JOURNAL OF CURRENT RESEARCH IN SCIENCE (ISSN 2322-5009)
CODEN (USA): JCRSDJ
2014, Vol. 2, No. 2, pp:285-290
Available at www.jcrs010.com

ORIGINAL ARTICLE

THE EFFECT OF SEASONAL VARIATION ON CITRON (*CITRUS MEDICA*) LEAF COMPONENTS

Behzad Babazadeh Darjazi
Department of Horticulture, College of Agriculture, Roudehen Branch, Islamic Azad University (I A U), Roudehen, Iran

ABSTRACT: Studies have shown that oxygenated compounds are important in food products. It seems that seasonal variation has a profound influence on this factor. The goal of the present study is to investigate on seasonal variation of citron leaf components. About 500 g of leaves were collected from many parts of the same trees during the vegetation season (in March, June, September and December 2012). Leaf components were extracted using water distillation method and then analyzed using GC and GC-MS. Data were analyzed using one-way analysis of variance (ANOVA) and Duncan's multiple range tests. The amount of oxygenated compounds ranged from 40.67% to 47.87%. Among four seasons examined, September showed the highest content of oxygenated compounds. As a result of our study, we can conclude that the seasonal variation can influence the quantity of oxygenated compounds present in the oil.
KEYWORDS: Flavor Components, Leaf Oil, Seasonal Variation, Water-distillation.

INTRODUCTION

Citrus is one of the most economically important crops in Iran. In the period 2009- 2010, the total Citrus production of Iran was estimated at around 87000 tonnes (FAO, 2012). The citron (*Citrus medica L.*) was first citrus fruit cultivated in Mediterranean region. It is considered as a parent plant in citrus evolution (Vekiari *et al.*, 2002a). It is one of the most important Citrus used in world. Although it is as important Citrus, the leaf components of citron have been investigated very little previously.

Citrus oils occur naturally in special oil glands in flowers, leaves, peel and juice. These valuable essential oils are composed of many compounds including: terpenes, sesquiterpenes, aldehydes, alcohols, esters and sterols. They may also be described as mixtures of hydrocarbons, oxygenated compounds and nonvolatile residues. Citrus oils are commercially used for flavoring foods, beverages, perfumes, cosmetics, medicines and etc. (Salem, 2003). In addition, recent studies have identified insecticidal, antimicrobial, antioxidative and antitumor properties for Citrus oils (Shahidi and Zhong, 2012).

The quality of an essential oil can be calculated from the quantity of oxygenated compounds present in the oil. The quantity of oxygenated compounds present in the oil, is variable and depends upon a number of factors including: seasonal variation (Boussaada and Chemli, 2007; Vekiari *et al.*, 2002b; Attaway *et al.*, 1967), rootstock (Babazadeh-Darjazi, 2011a), cultivars (Venturini *et al.*, 2010; Lota *et al.*, 1999), organ (Babazadeh-Darjazi, 2011b), extraction method (Babazadeh-Darjazi, 2011c) and etc.

Branched aldehydes and alcohols are important flavor compounds extensively used in food products (Salem, 2003). Several studies have shown that oxygenated terpenoids such as neral, geranial and geranyl acetate are important in citron flavor (Goshtasbi, 2005). The quality of a honey can be calculated from the amount of oxygenated components present in the honey (Alissandrakis *et al.*, 2003; Alistair *et al.*, 1993). In addition, type of flowers may influence the quality of volatile flavor components present in the honey. The effect of oxygenated compounds in the attraction of the pollinators has been proven. Therefore, the presence of oxygenated compounds can encourage the agricultural yield (Kite *et al.*, 1991; Andrews *et al.*, 2007). In this paper, we compared the leaf compounds isolated from Citron with the aim of determining whether the quantity of oxygenated compounds influenced by the season.

MATERIALS AND METHODS

2.1. Citron Scions

In 1989, citron scions that grafted on sour orange rootstock, were planted at 8×4 m with three replication at Ramsar research station [Latitude 36° 54′ N, longitude 50° 40′ E; Caspian Sea climate, average rainfall and temperature were 970 mm and 16.25°C per year respectively;

Corresponding Author: Behzad Babazadeh Darjazi**,** Department of Horticulture, College of Agriculture, Roudehen Branch, Islamic Azad University (I A U), Roudehen, Iran. **E-mail:** babazadeh@riau.ac.ir

soil was classified as loam-clay, pH ranged from 6.9 to 7)]. Citron was used as plant materials in this experiment (Table 1).

