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The Effect of Geographical Location on Mexican Lime (*Citrus aurantifolia*) Peel Components

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***Abstract* – Studies have shown that oxygenated compounds are important in food products. It seems that geographical location has a profound influence on this factor. The goal of the present study is to investigate on peel components of Mexican lime from two different locations. In the last week of December 2012, at least 50 mature fruit were collected and peel components were extracted using cold-press method, 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 5.74% to 7.14%. Between two locations examined, Jahrom showed the highest content of oxygenated compounds. As a result of our study, we can conclude that the geographical location can influence the quantity of oxygenated compounds present in the oil**.

***Keywords* – Cold-Press, Flavor Components, Geographical Location, Peel Oil.**

I. 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 [1]. Mexican lime is known as Key lime or West Indian lime. It originated in Asia and introduced to the Iran, Egypt and North Africa by Arab traders in the 900s [2]. It is one of the most important Citrus used in world. Although it is as important Citrus, the peel components of Mexican lime have been investigated very little previously [3].

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 [4]. In addition, recent studies have identified insecticidal, antimicrobial, antioxidative and antitumor properties for Citrus oils [5].

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: geographical location [6]-[7]-[8], seasonal variation [9], varieties [10], degree of maturity [11], extraction method [12] and etc.

Branched aldehydes and alcohols are important flavor compounds extensively used in food products [4]. Several studies have shown that oxygenated terpenoids such as neral, citral, geranial and geraniol are important in lime flavor [13]. The quality of a honey can be calculated from the amount of oxygenated components present in the honey [14]-[15]. 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 [16]-[17].

In this paper, we compared the peel compounds isolated from Mexican lime with the aim of determining whether the quantity of oxygenated compounds influenced by the location.

II. Materials and Methods

A. *Mexican Lime Trees*

In 1989, Mexican lime trees 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; soil was classified as loam-clay, pH ranged from 6.9 to 7]. Also, trees were planted at 8 × 4 m with three replication at an orchard around the Jahrom in 1989. [latitude 28° 50′ N, longitude 53° 33′ E; dry climate, average rainfall 200 mm per year and average temperature 21.0°C; soil was classified as loam-clay with lime and gypsum in some parts; pH=8.5]. Mexican lime was used as plant material in this experiment (Table 1, Fig1).

Fig.1. Fruit were collected from two geographical locations in Iran.

B. Preparation of Peel Sample

In the last week of December 2012, at least 50 mature fruit were collected from many parts of the same trees located in orchards of the Ramsar and Jahrom. About 150 g of fresh peel was cold-pressed and then the oil was separated from the crude extract by centrifugation (at 4000 RPM for 15 min at 4 °C). The supernatant was dehydrated with anhydrous sodium sulfate at 5 °C for 24h and then filtered. The oil was stored at -25 °C until analyzed [3].

C. GC and GC-MS

An Agilent 6890N gas chromatograph (USA) equipped with a DB-5 (30 m ×0.25 mm i.d ; film thickness =.25 μ m) fused silica capillary column (J&W Scientific) and a flame ionization detector (FID) was used. The column temperature was programmed from 60 ^{o} C (3min) to 250 ^{o} C (20 min) at a rate of 3 ^{o} C/ min. The injector and detector temperatures were 260 ^{o} 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.

D. 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 [18]-[19].

E. 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 peel 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.

III. RESULTS

A. Peel Compounds of the Mexican lime

GC-MS analysis of the flavor compounds extracted from Mexican lime peel using cold-press allowed identification of 39 volatile components (Table 2, Fig 2): 17 oxygenated terpenes [6 aldehydes, 8 alcohols, 3 esters] and 22 non oxygenated terpenes [10 monoterpens, 12 sesqiterpens].

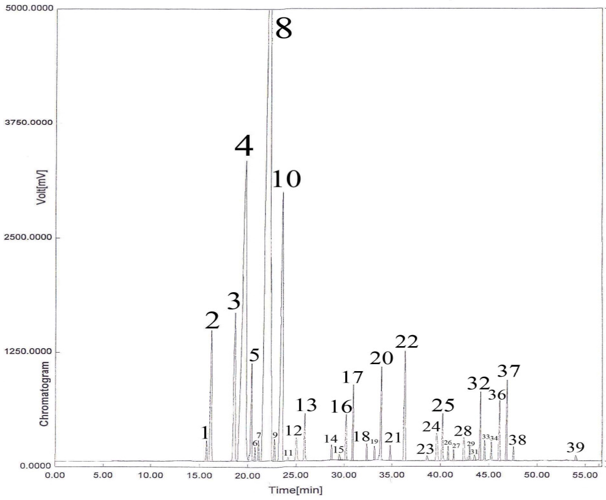

Fig.2. HRGC chromatograms peel oil of Mexican lime from Jahrom.

