



## RESEARCH ARTICLE

### Comparison of Peel Components of Sweet Orange (*Citrus sinensis*) Obtained using Cold-press and Hydro Distillation 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 orange obtained using cold-press and hydro distillation. In the last week of January 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 0.30 to 0.68%. 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.

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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 (FAO, 2012). Shahsavari orange is a native cultivar of orange that cultivated extensively in the Mazandaran province located in the north region of Iran (Zaare-Nahandi *et al.*, 2008, Ebrahimzadeh *et al.*, 2004). It has been regarded as a Citrus fruit with potential commercial value because of its pleasant aroma. It is one of the most important orange cultivars used in Iran. Although it is an important cultivar, the peel components of Shahsavari orange 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 (Swisher and Swisher, 1977). Citrus oils are commercially used for flavoring foods, beverages, perfumes, cosmetics, medicines and etc (Salem, 2003). 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 (Babazadeh Darjazi *et al.*, 2009), scion (Baaliouamer and Meklati, 1988), seasonal variation (Babazadeh Darjazi *et al.*, 2011a), organ (Babazadeh Darjazi, 2011b) and the technique of extraction (Bousbia *et al.*, 2009; Habashi *et al.*, 2009; Menichini *et al.*, 2011). The main techniques used at industrial scale are cold pressing (CP), hydro distillation (HD), extraction with organic solvent, extraction with compressed CO<sub>2</sub> 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 (Gaspar and Leeke, 2004).

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, sweet orange 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; soil was classified as loam-clay, pH ranged from 6.9 to 7]. Shahsavari orange were used as plant material in this experiment (Table 1).

### Preparation of peel sample

In the last week of January 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.

### 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) (Habashi *et al.*, 2009).

### 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) (Habashi *et al.*, 2009).

### 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.

### 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 & Stauffer, 1991).

### 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. Variations between two methods were analyzed using one-way analysis of variance (ANOVA). The correlation between pairs of characters was evaluated using Pearson's correlation coefficient.

## RESULTS

### Flavor compounds of the sweet orange obtained using cold-press (CP)

GC-MS analysis of the flavor compounds extracted from sweet orange using cold-press allowed identification of 50 volatile components (Table 2, Fig. 1): 23 oxygenated terpenes [11 aldehydes, 9 alcohols, 3 esters] and 27 non oxygenated terpenes [15 monoterpenes, 12 sesquiterpenes].

### Flavor compounds of the sweet orange obtained using hydro distillation (HD)

GC-MS analysis of the flavor compounds extracted from sweet orange using hydro distillation allowed identification of 44 volatile components (Table 2): 20 oxygenated terpenes [8 aldehydes, 9 alcohols, 3 esters] and 24 non oxygenated terpenes [13 monoterpenes, 11 sesquiterpenes].

### Aldehydes

Eleven aldehyde components that identified in this analysis were octanal, nonanal, citronellal, decanal, neral, geranial, perillaldehyde, undecanal, dodecanal, β-sinensal and α-sinensal (Table 3). In addition they were quantified from 0.30 to 0.68%. The concentrations of octanal and decanal were higher in our samples. Octanal has a citrus-like aroma (Buettner *et al.*, 2003) and is considered as one of the major contributors to orange flavor (Kostadinovic *et al.*, 2005). 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. perillaldehyde, undecanal and α-sinensal 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 to about 2.26 times (Table 3).

### Alcohols

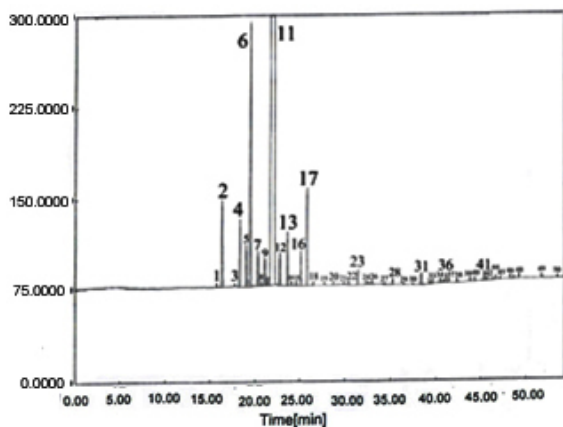
Nine alcoholic components identified in this analysis were octanol, linalool, terpinene-4-ol, α-terpineol, β-citronellol, nerol, geraniol, elemol and (E)-nerolidol (Table 3). The total amount of alcohols ranged from 1.00 to 1.81%. Linalool was identified as the major component in this study and was the most abundant. Linalool has been recognized as one of the most important components for Citrus flavor. Linalool has a flowery aroma (Buettner

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

Common name	botanical name	Parents	category
Sweet orange (scion)	<i>Citrus sinensis</i> cv. <i>shahsavari</i>	Unknown	Sweet orange
Sour orange (Rootstock)	<i>C. aurantium</i> (L.)	Mandarin × Pomelo	Sour orange