Table 1: Common and botanical names for citrus taxa used as scions and rootstock (Fotouhi and Fattahi, 2007).

Common name	botanical name	Parents	category
Citron(scion)	*Citrus medica* cv. *Etrog*	*Unknown*	Citron
Sour orange (Rootstock)	C. aurantium (L.)	*Mandarin ×Pomelo*	Sour orange

2.2. Preparation of Leaf Sample

About 500 g of leaves were collected from many parts of the same trees during the vegetation season (in March, June, September and December 2012), early in the morning (6 to 8 am) and only during dry weather.

2.3. Leaf Extraction Technique

In order to obtain the volatile compounds from the leaf, 500 g of fresh leaves were subjected to hydro distillation for 3 h using a Clevenger-type apparatus. N-hexane was used to isolate the oil layer from the aqueous phase. The hexane layer was dried over anhydrous sodium sulphate and stored at -4°C until used (Babazadeh-Darjazi, 2011a).

2.4. GC and GC-MS

An Agilent 6890N gas chromatograph (USA) equipped with a DB-5 (30 m × 0.25 mm i.d; film thickness = 0.25 μm) fused silica capillary column (J&W Scientific) and a flame ionization detector (FID) was used. The column temperature was programmed from 60 ° C (3min) to 250 ° C (20 min) at a rate of 3 ° C/ min. The injector and detector temperatures were 260 ° C and helium was used as the carrier gas at a flow rate of 1.00 ml/min and a linear velocity of 22 cm/s. The linear retention indices (LRIs) were calculated for all volatile components using a homologous series of n-alkanes (C9-C22) under the same GC conditions. The weight percent of each peak was calculated according to the response factor to the FID. Gas chromatography-mass spectrometry was used to identify the volatile components. The analysis was carried out with a Varian Saturn 2000R. 3800 GC linked with a Varian Saturn 2000R MS. The oven condition, injector and detector temperatures, and column (DB-5) were the same as those given above for the Agilent 6890 N GC. Helium was the carrier gas at a flow rate of 1.1 mL/min and a linear velocity of 38.7 cm/s. Injection volume was 1 μL.

2.5. Identification of Components

Components were identified by comparison of their Kovats retention indices (RI), retention times (RT) and mass spectra with those of reference compounds (Adams, 2001; McLafferty and Stauffer, 1991).

2.6. Data Analysis

SPSS 18 was used for analysis of the data obtained from the experiments. Analysis of variations was based on the measurements of 7 leaf components. Comparisons were made using one-way analysis of variance (ANOVA) and Duncan's multiple range tests. Differences were considered to be significant at $P < 0.01$. The correlation between pairs of components was evaluated using Pearson's correlation coefficient.

RESULTS

3.1. Leaf Compounds of the Citron during the Deferent Seasons

GC-MS analysis of the compounds extracted from citron leaf using water distillation allowed identification of 32 volatile components (Table 2, Figure 1): 16 oxygenated terpenes [5 aldehydes, 8 alcohols, 3 esters] and 16 non oxygenated terpenes [11 monoterpens, 5 sesqiterpens].

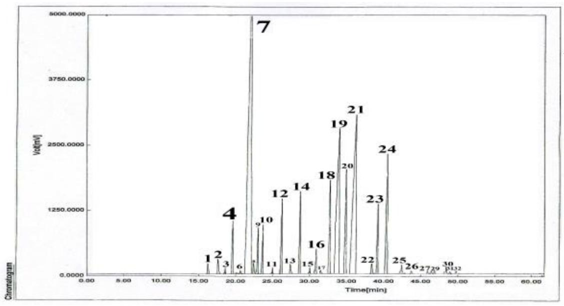

Figure 1: HRGC chromatogram of citron leaf oil in April

3.2. Aldehydes

Five aldehyde components that identified in this analysis were nonanal, citronellal, decanal, neral and geranial (Table 3). In addition they were quantified from 25.51% to 29.10%. The concentrations of neral and geranial were higher in our samples. Geranial has a green aroma (Sawamura *et al.,* 2004) and is considered as one of the major contributors to citron flavor (Goshtasbi, 2005). Among four seasons examined, September showed the highest content of aldehydes (Table 3). Since the aldehyde content of citrus oil is considered as one of the most important indicators of high quality, season apparently has a profound influence on this factor (Table 3).

