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Kinetics of extraction of *Citrus aurantium* essential oil by hydrodistillation: influence on the yield and the chemical composition

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Abstract
In order to assess the kinetics of extraction *Citrus aurantium* leaves underwent hydrodistillation with a Clevenger apparatus. Essential oil was collected every 15 minutes during the whole extraction period. Yield variation during extraction period revealed an exponential aspect curve with 95% of maximum yield at 150 minutes. Sixty one compounds were identified during extraction time. Chromatographic analysis showed notable differences between the different petitgrain samples especially for the major components: linalool (29.74-62.43%), α-terpineol (3.04-15.69%) and linalyl acetate (5.64-25.38%). Important variations were observed in petitgrain chemical composition according to extraction time and each component presented a specific kinetic according to extraction time. These variations may be the expression of variable chemical and physical phenomena that take place in Clevenger hydrodistillation batch.

Keywords: *Citrus aurantium*, essential oil, hydrodistillation, chemical composition, GC-MS.

Introduction
Essential oil extracted from sour orange *Citrus aurantium* flowers is extensively used in fragrance, flavour industry and aromatherapy. Sour orange leaves essential oil called Petitgrain is less famous although it's ancestral use in Chinese and Tunisian folk medicine [1]. Hydrodistillation is the most widely utilized physical method for isolating essential oils from the vegetal material [2,3]. The vegetal material is immersed in water, which is heated to boiling point using an external heat source. The hot water draws out the oils just when steam does. Later on, the vapours are allowed to condense and the essential oil is then separated from the aqueous phase [4,5]. Essential oils are the volatile organic constituents of fragrant plant matter. They are generally composed of a number of compounds with different chemical and physical properties. The aroma profile of the oil is a cumulative contribution from the individual compounds. The boiling points of most of these compounds ranged from 150 to 300°C at atmospheric pressure. If heated to this temperature, labile substances would be destroyed and strong resinification would occur. Hydrodistillation permits the safe recovery of these heat sensitive compounds from the plant matter [5,6]. The combination of different compounds determines essential oil chemical composition. Extraction procedure influences this chemical composition and thus food conservation quality when essential oil is used as a food additive [7]. Little is known about the impact of physical and chemical reactions that take place within the aqueous medium in presence of the vegetal material on the obtained essential oil. The aim of this study is to evaluate the chemical differences occurring on *C. aurantium* leaf essential oil composition according to extraction time during hydrodistillation.

2. Materials and methods
2.1. Chemicals
All chemicals used were of analytical reagent grade. All reagents were purchased from Sigma-Aldrich-Fluka (Saint-Quentin France).

2.2.Plant material
The plant materials used in this study were collected from a standard orchard plantation of *C. aurantium* L. ssp *aurantium* directed by Commissariat Régional du Développement Agricole in Nabel (Longitude 36°45'00'' North, Latitude 10°45'00'' East, Altitude 0 m), Tunisia. Fresh leaves were gathered during November 2008. They were dried in a shady place at room temperature until stable weight.

2.3. Isolation of essential oil

Six hundred grams of *C. aurantium* ground dried leaves were submitted to hydrodistillation with a Clevenger-type apparatus according to European Pharmacopoeia, and extracted with 3 liters of water for 3 hours. Every 15 minutes the obtained hydrolate and essential oil was collected than essential oil was separated and dried over anhydrous sodium sulfate and preserved in sealed dark vials at -20°C until further analysis. Yield was determined by weighting the obtained essential oil at each point.

2.4. Gas Chromatography and gas Chromatography-Mass Spectrometry

Quantitative and qualitative analysis of the essential oil was carried out by gas chromatography-flame ionization detection (GC-FID) and gas chromatography-mass spectrometry (GC-MS). Gas chromatography analyses were carried out on a Varian Star 3400 (Les Ulis, France) Cx chromatograph fitted with a fused silica capillary DB-5MS column (5% phenylmethylpolysyloxane, 30 m x 0.25 mm, film thickness 0.25 µm). Chromatographic conditions were 60°C to 260°C temperature rise with a gradient of 5°C/min and 15 minutes isotherm at 260°C. A second gradient was applied to 340°C at 40°C/min. Total analysis time was 57 minutes.

For analysis, essential oil was dissolved in petroleum ether. One microliter of sample was injected in the split mode ratio of 1:10. Helium (purity 99.999%) was used as carrier gas at 1 mL/min. The injector was operated at 200°C. The mass spectrometer (Varian Saturn GC/MS/MS 4D) was adjusted for an emission current of 10 µA and electron multiplier voltage between 1400 and 1500 V. Trap temperature was 220°C and that of the transfer line was 250°C. Mass scanning was from 40 to 650 amu.