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

Component	Cold-press	Hydro distillation	KI	Component	Cold-press	Hydro distillation	KI
1 $\alpha$ -thujene	*		928	27 Geraniol	*	*	1259
2 $\alpha$ -Pinene	*	*	935	28 Geranial	*	*	1274
3 Camphene	*	*	951	29 Perilla aldehyde	*		1280
4 Sabinene	*	*	975	30 Undecanal	*		1313
5 $\beta$ -pinene	*	*	979	31 $\delta$ -elemene	*	*	1344
6 $\beta$ -myrcene	*	*	991	32 Citronellyl acetate	*	*	1360
7 Octanal	*	*	1003	33 Neryl acetate	*	*	1368
8 $\alpha$ -phellandrene	*	*	1006	34 $\alpha$ -copaene	*		1384
9 $\delta$ -3-carene	*	*	1019	35 Geranyl acetate	*	*	1389
10 $\alpha$ -terpinene	*	*	1021	36 $\beta$ -elemene	*	*	1399
11 Limonene	*	*	1036	37 Dodecanal	*	*	1409
12 (E)- $\beta$ -ocimene	*	*	1057	38 (Z)- $\beta$ -caryophyllene	*	*	1416
13 $\gamma$ -terpinene	*	*	1061	39 (Z)- $\beta$ -farnesene	*	*	1451
14 Octanol	*	*	1069	40 $\alpha$ -humulene	*	*	1462
15 Cis-sabinene hydrate	*	*	1072	41 Germacrene D	*	*	1493
16 $\alpha$ -terpinolene	*	*	1091	42 Valencene	*	*	1499
17 Linalool	*	*	1100	43 Bicyclogermacrene	*	*	1504
18 Nonanal	*	*	1109	44 E,E, $\alpha$ -farnesene	*	*	1513
19 Trans-limonen oxide	*		1143	45 $\delta$ -cadinene	*	*	1530
20 Citronellal	*	*	1154	46 Elemol	*	*	1558
21 Terpinen-4-ol	*	*	1182	47 (E)-nerolidol	*	*	1567
22 $\alpha$ -terpineol	*	*	1195	48 Caryophyllene oxide	*	*	1588
23 Decanal	*	*	1208	49 $\beta$ -sinensal	*	*	1704
24 $\beta$ -citronellol	*	*	1229	50 $\alpha$ -sinensal	*		1762
25 Nerol	*	*	1237		50	44	
26 Neral	*	*	1244				

**Fig. 1:** HRGC chromatograms of sweet orange peel oil obtained using cold-press.

*et al.*, 2003) and its level is important to the characteristic favor of Citrus (Salem, 2003). Between two methods examined, hydro distillation showed the highest content of alcohols. Compared with cold-pressing, hydro distillation improved and increased alcohol components about 1.81 times (Table 3).

### Esters

Three ester components identified in this analysis were citronellyl acetate, neryl acetate and geranyl acetate. The total amount of esters ranged from 0.02% to 0.05%. Between two methods examined, cold-pressing showed the highest content of esters (Table 3).

### Monoterpene hydrocarbons

The total amount of monoterpene hydrocarbons ranged from 96.01 to 96.43%. 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 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 0.27 to 0.44%.  $\delta$ -elemene 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

Statistical analysis was performed on the peel data using SPSS 18. Comparisons were made using one-way analysis of variance (ANOVA) and Duncan's multiple range tests. Differences were considered to be significant at  $P < 0.01$ . These differences on the 1% level occurred in octanal and linalool. These differences on the 5% level occurred in  $\alpha$ -pinene and  $\gamma$ -terpinene. The non affected oil components were  $\beta$ -pinene,  $\beta$ -myrcene, sabinene and limonene (Table 3).

### Results of correlation

Simple intercorrelations between 8 components are presented in a correlation matrix (Table 4). The highest positive values or  $r$  (correlation coefficient) were observed

**Table 3:** Statistical analysis of variation in peel components of sweet orange obtained using cold-press and Hydro distillation. 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.

