



The Effect of Two Mandarin (*Citrus reticulata*) Cultivars on Peel Components and Juice Quality Parameters

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ABSTRACT

Studies have shown that oxygenated compounds and TSS are important in food products. It seems that cultivars have a profound influence on these factors. The goal of the present study is to investigate on flavor components and juice quality parameters of two mandarin cultivars. In the last week of January 2012, at least 10 mature fruit were collected and their peel oil was extracted using cold-press and eluted using n-hexane, then analyzed using GC and GC-MS. TSS were determined using a refractometer. Data were analyzed using one-way analysis of variance (ANOVA) and Duncan's multiple range tests. The amount of aldehydes ranged from 0.19% to 1.09%. TSS ranged from 7.6% to 9.8 %. Between two cultivars examined, Willow leaf showed the highest content of aldehydes and TSS. As a result of our study, we can conclude that the cultivars used can influence the quantity of oxygenated compounds present in the oil and TSS present in the juice.

Keywords: Flavor components, Juice quality, Peel oil, Mandarin cultivars.

1- 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). The King mandarin apparently originated in the indo-china region (Malaya, Vietnam, Cambodia) and introduced to California about 1880. Willow leaf seems that originated in china but it commonly known as Mediterranean mandarin because it is widely grown in that region. It called "Willow leaf" because of its leaves is similar to willow tree. It has been regarded as a Citrus fruit with potential commercial value because of its attractive and pleasant aroma (Ladanyia, 2008). They are two of the most important mandarin cultivars used in world. Although they are as important cultivars, the peel components of Willow leaf and king have been investigated very little before.

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 (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), scions or cultivars (Lota et al., 2000; Lota et al., 2001), seasonal variation (Babazadeh Darjazi et al., 2011a), organ (Bbazadeh Darjazi, 2011b), extraction method (Bbazadeh Darjazi, 2011c) and etc. Branched aldehydes and alcohols are important flavor compounds extensively used in food products (Salem, 2003). Several studies have shown that the tangerine-like smell is mainly a result of the presence of carbonyl compounds such as α -sinensal, geranial, citronellal, decanal and perilaldehyde (Buettner et al., 2003). The quality of a honey can be calculated from the amount of oxygenated components present in the honey (Alissandrakis et al., 2003; Alistair et al., 1993). 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 (Kite et al., 1991; Andrews et al., 2007). Citrus juice is the most popular beverage in the world because of the fantastic flavor and abundant nutrition. The quality of citrus juice is an important economic factor in an industry that buys its fruit based on the juice sugar content and processes over 95% (Rouse, 2000). The best juices are consumed by the food and beverage industries. The quality of citrus juice may be determined not only by the amount of oxygenated components present in the juice but also by the concentration of compositions such as TSS, acids and vitamin C (Babazadeh darjazi et al., 2009). Juice, TSS and TA content are the main internal parameters used to determine Citrus quality in the world (Antonucci et al., 2011). TSS content also forms the basis of payment for fruit by some juice processors in a number of countries, especially where the trade in juice is based on frozen concentrate (Hardy and Sanderson, 2010). The quantity of TSS, present in the juice, is variable and depends upon a number of factors including: rootstock, scion or cultivar, degree of maturity, seasonal effects, climate, nutrition, tree age and etc (Hardy and Sanderson, 2010). Several studies have shown that the cultivar used may influence the quantity of chemical compositions (TSS, TA and vitamin C) present in the juice (Nematollahi, 2005). Compared with orange juice, very little research has been carried out on mandarin juice. Therefore, it is very important to be able to assess the differences between mandarin cultivars in terms of quantity of compositions (TSS, acids and vitamin C). In this paper, we compare the peel compounds isolated from different cultivars with the aim of determining whether the quantity of oxygenated compounds influenced by the cultivars. Also the present study reports the effects of cultivars on the juice quality parameters.

2- MATERIALS AND METHODS

2-1) Mandarin cultivars

In 1989, mandarin cultivars 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 970 mm per year and average temperature 16.25°C; soil was classified as loam-clay, pH range (6.9 to 7)]. Willow leaf and King mandarin were used as cultivars in this experiment (Table 1).

2-2) Preparation of peel sample

In the last week of January 2012, at least 10 mature fruit were collected from many parts of the same trees located in Ramsar research station. 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.

