, put the synthesized raw materials' (*Capsicum chinense*) methanol-monoglyceride bioactive molecules solution in a beaker, add the icy-distillated water and mix this solution during minimally one minute. The second step was to extract the non-polar raw materials' (in this case *Capsicum chinense)* bioactive molecules R- by hexane solvent. Thus, add the hexane solvent into the previous beaker such as its quantities-moles was in excess against the moles of NaOH-0.05N used for titration (§2.2. – Table 2) (in this case of *Capsicum chinense,* hexane volume = 134.26[ml]). Mix the reached solution with magnetic stirrer then let allow to settle into a settling bulb for 15[mn] to 45[mn] maximally until having two phases such as the aqueous phase underneath and the organic-hexane phase above. Seeing that the raw materials' (*Capsicum chinense*) methanol-monoglyceride bioactive molecules solution contained again the rest of methanol in excess (§3.1. – Table 5), the polar R- bioactive molecules stay dissolved in the aqueous phase. Thus, in this case of *Capsicum chinense* raw materials, two phases were reached at the end of the second step such as the white organic-hexane phase above overhang by a yellow thin layer probably composed respectively with methanol/monoglyceride of spicy-*Capsicum chinense's* fatty-acids (figure 10 – figure 11) and R-Capsaicinoïdes (figure 8 – figure 9) and on top the (luteolin/quercetin)-R-O-fatty-acid formed by ether cleavage between the (luteolin/quercetin)-R-O-CH_3 molecules (Figure 8) and the spicy-*Capsicum chinense's* fatty-acids (figure 11) according to the mechanism on the figure 12. Recover the aqueous phase then the organic-hexane phase which was directly concentrated using an evaporator-rotavapor and could be analyzed by chromatography. The third step was to add dichloromethane polar-solvent the recovered aqueous phase such as its quantities-moles was in excess against the used quantities-moles methanol (§3.1. – Table 5) (in this case of *Capsicum chinense,* dichloromethane volume = 307.03[ml] but only 90[ml] was used experimentally). Mix the reached solution with magnetic stirrer then let allow to settle into a settling bulb for 15[mn] to 45[mn] maximally until having two phases such as the aqueous phase above and the organic-dichloromethane phase underneath which was orange-yellow color in this case of *Capsicum chinense* raw material. Thus, this organic-dichloromethane phase contained certainly all methanol/monoglyceride of raw materials bioactive molecules R-products of reactions showed on the figure 8 to the figure 20 without citric acid and its methanol-monoglyceride derivatives products of reactions showed on the figure 8 to the figure 20. In this case of *Capsicum chinense* raw material R- was equivalent to capsaicinoïdes, luteolin and quercetin. Once recovered, this organic-dichloromethane phase was directly concentrated using an evaporator-rotavapor and could be analyzed by chromatography.

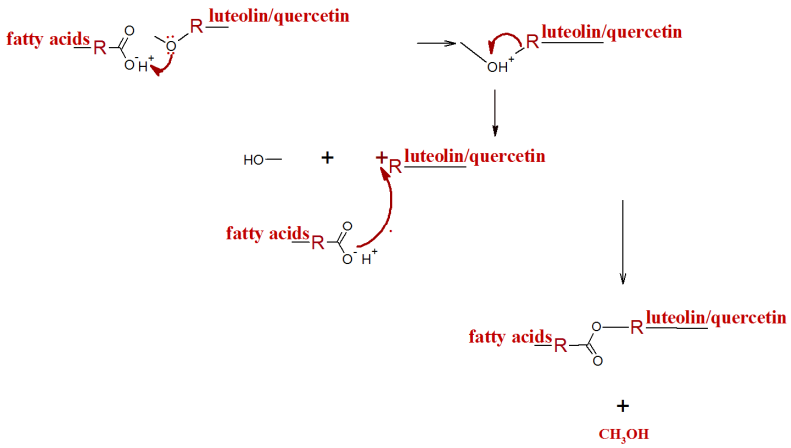

Figure 21. *Ether cleavage between the (luteolin/quercetin)-R-O-CH_3 molecules and fatty-acids (spicy-Capsicum chinense's).*

4. Calcium Salts of Raw Materials' Citric Acid Ester Synthesis Procedure

The objective was to synthesize gel-solutions and solid-crystals calcium salts of raw materials' (in this case *Capsicum chinense*'s) bioactive molecules from its citric acid esters solutions (§2.2.) and calcium hydroxide ($Ca(OH)_2$) without adding distillated water. The global reaction of this synthesis was shown on the following figure 22 such as R- was the raw materials' (in this case *Capsicum chinense)* bioactive molecules; "a", "b" and "c" were stoichiometric coefficients of the reaction.

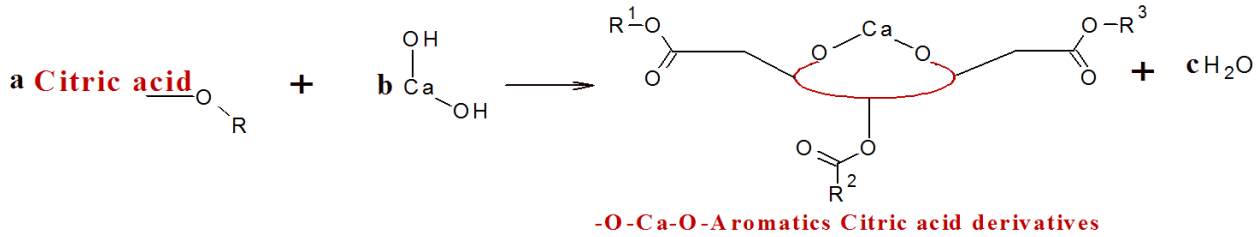

Figure 22. *Global reactions of the gel-solution and solid-crystals calcium salts of raw materials' (in this case Capsicum chinense's) bioactive molecules formation-synthesis.*

Trying to inventory few cases of this global reactions (figure 22), these following aromatics citric acid derivatives structures were reached according to the pH of the raw materials' (in this case *Capsicum chinense*'s) bioactive molecules of citric acid esters solutions without adding distillated water i.e. according to the citric acid esters solutions' pH-synthesis. And referring to these cases, it was noticed that the molar ratio between esters organic functions and calcium atom was in majority equal to two-2.

