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Comparison of Peel Components of Pummelo (Citrus Grandis) 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 pummelo obtained using cold-press and hydro distillation. In the last week of January 2012, at least 40 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 0.14% to 0.28%. 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.

Key Words: 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 Citrus grandis Osbeck commonly known as pummelo is native to Southeastern Asia. The main areas of pummelo production in the orient are Southern China, Southern Japan, Thailand, Vietnam, Malaysia, Korea and Indonesia [2]. It is one of the most important Citrus species used in world. Although it is as important species, the peel components of pummelo 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 [4]. Citrus oils are commercially used for flavoring foods, beverages, perfumes, cosmetics, medicines and etc [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: rootstock [6], varieties [7], seasonal variation [8], organ [9] and the technique of extraction [10-12].

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 [13].

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 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

Citrus scions: In 1989, pummelo 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,

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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]. Pummelo were used as plant material in this experiment (Table 1).

Table 1. Common and botanical names for citrus taxa used as scions and rootstock

Common name	Botanical Name	Parents	Category
Pummelo(scion)	*Citrus grandis* (shaddock)	*Unknown*	Pummelo
Sour orange (Rootstock)	*C. aurantium (L.)*	*Mandarin ×Pomelo*	Sour orange

Preparation of peel sample

In the last week of January 2012, at least 40 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 .

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) [11].

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) [11].

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 m) fused silica capillary column (J&W Scientific) and a flame ionization detector (FID) was used. The column temperature was programmed from 60 to 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 [14, 15].

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 pummelo obtained using cold-press (CP)

GC-MS analysis of the flavor compounds extracted from pummelo using cold-press allowed identification of 47 volatile components (Table 2, Fig. 1): 23 oxygenated terpenes [8 aldehydes, 10 alcohols, 4 esters, 1 ketone] and 24 non oxygenated terpenes [15 monoterpens, 9 sesqiterpens].

Flavor compounds of the pummelo obtained using hydro distillation (HD): GC-MS analysis of the flavor compounds extracted from pummelo using hydro distillation allowed identification of 40 volatile components (Table 2): 19 oxygenated terpenes [5 aldehydes, 11 alcohols, 2 esters, 1 ketone] and 21 non oxygenated terpenes [12 monoterpens, 9 sesqiterpens].

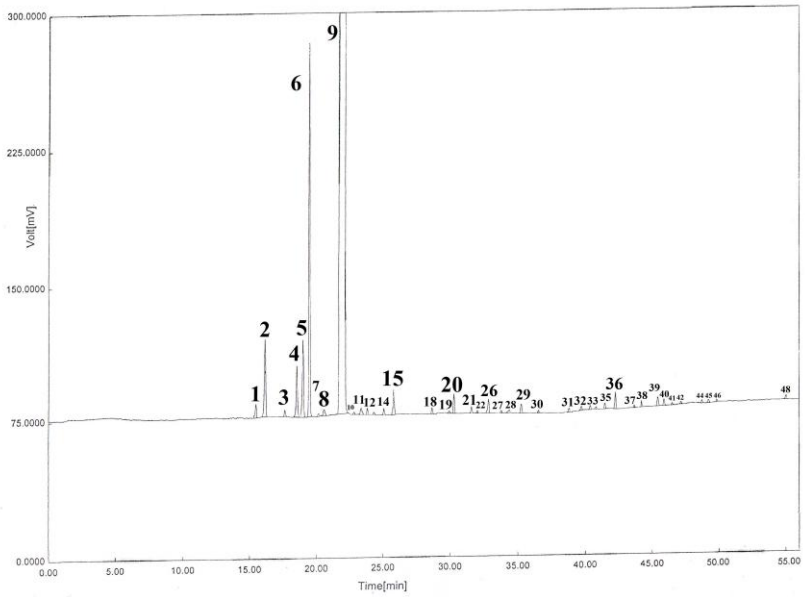

Fig1. HRGC chromatograms of pummelo peel oil obtained using cold-press

Table 2. Peel components of pummelo obtained using cold-press and hydodistillation. (*There is in oil)

