JOURNAL OF CURRENT RESEARCH IN SCIENCE (ISSN 2322-5009)
CODEN (USA): JCRSDJ
2014, Vol. 2, No. 3, pp: 375-380
Available at www.jcrs010.com

ORIGINAL ARTICLE

COMPARISON OF PEEL COMPONENTS OF GRAPEFRUIT (CITRUS PARADISI) 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 grapefruit 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 0.37% to 0.70%. 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.

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 tons (FAO, 2012). Grapefruit resulted from a natural cross between the pummelo and the sweet orange (Fotouhi and Fattahi, 2007). It is one of the most important Citrus species used in world. Although it is as important species, the peel components of grapefruit have been investigated very little previously (Babazadeh, 2013a).

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 (Verzera *et al.*, 2003), scion (Babazadeh, 2013b), seasonal variation (Babazadeh *et al.*, 2011a), organ (Babazadeh, 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 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 (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

2.1. Grapefruit Scions

In 1989, grapefruit 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]. Grapefruit was

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

2.2. 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.

2.3. 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).

2.4. 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).

2.5. 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 1µL.

2.6. 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).

2.7. 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

3.1. Flavor Compounds of the Grapefruit Obtained Using Cold-press (CP) and Hydro distillation (HD) Method

GC-MS analysis of the flavor compounds extracted from grapefruit using cold-press and hydro distillation allowed identification of 33 volatile components (Table 2, Fig. 1): 19 oxygenated terpenes [8 aldehydes, 7 alcohols, 3 esters, 1 ketone] and 14 non oxygenated terpenes [9 monoterpens, 5 sesqiterpens].

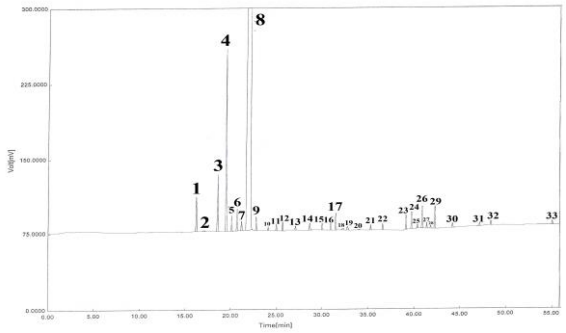

Figure 1: HRGC chromatograms of grapefruit peel oil obtained using cold-press.

3.2. Aldehydes

Eight aldehyde components that identified in this analysis were octanal, nonanal, citronellal, decanal, neral, geranial, perillaldehyde and dodecanal (Table 3). In addition they were quantified from 0.37% to 0.70%. The concentration of decanal was higher in our samples. Decanal has a lemon-like aroma and is considered as one of the major contributors to grapefruit flavor (Lin and Rouseff, 2001). 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.
Compared with hydro distillation, the cold-pressing improved and increased aldehyde components about 1.89 times (Table 3).

3.3. Alcohols
Seven alcoholic components identified in this analysis were octanol, linalool, terpinene-4-ol, α-terpineol, nerol, geraniol and elemol (Table 3). The total amount of alcohols ranged from 0.40% to 1.01%. 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 *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 2.52 times. (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 0.39% to 0.57%. Between two methods examined, cold-pressing showed the highest content of esters (Table 3).

3.5. Ketones
One component identified in this analysis was Nootkatone. The total amount of ketones ranged from 0.02% to 0.04%. Between two methods examined, cold-pressing showed the highest content of ketones (Table 3).

3.6. Monoterpene hydrocarbons
The total amount of monoterpene hydrocarbons ranged from 95.63 % to 97.64%. 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).

3.7. Sesquiterpene hydrocarbons
The total amount of sesquiterpene hydrocarbons ranged from 0.30 % to 0.42%. (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).

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

Common name	botanical name	Parents	category
Grapefruit (scion)	*Citrus paradise cv. Marsh*	pummelo ×sweet orange	Grapefruit
Sour orange (Rootstock)	*C. aurantium (L.)*	*Mandarin ×Pomelo*	Sour orange

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

	Component	Cold-press	Hydro distillation	KI		Component	Cold-press	Hydro distillation	KI
1	α-Pinene	*	*	935	18	Nerol	*	*	1235
2	Camphene	*	*	951	19	Neral	*	*	1244
3	Sabinene	*	*	975	20	Geraniol	*	*	1258
4	β-myrcene	*	*	991	21	Geranial	*	*	1275
5	Octanal	*	*	1003	22	Perilla aldehyde	*	*	1280
6	α-phellandrene	*	*	1006	23	Citronellyl acetate	*	*	1350
7	α-terpinene	*	*	1014	24	Neryl acetate	*	*	1356
8	Limonene	*	*	1036	25	α-copaene	*	*	1373
9	(E)-β-ocimene	*	*	1049	26	Granyl acetate	*	*	1382
10	γ-terpinene	*	*	1061	27	β-elemene	*	*	1398
11	Octanol	*	*	1070	28	Dodecanal	*	*	1409
12	Linalool	*	*	1100	29	(Z)-β-caryophyllene	*	*	1415
13	Nonanal	*	*	1108	30	α-humulene	*	*	1463
14	Citronellal	*	*	1154	31	δ-cadinene	*	*	1522
15	Terpinene-4-ol	*	*	1182	32	Elemol	*	*	1558
16	α-terpineol	*	*	1195	33	Nootkatone	*	*	1815
17	Decanal	*	*	1205			33	33	