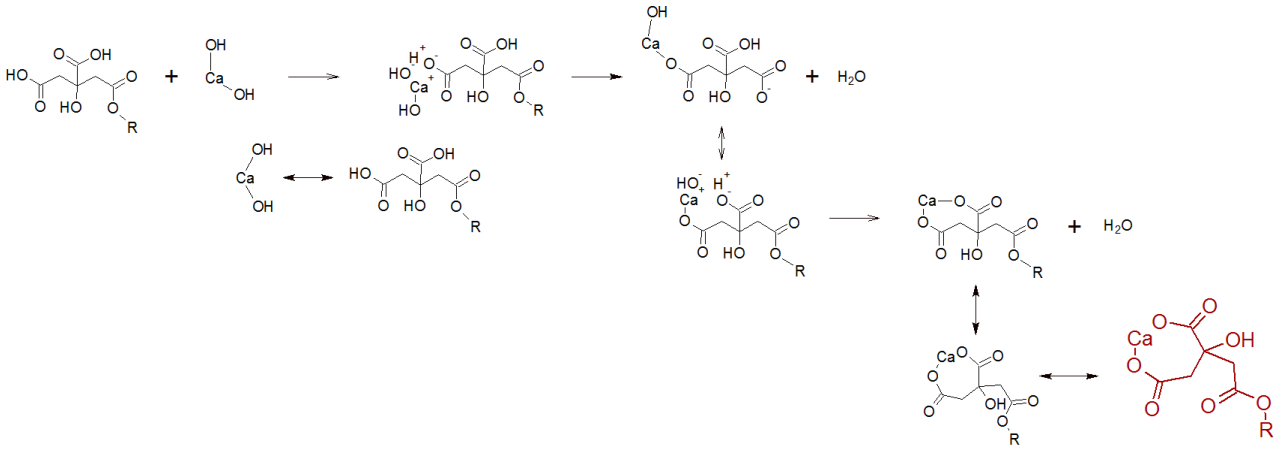

Figure 23. *First case of synthesized calcium citric acid aromatics with six (6) faces.*

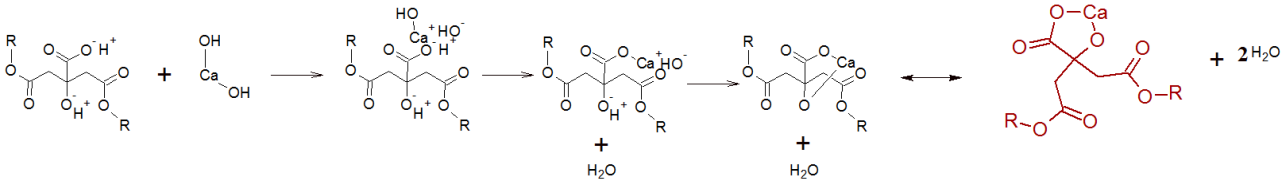

Figure 24. *Second case of synthesized calcium citric acid aromatics with five (5) faces.*

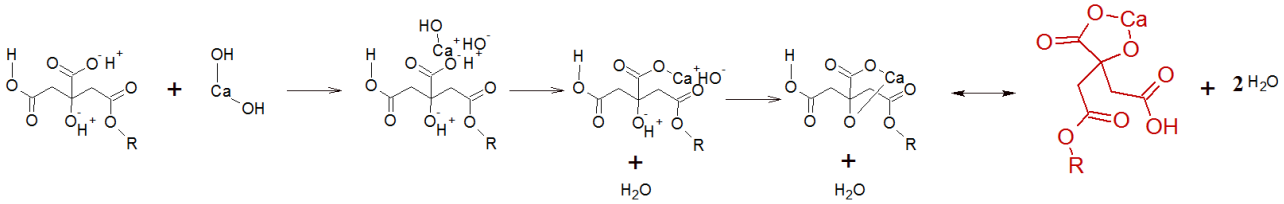

Figure 25. *Third case of synthesized calcium citric acid aromatics with five (5) faces.*

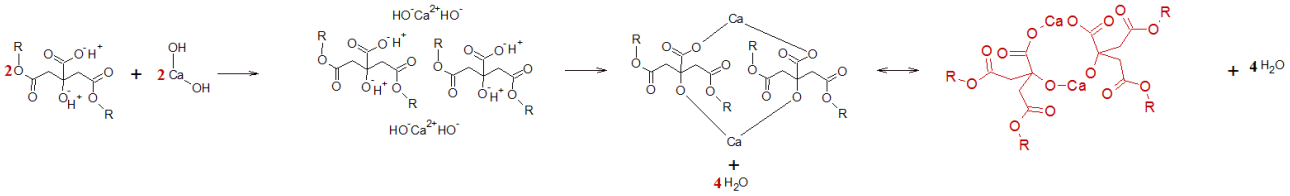

Figure 26. *Fourth case of synthesized calcium citric acid aromatics with five (10) faces.*

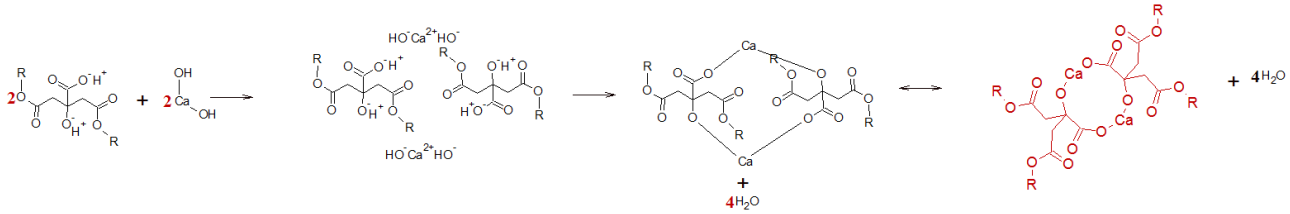

Figure 27. *Fifth case of synthesized calcium citric acid aromatics with five (10) faces.*

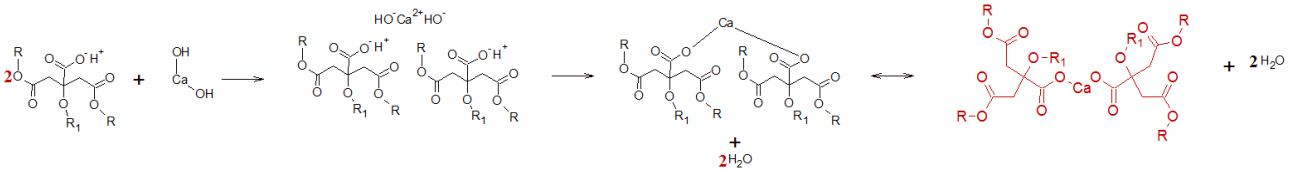

Figure 28. *Sixth case of synthesized calcium citric acid non-aromatics.*

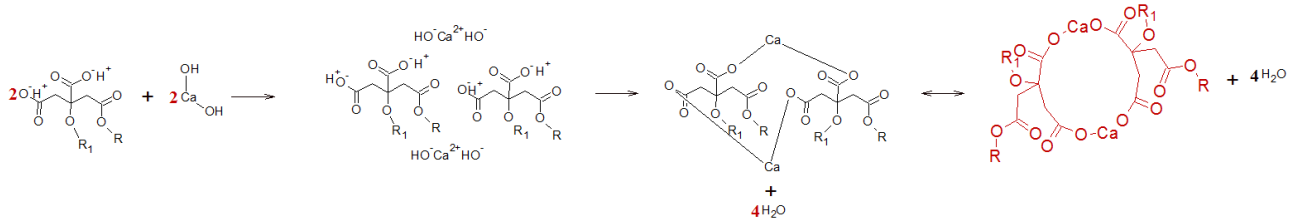

Figure 29. *Seventh case of synthesized calcium citric acid aromatics with five (14) faces.*