2-3) Preparation of juice sample

In the last week of January 2012, at least 10 mature fruit were collected from many parts of the same trees located in Ramsar research station. Juice was obtained using the Indelicate Super Automatic, Type A2 104 extractor. After extraction, juice was screened to remove peel, membrane, pulp and seed pieces according to the standard operating procedure. Three replicates were carried out for the quantitative analysis (n=3). Ten fruits were used for each replicate.

2-4) Chemical methods

The total titratable acidity was assessed by titration with sodium hydroxide (0.1 N) and expressed as % citric acid. Total soluble solids, expressed as Brix, were determined using a Carl Zeiss, Jena (Germany) refractometer. The pH value was measured using a digital pH meter (WTW Inolab pH-L1, Germany). Ascorbic acid was determined by titration with Potassium iodide. The density of the juice was measured using a pycnometer and ash was determined by igniting a weighed sample in a muffle furnace at 550 °C to a constant weight ([Majedi, 1994](#)).

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 1 μL.

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 & 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 7 peel component and 6 juice characteristics. Variations between cultivars were analyzed using one-way analysis of variance (ANOVA). The Correlation between pairs of characters was evaluated using Pearson's correlation coefficient.

3- RESULTS

3-1) Flavor compounds of the Willow leaf mandarin peel

GC-MS analysis of the flavor compounds extracted from Willow leaf mandarin peel using cold-press allowed identification of 38 volatile components (Table 2) : 18 oxygenated terpenes [12 aldehydes , 6 alcohols] and 20 non oxygenated terpenes [11 monoterpenes, 9 sesquiterpenes].

3-2) Flavor compounds of the King mandarin peel

GC-MS analysis of the flavor compounds extracted from King mandarin peel using cold-press allowed identification of 21 volatile components (Table 2, Fig. 1): 9 oxygenated terpenes [4 aldehydes, 3 alcohols, 2 esters] and 12 non oxygenated terpenes [6 monoterpenes, 6 sesquiterpenes].

3-3) Aldehydes

Twelve aldehyde components that identified in this analysis were octanal, nonanal, citronellal, decanal, (E)-2-decanal, geranial, perillaldehyde, undecanal, , dodecanal, (E)-2-decenal, tetradecanal and α -sinensal (Table 3). In addition they were quantified from 0.19% to 1.09%. The concentration of octanal was higher in our samples. Octanal has a citrus-like aroma and is considered as one of the major contributors to mandarin flavor (Buettner et al., 2003). Between two cultivars examined, Willow leaf showed the highest content of aldehydes (Table 3). Since the aldehyde content of citrus oil is considered as one of the more important indicators of high quality, cultivar apparently has a profound influence on this factor. Willow leaf aldehydes were also compared to those of King in this study. Nonanal, (E)-2-decanal, perillaldehyde, undecanal, dodecanal, (E)-2-decenal, tetradecanal and α -sinensal were identified in Willow leaf, while they were not detected in King. Compared with King, the Willow leaf improved and increased aldehyde components about 5.73 times (Table 3).

3-4) Alcohols

Seven alcohol components identified in this analysis were linalool, terpinene-4-ol, α -terpineol, β -citronellol, thymol, P-menth-1-en-9-ol and elemol (Table 3). The total amount of alcohols ranged from 0.11% to 0.40%. 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 mandarin flavor (Buettner et al., 2003). Linalool has a flowery aroma (Buettner et al., 2003) and its level is important to the characteristic favor of tangerine (Salem, 2003). Between two cultivars examined, Willow leaf showed the highest content of alcohols (Table 3).

Willow leaf alcohols were also compared to those of King in this study. Terpinene-4-ol, β -citronellol, thymol and P-menth-1-en-9-ol were identified in Willow leaf, while they were not detected in King. Compared with King, Willow leaf improved and increased alcohol components about 3.63 times. (Table 3).

3-5) Esters

Two ester components identified in this analysis were citronellyl acetate and neryl acetate. The total amount of esters ranged from 0.00% to 0.03%. Between two cultivars examined, King showed the highest content of esters (Table 3).

3-6) Monoterpenes Hydrocarbons

The total amount of monoterpene hydrocarbons ranged from 92.5 % to 98.05 %. 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 mandarin flavor. Between two cultivars examined, king showed the highest content of monoterpenes (Table 3).

3-7) Sesquiterpenes Hydrocarbons

The total amount of sesquiterpene hydrocarbons ranged from 0.09 % to 0.15 %. (Z)- β -caryophyllene was the major component among the sesquiterpen hydrocarbons of Willow leaf. Between two cultivars examined, Willow leaf showed the highest content of sesquiterpenes (Table 3).