Compounds were identified by comparison of their KI (rentention indices) relative to C5-C24 *n*-alkanes obtained on a nonpolar DB-5MS column, with those provided in the literature, by comparison of their mass spectra with those recorded in NIST 08 (National Institute of Standards and Technology) and reported in published articles and by co-injection of available reference compounds (α-pinene (98%, Aldrich); p-cymene (99%, Aldrich); limonene (≥99.0%, Fluka); 1,8-cineole (99%, Aldrich); γ-terpinene (97%, Aldrich); α-terpinolene (≥95.0%, Aldrich); fenchol (≥99.0%, Fluka); borneol (97%, Aldrich); myrtenal (98%, Aldrich); myrtenol (≥95.0%, Aldrich); verbenone (94%, Aldrich); pulegone (≥98.5%, Fluka); cuminaldehyde (98%, Aldrich); p-cymen-7-ol (97%, Aldrich); globulol (≥98.5%, Aldrich); guaiol (97%, Aldrich); β-eudesmol (>90%, Sigma). The samples were analyzed in duplicate.

The percentage composition of the essential oil was computed by the normalization method from the GC peak areas, assuming identical mass response factor for all compounds. Results were calculated as mean values of two injections from essential oil, without using correction factors. All determinations were performed in triplicate and averaged.

3. Results and discussion

3.1. Yield and extraction kinetic

The first droplet fall occurred 30 minutes after the beginning of the experience. This duration was the required period to reach the boiling point of water (100°C, if the operation is performed at atmospheric pressure). The timing for the kinetic started at the first droplet. Figure 1 shows the variation of the extraction yield according to the extraction time. The obtained figure has a logarithmic aspect with three phases. The first step ends at 30 min when approximately 64% of the yield is obtained. The second step is characterized by a lower slope leading to 95% of the yield into 90 min. The last step is represented by an almost horizontal line marking the end of the extraction process. To the best of our knowledge, no previous study concerning *Citrus* kinetics of extraction by hydrodistillation is reported in the literature. The obtained kinetic is similar to the one reported by Bousbia et al.[8] during *Rosmarinus officinalis* leaves hydrodistillation. However, some differences were noted: in fact after 30 min of extraction 64% of the yield was recovered for *C. aurantium* leaves whereas 93 min were needed to reach 60% of the yield for *R. officinalis* ones. Moreover, the end of the extraction process required 180 min for *R. officinalis* while only 90 min were needed for *C. aurantium*. It comes out that essential oil recovery of *C. aurantium* hydrodistillation is faster than *R. officinalis* one. Within 15 minutes of extraction 35% of the final extraction yield was recovered, this fact may be due to the presence of essential oils glands at the surface of the leaves. Indeed, the external layer which is the only barrier that imprisons essential oil is easily broken when boiling point is reached [9]. The mixture water and vegetal material is the siege of a variety of transfer phenomena (figure 2) leading to essential oil extraction. The first process (1) corresponds to boiling water mass transfer into vegetal cells. This water diffuses (2) in internal milieu enabling the dissolution of essential oil molecules among other vegetal cell components, this water-oil solution permeates by osmosis (3) and finally reaches the outer surface where the oil is vaporized by passing stream and driven in azeotropic mixture (4) [10,11]. The vapor mixture of water and oil is submitted to hydro diffusion. The speed of oil vaporization is not influenced by the volatility of the oil components but by their degree of solubility in water indeed, when a molecule is hydro soluble it sticks to the water one and diffuses into gas phase but when not, it will remain in essential oils glands.

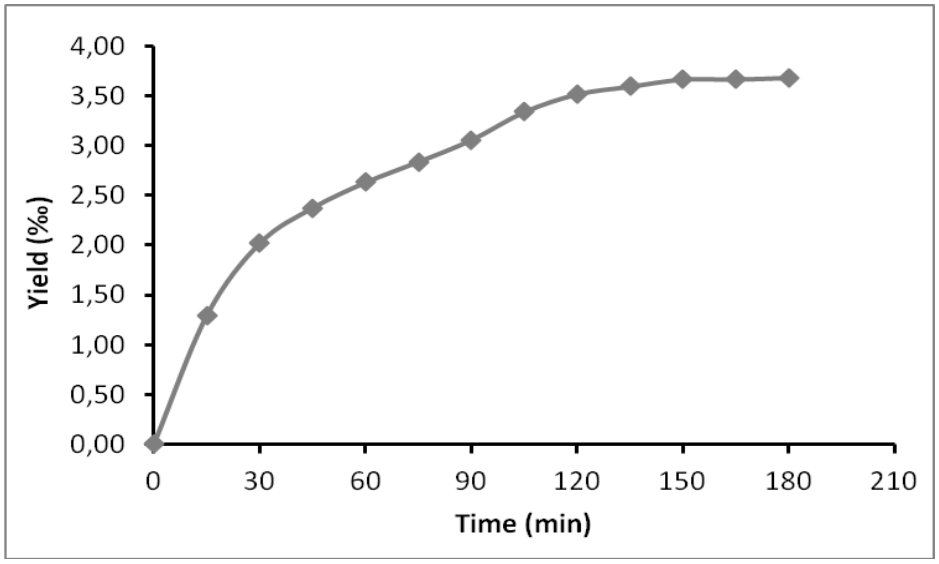

Figure 1: Variation of *C. aurantium* leaf essential oils yields according to extraction time.