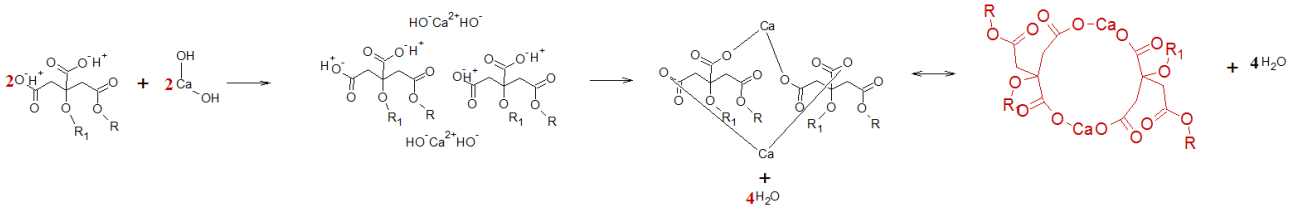

Figure 30. *Eighth case of synthesized calcium citric acid aromatics with five (14) faces.*

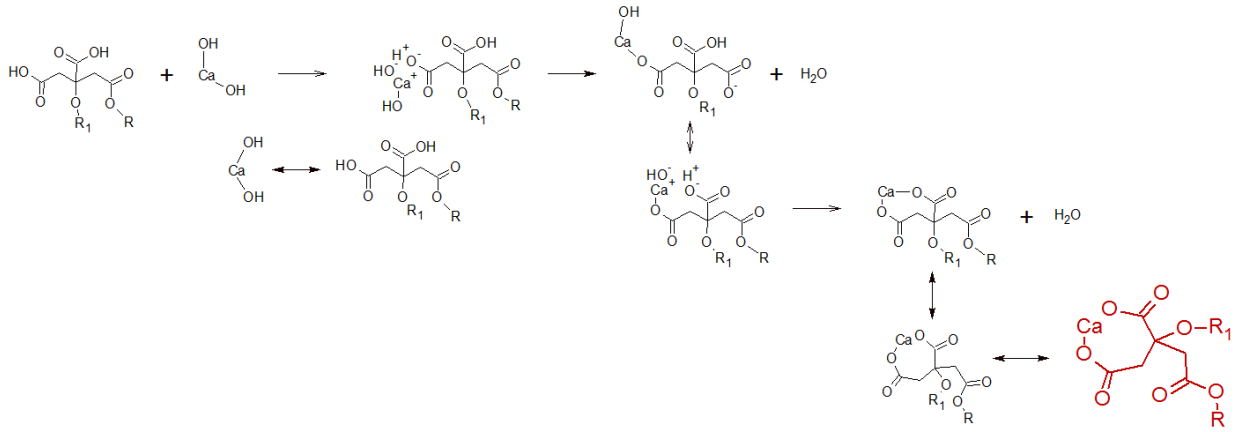

Figure 31. *Ninth case of synthesized calcium citric acid aromatics with five (7) faces.*

4.1. Gel and Crystal Calcium Salts of Raw Materials' Citric Acid Ester Synthesis Procedure – Case of Capsicum Chinense

First, the raw materials' citric acid esters solution was prepared and characterized by NaOH-0.05N titration in order to determine the moles-quantities of NaOH titrants then its citric acid (unreacted and reacted) concentration (§2.2) [1]. Determine the esters volume used for the synthesis and deduced the equivalent citric acid moles with the equivalent moles-quantities of NaOH titrants. Then, calculate the calcium hydroxide-$Ca(OH)_2$weight-quantities sufficient for this calcium salts of raw materials' (*Capsicum chinense*'s) citric acid ester synthesis. Once determined, the quantities of the esters solutions (in this case the *Capsicum chinense's* citric acid esters volume = 140[ml]) and the calcium hydroxide-$Ca(OH)_2$weight (in this case equals to 0.0389[g]) were putted into a 250[ml] flask-balloon which was overhang by straight condenser and placed this reflux-assembly into a balloon heater. Start the heat and the chronometer in the same time; the temperature was in the vicinity of 141[°C] during the calcium salts synthesis. When the reaction time more than one hour was over, stop the balloon heater and withdraw the flask-balloon in order to decrease rapidly its temperature then to freeze reactions. Thus, a solution of calcium salts of raw materials' (*Capsicum chinense*'s) citric acid ester was reached. The second step was to evaporate the previous synthesized solution either firstly at high-100[°C] temperature with agitation either secondly at low-40[°C] temperature under vacuum using an evaporator-rotavapor. In this case, the synthesized *Capsicum chinense*'s calcium salt solution was divided in two portions such as after the first process of evaporation (high-100[°C] temperature) a gel which became sticky-solid at high evaporation was reached with the one portion; it smelt and tasted very spicy and very nice in mouth, though under low temperature with vacuum evaporation a concentrated gel which was also very spicy and very nice in mouth was reached. The third step was to undergo the previous gel on a thermic treatment in an oven at 100[°C] during minimally one hour. At the end, a hygroscopic solid crystals which smelt and tasted very spicy and very nice in mouth was reached. Once recovered, it could be also characterized by its density, by its calcium content, by its citric acid content and eventually by its alkene content according to its pH-synthesis and its thermic treatment.

4.2. Gel and Crystal Calcium Salts of Raw Materials' Citric Acid Ester EDTA-0.01N Complexometric Titration Procedure – Calcium Concentration

Take approximately 0.25[g] and 0.05[g] weight samples (m_{ech}) of respectively gel and crystals calcium salts of raw materials' (*Capsicum chinense*'s) citric acid ester. Dissolve the samples in 10[ml] of distillated water. Add 20[ml] of pH-Tampon 10. Add three to five drops of the NET-color indicator. The solution to be titrated became "pink" or other colors in function of the samples color. In this case, the gel and crystals color was orange-yellow consequently the solution to be titrated became not clearly pink after addition of the NET-color indicator. Put the EDTA-0.01N into the oilcan and start the titration. Look carefully to the previous solution to be titrated color such as when its turned to blue or turned firstly to other color in function of the gel and crystals samples color, the EDTA-0.01N volume recorded on the oilcan corresponded to the volume on the equivalent point (V_e).