	Component	Cold-press	Hydro distillation	KI		Component	Cold-press	Hydro distillation	KI
1	α - thujene	*	*	928	25	Nerol	*	*	1237
2	α - Pinene	*	*	935	26	Neral	*	*	1244
3	Camphene	*	*	951	27	geraniol	*	*	1259
4	Sabinene	*	*	975	28	Linalyl acetate	*		1262
5	β -pinene	*	*	979	29	Geranial	*	*	1274
6	β -myrcene	*	*	991	30	Perilla aldehyde	*		1280
7	octanal	*		1003	31	α -terpinyl acetate	*		1351
8	α - phellandrene	*	*	1006	32	Neryl acetate	*	*	1356
9	Limonene	*	*	1036	33	α -copaene	*	*	1384
10	(Z)- β - ocimene	*	*	1044	34	Granyl acetate	*	*	1389
11	(E)- β - ocimene	*	*	1055	35	Dodecanal	*	*	1409
12	γ - terpinene	*	*	1061	36	(Z)- β -caryophyllene	*	*	1416
13	cis-sabinene hydrate	*		1065	37	β -copaene	*	*	1439
14	α -terpinolene	*	*	1091	38	α - humulene	*	*	1462
15	Linalool	*	*	1100	39	Germacrene D	*	*	1493
16	cis-limonen oxide	*		1137	40	Bicyclogermacrene	*	*	1504
17	trans-limonen oxide	*		1141	41	E,E, α - farnesene	*	*	1513
18	Citronellal	*	*	1154	42	δ-cadinene	*	*	1531
19	terpinen-4-ol	*	*	1182	43	elemol		*	1559
20	α - terpineol	*	*	1195	44	germacrene B	*	*	1567
21	Decanal	*	*	1205	45	(E)-nerolidol	*	*	1571
22	trans-carveol	*	*	1219	46	Tetradecanal	*		1621
23	β -citronellol	*	*	1229	47	E,E-cis-farnesol	*	*	1732
24	cis-carveol	*	*	1234	48	Nootkatone	*	*	1814
							47	40	

Aldehydes

Eight aldehyde components that identified in this analysis were octanal, citronellal, decanal, neral, geranial, perillaldehyde, dodecanal, and tetradecanal (Table 3). In addition they were quantified from 0.14% to 0.28%. The concentration of neral was higher in our samples. Neral has a lemon-like aroma and is considered as one of the

major contributors to pummelo flavor [16]. Between two methods examined, cold-pressing 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, method apparently has a profound influence on this factor. Peel aldehydes obtained using cold-pressing was also compared to those of hydro distillation in this study. Octanal, perillaldehyde and tetradecanal were identified in cold-pressing, while they were not detected in hydro distillation. Compared with hydro distillation, the cold-pressing improved and increased aldehyde components about 2 times (Table 3).

Table 3. Statistical analysis of variation in peel components of pummelo obtained using cold-press and Hydro distillation.

Compounds	Cold-press		Hydrodistillation		
	Mean	St.err	Mean	St.err	F value
Oxygenated compounds					
a) Aldehyds					
1) Octanal	0.01	0.006	0	0	
2) Citronellal	0.03	0.006	0.01	0.006	
3) Decanal	0.04	0.006	0.03	0.006	
4) Neral	0.1	0	0.05	0.01	F**
5) Geranial	0.06	0.01	0.04	0.01	
6) Perilla aldehyde	0.01	0	0	0	
7) Dodecanal	0.02	0.006	0.01	0	
8) Tetradecanal	0.01	0	0	0	
total	0.28	0.03	0.14	0.03	
b) Alcohols					
1) Linalool	0.13	0.05	0.3	0.02	F**
2) Terpinen-4-ol	0.01	0	0.08	0.006	
3) α-terpineol	0.1	0	0.23	0.02	F**
4) Trans-carveol	0.01	0	0.02	0.006	
5) β-citronellol	0.002	0.001	0.01	0.006	
6) cis-carveol	0.001	0.001	0.01	0	
7) Nerol	0.001	0	0.01	0.006	
8) Geraniol	0.01	0	0.03	0.006	
9) Elemol	0	0	0.02	0.006	
10) (E)-Nerolidol	0.02	0.006	0.03	0.006	
11) E,E-α-farnesol	0.003	0.001	0.01	0.006	
total	0.28	0.05	0.75	0.08	
c) Esters					
1) linalyl acetate	0.02	0	0	0	
2) α -terpinyl acetate	0.02	0.006	0	0	
3) Neryl acetate	0.02	0.006	0.01	0.006	
4) Geranyl acetate	0.01	0	0.01	0.006	
total	0.07	0.01	0.02	0.01	
d) Ketones					
1) Nootkatone	0.02	0.006	0.01	0	
Monoterpenes					
1) α-thujene	0.080	0.006	0.04	0.006	
2) α-pinene	0.5	0.1	0.6	0.1	NS
3) Camphene	0.03	0.006	0.01	0	
4) Sabinene	0.37	0.05	0.45	0.03	NS
5) β-pinene	0.47	0.05	0.64	0.04	F*
6) β-myrcene	1.9	0.2	1.96	0.11	NS
7) α-phellandrene	0.06	0.01	0.13	0.02	
8) Limonene	94.17	0.97	94.59	0.52	NS
9) (Z)-β-ocimene	0.01	0.006	0.01	0	
10) (E)-β-ocimene	0.07	0.006	0.08	0.006	
11) γ-terpinene	0.04	0.006	0.12	0.02	
12) Cis-sabinene hydrate	0.01	0	0	0	
13) α-terpinolene	0.03	0.006	0.08	0.01	
14) cis-limonen oxide	0.004	0.001		0	
15) Trans-limonen oxide	0.003	0.001	0	0	
total	97.74	1.41	98.71	0.86	
Sesquiterpenes					
1) α-copaene	0.02	0	0.01	0	
2) (Z)-β-caryophyllene	0.09	0.01	0.07	0.01	
3) β -copaene	0.02	0.006	0.02	0	
4) α-humulene	0.02	0.006	0.01	0	
5) Germacrene D	0.07	0	0.04	0.006	
6) Bicyclogermacrene	0.03	0.006	0.02	0.006	
7) E,E-α-farnesene	0.01	0	0.01	0.006	
8) δ-cadinene	0.01	0	0.01	0.006	
9) Germacrene B	0.01	0	0.008	0.001	
total	0.28	0.028	0.19	0.03	
Total oxygenated compounds	0.65	0.11	0.9	0.12	
Total	98.68	1.55	99.82	1.02	