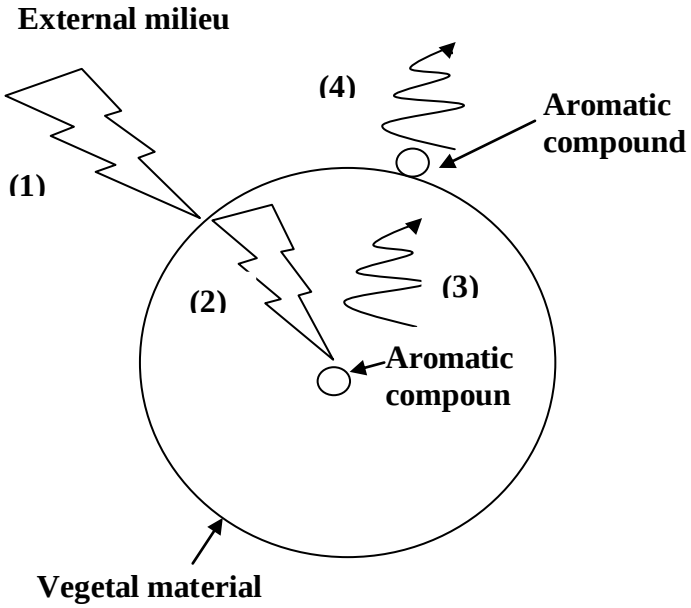

Figure 2: Transfer phenomena taking place in the mixture vegetal material and water during hydrodistillation.

3.1. Chemical composition and kinetic evolution

3.1.1. Chemical composition

Sixty one components were identified for all obtained essential oils during hydrodistillation of *C. aurantium* leaves (Table 1). Major components were linalool (62.57 – 22.35%), linalyl acetate (25.38 – 5.64%) and α-terpineol (3.04 – 15.69%). Previous studies on sour orange leaves essential oil all over the world identified that the dominant chemotype of *C. aurantium* is linalool/linalyl acetate one [12-16]. Their main component was mostly linalyl acetate (36.8-73.1%) whereas for our results it was linalool (22.35-62.43%). The Italian previously reported petitgrain essential oil was characterized by higher amounts of linalyl acetate 0.3-73.1%, 50.68-62.57% and 50.68-62.57% and lower quantities of linalool 8.7-16.7%, 21.70-32.55% and 21.70-32.55% respectively De Pasquale et al. [15], Dugo et al. [12] and Mondello et al. [13]. The noticed differences between Tunisian and Italian petitgrain may be attributed to geographical influence on EO chemical composition. Eleven components were identified for the first time in our EOs, namely trans-isolimonene, sylvestrene, lavandulol, Z-β-farnesene, trans-isolongiflanone, 14-hydroxy-9-epi-(E)-caryophyllene, Z-α-trans-bergamotol, cis-β-santalol, phytone, E-E-farnesyl acetone and Z-phytol.

Table 1: Variation of chemical composition (%) of leaf *C. aurantium* essential oils during hydrodisillation.