Thus, in the sample there was

$$n_{Ca^{2+}} = V_e \times C_{EDTA}(0.01[mol.l^{-1}]) \qquad (15)$$

And the Ca^{2+} weight concentration of the synthesized gel or crystals was

$$[Ca^{2+}]_{Gel/crystals}[mol.g^{-1}] = \frac{n_{Ca^{2+}}}{m_{ech}} \qquad (16)$$

The following table 6 showed the results of the gel and crystals calcium salts of *Capsicum chinense*'s citric acid ester EDTA-0.01N complexometric titration.

Table 6. *Calcium rate of gel and crystals calcium salts of Capsicum chinense's citric acid ester.*

	Crystals calcium salts	**Gel calcium salts**
Sample weight (m_{ech}) [g]	0.0504	0.2314
$n_{Ca^{2+}}$ $[mol.l^{-1}]$	1.13E-5	2E-5
$[Ca^{2+}]_{Gel/crystals}[mol.g^{-1}]$	2.2421E-4	8.6430E-5
Recorded Total salts synthesized [g] from 70[ml]	1.1515	2.8720
Evaluated Total moles of Ca^{2+} [moles] in 140[ml]	5.1636E-4	4.9645E-4
Equivalent Total $Ca(OH)_2$ weight [g]	3.8258E-2	3.6783E-2
Initial $Ca(OH)_2$ used for synthesis [g] (§4.1.)	0.0389	
Relative error ($\frac{\Delta C}{C}$)	0.02 (2[%])	0.05 (5[%])

4.3. The Rest of Equivalent-Citric Acid Molecules in the Synthesized Gel-Crystal Calcium Salts Titration with NaOH-0.05N Procedure

Once synthesized, the rest of citric acid molecules in synthesized crystals calcium salts of raw materials' (in this case the raw material was *Capsicum chinense)* citric acid ester could be titrated with NaOH-0.05N according the following procedure: first take approximately 0.05[g] or 0.25[g] of samples to be titrated respectively for a crystal salts or gel salts in a beaker 250[ml]. In this case of *Capsicum chinense's* calcium crystal salt the sample weight was 0.0507[g]. Wash the sample in 15[ml] of dichloromethane during one minute in order to extract the rest of citric acid molecules. Thus, add 15[ml] of distillated water and agitate the solution using a magnetic stirrer during one minute in order to solubilize the rest of citric acid and calcium which were very soluble in water. Then, let allow to settle into a settling bulb

for 15[mn] maximally the reached solution until having two phases: the aqueous phase on top and the organic phase at the bottom. Recover firstly the organic phase in a beaker just slightly before the aqueous phase, then secondly recover the remainder solution in another beaker-250[ml] without forgetting to record its pH and volume. Thus, add two or three drops of helianthine color indicator in this second beaker-250[ml] solution which turn to red or other color according to the raw materials' calcium gels or crystals colors. In this case of *Capsicum chinense* raw material, this solution was very slightly orange-yellow. Put the NaOH-0.05N titration solution in the oil can and start the titration. Look carefully to the previous solution to be titrated color such as when it turned to net orange-yellow or turned firstly to other color, the NaOH-0.05N volume recorded on the oilcan corresponded to the volume on the equivalent point (V_e). The results of this titration was shown on the following table 7. Noticed that the ratio (2.11 – Table 7) between the initial total reactant equivalent-citric acid moles on *Capsicum chinense*'s citric acid ester (b) and the total equivalent-citric acid moles of synthesized crystals calcium salts of *Capsicum chinense*'s citric acid ester (b) was equal to the molar ratio (in majority equal to 2) between esters organic functions and calcium atom used during crystals synthesis; this equality announced and confirmed that the great majority of the synthesized solid-crystals calcium salts of raw materials' (in this case *Capsicum chinense*'s) bioactive molecules from its citric acid esters solutions, following the established procedure on paragraph-4, had and retained the structures inventoried on figure 23 to figure 31.

Table 7. *Equivalent citric acid rate of crystals calcium salts of Capsicum chinense's citric acid ester.*

Equivalent-Citric acid molecules rate in synthesized crystals calcium salts of capsicum chinense's citric acid ester	
Sample weight [g]	0,0507
Sample total moles of citric acid [moles]	1.90E-05
Citric acid weight concentration [mol/g]	3.74E-4
Total synthesized crystals calcium salts of *Capsicum chinense's* citric acid ester weight [g] (Table 6)	2.8720
Total equivalent-citric acid moles of synthesized crystals calcium salts of *Capsicum chinense's* citric acid ester [mol] (a)	1.08E-3
Initial total reactant equivalent-citric acid moles on *Capsicum chinense's* citric acid ester [mol] (b)	2.28E-3
Rate of equivalent-citric acid moles on synthesized crystals calcium salts of *Capsicum chinense*'s citric acid ester [%]	47.37
(b)/(a) ratio	2.11

In the other words, these results ratio (2.11 – Table 7) allowed to affirm that the addition of distillated water having maximally ambient temperature during these rest of equivalent-citric acid molecules in synthesized gel-crystals calcium salts of raw materials' (*Capsicum chinense's*) citric acid ester NaOH-0.05N titration procedure corresponded to a mild hydrolysis of this raw material (*Capsicum chinense*'s) citric acid ester's gel-crystals calcium salts which included the hydrolysis of the esters obtained on figure 23 to the figure 31 without disturbing or breaking the -O-Ca-O- linked as well as the corresponding aromatic cycles. In general it corresponded to the inverse reaction of the figure 22 only the products were raw materials molecules (R^i), citric acid molecules and the –O-Ca-O- calcium cycles according to this following figure 32.