Table 2: Seasonal variations on citron leaf components. (*There is in oil)

No.	Component	March	June	September	December	KI
1	α-Pinene	*	*	*	*	935
2	Sabinene	*	*	*	*	974
3	β-pinene	*	*	*	*	978
4	β- myrcene	*	*	*	*	989
5	α- terpinene	*	*	*	*	1012
6	p-cymene	*	*	*	*	1027
7	Limonene	*	*	*	*	1031
8	(Z)- β-ocimene	*	*	*	*	1042
9	(E)- β- ocimene	*	*	*	*	1052
10	γ- terpinene	*	*	*	*	1061
11	α- terpinolene	*	*	*	*	1091
12	Linalool	*	*	*	*	1100
13	Nonanal	*	*	*	*	1107
14	Citronellal	*	*	*	*	1154
15	Terpinene-4-ol	*	*	*	*	1182
16	α - terpineol	*	*	*	*	1194
17	Decanal	*	*	*	*	1205
18	Nerol	*	*	*	*	1233
19	Neral	*	*	*	*	1240
20	Geraniol	*	*	*	*	1257
21	Geranial	*	*	*	*	1269
22	Citronellyl acetate	*	*	*	*	1355
23	Neryl acetate	*	*	*	*	1365
24	Geranyl acetate	*	*	*	*	1384
25	(Z)- β- caryophyllene	*	*	*	*	1416
26	(Z)-α-bergamotene	*	*	*	*	1439
27	Bicyclogermacrene	*	*	*	*	1504
28	(Z)-a-Bisabolene	*	*	*	*	1510
29	β - Bisabolene	*	*	*	*	1512
30	(E)-Nerolidol	*	*	*	*	1567
31	Spathulenol	*	*	*	*	1582
32	α – Bisabolol	*	*	*	*	1591

3.3. Alcohols

Eight alcohol components identified in this analysis were linalool, terpinene-4-ol, α-terpineol, nerol, geraniol, (E)-Nerolidol, spathulenol and α-Bisabolol (Table 3). The total amount of alcohols ranged from 8.17% to 11.25%. Geraniol was identified as the major component in this study and was the most abundant. Geraniol has been recognized as one of the most important components for citron flavor (Goshtasbi, 2005). Geraniol has a nutty aroma (Sawamura *et al.,* 2004) and its level is important to the characteristic favor of citron. Among four seasons examined, September showed the highest content of alcohols (Table 3).

3.4. Esters

Three ester components identified in this analysis were citronellyl acetate, neryl acetate and geranyl acetate. The total amount of esters ranged from 5.55% to 7.52%. Among four seasons examined, September showed the highest content of esters (Table 3).

3.5. Monoterpene Hydrocarbons

The total amount of monoterpene hydrocarbons ranged from 44.08 % to 53.56 %. Limonene was identified as the major component in this study and was the most abundant. Limonene has a weak citrus-like aroma (Buettner *et al.,* 2003) and is considered as one of the major contributors to citron flavor. Among four seasons examined, December showed the highest content of monoterpenes (Table 3).

3.6. Sesquiterpene Hydrocarbons

The total amount of sesquiterpene hydrocarbons ranged from 0.73 % to 1.29 %. (Z)-β-caryophyllene was the major component among the sesquiterpen hydrocarbons. Among four seasons examined, March showed the highest content of sesquiterpenes (Table 3).

3.7. Results of Statistical Analyses

Differences were considered to be significant at $P < 0.01$. These differences on the 1% level occurred in citronellal, geranial, nerol, geraniol, geranyl acetate and limonen. The non-affected oil component was neral (Table 3).

3.8. Results of Correlation

Simple intercorrellations between 7 components are presented in a correlation matrix (Table 4). The highest positive values or r (correlation coefficient) were observed between [geranyl acetate and citronellal (90%)]; [nerol and citronellal (87%)]. The highest significant negative correlations were observed between [limonene and nerol (81%)] (Table 4).

Table 3: Statistical analysis of variation in leaf flavor Components during different seasons.