			Extraction time (min)										
N°	Compounds	RI	15	30	45	60	75	90	120	135	150	165	180
1	**α-pinene**	**928**	**0.02**	**-**	**0.02**	**0.06**	**0.02**	**-**	**0.02**	**0.04**	**-**	**0.03**	**1.15**
2	**β-pinene**	**962**	**-**	**0.21**	**0.01**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
3	**Trans-isolimonene**	**968**	**0.20**	**-**	**0.18**	**0.02**	**0.12**	**-**	**-**	**-**	**-**	**-**	**-**
4	**Sabinene**	**972**	**-**	**-**	**-**	**-**	**-**	**-**	**0.11**	**0.10**	**0.18**	**0.05**	**0.68**
5	**Myrcene**	**981**	**0.04**	**-**	**0.02**	**0.42**	**0.02**	**-**	**0.24**	**0.16**	**0.35**	**0.09**	**1.57**
6	**Decane**	**989**	**0.04**	**-**	**0.04**	**0.02**	**0.02**	**-**	**0.03**	**-**	**-**	**0.02**	**0.88**
7	**Pseudolimonene**	**999**	**-**	**-**	**-**	**0.03**	**-**	**-**	**-**	**0.05**	**-**	**0.04**	**1.32**
8	**α-phellandrene**	**1006**	**0.07**	**-**	**0.02**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
9	**Isobutylbenzene***	**1014**	**-**	**-**	**0.02**	**0.02**	**0.02**	**-**	**-**	**-**	**-**	**-**	**-**
10	**o-cymene**	**1018**	**2.78**	**1.56**	**1.01**	**0.01**	**0.55**	**0.17**	**0.02**	**-**	**-**	**0.03**	**0.82**
11	**α-terpinene**	**1022**	**0.10**	**0.20**	**0.17**	**0.87**	**0.13**	**-**	**0.32**	**0.42**	**0.56**	**0.22**	**4.20**
12	**eucalyptol**	**1026**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**0.05**	**-**	**0.05**	**0.78**
13	**sylvestrene**	**1028**	**-**	**-**	**-**	**0.22**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
14	**limonene**	**1036**	**0.16**	**0.42**	**0.39**	**-**	**0.28**	**-**	**-**	**-**	**-**	**-**	**-**
15	**Cis-β-ocymene**	**1040**	**-**	**-**	**-**	**0.35**	**-**	**-**	**0.07**	**0.11**	**0.24**	**0.07**	**0.50**
16	**Trans-β-ocymene**	**1048**	**-**	**-**	**0.03**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
17	**Cis-linalool oxide**	**1067**	**0.09**	**-**	**-**	**0.20**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
18	**Trans-linalool oxide**	**1084**	**0.17**	**0.37**	**0.38**	**0.23**	**0.30**	**-**	**-**	**0.15**	**0.34**	**0.11**	**0.53**
19	**linalool**	**1096**	**56.65**	**59.24**	**55.14**	**62.43**	**51.34**	**44.55**	**36.79**	**28.14**	**36.17**	**22.35**	**29.74**
20	**lavandulol**	**1166**	**0.05**	**0.08**	**0.08**	**-**	**0.13**	**-**	**-**	**0.03**	**-**	**0.03**	**-**
21	**Terpinen-4-ol**	**1175**	**0.10**	**0.06**	**-**	**0.09**	**0.05**	**-**	**0.07**	**0.06**	**-**	**0.06**	**0.15**
22	**α-terpineol**	**1188**	**3.04**	**-**	**9.43**	**10.47**	**-**	**11.80**	**13.42**	**11.51**	**15.69**	**12.74**	**14.87**
23	**E-dihydrocarvone**	**1201**	**-**	**-**	**-**	**-**	**-**	**-**	**0.06**	**-**	**-**	**-**	**-**
24	**nerol**	**1223**	**0.25**	**0.59**	**0.75**	**-**	**1.97**	**-**	**2.52**	**2.18**	**2.26**	**2.36**	**1.17**
25	**Linalyl acetate**	**1251**	**25.38**	**24.81**	**16.23**	**16.07**	**16.90**	**10.12**	**10.04**	**8.65**	**8.20**	**8.17**	**5.64**
26	**2,6-Dimethoxy-1-methylbenzene**	**1266**	**-**	**-**	**-**	**-**	**-**	**-**	**0.08**	**-**	**-**	**-**	**-**
27	**2-methylnaphtalene**	**1286**	**0.08**	**-**	**0.08**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
28	**Dihydrocarveol acetate**	**1344**	**0.16**	**0.16**	**0.15**	**0.12**	**0.22**	**-**	**0.07**	**-**	**-**	**-**	**-**
29	**2,6-dimethoxyphenol**	**1351**	**0.46**	**1.11**	**1.20**	**-**	**2.10**	**-**	**-**	**-**	**-**	**-**	**-**
30	**α-cyclogeraniol**	**1357**	**-**	**-**	**-**	**1.69**	**-**	**-**	**0.96**	**0.65**	**1.08**	**0.56**	**1.17**
31	**Geranyl acetate**	**1376**	**0.83**	**2.20**	**2.40**	**-**	**4.51**	**1.23**	**1.86**	**1.27**	**1.91**	**0.92**	**2.20**
