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ORIGINAL ARTICLE

Comparison of Peel Components of Sweet lime (*Citrus limetta Risso*) Obtained using Cold-press and Hydrodistillation Method

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ABSTRACT
Studies have shown that oxygenated compounds are important in food products. It seems that extraction methods have a profound influence on this factor. The goal of the present study is to investigate on flavor components of sweet lime obtained using cold-press and hydro distillation. In the last week of December 2012, at least 50 mature fruit were collected from many parts of the same trees. Peel components were extracted using cold-press and hydro distillation method. Then all 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 aldehydes ranged from 1.71% to 2.98%. Between two methods examined, cold-press showed the highest content of aldehydes. As a result of our study, we can conclude that the extraction methods used can influence the quantity of oxygenated compounds present in the oil.
Keywords: *Cold-press, Extraction method, Flavor components, Hydro distillation, Peel oil.*

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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]. The sweet lime (Citrus limetta) is a very close relative of the lemon (Citrus limon), though some botanists believe that the sweet lime is actually biologically identical to the lemon and is really a hybrid or mutation of lemon [2]. It is one of the most important Citrus used in world. Although it is as important Citrus, the peel components of sweet lime 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 [3]. Citrus oils are commercially used for flavoring foods, beverages, perfumes, cosmetics, medicines and etc [4]. The quality of an essential oil can be calculated from the quantity of aldehyde compounds present in the oil. The quantity of aldehyde compounds present in the oil, is variable and depends upon a number of factors including: the technique of extraction [5], seasonal variation [6] and etc.

The main techniques used at industrial scale are cold pressing (CP), hydro distillation (HD), extraction with organic solvent, extraction with compressed CO2 and extraction with ultrasound-assisted extraction (UAE).

Hydro distillation (HD) enable the isolation of the essential oil borne in the plant, however, it has disadvantages. Hydro distillation needs a large amount of plant material and the time for extraction is quite long (around 3 hours). Because of the long time for extraction, the energy consumption is quite high. Also it can thermally degrade, hydrolyze and distort some of the oil components [7].

One of the simplest extraction techniques is the cold-pressing (CP) that is easy to perform in common laboratory equipment. In this method, the extraction of essential oils occurs at room temperature so degradation at high temperature does not happen. Cold-pressing (CP) is a good extraction method in comparison with the more traditional approaches due to its high efficiency. Also it does not need to heating equipment and the operation is easy.

In this paper, we compared the peel compounds obtained using cold press (CP) with those obtained using hydro distillation (HD).

MATERIALS AND METHODS

Sweet Lime Trees

In 1989, Sweet 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]. Sweet lime was used as plant material in this experiment (Table 1).

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 Ramsar research station, early in the morning (6 to 8 am) and only during dry weather. The selection method of all samples was on a random basis.

Cold-pressing Extraction Technique

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. Three replicates were carried out for the quantitative analysis (n=3) (5).

Hydro distillation Extraction Technique

In order to obtain the volatile compounds from the peel, 250 g of fresh peel 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. Three replicates were carried out for the quantitative analysis (n=3) (5).

GC and GC-MS

An Agilent 6890N gas chromatograph (USA) equipped with a DB-5 (30 m $\times$0.25 mm i.d ; film thickness = 0.25 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.

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 [8, 9].

Data Analysis

SPSS 18 was used for analysis of the data obtained from the experiments. Analysis of variations was based on the measurements of 8 peel component. 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 characters was evaluated using Pearson's correlation coefficient.

RESULTS

Flavor Compounds of the Sweet Lime Obtained Using Cold-press (CP)

GC-MS analysis of the flavor compounds extracted from sweet lime using cold-press allowed identification of 29 volatile components (Table 2, Fig. 1): 13 oxygenated terpenes [6 aldehydes, 5 alcohols, 2 esters] and 16 non oxygenated terpenes [12 monoterpens, 4 sesqiterpens].