Compounds	March		June		September		December		F value
	Mean	St.err	Mean	St.err	Mean	St.err	Mean	St.err	
Oxygenated compounds									
a) Aldehyds									
1) Nonanal	0.21	0.02	0.30	0.02	0.34	0.03	0.20	0.02	
2) Citronellal	4.43	0.26	1.88	0.11	2.51	0.19	3.33	0.19	F**
3) Decanal	0.006	0.001	0.10	0.01	0.14	0.02	0.005	0.001	
4) Neral	10.24	0.86	11.16	0.22	11.41	0.96	10.45	0.34	NS
5) Geranial	12.05	0.94	14.55	0.30	14.70	0.27	11.53	0.25	F**
Total	26.95	2.08	27.98	0.65	29.10	1.46	25.51	0.80	
b) Alcohols									
1) Linalool	1.09	0.09	1.61	0.16	2.24	0.10	1.23	0.07	
2) Terpinen-4-ol	0.17	0.02	0.20	0.02	0.18	0.02	0.17	0.02	
3) α-terpineol	0.36	0.03	0.40	0.04	0.66	0.06	0.35	0.04	
4) Nerol	2.89	0.11	3.11	0.17	3.40	0.27	2.66	0.12	F**
5) Geraniol	3.31	0.10	3.42	0.10	4.31	0.29	3.85	0.29	F**
6) (E)-Nerolidol	0.18	0.02	0.22	0.02	0.27	0.03	0.15	0.01	
7) Spathulenol	0.07	0.006	0.08	0.01	0.09	0.01	0.08	0.01	
8) α - Bisabolol	0.09	0.01	0.10	0.01	0.11	0.01	0.08	0.006	
Total	8.17	0.37	9.13	0.52	11.25	0.78	8.57	0.57	
c) Esters									
1) Citronellyl acetate	0.31	0.03	0.35	0.02	0.39	0.03	0.33	0.02	
2) Neryl acetate	1.88	0.15	1.90	0.07	2.11	0.15	2.36	0.13	
3) Geranyl acetate	3.36	0.23	4.12	0.22	5.01	0.29	4.81	0.31	F**
Total	5.55	0.41	6.37	0.31	7.52	0.46	7.50	0.46	
Monoterpenes									
1) α-pinene	0.71	0.04	0.50	0.05	0.42	0.02	0.74	0.06	
2) Sabinene	0.92	0.08	0.70	0.06	0.57	0.04	0.91	0.06	
3) β- pinene	0.25	0.02	0.20	0.02	0.17	0.02	0.22	0.02	
4) β-myrcene	2.26	0.12	1.92	0.11	1.55	0.15	2.49	0.11	
5) α-terpinene	0.15	0.02	0.08	0.01	0.07	0.01	0.13	0.02	
6) p-cymene	0.30	0.03	0.20	0.02	0.12	0.02	0.30	0.03	
7) Limonene	43.37	1.31	41.15	1.02	38.22	2.05	44.82	0.94	F**
8) (Z)-β-ocimene	0.41	0.02	0.48	0.04	0.40	0.04	0.45	0.04	
9) (E)-β-ocimene	1.37	0.06	1.35	0.07	1.13	0.18	1.31	0.12	
10) γ-terpinene	1.97	0.08	1.45	0.09	1.26	0.13	1.86	0.09	
11) α-terpinolene	0.30	0.03	0.20	0.02	0.17	0.02	0.32	0.04	
Total	52.02	1.80	48.23	1.51	44.08	2.67	53.56	1.51	
Sesquiterpenes									
1) (Z)-β-caryophyllene	0.82	0.07	0.57	0.05	0.31	0.02	0.71	0.05	
2) (Z)-α-bergamotene	0.12	0.02	0.10	0.01	0.11	0.01	0.13	0.01	
3) Bicyclogermacrene	0.11	0.01	0.10	0.02	0.10	0.01	0.11	0.01	
4) α -Bisabolene	0.13	0.02	0.10	0.01	0.10	0.01	0.12	0.01	
5) β - Bisabolene	0.11	0.01	0.10	0.01	0.11	0.01	0.12	0.01	
Total	1.29	0.13	0.97	0.10	0.73	0.06	1.19	0.09	
Total oxygenated compounds	40.67	2.86	43.48	1.48	47.87	2.7	41.58	1.83	
Total	93.98	4.79	92.68	3.09	92.68	5.43	96.33	3.43	

Mean is average composition in % over the different seasons used with three replicates. St. err = standard error. F value is accompanied by its significance, indicated by: NS = not significant, * = significant at P = 0.05, ** = significant at P = 0.01.

Table 4: Correlation matrix (numbers in this table correspond with main components mentioned in Table 3).