32	**β-caryophyllene**	**1419**	**1.97**	**2.75**	**2.23**	**1.11**	**2.65**	**-**	**0.62**	**0.77**	**1.55**	**0.63**	**4.52**
33	**α-ionone**	**1424**	**-**	**-**	**-**	**-**	**-**	**-**	**0.08**	**-**	**-**	**0.05**	**-**
34	**Z-β-farnesene**	**1445**	**0.21**	**0.31**	**0.36**	**-**	**0.49**	**-**	**0.09**	**0.10**	**-**	**-**	**-**
35	**α-caryophyllene**	**1453**	**-**	**-**	**-**	**0.31**	**-**	**-**	**0.07**	**0.10**	**0.30**	**0.08**	**-**
36	**β-ionone**	**1483**	**-**	**0.45**	**-**	**-**	**-**	**-**	**0.16**	**0.12**	**-**	**0.10**	**-**
37	**α-zingiberene**	**1496**	**0.16**	**-**	**0.52**	**0.38**	**1.02**	**0.18**	**0.22**	**0.27**	**0.48**	**0.23**	**0.92**
38	**2,6-dibutyl-4me-phenol**	**1508**	**0.11**	**0.26**	**0.27**	**-**	**-**	**-**	**-**	**0.15**	**-**	**0.16**	**4.89**
39	**δ-cadinene**	**1521**	**-**	**-**	**-**	**0.28**	**0.64**	**-**	**0.25**	**0.34**	**0.24**	**0.36**	**0.47**
40	**8,14-cedranoxide**	**1537**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**0.10**	**-**	**-**	**-**
41	**α-calacorene**	**1553**	**-**	**0.27**	**-**	**-**	**1.03**	**-**	**-**	**-**	**0.95**	**-**	**0.43**
42	**Germacrene B**	**1558**	**-**	**-**	**-**	**-**	**9.37**	**4.23**	**3.01**	**3.93**	**3.23**	**5.15**	**1.25**
43	**spathulenol**	**1571**	**1.60**	**3.07**	**2.00**	**-**	**-**	**-**	**-**	**0.06**	**-**	**-**	**-**
44	**Caryophyllene oxide**	**1585**	**-**	**-**	**1.50**	**-**	**2.04**	**21.96**	**16.36**	**20.73**	**17.30**	**27.04**	**8.63**
45	**β-oplopenone**	**1588**	**-**	**-**	**-**	**1.16**	**-**	**-**	**3.51**	**4.65**	**2.42**	**-**	**-**
46	**viridiflorol**	**1590**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**5.02**	**-**	**3.97**	**-**
47	**widdrol**	**1597**	**-**	**-**	**-**	**-**	**-**	**-**	**0.18**	**0.25**	**0.17**	**0.32**	**-**
48	**Cis-isolongifolanone**	**1604**	**-**	**-**	**-**	**-**	**-**	**-**	**0.31**	**0.11**	**-**	**-**	**-**
49	**1,2-epoxyde-humulène**	**1609**	**-**	**-**	**-**	**-**	**0.24**	**-**	**-**	**0.42**	**0.21**	**0.54**	**-**
50	**Trans-isolongifolanone**	**1617**	**-**	**-**	**-**	**0.14**	**0.32**	**0.51**	**0.42**	**0.95**	**0.33**	**0.11**	**-**
51	**cedrenol**	**1636**	**-**	**-**	**-**	**0.13**	**0.65**	**1.17**	**0.81**	**1.10**	**1.20**	**1.58**	**-**
52	**14-hydroxy-1-epi-caryophyllene**	**1647**	**-**	**-**	**-**	**0.33**	**-**	**2.73**	**1.85**	**2.44**	**0.18**	**3.47**	**1.03**
53	**14-hydroxy-9-epi-(E)-caryophyllene**	**1654**	**-**	**-**	**-**	**-**	**0.25**	**-**	**0.34**	**0.34**	**0.39**	**-**	**-**
54	**1-(2,3,4,5-tetramethylphenyl)-butan-1-one***	**1664**	**-**	**-**	**-**	**0.12**	**-**	**-**	**0.87**	**1.18**	**1.44**	**1.80**	**0.70**
55	**8-cedren-13-ol**	**1677**	**-**	**-**	**-**	**-**	**0.18**	**-**	**0.17**	**-**	**-**	**-**	**-**
56	**Cis-α-santalol**	**1683**	**-**	**-**	**-**	**-**	**-**	**-**	**0.64**	**0.88**	**1.02**	**1.37**	**0.60**
57	**Z-α-trans-bergamotol**	**1700**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**0.25**	**0.14**	**-**	**-**
58	**Cis-β-santalol**	**1718**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**0.15**	**-**	**-**	**-**
59	**Phytone**	**1849**	**-**	**-**	**-**	**-**	**-**	**0.24**	**0.16**	**0.24**	**0.31**	**0.38**	**0.29**
60	**E,E-farnesyl acetone**	**1926**	**-**	**-**	**-**	**-**	**-**	**0.15**	**-**	**0.15**	**0.20**	**0.28**	**0.50**
61	**Z-phytol**	**2117**	**-**	**-**	**-**	**-**	**-**	**0.38**	**0.25**	**0.34**	**0.50**	**0.69**	**6.50**
	Total		**94.72**	**98.10**	**94.63**	**97.30**	**97.60**	**99.40**	**96.90**	**98.90**	**99.50**	**97.20**	**98.10**