3-8) juice quality parameters

Juice quality parameters are given in table 4. Brix (total soluble solids) ranged from 7.6% (King) to 9.8 % (Willow leaf). The content of total acidity ranged from 0.51% (Willow leaf) to 2.03% (King). TSS/TA rate ranged from 3.74 (King) to 19.21 (Willow leaf). Ascorbic acid ranged from 20.06% (Willow leaf) to 28.34% (King). The pH value ranged from 2.88 (King) to 3.79 (Willow leaf). The juice yield ranged from 61.27% (King) to 62.53% (Willow leaf). Total dry matter ranged from 14.01% (Willow leaf) to 16.59% (King). Ash ranged from 2% (Willow leaf) to 4% (King). Between two cultivars examined, Willow leaf showed the highest content of TSS, TSS /TA and pH. (Table 4).

3-9) Results of statistical analyses

Statistical analysis was performed on the peel and juice 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 linalool, α -pinen, limonen, γ -terpinene TSS, TA, TSS /TA, ascorbic acid, pH and juice yield. These differences on the 5% level occurred in β -myrcene. The non affected oil components were octanal and sabinene (Table 3 and 4).

3-10) Results of correlation

Simple intercorrelations between 7 components are presented in a correlation matrix (Table 5). The highest positive values or r (correlation coefficient) were observed between [γ -terpinene and α -pinene (99%)]; [γ -terpinene and linalool (95%)]; [limonene and β -myrcene (93%)]. The highest significant

negative correlations were observed between [limonene and linalool (97%)]; [γ -terpinene and limonene (97%)]; [limonene and α -pinene (92%)] (Table 5). Also simple intercorrelations between 6 juice characteristics are presented in a correlation matrix (Table 6). The highest positive values or r (correlation coefficient) were observed between [pH and TSS (100%)]; [Ascorbic acid and TA (100%)]; [TSS /TA and TSS (99%)]; [pH and TSS /TA (99%)]; [juice and TSS (99%)]; [juice and pH (99%)]. The highest significant negative correlations were observed between [TA and TSS (99%)]; [TSS /TA and TA (99%)]; [Ascorbic acid and TSS (99%)]; [Ascorbic acid and TSS /TA (99%)]; [pH and TA (99%)]; [pH and Ascorbic acid (99%)] (Table 6).

4- DISCUSSION

Our observation that different cultivars have an effect on some of the components of mandarin oil is in accordance with previous findings (Lota et al., 2000; Lota et al., 2001). The compositions of the peel oils obtained by cold pressing from different cultivars of mandarin were very similar. However, the relative concentration of compounds was different according to the type of cultivar.

Comparison of our data with those in the literatures revealed some inconsistencies with previous studies (Lota et al., 2001). It may be related to rootstock and environmental factors that can influence the compositions. However, it should be noticed that the extraction methods also may influence the results. Fertilizer (Rui et al., 2006) and irrigation (Al-Rousan et al., 2012) affects the content of compositions present in citrus juice. Fertilization, irrigation and other operations were carried out uniform in this study so we do not believe that this variability is 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 \rightarrow Isopentenyl Pyrophosphate \rightarrow 3.3-dimethylallylpyrophosphate \rightarrow geranyl pyrophosphate \rightarrow Alcohols and Aldehyds

This reaction pathway catalyzed by isopentenyl pyrophosphate isomerase and geranyl pyrophosphate synthase, respectively (Hay and Waterman, 1995). The pronounced enhancement in the amount of oxygenated compounds, when Willow leaf used as the scion, showed that either the synthesis of geranyl pyrophosphate is enhanced or activities of both enzymes increased. High positive correlations between pairs of terpenes [γ -terpinene and α -pinene (99%)]; [γ -terpinene and linalool (95%)]; [limonene and B-myrcene (93%)] 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 [limonene and linalool (97%)]; [γ -terpinene and limonene (97%)]; [limonene and α -pinene (92%)] 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 [γ -terpinene and α -pinene (99%)]. This result indicates that these compounds should be under the control of a single dominant gene (Scora et al., 1976). 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 Willow leaf. Our results showed that there was a positive correlation between pH and TSS/TA. This finding was similar to previous studies (Baldwin, 2002).