Flavor Compounds of the Sweet Lime Obtained Using Hydro distillation (HD)

GC-MS analysis of the flavor compounds extracted from sweet lime using hydro distillation allowed identification of 34 volatile components (Table 2): 14 oxygenated terpenes [6 aldehydes, 6 alcohols, 2 esters] and 20 non oxygenated terpenes [15 monoterpens, 5 sesqiterpens].

Aldehydes

Six aldehyde components that identified in this analysis were octanal, nonanal, citronellal, decanal, neral and geranial (Table 3). In addition they were quantified from 1.71% to 2.98%. The concentrations of neral and geranial were higher in our samples. Geranial has a grassy-like aroma [10] and is considered as one of the major contributors to Citrus flavor. Between two methods examined, cold-pressing showed the

highest content of aldehydes. Since the aldehyde content of citrus oil is considered as one of the most important indicators of high quality, method apparently has a profound influence on this factor.
Compared with hydro distillation, the cold-pressing improved and increased aldehyde components about 1.74 times (Table 3).

Alcohols

Six alcoholic components identified in this analysis were linalool, terpinen-4-o1, α-terpineol, nerol, geraniol and α-bisabolol (Table 3). The total amount of alcohols ranged from 0.70% to 1.36%. 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 [10] and its level is important to the characteristic favor of Citrus [4].
Between two methods examined, hydro distillation showed the highest content of alcohols. Compared with cold-pressing, hydro distillation improved and increased alcohol components about 1.94 times. (Table 3)

Esters

Two ester components identified in this analysis were neryl acetate and geranyl acetate. The total amount of esters ranged from 0.66% to 0.83%. Between two methods examined, cold-pressing showed the highest content of esters (Table 3).

Monoterpene Hydrocarbons

The total amount of monoterpene hydrocarbons ranged from 86.95 % to 89.14 %. Limonene was identified as the major component in this study and was the most abundant. Limonene has a lemon-like aroma [10] and is considered as one of the major contributors to Citrus flavor. Between two methods examined, hydro distillation showed the highest content of monoterpenes (Table 3).

Sesquiterpene Hydrocarbons

The total amount of sesquiterpene hydrocarbons ranged from 0.82% to 0.91 %. β-bisabolene was identified as the major component in this study and was the most abundant. Between two methods, cold-pressing showed the highest content of sesquiterpenes (Table 3).

RESULTS

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

Results of Correlation

Simple intercorrellations between 8 components are presented in a correlation matrix (Table 4). The highest positive values or r (correlation coefficient) were observed between geranial and neral (98%); β-myrcene and β- pinene (97%); γ- terpinene and β- pinene (95%) . (Table 4).

Table 1. Common and botanical names for citrus taxa used as plant material.

Common name	Botanical name	Parents	Category
Palestine sweet lime	*Citrus limetta Risso*	*Unknown*	lime

Table2. Peel components of Sweet lime obtained using cold-press and hydro distillation.

	Component	Cold-press	Hydro distillation	KI		Component	Cold-press	Hydro distillation	KI
1	α -thujene	*	*	927	18	Nonanal	*	*	1110
2	α - Pinene	*	*	935	19	Citronellal	*	*	1155
3	Camphene		*	952	20	Terpinene-4-ol	*	*	1183
4	Sabinene	*	*	975	21	α-terpineol	*	*	1195
5	β - Pinene	*	*	980	22	Decanal	*	*	1205
6	β -myrcene	*	*	992	23	Nerol	*	*	1236
7	Octanal	*	*	1003	24	Neral	*	*	1242
8	α -phellandrene	*	*	1006	25	Geraniol	*	*	1258
9	α -terpinene	*	*	1018	26	Geranial	*	*	1270
10	p-cymene	*	*	1026	27	Neryl acetate	*	*	1361
11	Limonene	*	*	1032	28	Geranyl acetate	*	*	1384
1	(Z)-β-ocimene		*	1037	29	(Z)-β-caryophyllene	*	*	1417