B. Aldehydes

Six aldehyde components that identified in this analysis were octanal, citronellal, decanal, neral, geranial and dodecanal (Table 3). In addition they were quantified from 2.74% to 3.60%. The concentrations of neral and geranial were higher in our samples. Geranial has a grassy-like aroma [20] and is considered as one of the major contributors to lime flavor [13]. Between two locations examined, Jahrom 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, location apparently has a profound influence on this factor (Table 3).

C. Alcohols

Eight alcoholic components identified in this analysis were octanol, linalool, borneol, terpinen-4-o1, α-terpineol, nerol, geraniol and α-bisabolol (Table 3). The total amount of alcohols ranged from 1.82% to 2.44%. α-terpineol was identified as the major component in this study and was the most abundant. α-terpineol has been recognized as one of the most important components for Citrus flavor.

α-terpineol has a tea-like aroma [20] and its level is important to the characteristic favor of Citrus [4]. Between two locations examined, Jahrom showed the highest content of alcohols (Table 3).

D. Esters

Three ester components identified in this analysis were citronellyl acetate, neryl acetate and geranyl acetate. The total amount of esters ranged from 0.91% to 1.10%. Between two locations examined, Jahrom showed the highest content of esters (Table 3).

E. Monoterpene Hydrocarbons

The total amount of monoterpene hydrocarbons ranged from 85.09 % to 87.9 %. Limonene was identified as the major component in this study and was the most abundant. Limonene has a lemon-like aroma [20] and is considered as one of the major contributors to Citrus flavor. Between two locations examined, Ramsar showed the highest content of monoterpenes (Table 3).

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Table 1: Common and botanical names for citrus taxa used as plant material [2].

Common name	botanical name	Parents	category
Mexican lime	*Citrus aurantifolia*	Unknown	Lime

Table 2: Peel components of Mexican lime from two geographical locations.

	Component	Ramsar	Jahrom	KI		Component	Ramsar	Jahrom	KI
1	α -thujene	*	*	925	21	Geraniol	*	*	1255
2	α - Pinene	*	*	933	22	Geranial	*	*	1267
3	Sabinene		*	975	23	Citronellyl acetate	*	*	1350
4	β - Pinene	*	*	978	24	Neryl acetate	*	*	1365
5	β -myrcene	*	*	989	25	Geranyl acetate	*	*	1384
6	Octanal	*	*	1003	26	β-elemene	*	*	1398
7	α -terpinene	*	*	1017	27	Dodecanal		*	1409
8	Limonene	*	*	1030	28	(Z)-β-caryophyllene	*	*	1417
9	(E)-β-ocimene	*	*	1052	29	Cis-α-bergamotene	*	*	1419
10	γ- terpinene	*	*	1057	30	Trans-α-bergamotene	*	*	1441
11	Octanol	*	*	1070	31	γ-elemene	*		1444
12	α- terpinolene	*	*	1088	32	(Z)- β -farnesene	*	*	1451
13	Linalool	*	*	1100	33	(E)- β -farnesene		*	1457
14	Citronellal	*	*	1154	34	Germacrene D	*	*	1493
15	Borneol	*	*	1170	35	Valencene	*		1499
16	Terpinene-4-ol	*	*	1182	36	E,E, α-farnesene	*	*	1509
17	α-terpineol	*	*	1195	37	β -bisabolene	*	*	1513
18	Decanal	*	*	1205	38	(z)-α-bisabolene	*	*	1515
19	Nerol	*	*	1235	39	α-bisabolol	*	*	1693
20	Neral	*	*	1240					

*There is in oil

Table 3: Statistical analysis of variation in peel flavor Components from different locations.

Compounds	Ramsar		Jahrom		F value
	Mean	St.err	Mean	St.err	
a) Aldehyds					
1) Octanal	0.07	0.006	0.15	0.01	
2) Citronellal	0.15	0.01	0.18	0.02	
3) Decanal	0.11	0.01	0.21	0.02	
4) Neral	0.91	0.02	1.21	0.13	F*
5) Geranial	1.5	0.04	1.74	0.17	F*
6) Dodecanal			0.11	0.01	
total	2.74	0.08	3.60	0.36	
b) Alcohols					
1) Octanol	0.03	0	0.04	0.006	
2) Linalool	0.31	0.02	0.52	0.06	
3) Borneol	0.05	0.006	0.07	0.006	
4) Terpinen-4-ol	0.44	0.03	0.51	0.06	
5) α-terpineol	0.72	0.04	0.88	0.08	
6) Nerol	0.11	0.02	0.15	0.02	
7)Geraniol	0.12	0.01	0.18	0.02	
8) α-bisabolol	0.04	0.006	0.09	0.01	
total	1.82	0.13	2.44	0.26	
d) Esteres					
1) Citronellyl acetate	0.04	0.006	0.06	0.01	