	Citronellal	Neral	Geranial	Nerol	Geraniol	Geranyl acetate
Neral	-0.45					
Geranial	0.78**	-0.72**				
Nerol	0.87**	0.82**	-0.42			
Geraniol	0.55	0.35	0.59*	-0.24		
Geranyl acetate	0.90**	0.36	0.32	0.56	-0.45	
Limonene	-0.26	-0.37	-0.71**	-0.81**	-0.48	0.55

*=significant at 0.05

**=significant at 0.01

DISCUSSION

Our observation that seasonal variations had an effect on some of the components of citron oil was in accordance with previous findings (Boussaada and Chemli, 2007; Vekiari *et al.*, 2002b; Attaway *et al.*, 1967). The compositions of the leaf oils obtained by water distillation during different seasons were very similar. However, the relative concentration of compounds was different according to the type of season.

Comparison of our data with those in the literatures revealed some inconsistencies with previous studies (Boussaada and Chemli, 2007;

Vekiari *et al.,* 2002b). It may be related to rootstock and environmental factors that can influence the compositions. However, it should be kept in mind that the extraction methods also may influence the results. Fertilizer and irrigation affects the content of oil present in Citrus (Kesterson *et al.,* 1974). Fertilization, irrigation and other operations were carried out uniform in this study so we did not believe that this variability was a result of these factors.

The discovery of geranyl pyrophosphate (GPP), as an intermediate between mevalonic acid and oxygenated compounds (Alcohols and aldehyds), led to a rapid description of the biosynthetic pathway of oxygenated compounds. The biosynthetic pathway of oxygenated compounds in higher plants is as below:

Mevalonic acid → Isopentenyl Pyrophosphate → 3.3-dimethylallylpyrophosphate→ geranyl pyrophosphate→ Alcohols and Aldehyds

This reaction pathway catalyzed by isopentenyl pyrophosphate isomerase and geranyl pyrophosphate synthase, respectively (Hay and Waterman, 1995). The pronounced enhancement in the amount of oxygenated compounds, when September used as the season, showed that either the synthesis of geranyl pyrophosphate was enhanced or activities of both enzymes increased.

Also, the higher proportion of the detected oxygenated compounds in leaf was probably due to seasonal temperature (Sekiya *et al.,* 1984), which is the most important environmental factor in the control of endogenous enzymes. High positive correlations between pairs of terpenes suggest a genetic control (Scora *et al.,* 1976) and such dependence between pairs of terpenes was due to derivation of one from another that was not known. Similarly, high negative correlations between pairs of terpenes suggest that one of the two compounds have been synthesized at the expense of the other or of its precursor. Non-significant negative and positive correlations can imply genetic and/or biosynthetic independence. However, without an extended insight into the biosynthetic pathway of each terpenoid compound, the true significance of these observed correlations is not clear. Considering that acetate is necessary for the synthesis of terpenes, it can be assumed that there is a specialized function for this molecule and it may be better served in September.

CONCLUSION

In the present study we found that the amount of leaf compositions was significantly affected by seasons and there was a great variation in most of the measured characters among four seasons. The present study demonstrated that volatile compounds in leaf can vary when different seasons are utilized. Among four seasons examined, September showed the highest content of oxygenated compounds. The lowest of oxygenated compounds content were produced by March. Studies like this are very important to determine the amount of chemical compositions existing during different seasons. Further research on the relationship between seasonal variations and oxygenated compounds is necessary.

ACKNOWLEDGEMENTS

The author would like to express his gratitude to Z.Kadkhoda from Institute of Medicinal Plants located at Supa blvd-Km 55 of Tehran – Qazvin (Iran) for her help in GC-MS and GC analysis.

REFERENCES

Adams RP. Identification of essential oil components by gas chromatography/mass spectrometry. Allured Publishing Corporation, Carol Stream. Illinois, USA 2001.

Alissandrakis E, Daferera D, Tarantilis PA, Polissiou M, Harizanis PC. Ultrasound assisted extraction of volatile compounds from Citrus flowers and Citrus honey. Food Chem 2003;82:575-582.

Alistair LW, Yinrong LU, Seng-To T. Extractives from New Zealand honey 4.linalool derivatives and other components from nodding thistle (*Corduus nutans*) honey. J Agric Food Chem 1993;41(6):873-878.

Andrews ES, Theis N, Alder LS. Pollinator and herbivore attraction to cucurbita floral volatiles. J Chem Ecol 2007;33:1682-1691.