12								
13	(E)-β-ocimene	*	*	1052	30	(Z)-α-bergamotene	*	*	1441
14	γ- terpinene	*	*	1062	31	α -humulene		*	1458
15	(Z)-sabinene hydrate		*	1070	32	(E)- β -farnesene	*	*	1461
16	α- terpinolene	*	*	1090	33	β -bisabolene	*	*	1515
17	Linalool	*	*	1102	34	α-bisabolol		*	1690

*There is in oil

Table 3. Statistical analysis of variation in peel components of sweet lime obtained using cold-press and hydro distillation.

Compounds	Cold-press		Hydro distillation		
	Mean	St.err	Mean	St.err	F value
a) Aldehyds					
1) Octanal	0.07	0.01	0.02	0.00	
2) Nonanal	0.11	0.01	0.04	0.01	
3) Citronellal	0.10	0.006	0.04	0.01	
4) Decanal	0.13	0.02	0.05	0.01	
5) Neral	1.00	0.13	0.58	0.09	F**
6) Geranial	1.57	0.11	0.98	0.19	F**
total	2.98	0.29	1.71	0.31	
b) Alcohols					
1) Linalool	0.22	0.03	0.37	0.05	
2) Terpinen-4-ol	0.11	0.02	0.23	0.02	
3) α-terpineol	0.24	0.02	0.50	0.05	
4) Nerol	0.07	0.01	0.12	0.02	
5)Geraniol	0.06	0.01	0.11	0.02	
6) α-bisabolol			0.03	0.00	
total	0.70	0.09	1.36	0.16	
d) Esteres					
1) Neryl acetate	0.43	0.03	0.35	0.03	
2) Granyl acetate	0.40	0.03	0.31	0.02	
total	0.83	0.06	0.66	0.05	
Monoterpenes					
1) α-thujene	0.34	0.03	0.35	0.03	
2) α-pinene	1.70	0.10	2.03	0.17	F*
3) Camphene			0.06	0.01	
4) Sabinene	1.80	0.08	2.00	0.23	NS
5) β- pinene	10.98	1.08	11.76	0.98	NS
6) β-myrcene	1.40	0.10	1.45	0.11	NS
7) α- phellandrene	0.06	0.01	0.09	0.01	
8) α-terpinene	0.20	0.02	0.28	0.04	
9) p-cymene	0.41	0.04	0.50	0.05	
10) Limonene	62.01	0.99	60.81	1.17	NS
11) (Z)-β-ocimene			0.09	0.01	
12) (E)-β-ocimene	0.16	0.02	0.25	0.03	
13) γ-terpinene	7.49	1.05	8.94	1.02	NS
14) (Z)-sabinene hydrate			0.06	0.01	
15) α-terpinolene	0.40	0.04	0.47	0.05	
total	86.95	3.56	89.14	3.92	
Sesquiterpenes					
1) (Z)-β-caryophyllene	0.25	0.03	0.21	0.02	
2) (Z)-α-bergamotene	0.07	0.01	0.05	0.01	
3) α- humulene			0.03	0.00	

Compounds	Cold-press		Hydro distillation		
	Mean	St.err	Mean	St.err	F value
4) (E)- β -farnesene	0.05	0.006	0.03	0.00	
5) β-bisabolene	0.54	0.05	0.50	0.05	
Total	0.91	0.09	0.82	0.08	
Total oxygenated compounds	4.51	0.43	3.73	0.52	
Total	92.37	4.09	93.69	4.52	

Mean is average composition (%) in two methods 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	Sabinene	β- pinene	β-myrcene	Limonene
Geranial	0.98**						
α-pinene	-0.57	-0.54					
Sabinene	-0.28	-0.21	0.92**				
β- pinene	-0.04	-0.05	0.83*	0.89*			
β-myrcene	0.11	0.12	0.75	0.88*	0.97**		
Limonene	0.82*	0.83*	-0.01	0.30	0.50	0.64	
γ- terpinene	-0.30	-0.31	0.94**	0.93**	0.95**	0.90*	0.26