**** Tentatively identified according to the mass spectrum and by comparison of KI with the literature. "-" not detected.***

3.1.2. Kinetic evolution

During the first (0-30 min) and second (30-90 min) step an average of 22 and 25 components were identified respectively whereas for the last step (90-180 min) 36 ones were detected. In fact, a variety of components were noticed only in essential oils obtained during the last step such as eucalyptol, cis-β-ocimene, germacrene B,… these components are particularly heavy and eluted at high retention time. An important variation was noted for aromatic profiles according to the extraction time (Figure 3). Indeed, for the major component (linalool), had an hyperbolic form: a first period was characterized by an increase reaching a maximum of 62.43% corresponding to 199.78 mg/Kg dry matter at 60 min; later on an important decrease was registered at 180 min linalool amount was 29.74% corresponding to 124.91 mg/Kg dry matter). An important decrease was noted for linalyl acetate (from 24.81 to 5.64% corresponding to 62.03 and 23.69 mg/Kg dry matter at respectively 30 and 180 min), whereas for α-terpineol an important increase was detected from 3.04 to 15.69% corresponding to 5.47 and 62.45 mg/Kg dry matter at respectively 15 and 180 min. During the last step of the kinetic, an important increase in caryophyllene oxide was registered: 16.36 to 27.04% corresponding to 65.44 and 113.57 mg/Kg dry matter at respectively 120 and 180 min. It seemed that with increasing yield, amounts of main components (expressed in mg/Kg dry matter) stay unchanged although their percentage vary, indeed at 180 min linalool represents 28.14% of total components of essential oil corresponding to 124.91 mg/Kg dry matter while at 15 min it represents 56.65% corresponding to 101.97 mg/Kg dry matter .This fact may be caused by extraction of new compounds.

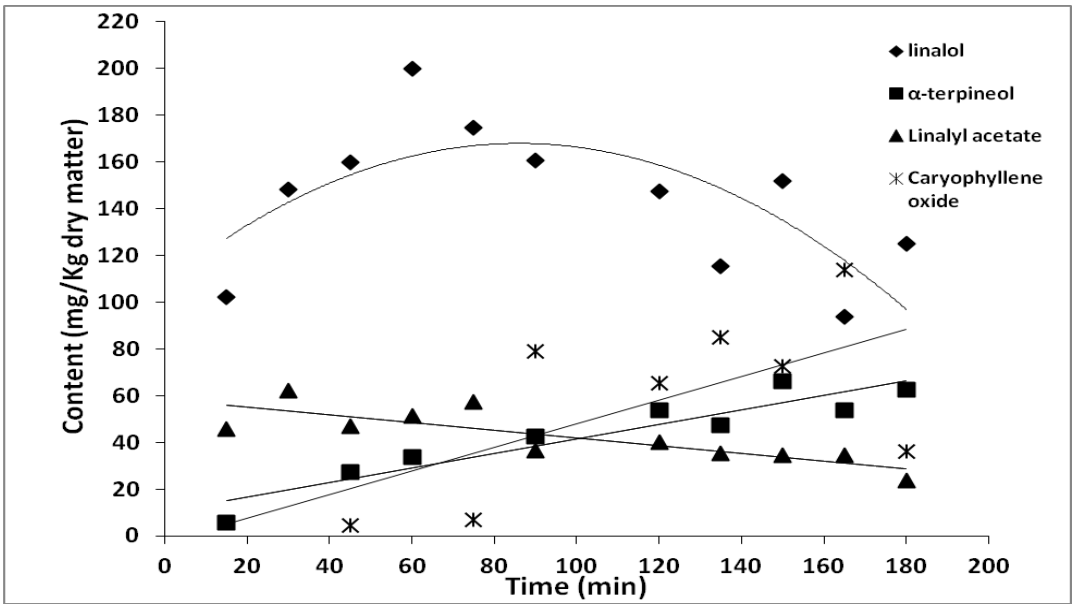

Figure 3: Variation of linalol, linalyl acetate, caryophyllene oxide and α-terpineol amounts according to extraction time.

Moreover, enrichment in oxygenated component (monoterpene + sesquiterpene) was noticed: 90.13% at the beginning of extraction while at 60 min 94.23% was reported corresponding respectively to 162.23 and 293.26 mg/Kg dry matter. This enrichment was followed by a great loss of these compounds expressed by 77.52% of oxygenated components at 180 min corresponding to 55.89 mg/Kg dry matter. The gain in oxygenated component may be due to the hydro diffusion that took place during hydrodistillation and is always highly related to aromatic component's water solubility [17]. Hydro diffusion is defined by Von Rechenber [18] as the transfer phenomenon that combines the effects of diffusion to that of water presence. Many other molecule characteristics interfere in hydro diffusion and their extraction order; especially their molar volume (sesquiterpene < monoterpene) and their diffusivity (hydrocarbon < polar component) [17]. If hydro diffusion was the restricting step of hydrodistillation, the order of release of different components would be induced by their polarity and not their volatility. In the case of components with intermediate polarity such as monoterpenic esters neither hydro diffusion nor vaporization rate are to neglect.