Compounds	Cold-press		Hydrodistillation		F value
	Mean	St.err	Mean	St.err	
Oxygenated compounds					
a) Aldehyds					
1) Octanal	0.26	0.04	0.09	0.01	F**
2) Nonanal	0.05	0.006	0.02	0	
3) Citronellal	0.05	0.01	0.02	0.006	
4) Decanal	0.14	0.01	0.11	0.01	
5) Neral	0.04	0.006	0.02	0.01	
6) Geranial	0.07	0.01	0.03	0.01	
7) Perilla aldehyde	0.01	0	0	0	
8) Undecanal	0.01	0	0	0	
9) Dodecanal	0.02	0	0.01	0	
10) $\beta$ -sinensal	0.02	0.006	0.007	0.001	
11) $\alpha$ -sinensal	0.01	0.006	0	0	
total	0.68	0.09	0.30	0.04	
b) Alcohols					
1) Octanol	0.04	0.006	0.05	0.01	
2) Linalool	0.8	0.1	1.44	0.11	F**
3) Terpinen-4-ol	0.01	0	0.06	0.01	
4) $\alpha$ -terpineol	0.07	0.01	0.15	0.02	
5) $\beta$ -citronellol	0.02	0	0.03	0.006	
6) Nerol	0.01	0	0.02	0.006	
7) Geraniol	0.01	0	0.03	0.006	
8) Elemol	0.03	0.006	0.01	0.006	
9) (E)-Nerolidol	0.01	0	0.02	0.006	
total	1.00	0.12	1.81	0.18	
c) Esters					
1) Citronellyl acetate	0.01	0	0.004	0.001	
2) Neryl acetate	0.02	0.006	0.01	0	
3) Geranyl acetate	0.02	0.006	0.01	0.006	
total	0.05	0.012	0.02	0.007	
Monoterpenes					
1) $\alpha$ -thujene	0.02	0	0	0	
2) $\alpha$ -pinene	0.73	0.07	0.88	0.06	F*
3) Camphene	0.01	0	0.02	0.006	
4) Sabinene	0.5	0.1	0.61	0.05	NS
5) $\beta$ -pinene	0.35	0.05	0.41	0.04	NS
6) $\beta$ -myrcene	1.7	0.1	1.75	0.13	NS
7) $\alpha$ -phellandrene	0.02	0.006	0.04	0.006	
8) $\delta$ -3-carene	0.2	0.02	0.24	0.02	
9) $\alpha$ -terpinene	0.03	0.006	0.05	0.006	
10) Limonene	91.47	1.45	91.22	1.42	NS
11) (E)- $\beta$ -ocimene	0.24	0.02	0.28	0.02	
12) $\gamma$ -terpinene	0.43	0.04	0.55	0.05	F*
13) Cis-sabinene hydrate	0.03	0.006	0.01	0	
14) $\alpha$ -terpinolene	0.27	0.03	0.37	0.03	
15) Trans-limonen oxide	0.01	0.006	0	0	
total	96.01	1.904	96.43	1.838	
Sesquiterpenes					
1) $\delta$ -elemene	0.09	0.006	0.06	0.01	
2) $\alpha$ -copaene	0.03	0	0	0	
3) $\beta$ -elemene	0.08	0.006	0.04	0.006	
4) (Z)- $\beta$ -caryophyllene	0.02	0.006	0.03	0.006	
5) (Z)- $\beta$ -farnesene	0.02	0.006	0.01	0.006	
6) $\alpha$ -humulene	0.02	0.006	0.01	0	
7) Germacrene D	0.08	0.01	0.04	0.006	
8) Valencene	0.04	0.006	0.05	0.006	
9) Bicyclogermacrene	0.01	0	0.006	0.001	
10) E,E- $\alpha$ -farnesene	0.02	0.006	0.01	0.006	
11) $\delta$ -cadinene	0.01	0	0.01	0	
12) Caryophyllene oxide	0.02	0.006	0.01	0	
total	0.44	0.05	0.27	0.04	
Total oxygenated compounds	1.73	0.22	2.14	0.23	
Total	98.18	2.19	98.84	2.11	

between B-pinene and sabinene (98%);  $\gamma$ -terpinene and  $\alpha$ -pinene (98%); B-pinene and  $\alpha$ -pinene (96%). The highest significant negative correlations were observed between linalool and octanal (87%) (Table 4).

## DISCUSSION

Our observation that different methods have an effect on some of the components of citrus oil is in accordance with previous findings (Bousbia *et al.*, 2009; Habashi *et al.*, 2009; Menichini *et al.*, 2011). 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 aldehyde components in HD method was probably due to the use a large quantity of water (Porto and Decorti, 2009) 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 (Swisher and Swisher, 1977).

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 (Gontaru, 2009).

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 (Atal and Kapur, 1982; Guenther, 1972). 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). (Silva TD, 1995).

High positive correlations between pairs of terpenes such as B-pinene and sabinene (98%);  $\gamma$ -terpinene and  $\alpha$ -pinene (98%); B-pinene and  $\alpha$ -pinene (96%) suggest the presence of a genetic control (Scora *et al.*, 1976) 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 linalool and octanal (87%) 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 (98%);  $\gamma$ -terpinene and  $\alpha$ -pinene (98%). This result indicates that these compounds should be under the control of a single dominant gene (Scora *et al.*, 1976).

## 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

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

	Octanal	Linalool	$\alpha$ -pinene	Sabinene	B-pinene	B-myrcene	Limonene
Linalool	-0.87*						
$\alpha$ -pinene	-0.66	0.93**					
Sabinene	-0.43	0.80	0.94**				
B-pinene	-0.45	0.82*	0.96**	0.98**			
B-myrcene	-0.06	0.49	0.74	0.81*	0.87*		
Limonene	0.32	0.15	0.46	0.63		0.91*	
$\gamma$ -terpinene	-0.67	0.93**	0.98**	0.90*	0.94**	0.74	0.47

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

can cause a lesser damage to thermal-sensitive molecules, so can be a good technique to recovery 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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