*=significant at 0.05, **=significant at 0.01

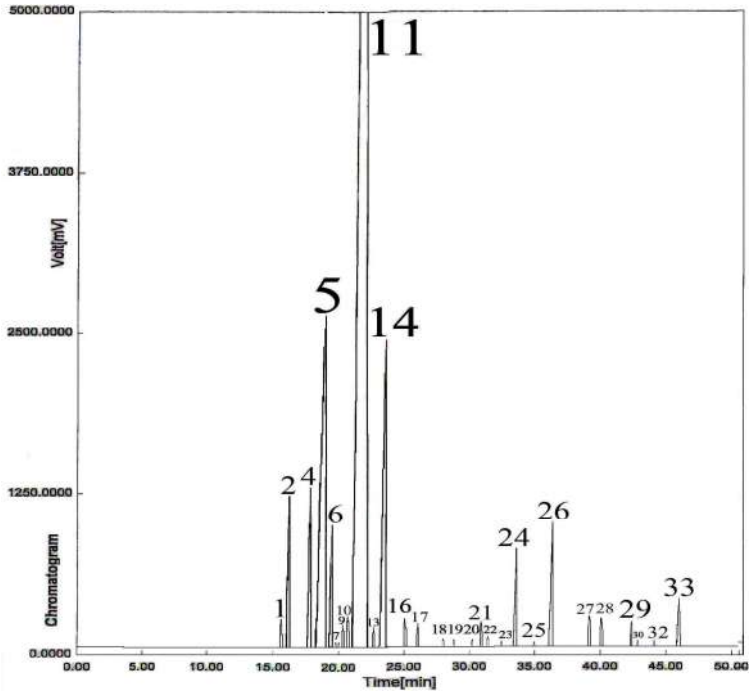

Fig1. HRGC chromatogram of sweet lime peel oil obtained using cold-press.

DISCUSSION

Our observation that different methods had an effect on some of the components of citrus oil was in accordance with previous findings [5]. The concentrations of aldehyd components obtained by HD method were low because of the application of heating for long time resulting in thermal degradation of labile compounds.

The lower proportion of the detected aldehyd components in HD method was probably due to the use a large quantity of water [11] and was due to solubility of those compounds in the water phase. However,

the losses may be readily explained by selective absorption of these compounds on the pulp particles by the factor of solubility [3].
The higher proportion of the detected alcohol components in HD method was probably due to hydrolysis of some components that can react with water at high temperature and provide alcohols and acids [12].
Esters are constituents of essential oils and, in the presence of water, especially at high temperatures; they tend to react with water to form acids and alcohols [13]. Oil components like esters are sensitive to hydrolysis while others like acyclic monoterpene hydrocarbons and aldehydes are susceptible to polymerization (since the pH of water is often reduced during distillation, hydrolytic reactions are facilitated).[14]
High positive correlations between pairs of terpenes suggest a genetic control [15] 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. The highest positive value (correlation) was observed between geranial and neral (98%). This result indicates that these compounds should be under the control of a single dominant gene [15].

5- CONCLUSION

The recovery percentage of flavor compounds depends on method. Between two methods examined, cold-pressing showed the highest content of aldehydes. It is easy to observe the significant variations between HD and CP method, mainly in terms of the quantities of oxygenated compounds. The application of CP method can cause a lesser damage to thermal-sensitive molecules, so can be a good technique to recovery of Citrus compounds. The CP method can reduce the danger of thermal degradation of sensitive compounds. Also it is easy to carry out and can be applicable to large industrial scale. Further research on the relationship between extraction method and oxygenated terpenes is necessary.

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