Attaway JA, Pieringer AP, Barabas LJ. The origin .of citrus flavor components. III. A study of the percentage variation in peel and leaf oil terpenes during one season. Photochemistry 1967;6:25-32.

Babazadeh-Darjazi B. The effects of rootstock on the volatile mandarin flavor components of page mandarin flower and leaf. Afr J Agric Res 2011a;6(7):1884-1896.

Babazadeh-Darjazi B. Comparison of volatile components of flower, leaf, peel and juice of 'Page' mandarin. Afr J Biotechnol 2011b;10(51):10437-10446.

Babazadeh-Darjazi B . A comparison of volatile components of flower of page mandarin obtained by ultrasound-assisted extraction and hydrodistillation. J Med Plant Res 2011c;5(13):2840-2847.

Boussaada O, Chemli R. Seasonal variation of essential oil composition of Citrus aurantium L. var. amara. J Essential Oil Bearing Plants 2007;10(2):109-120.

Buettner A, Mestres M, Fischer A, Guasch J, Schieberie P. Evaluation of the most odor-active compounds in the peel oil of clementines (*Citrus reticulate Blanco cv. Clementine*). Eur Food Res Technol 2003;216:11-14.

FAO. Statistical Database, 2012. Available from: http://faostat.fao.org/site/567/default.aspx#ancor.

Fotouhi R, Fattahi J. Citrus growing in Iran. 2nd Edition, Gilan University, Rasht 2007.

Goshtasbi-Irani A. Extraction and identification of essential oil components of the peel, leaf and flower of citron *"Citrus medica L."* cultivated at the north of Iran. Master of Science thesis, Islamic Azad University, Pharmaceutical sciences branch 2005.

Hay RKM, Waterman P. Volatile oils crops; their biology, biochemistry, and production. 3rd Edition, Wiley, New Jersey, USA 1995.

Kesterson JW, Braddock RJ , Koo RCJ. The effect of bud wood, rootstock, irrigation and fertilization on the yield of Florida lemon oil. Proc Fla State Hort Soc 1974;87:6-9.

Kite G, Reynolds T, Prance T. Potential pollinator–attracting chemicals from Victoria (Nymphaeaceae). Biochem Syst Ecol 1991;19(7):535-539.

Lota ML, Serra DR, Tomi F, Bessiere JM, Casanova J. Chemical composition of peel and leaf essential oils of Citrus medica L. and C. limonimedica Lush. Flavour Fragr J 1999;14:161–166.

McLafferty FW, Stauffer DB. The important peak index of the registry of mass spectral data. Wiley, New York, USA 1991.

Salem A. Extraction and identification of essential oil components of the peel, leaf and flower of tangerine '*Citrus nobilis loureior var deliciosa swingle*' cultivated at the north of Iran. Master of Science thesis, Islamic Azad University, Pharmaceutical sciences branch 2003.

Sawamura M, Minh-Tu NT, Onishi Y, Ogawa E, Choi HS. Characteristic odor components of *Citrus reticulata* Blanco (ponkan) cold pressed oil. Biosci Biotechnol Biochem 2004;68(8):1690-1697.

Scora RW, Esen A, Kumamoto J. Distribution of essential oils in leaf tissue of an F2 population of Citrus. Euphytica 1976;25:201-209.

Sekiya J, Kajiwara T, Hatanaka A. Seasonal changes in activities of enzymes responsible for the formation of C6-aldehydes and C6-alcohols in tea leaves, and the effects of environmental temperatures on the enzyme activities. Plant Cell Physiol 1984;25(2):269-280.

Shahidi F, Zhong Y. Citrus oils and essences. Kirk-Othmer Encyclopedia of Chemical Technology 2012; pp:1-17.

Vekiari SA, Protopapadakis EE, Papadopoulou P. Gas chromatography-mass spectroscopy analysis of aromatic compounds of leaves and peel from healthy and viroid-infected citron plants. Fifteenth IOCV conference, Viroids 2002a; pp:272-277.

Vekiari SA, Protopapadakis EE, Papadopoulou P, Papanicolaou D, Panou C, Vamvakias M. composition and seasonal variation of the essential oil from leaves and peel of a cretan lemon variety. J Agric Food Chem 2002b;50:147-153.

Venturini N, Curk F, Desjobert J, Karp D, Costa J, Paolini J. Chemotaxonomic Investigations of Peel and Petitgrain Essential Oils from 17 Citron Cultivars. Chem Biodiver 2010;7:736-750.