During hydrodistillation a decrease of monterpenes (89.93 to 47.32% at respectively 15 and 165 min) was observed while sesquiterpenes amounts increased (Table 2). While looking at their weight, we notice that monoterpene amounts increased from 162.23 to 293.26 mg/Kg dry matter at 15 and 60 min respectively. Simultaneously sesquiterpenes amounts rose up to 193.62 mg/Kg dry matter at 165 min. Obviously, new compounds that were eluted at later periods of hydrodistillation make percentages of the previous ones fewer. This

is why the firstly eluted compounds during hydrodistillation decrease in percentage and raise up in mass. On the other hand, it is important to take in account the set of chemical phenomena that took place within aqueous media during hydrodistillation. In fact, the aqueous media pH ranged between 4 and 7. Throughout longer distillation process, the acidity of the distillation water also influences the composition of the obtained oil. The acid medium enables various chemical conversions (hydrolysis, cyclization…) to occur on native essence components [10]. Monoterpenes, oxygenated monoterpenes and oxygenated sesquiterpens are the most attacked components [19]. This hypothesis can explain the noticed variation of monoterpenes hydrocarbon for our results (Table 2). In vapor phase, co-distillation took place; this phenomenon is highly related to components volatility that depends on their physico-chemical properties [6,20]. Therefore, components order of release determines treatment duration especially when cohobation is applied. Cohobation uses the practice of returning the distillate water to the still after the oil has been separated from it so that it can be re-boiled. The principal behind it is to minimize the losses of oxygenated components, particularly phenols which dissolve to some extent in the distillate water. This may explain the highest amount of phenols for the last extracted sample (4.89%) at 180 min and the increase of oxygenated sesquiterpenes during extraction time both in amounts and percentage. As this material is being constantly re-vaporized, condensed and re-vaporized again, any dissolved oxygenated constituents will promote hydrolysis and degradation of themselves or other oil constituents. Similarly, if any oxygenated component is constantly brought in contact with a direct heat source or side of a still, then the chances of degradation are enhanced. However, essential oil components evolution according to extraction time is tightly related to decantation that depends on the speed of transfer between two liquid phases. Indeed, the solubility of each component in water determines how much time it requires to separate him from distillate water [21]. However, leaves in this study submitted a grinding, parameter that would enhance volatility as a separation determinant factor for components recovering [6].

Table 2: Variations of chemical families (%) during leaf *C. aurantium* essential oil hydrodistillation**.**

	Extraction Time (min)										
	15	30	45	60	75	90	120	135	150	165	180
Monoterpene hydrocarbons	**3.37**	**2.39**	**1.85**	**1.99**	**1.13**	**0.17**	**0.78**	**0.88**	**1.33**	**0.52**	**10.24**
Monoterpene oxygenated	**86.56**	**87.34**	**84.41**	**89.50**	**45.27**	**67.69**	**64.76**	**52.05**	**64.57**	**46.80**	**55.08**
Sesquiterpene hydrocarbons	**0.37**	**0.58**	**0.88**	**0.80**	**12.88**	**4.92**	**4.31**	**5.79**	**5.22**	**6.84**	**3.07**
Sesquiterpene oxygenated	**3.57**	**5.82**	**5.73**	**4.73**	**6.01**	**26.24**	**25.60**	**38.32**	**26.44**	**40.26**	**22.44**
Phenolics	**0.57**	**1.37**	**1.47**	**0.00**	**2.10**	**0.00**	**0.00**	**0.15**	**0.00**	**0.16**	**4.89**
Others	**0.28**	**0.60**	**0.29**	**0.28**	**0.26**	**0.38**	**1.45**	**1.70**	**1.94**	**2.63**	**2.37**

The instant t=75 min seemed to be determinant to a wide range of compounds: linalyl acetate, geranyl acetate, limonene, α-terpineol, α-cyclogeraniol,... (Table 1). In fact, for linalyl acetate a variable increase in its amounts is registered until t=75 min, beyond this instant these values became stable around 3.3 mg/Kg dry matter. While an important slope characterizes the increase of geranyl acetate values to a maximum at t=75 min, an important fluctuation is registered after this time. Limonene kinetic highlighted a total disappear beyond t=75 min whereas α-terpineol amount rose up considerably beyond this moment. α-terpineol may be produced from limonene as reported by Robles-Dutenhefner et al. [22], this chemical reaction (Figure 4a) is favored in acid medium especially with long treatment periods that induce more important quantities of α-terpineol.