2) Neryl acetate	0.47	0.03	0.5	0.03	
3) Granyl acetate	0.4	0.03	0.54	0.04	
total	0.91	0.06	1.10	0.08	
Monoterpenes					
1) α-thujene	0.35	0.05	0.33	0.02	
2) α-pinene	1.66	0.06	2.32	0.27	F*
3) Sabinene			2.72	0.23	
4) β- pinene	10.19	0.36	16	1	F**
5) β-myrcene	1.45	0.13	1.34	0.11	NS
6) α-terpinene	0.48	0.03	0.23	0.02	
7) Limonene	64	2	52	1	F**
8) (E)-β-ocimene	0.24	0.02	0.37	0.02	
9) γ-terpinene	8.83	0.47	9.3	0.87	NS
10) α-terpinolene	0.7	0.04	0.48	0.05	
total	87.9	3.16	85.09	3.59	
Sesquiterpenes					
1) β-elemene	0.09	0.01	0.15	0.02	
2) (Z)-β-caryophyllene	0.3	0.02	0.46	0.05	
3) Cis-α-bergamotene	0.35	0.02	0.14	0.01	
4) γ-elemene	0.01	0			
5) (Z)-β-farnesene	0.05	0.006	0.08	0.01	
6) Trans-α-bergamotene	0.54	0.02	0.77	0.06	
7) (E)- β -farnesene			0.23	0.03	
8) Germacrene D	0.13	0.02	0.21	0.02	
9) Valencene	0.03	0			
10) E,E-α-farnesene	0.44	0.03	0.75	0.05	
11) β-bisabolene	0.62	0.03	0.91	0.07	
12) (Z)-α-bisabolene	0.07	0.006	0.13	0.01	
Total	2.63	0.16	3.83	0.33	
Total oxygenated compounds	5.47	0.28	7.14	0.70	
Total	96	3.60	96.06	4.62	

Mean is average composition in % over the different locations 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).

	Neral	Geranial	α-pinene	β- pinene	β-myrcene	Limonene
Geranial	0.96**					
α-pinene	0.99**	0.96**				
β- pinene	0.96**	0.87*	0.96**			
β-myrcene	-0.12	0.11	-0.12	-0.31		
Limonene	-0.82*	-0.66	-0.83*	-0.93**	0.59	
γ-terpinene	0.70	0.83*	0.71	0.54	0.52	-0.24

*=significant at 0.05; **=significant at 0.01

F. Sesquiterpene Hydrocarbons

The total amount of sesquiterpene hydrocarbons ranged from 2.63% to 3.83 %. β -bisabolene was identified as the major component in this study and was the most abundant. Between two locations examined, Jahrom showed the highest content of sesquiterpenes (Table 3).

G. Results of Statistical Analyses

Differences were considered to be significant at $P < 0.01$. These differences on the 1% level occurred in β-pinene and limonene. These differences on the 5% level occurred in neral, geranial and α-pinene. The non affected oil components were β-myrcene and γ-terpinene (Table 3).

H. 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 α-pinene and neral (99%). The highest significant negative correlations were observed between limonene and β- pinene (93%) (Table 4).

IV. Discussion

Our observation that geographical locations had an effect on some of the components of Mexican lime oil was in accordance with previous findings [6]-[7]-[8]. The compositions of the peel oils obtained by cold-press from different locations were very similar. However, the relative concentration of compounds was different according to the type of location.

Comparison of our data with those in the literatures revealed some inconsistencies with previous studies [10]. It may be related to environmental factors such as altitude, insolation (solar radiation), average temperature, humidity and chemical composition of soil that can influence the oil compositions [21]. 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 [22]. 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 [23]. The pronounced enhancement in the amount of oxygenated compounds, when Jahrom used as the location, 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 Jahrom was probably due to seasonal temperature [24], which is the most important environmental factor in the control of endogenous enzymes. Solar radiations can also be involved in activation or inactivation of certain enzymatic groups, leading to the predominance of a particular biosynthetic pathway [25].

High positive correlations between pairs of terpenes suggest a genetic control [26] 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 indicated that one of the two compounds had 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 by Jahrom.

V. Conclusion

In the present study we found that the amount of peel compositions was significantly affected by locations and there was a great variation in most of the measured characters between two locations. The present study demonstrated that volatile compounds in peel can vary when different locations are utilized. Between two locations examined, Jahrom showed the highest content of oxygenated compounds. The lowest of oxygenated compounds content were produced by Ramsar. Studies like this are very important to determine the amount of chemical compositions existing in different locations. Further research on the relationship between geographical locations and oxygenated compounds is necessary.

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