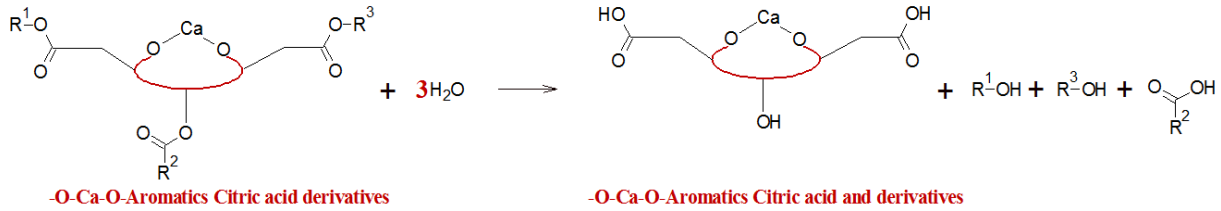

Figure 32. *Global reactions of the gel-solution and solid-crystals calcium citric acid salts of raw materials' (in this case Capsicum chinense's) bioactive molecules hydrolysis.*

4.4. The Equivalent-Alkene in the Synthesized Gel-Crystal Calcium Salts Titration with HF-0.0026N Procedure

Take and put approximately 0.3779 [g] sample of the synthesized gel-crystals calcium salts of raw materials' (*Capsicum chinense's*) citric acid ester in a beaker-250[ml]. Add 150[ml] of distillated water which was in excess with respect to the evaluated –O-R^i links (Table 5). Agitate in hand for 10 seconds and with a magnetic stirrer during one minute to hydrolyze (figure 32). Decant this solution into a settling bulb and let allow to settle for 15[mn] maximally. A slight white suspension was noticed. Then, add 100[ml] of hexane in the previous solution into the settling bulb followed by hand agitation for 10 seconds and by a magnetic stirrer during one minute. After a few decantation three phases were observed; then add again 60[ml] of hexane which in total molarly in excess with respect to the evaluated –O-R^i links (Table 5). After maximally 15[mn] of decantation, three phases were observed, and recovered separately without forgotten to record their volumes such as 155[ml], 15[ml] and 140[ml] of respectively the hexane-on top phase, the middle-white phase and the aqueous-bottom phase. Then, take exactly 1[ml] sample of the first and second solutions, which certainly contained alkenes organic functions from raw materials' (*Capsicum chinense's*) fatty-acids molecules, into two different beaker-250[ml]. Add in each beaker 15[ml] of distillated water and three drops of bromophenol-blue color indicator. The solution to be titrated turned to blue-purple. Put the HF-0.0026N titration solution into the oil can and titration could begin. When the solution color turned into

transparent-yellow, close the oil can and record the correspondent volume which correspond to the equivalent point volume. Deduce the moles of alkene organic function and their concentration in each recovered phases. The results of the alkenes organic function titrations were shown in the following table 8.

Table 8. *Equivalent alkene organic function rate of gel calcium salts of raw materials'-Capsicum chinense's citric acid ester.*

Phases	On top phase	Middle phase
Volume [ml]	155	15
Total alkene moles [mol]	3.12E-06	2.08E-06
Alkene concentrations [mol/l]	2.013E-05	1.39E-04

These results and the previous explanations on paragraph §-4.3 (figure 32) and paragraph §-4.4 allowed to affirm that first the alkenes organic function titrated on the on top phase belongs to the raw materials'-*Capsicum chinense's fatty-acids* (figure 32 – -R^2); but second the alkenes organic function titrated on the middle phase belongs to the first case of synthesized calcium citric acid aromatics with six (6) faces –O-Ca-O–aromatics citric acid (figure 23), these alkenes were certainly formed by their dehydration during the vaporization procedure (§-4.1) according the following mechanism on the figure 33. Noticed that normally the recovered on top phase could be analyzed by cpg-chromatography to detect the fatty-acids natures and structures.

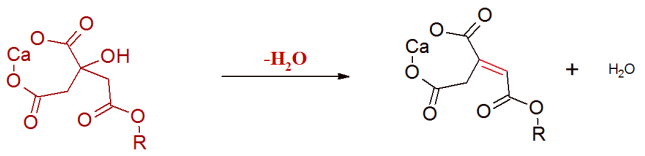

Figure 33. *First case of synthesized calcium citric acid aromatics with six (6) faces dehydration.*

4.5. Hplc-Analysis of the Raw Materials'- Capsicum Chinense'S Extracted Organic-Dichloromethane Phase and Their Derivatives (§-3.2.)

First, remind that once synthesized the raw materials'-*Capsicum chinense's* citric acid esters solutions was trans-esterified with methanol (§-3.1.). Second, this solution suffered an extraction procedure in order to extract molecules soluble in organic phases (hexane and dichloromethane) (§-3.2.) then their analysis by chromatography trying to confirm and eventually to identify their probable nature and their probable structure according bibliographies notes about the main molecules-components of the raw materials'- *Capsicum chinense*. In this case the raw material was the *Capsicum chinense* and its dichloromethane extracted molecules-components (capsaïcine-luteolin-quercetin) and their derivatives, reached after their citric acid's esters forms trans-esterified with methanol (§-3.2.), concentrated solution was analyzed with hplc-chromatography. The following table 9 shew the hplc experimental conditions of these analysis [24, 25].

Table 9. *hplc-chromatography analysis experimental conditions of Capsaicinoïdes, luteolin, quercetin and their derivatives.*

Bioactive molecules hplc – Analysis conditions	Capsaicinoïdes and derivatives	Luteolin and derivatives	Quercetin and derivatives
Temperature column-C18 [°C]	40	35	35
Eluent	water/acetonitrile (50/50) pH=2.49	methanol/0.1%orthophosphoric acid (65/35) pH=2.92	methanol/0.1%orthophosphoric acid (65/35) pH=2.92
Flow rate [ml/mn]	1	1	1
Wavelength [nm]	225	317	350
Analysis duration [s]	8,200	3,800	5,088

The following figure 34 showed the chromatogram of hplc-chromatography analysis, according the experimental conditions on table 8 (luteolin and derivatives), of luteolin and its derivatives extracted in the dichloromethane (§.3.2.) after methanol trans-esterification of raw material's-*Capsicum chinense's* citric acid ester solution (§.3. - §.3.1.). Thus, three main components were detected such as the [(OH) and/or CH_3O-quercetin-OH], the luteolin [luteolin (-C=O)], its derivative molecules including [(OH) and/or CH_3O-luteolin-OH] reached by the mechanisms on figure 15 and figure 16 and [CH_3O-luteolin] reached by luteolin transesterification with methanol by the global mechanism (figure 8) whose evaluated concentrations were respectively 2,5717[mol.l^{-1}], 0.4857[mol.l^{-1}], 53.42E-3 [mol.l^{-1}] and 11.51E-3[mol.l^{-1}].