Alcohols: Eleven alcoholic components identified in this analysis were linalool, terpinene-4-ol, -terpineol, trans-carveol, -citronellol, cis-carveol, nerol, geraniol, elemol, (E)-nerolidol and E, E-α-farnesol (Table 3). The total amount of alcohols ranged from 0.28% to 0.75%. Linalool was identified as the major component in this study and was the most abundant. Linalool has been recognized as one of the most important components for Citrus flavor. Linalool has a flowery aroma [17] and its level is important to the characteristic favor of Citrus [5]. Between two methods examined, hydro distillation showed the highest content of alcohols.

Compared with cold-pressing, hydro distillation improved and increased alcohol components about 2.50 times. (Table 3)

Esters: Four ester components identified in this analysis were linalyl acetate, terpinyl acetate, neryl acetate and geranyl acetate. The total amount of esters ranged from 0.02% to 0.07%. Between two methods examined, cold-pressing showed the highest content of esters (Table 3).

Ketones: One component identified in this analysis was Nootkatone. The total amount of ketones ranged from 0.01% to 0.02%. Between two methods examined, cold-pressing showed the highest content of ketones (Table 3).

Monoterpene hydrocarbons: The total amount of monoterpene hydrocarbons ranged from 97.74 % to 98.71 %. Limonene was identified as the major component in this study and was the most abundant. Limonene has a weak citrus-like aroma [17] 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 0.19 % to 0.28 %. (Z)-β-caryophyllene 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 of statistical analyses: Differences were considered to be significant at $P < 0.01$. These differences on the 1% level occurred in neral, linalool and α-terpineol. This difference on the 5% level occurred in β-pinene. The non-affected oil components were α-pinene, sabinene, β-myrcene and limonene (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 B-pinene and sabinene (95%); B-pinene and α-terpineol (93%); B-pinene and linalool (92%); α-terpineol and linalool (92%). The highest significant negative correlations were observed between α-terpineol and neral (90%); linalool and neral (86%); B-pinene and neral (81%) (Table 4).

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

Item	Neral	Linalool	α-terpineol	α-pinene	Sabinene	B-pinene	B-myrcene
Linalool	-0.86*						
α-terpineol	-0.90*	0.92**					
α-pinene	-0.37	0.75	0.63				
Sabinene	-0.65	0.83*	0.79	0.57			
B-pinene	-0.81*	0.92**	0.93**	0.63	0.95**		
B-myrcene	-0.12	0.54	0.32	0.91**	0.31	0.33	
Limonene	-0.21	0.04	0.38	-0.08	0.25	0.34	-0.42

*=significant at 0.05 **=significant at 0.01

DISCUSSION

Our observation that different methods have an effect on some of the components of citrus oil is in accordance with previous findings [10- 12]. The 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 [18] and was due to solubility of those compounds in the water phase. However, the losses may be as readily explained by selective absorption of these compounds on the pulp particles by the factor of solubility [4].

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 [19].

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 [20]. 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).[21]

High positive correlations between pairs of terpenes such as B-pinene and sabinene (95%); B-pinene and α-terpineol (93%); B-pinene and linalool (92%); α-terpineol and linalool (92%). suggest the presence of a genetic control [22] and such dependence between pairs of terpenes is due to derivation of one from another that is not known. Similarly, high negative correlations observed between α-terpineol and neral (90%); linalool and neral (86%); B-pinene and neral (81%) suggest that one of the two compounds is being 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 B-pinene and sabinene (95%) This result indicates that these compounds should be under the control of a single dominant gene [22].

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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