Nerol kinetic presented a constant increase according to treatment period until t=180 min when it showed a diminution. Furthermore, α-cyclogeraniol is detected at t=60 min exactly when nerol was not, later this variation showed important fluctuations. It is possible that those variations were caused by the chemical reaction in Figure 4b. Linares-Palomino et al. [23] underlined variable chemical reactions starting with nerol, the one that leads to α-cyclogeraniol is highly dependent of milieu hydration and his pH. Both phytone an E-E-farnesyl acetone appeared at t=90 min and presented similar variations according to treatment time. But at t=180 min a more important quantity of E-E-farnesyl acetone was registered, this fact may be induced by the chemical reaction presented by Figure 4c. This reaction is enhanced by aqueous medium but was not reported for EOs obtained by hydrodistillation.

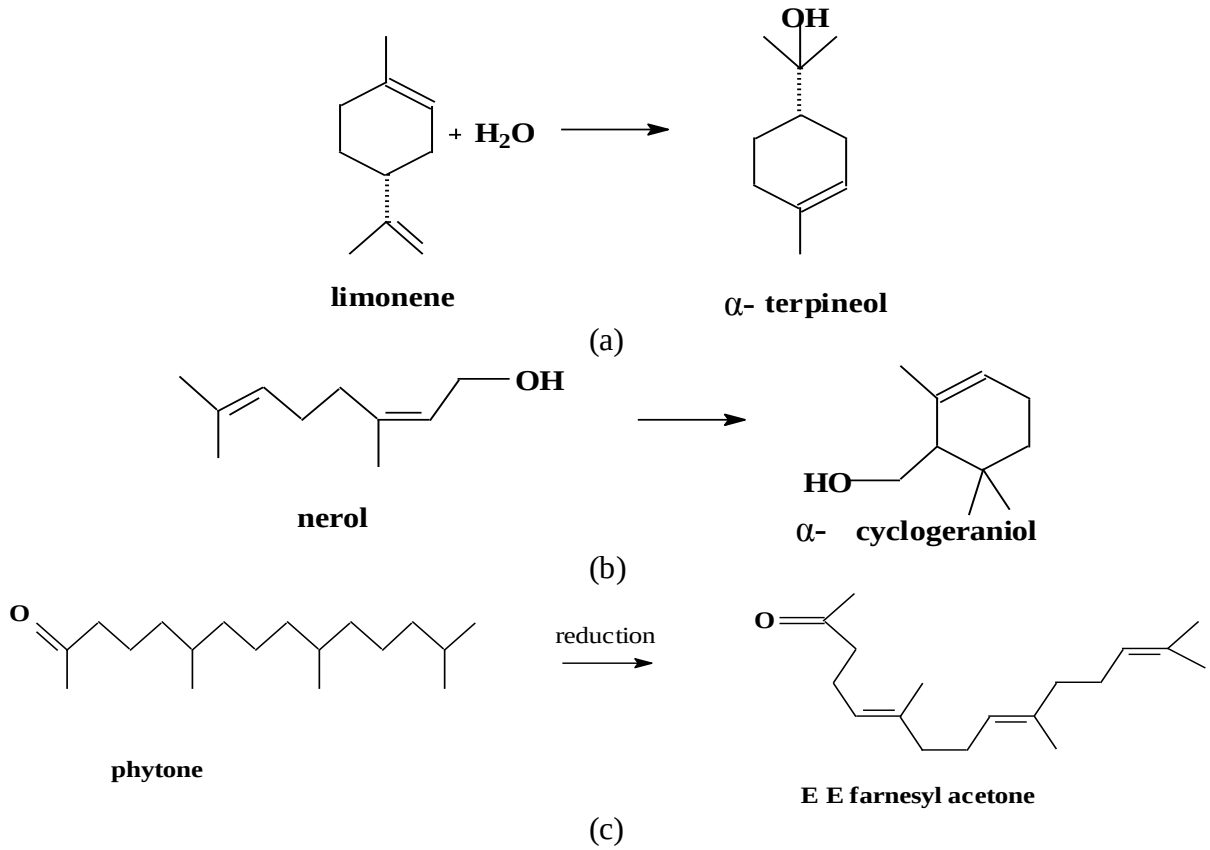

Figure 4: Chemical reaction leading to: α-terpineol from limonene (a), α-cyclogeraniol from nerol (b) and E-E-farnesyl acetone from phytone (c).

Conclusion

The yield of essential oil according to extraction time presented a kinetic with 3 phases. The most important yield was obtained during the first one. Chromatographic analysis allowed the identification of 61 components during the hydrodistillation of *C. aurantium* leaves and their classification among linalool/linalyl acetate chemotype. Each compound presented a specific kinetic during hydrodistillation treatment: for linalool it was a hyperbolic curve while linalyl acetate showed an important linear decrease. These variations were the expression of multiple physical laws governing mass transfer within liquid, gas phases and across the phase interfaces, and chemical reactions taking place in the wet vegetable material matrix.

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