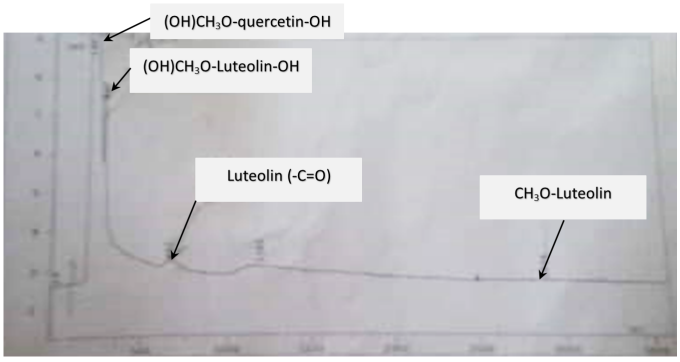

Figure 34. *hplc-chromatogram of luteolin and its derivatives extracted in the dichloromethane solvent after methanol trans-esterification of Capsicum chinense's citric acid ester solution.*

Then, the following figure 35 showed the chromatogram of hplc-chromatography analysis, according to the experimental conditions on table 8 (quercetin and derivatives), of quercetin and its derivatives extracted in the dichloromethane (§.3.2.) after methanol trans-esterification of raw material's-*Capsicum chinense's* citric acid ester solution (§.3. - §.3.1.). Like on the luteolin and derivatives hplc-chromatogram, a couple of peak was noticed for each detected molecules such as the first peak was their oxygenated-derivative and the second peak of the couple was the molecules (figure 35). Noticed that this experimental conditions on table 8 (quercetin and derivatives) allowed also to detect flavonoids which were represented here by the couple of flavonol and/or flavone 1-2-3-4-5-6-7 on the figure 35. And, the molecules trans-esterified molecules-forms (CH_3O-molecules) were detected at the end of the chromatogram (figure 35). The evaluated concentration of these molecules and their derivatives were respectively [(OH) and/or CH_3O-quercetin-OH]=1.10[mol.l^{-1}] / [quercetin (-C=O)]=0.63[mol.l^{-1}] / [(OH) and/or CH_3O-luteolin-OH]=0.18[mol.l^{-1}] / [luteolin (-C=O)]=0.05[mol.l^{-1}] / [(OH) and/or CH_3O- flavonol and/or flavone -OH]=0.27[mol.l^{-1}] / [flavonol and/or flavone (-C=O)]=0.28[mol.l^{-1}] / [CH_3O-quercetin]= 0.09[mol.l^{-1}] / [CH_3O- flavonol and/or flavone]= 0.50[mol.l^{-1}] / [CH_3O-luteolin]= 0.06[mol.l^{-1}].

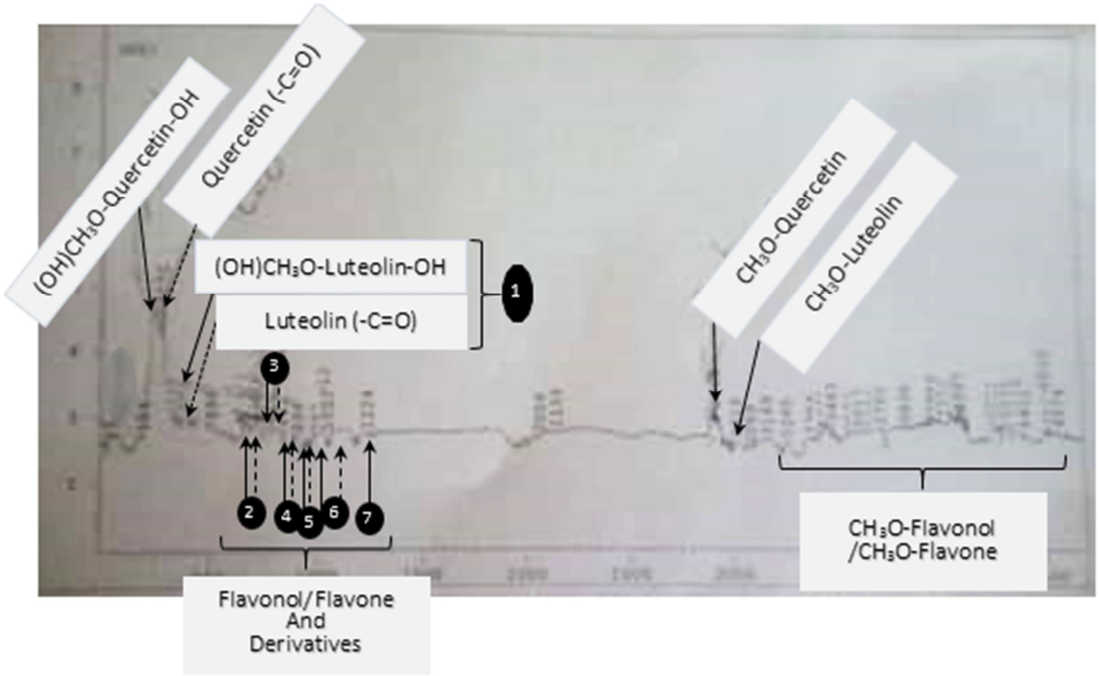

Figure 35. *hplc-chromatogram of quercetin and its derivatives with other flavonoids (flavonol and/or flavone) and their derivatives extracted in the dichloromethane solvent after methanol trans-esterification of Capsicum chinense's citric acid ester solution.*

And, the following figure 36 showed the chromatogram of hplc-chromatography analysis, according to the experimental conditions on table 8 (capsaicinoïdes and derivatives), of capsaicinoïdes and its derivatives extracted in the dichloromethane (§.3.2.) after methanol trans-esterification of raw material's-*Capsicum chinense's* citric acid ester solution (§.3. - §.3.1.). Like on the luteolin/quercetin and derivatives hplc-chromatogram, a couple of peak was generally noticed for each detected molecules such as the first peak was their oxygenated-derivative and the second peak of the couple was the molecules (figure 36). The evaluated concentration of these molecules and their derivatives were respectively [(OH) and/or CH_3O-capsaïcinoïdes-OH (figure 15-figure 16)]=2.08[mol.l^{-1}] / [Capsaïcine-OH/($C^{=}$) (figure 12-figure13)]=86.57E-3[mol.l^{-1}] – [Capsaïcine- OCH_3 /Capsaïcine-OH/($C^{=}$) (figure 8)]=79.61E-3[mol.l^{-1}] / [Homocapsaïcine-OH/($C^{=}$)-OCH_3 (figure12-figure13+figure8)]=33.51E-3[mol.l^{-1}]/[(Dihydro+Nordihydro)-capsaïcine]=174.07E-3[mol.l^{-1}]/[capsaïcine]=353.49E-3[mol.l^{-1}]/ [(Homo+Homodihydro)-capsaïcine]=2.25E-3[mol.l^{-1}] / [HO-Amine-OCH_3(OH)(figure19+figure15-16)]=24.9E-3[mol.l^{-1}] / [CH_3O-Amine-OCH_3(OH)(figure19+figure8+figure15-16)=50.14E-3[mol.l^{-1}] / [HO-Amine(Figure 19)]=18.91E-3[mol.l^{-1}] / [CH_3O-Amine /(OH)(figure 8)]=11.06E-3[mol.l^{-1}] / [4-(aminomethyl)-2-methoxyphenol]=74.86E-3[mol.l^{-1}] / [1-(3,4-dimethoxyphenyl)methanamine]=20.39E-3[mol.l^{-1}] / [Fatty acid-Ether cleavage-Quercetin (figure 21)] =11.39E-3[mol.l^{-1}] / [Fatty acid-Ether cleavage-Luteolin] (figure 21)= =31.87E-3[mol.l^{-1}] / [Ether(OCH_3)-Capsaïcinoïdes/(OH)(OCH_3)]=8.23E-3[mol.l^{-1}] /[Ether(OCH_3)-capsaïcine]=16.82E-3[mol.l^{-1}] /[Ether(OCH_3)-homocapsaïcine]=8.06E-3[mol.l^{-1}] /[Ether(OCH_3)-(Nordihydr+Dihidro)capsaïcine]=13.03E-3[mol.l^{-1}]