Table 3*:* Statistical analysis of variation in peel components of grapefruit 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		Hydro distillation		
	Mean	St. err	Mean	St. err	F-value
Oxygenated compounds					
Aldehyds					
1) Octanal	0.15	0.01	0.09	0.01	
2) Nonanal	0.04	0.006	0.02	0	
3) Citronellal	0.12	0.01	0.07	0.01	
4) Decanal	0.19	0.02	0.11	0.02	F**
5) Neral	0.05	0.006	0.02	0	
6) Geranial	0.06	0.006	0.03	0.006	
7) Perilla aldehyde	0.07	0.006	0.02	0	
8) Dodecanal	0.02	0	0.01	0	
total	0.70	0.06	0.37	0.04	
Alcohols					
1) Octanol	0.07	0.006	0.11	0.02	
2) Linalool	0.1	0.01	0.23	0.03	F**
3) Terpinen-4-ol	0.07	0.01	0.32	0.04	F**
4) α-terpineol	0.07	0.006	0.19	0.02	
5) Nerol	0.04	0.006	0.07	0.01	
6)Geraniol	0.008	0.001	0.02	0.006	
7) Elemol	0.05	0.006	0.07	0.01	
total	0.40	0.04	1.01	0.13	
Esters					
1) Citronellyl acetate	0.16	0.01	0.11	0.02	
2) Neryl acetate	0.18	0.01	0.13	0.03	
3) Granyl acetate	0.23	0.02	0.15	0.02	F**
total	0.57	0.04	0.39	0.07	
Ketones					
1) Nootkatone	0.04	0.006	0.02	0.006	
Monoterpenes					
1) α-pinene	0.39	0.03	0.55	0.05	F*
2) Camphene	0.005	0.001	0.01	0	
3) Sabinene	0.62	0.04	0.91	0.08	F**
4) β-myrcene	1.72	0.07	1.88	0.12	NS
5) α-phellandrene	0.24	0.03	0.49	0.07	
6) α-terpinene	0.1	0.006	0.17	0.02	
7) Limonene	92.39	1.45	93.38	0.73	NS
8) (E)-β-ocimene	0.14	0.01	0.16	0.02	
9) γ-terpinene	0.03	0.006	0.09	0.01	
total	95.63	1.64	97.64	1.10	
Sesquiterpenes					
1) α-copaene	0.04	0.006	0.03	0.006	
2) β-elemene	0.06	0.01	0.03	0.006	
3) (Z)-β-caryophyllene	0.25	0.02	0.20	0.02	
4) α-humulene	0.05	0.006	0.03	0.006	
5) δ-cadinene	0.02	0	0.01	0.006	
total	0.42	0.04	0.30	0.04	
Total oxygenated compounds	1.71	0.15	1.79	0.25	
Total	97.77	1.84	99.73	1.40	

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

Component	Decanal	Linalool	Terpinen-4-ol	Granyl acetate	α-pinene	Sabinene	B-myrcene
Linalool	-0.76						
Terpinen-4-ol	-0.84*	0.99**					
Granyl acetate	0.99**	-0.78	-0.85*				
α-pinene	-0.72	0.99**	0.96**	-0.74			
Sabinene	-0.73	0.99**	0.98**	-0.74	0.97**		
B-myrcene	-0.38	0.86*	0.81	-0.38	0.85*	0.90*	
Limonene	-0.23	0.66	0.57	-0.29	0.75	0.60	0.63

*=significant at 0.05
**=significant at 0.01

3.8. Results of statistical analyses

Differences were considered to be significant at $P < 0.01$. These differences on the 1% level occurred in decanal, linalool, terpinen-4-ol, granyl acetate and sabinene. This difference on the 5% level occurred in α-pinene. The non-affected oil components were β-myrcene and limonene (Table 3).

3.9. 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 terpinen-4-ol and linalool (99%); granyl acetate and decanal (99%); α-pinene and linalool (99%); sabinene and linalool (99%). The highest significant negative correlations were observed between granyl acetate and terpinen-4-ol (85%); terpinen-4-ol and decanal (84%) (Table 4).

DISCUSSION

Our observation that different methods had an effect on some of the components of citrus oil was in accordance with previous findings (Bousbia *et al.*, 2009; Habashi *et al.*, 2009; Menichini *et al.*, 2011). 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 (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 (Handa, 2008). 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)(Lawrence, 1995).

High positive correlations between pairs of terpenes such as terpinen-4-ol and linalool (99%); granyl acetate and decanal (99%); α-pinene and linalool (99%); sabinene and linalool (99%) 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 granyl acetate and terpinen-4-ol (85%); terpinen-4-ol and decanal (84%) 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 terpinen-4-ol and linalool (99%); granyl acetate and decanal (99%); α-pinene and linalool (99%); sabinene and linalool (99%). 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 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.

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.

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