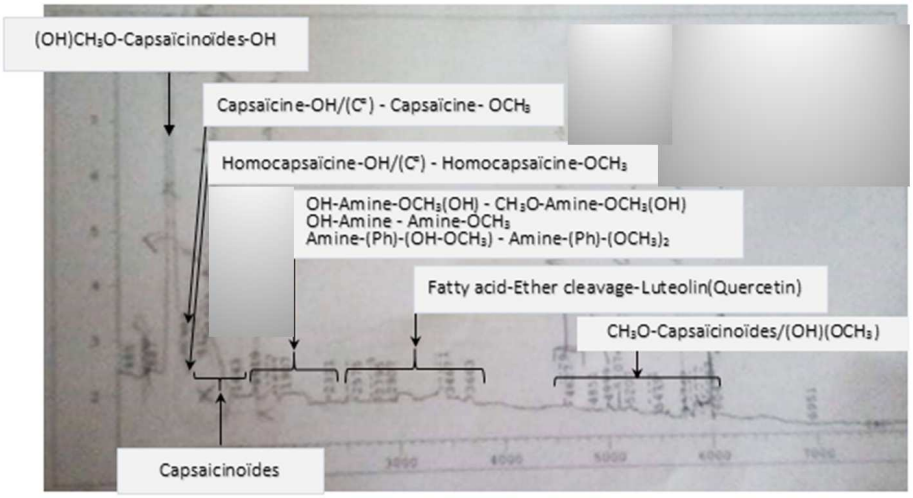

Figure 36. *hplc-chromatogram of capsaicinoïdes and its derivatives extracted in the dichloromethane solvent after methanol trans-esterification of Capsicum chinense's citric acid ester solution.*

5. Conclusion

The esterification between citric acid molecules and spicy-*Capsicum chinense*'s organic molecules in excess at 137°C-410.15°K was second order in comparison with citric acid. The established procedure of trans-esterification in an reflux assembly at 137°C-410.15°K between methanol and *Capsicum chinense*'s citric acid esters solutions followed by the established procedure of extraction using hexane and dichloromethane solvents allowed to bring to the fore the raw materials'-*Capsicum chinense's* main components including luteolin, capsaïcine, quercetin and their derivatives synthesized during this trans-esterification reaction where citric acid's protonic acid-H^+ sites functioned as catalyst. As shown in this manuscript, the products inventory of the raw materials' bioactive molecules esterification with citric acid followed by the products inventory of the methanol trans-esterification was necessary for each raw materials' study in order to determine the probably derivatives and exceptional derivatives products. In this case of study, among these exceptional derivatives were the raw materials'-*Capsicum chinense's* amine derivatives, the raw materials'-*Capsicum chinense's* ketone derivatives, those reached by the raw materials'-*Capsicum chinense's* bioactive molecules ether cleavage. Thus, the hplc analysis results bring to the fore not only the identification of peaks corresponding to these previous products but also the etherification of alkene function which quantities decreased after a while. In addition, the evaluated *Capsicum chinense* sample main components concentrations were capsaïcine-2.8426[mol.l^{-1}], quercetine-1.82[mol.l^{-1}] and luteolin-0.5506[mol.l^{-1}]. Then, it was confirmed that the synthesis of gels and crystals calcium salts procedure described on this manuscript was efficiency and allowed to synthesize an excellent gels then crystals which structure was stable and could be characterized by its calcium and the remains of citric acid molecules weight concentrations ratio approximately equals to 1/2 titrated respectively by EDTA-0.01N and NaOH-0.05N procedures. It was noticed that they still have the characteristics of the used raw material such as the gels and solid crystals of the *Capsicum chinense* smelt and tested very spicy and excellently nice in mouth.

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References

[1] Paulin Merix Raharilaza, Nambinina Richard Randriana, Andry Tahina Rabeharitsara – "Esterification Between Citric acid and Pumpkin Pips' Organic Molecules – Esters Hydrolysis And Esters Used as Hydrocarbons Additives" - *American Journal of Applied Chemistry. Vol. 6, No. 3, 2018, pp. 78-95.* doi: 10.11648/j.ajac.20180603.12

[2] Sedraniaina Domoina Marie Esperance, Ny Idealy Elite Randriamanantena, Raïssa Faneva Mampitefa, Nambinina Richard Randriana, Andry Tahina Rabeharitsara – "Esterification Between Citric Acid and Callistemon citrinus, Rice-Husk, Garcinia dulcis Catalysed by Citric Acid's-H^+-Monomers and Polymers Formation Mechanism" - *American Journal of Applied Chemistry. Vol. 8, No. 2, 2020, pp. 31-54.* doi: 10.11648/j.ajac.20200802.11

[3] Nambinina Richard Randriana, Ernestine Ravomialisoa, Andry Tahina Rabeharitsara – "Washing-Disinfectant Product Synthesis Tested During the Production of the "Speb-Pebe" - Spicy Product Energy Booster Characterized by Established Titration Procedures" - *American Journal of Applied Chemistry. Vol. 9, No. 3, 2021, pp. 53-64.* doi: 10.11648/j.ajac.20210903.11

[4] DJ Bennett and GW Kirby (1968) Constitution and biosynthesis of capsaicin. *Journal of Chemical Society, 442-446.*

[5] Département de l'Agriculture des États-Unis, «USDA Database for the Flavonoid Content of Selected Foods» [archive], sur http://www.nal.usda.gov [archive], *National Agricultural Library, 2003*

[6] Phenol Explorer INRA

[7] Lee Y., Howard L. R., Villalon B. (1995) Flavonoids and antioxidant activity of fresh pepper (Capsicum annuum) cultivars. *Journal of Food Science 60:473-476*

[8] Howard L. R., Talcott S. T., Brenes C. H., Villalon B. (2000) "Changes in phytochemical and antioxidant activity of selected pepper cultivars (Capsicum species) as influenced by maturity". *Journal of Agricultural and Food Chemistry 48:1713-1720 PubMed (10820084)*

[9] J. King, H. Wickes Felter, J. Uri Lloyd (1905) A King's American Dispensatory. *Eclectic Medical Publications* (ISBN 1888483024)

[10] *"Quercetin dihydrate safety sheet"*. Archived from *the original* on September 16, 2011.

[11] Chevreul, M. E. (1829). "30e Leçon, Chapitre XI. De la Gaude. [30th lesson. Chapter 11. On Weld (i.e., the plant Reseda luteola, which provides a yellow dye)]". *Leçons de Chimie Appliquée à la Teinture [Lessons on Chemistry Applied to Dyeing] (in French). Paris, France: Pichon et Didier. pp. 143–148.* Chevreul named luteolin on p. 144: "J'ai fait des recherches sur la composition de la gaude, j'ai obtenu le principe colorant critalisé par sublimation; je l'ai nommé lutéolin." (I have done some research on the composition of weld; I obtained the principal colorant [which I] crystallized via sublimation; I have called it "luteolin".)

[12] Thomson, Thomas (1838). *Chemistry of Organic Bodies. Vegetables. London, England: J. B. Baillière. pp. 415–416.*

[13] However, Perkin claimed (without citing a source) that Chevreul had isolated luteolin as early as 1814–1815. See: Perkin, Arthur George; Everest, Arthur Ernest (1918). *The Natural Organic Colouring Matters. London, England: Longmans, Green and Co. p. 4*

[14] Capsaïcine - Chemical Trading Guidchem – Hubei Xin Runde Chemical Co., Ltd - https://www.guidechem.com/trade/capsaicin-cas-404-86-4-id3562256.html

[15] Dihydrocapsaïcine - © 2015 ChemBK.com All Rights Reserved | Build: 20150530002 - https://www.chembk.com/en/chem/dihydrocapsaicin

[16] Mehdi Bagheri Farhoush Kiani Fardad Koohyar Nguyen Truong Khang Fatemeh Zabihi, Department of Chemistry, Faculty of Science, Ayatollah Amoli Branch, Islamic Azad University, Amol, Iran, Division of Computational Physics, Institute for Computational Science, Ton Duc Thang University, Ho Chi Minh City, Vietnam, Faculty of Applied Sciences, Ton Duc Thang University, Ho Chi Minh City, Vietnam, Department of Physics, Ayatollah Amoli Branch, Islamic Azad University, Amol, Iran: "Measurement of refractive index and viscosity for aqueous solution of sodium acetate, sodium carbonate, trisodium citrate, (glycerol + sodium acetate), (glycerol + sodium carbonate), and (glycerol + trisodium citrate) at T = 293.15 to 303.15 K and atmospheric pressure" - https://www.sciencedirect.com/science/article/abs/pii/S0167732219363135

[17] Andry Tahina Rabeharitsara, Behevitra Rovatahianjanahary, Nambinina Richard Randriana, «Pine Wood Powder Treatment to B_XH^+ Homogeneous Catalyst (H^+/H_2SO_4) Supported on Its Aromatics' and PNA' Alkenes – Application in Black Citric Acid Polymer Synthesis», *American Journal of Polymer Science and Technology. Vol. 4, No. 1, 2018, pp. 1-27.* doi: 10.11648/j.ajpst.20180401.11

[18] Behevitra Rovatahianjanahary - «Traitement De La Poudre Du Bois Du Pin Par De L'acide Sulfurique (H_2SO_4) Pour Obtenir Le Catalyseur Homogene BxH^+ Supporte Sur Les Alcenes Des Aromatiques Et Des Polynucleaires Aromatiques Oxygenes Composant Le Bois- Application Dans La Synthese De Polymere Noire D'acide Citrique» - *Mémoire De Fin d'Etudes En Vue De L'obtention Du Diplôme De Master Titre Ingénieur En Génie Des Procédés Chimiques Et Industriels* - Ecole Supérieure Polytechnique Antananarivo. Université d'Antananarivo – Promotion 2017.

[19] Phandry Nomena Ndjiva Rabearimihaja, Andry Tahina Rabeharitsara – "Betacarotenes Dosage by Hydrofluoric Acid Solution and Validation of This New Process by SPC". *American Journal of Applied Chemistry. Vol. 4, No. 3, 2016, pp. 91-96.* doi: 10.11648/j.ajac.20160403.14

[20] Rabearimihaja Phandry Taryh Nomena Ndjiva - «Valorisation Du Betacarotene Extrait De L'huile De Palme Brute Par De L'acide Acetique Pour La Determination Des Teneurs En Eau De Produits Petroliers (Gazole /Fuel-Oil) Et Dosage Des Teneurs En Betacarotene Par De L'acide Fluorhydrique» - *Mémoire de fin d'Etudes en vue de l'obtention du Diplôme de Master Titre Ingénieur en Génie des Procédés chimiques et Industriels* - Ecole Supérieure Polytechnique Antananarivo. Université d'Antananarivo – Promotion 2015.

[21] Nambinina Richard Randriana, Raherimandimby Jaochim, Andry Tahina Rabeharitsara: "Trans-Esterification Between Citric Acid and Peanut Oil at Low pH and Ambient Temperature Catalyzed by Citric Acid and Sulfuric Acid Protonic Acid-H^+", *American Journal of Applied Chemistry - Volume 8, Issue 4, August 2020, Pages: 100-116-* doi: 10.11648/j.ajac.20200804.12 - Chemical Process Engineering Department (E. S. P. A), Antananarivo University, Antananarivo, Madagascar.

[22] George F. Antonious "Capsaicinoids and Vitamins in Hot Pepper and Their Role in Disease Therapy"Submitted: January 16th 2018Reviewed: May 2nd 2018Published: November 5th 2018, DOI: 10.5772/intechopen.78243.

[23] Nambinina Richard Randriana, Ernestine Ravomialisoa, Andry Tahina Rabeharitsara, "Speb-Pebe" - Spicy Product Energy Booster Consumer Test and Its Effects on the Consumers' Health, *American Journal of Applied Chemistry*. Vol. 9, No. 6, 2021, pp. 186-201. doi: 10.11648/j.ajac.20210906.12

[24] Masao Bounashita - "HPLC Analysis of Capsaicin and Dihydrocapsaicin in Capsicum" - https://jascoinc.com/applications/analysis-capsaicin-dihydrocapsaicin/

[25] Roxana-Mădălina STOICA, Ovidiu POPA, Liliana-Claudia BLASS, Narcisa BĂBEANU – "Capsaicinoids Extraction From Several Capsicum Species Cultivated In Romania" - *Scientific Bulletin. Series F. Biotechnologies, Vol. XIX, 2015* ISSN 2285-1364, CD-ROM ISSN 2285-5521, ISSN Online 2285-1372, ISSN-L 2285-1364 University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăşti Blvd, District 1, 011464, Bucharest, Romania - Faculty of Biotechnologies.