5- CONCLUSION

In the present study we found that the amount of peel and juice compositions were significantly affected by cultivars and there was a great variation in most of the measured characters between two cultivars. The present study demonstrated that volatile compounds in peel and quality parameters in juice can vary when different cultivars are utilized. Between two cultivars examined, Willow leaf showed the highest content of TSS, TSS /TA and pH. The lowest of TSS, TSS /TA, pH content were produced by king. These results show that there is a positive correlation between pH and TSS/TA. Studies like this is very important to determine the amount of chemical compositions existing in the cultivars that we want to use, before their fruits can be utilized in food industries, aromatherapy, pharmacy, cosmetics, hygienic products and other areas. Further research on the relationship between cultivars and quality parameters is necessary.

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Table 1. Common and botanical names for citrus taxa used as scions and rootstock (Ladanya, 2008; Lota et al., 2001)

Common name	botanical name	Parents	category
Willow leaf (scion)	<i>Citrus deliciosa</i>	<i>Unknown</i>	Mandarin
King (scion)	<i>Citrus nobilis</i>	<i>Unknown</i>	Mandarin
Sour orange (Rootstock)	<i>C. aurantium (L.)</i>	<i>Mandarin × Pomelo</i>	Sour orange

Table 2. Peel volatile components of mandarin cultivars. (*There is in oil)

	Component	Willow leaf	King	KI		Component	Willow leaf	King	KI
1	α -thujene	*		928	23	Perilla aldehyde	*		1280
2	α -Pinene	*	*	935	24	Thymol	*		1291
3	Sabinene	*	*	975	25	P-menth-1-en-9-ol	*		1301
4	β -pinene	*		979	26	Undecanal	*		1307
5	β -myrcene	*	*	991	27	Citronellyl acetate		*	1350
6	octanal	*	*	1003	28	Neryl acetate		*	1356
7	α -terpinene	*		1012	29	α -copaene	*	*	1373
8	Limonene	*	*	1036	30	β -cubebene		*	1388
9	(Z)- β -ocimene	*		1042	31	β -elemene	*		1399
10	(E)- β -ocimene		*	1049	32	Dodecanal	*		1409
11	γ -terpinene	*	*	1061	33	(Z)- β -caryophyllene	*		1418
12	(E)sabinene hydrate	*		1065	34	(Z)- β -farnesene	*	*	1452
13	α -terpinolene	*		1091	35	α -humulene	*		1464
14	Linalool	*	*	1100	36	(E)-2-dodecenal	*		1474
15	Nonanal	*		1109	37	Germacrene D	*	*	1493
16	Citronellal	*	*	1154	38	α -selinene	*		1501
17	Terpinene-4-ol	*		1182	39	Bicyclogermacrene		*	1504
18	α -terpineol	*	*	1195	40	E,E, α -farnesene	*		1515
19	Decanal	*	*	1205	41	δ -cadinene	*	*	1531
20	β -citronellol	*		1229	42	Elemol		*	1558
21	(E)-2-decenal	*		1263	43	Tetradecanal	*		1612
22	Geranial	*	*	1275	44	α -sinensal	*		1756
							38	21	

Table 3- Statistical analysis of variation in peel flavor Components of mandarin cultivars (see Materials and methods). Mean is average composition in % over the different cultivars 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	Willow leaf		King		F value
	Mean	St.err	Mean	St.err	
Oxygenated compounds					
a) Aldehyds					
1) Octanal	0.16	0.01	0.14	0.01	NS
2) Nonanal	0.04	0			
3) Citronellal	0.02	0.01	0.01	0.006	
4) Decanal	0.08	0.01	0.04	0.006	
5) (E)-2-decanal	0.01	0.001			
6) Geranial	0.01	0.001	0.009	0.002	
7) Perilla aldehyde	0.03	0.01			
8) Undecanal	0.01	0			
9) Dodecanal	0.52	0.08			
10) (E)-2-decenal	0.01	0.006			
11) Tetradecanal	0.01	0.001			
12) α -sinensal	0.2	0.02			
total	1.09	0.14	0.19	0.02	
b) Alcohols					
1) Linalool	0.13	0.01	0.07	0.01	F**
2) Terpinen-4-ol	0.03	0.01			
3) α -terpineol	0.2	0.01	0.03	0	
4) β -citronellol	0.01	0.006			
5) Thymol	0.02	0.01			
6) P-menth-1-en-9-ol	0.01	0.002			
7) Elemol			0.01	0.006	
total	0.4	0.04	0.11	0.01	
c) Esters					
1) Citronellyl acetate			0.02	0.006	
2) Neryl acetate			0.01	0	
total			0.03	0.006	
Monoterpenes					
1) α -thujene	0.61	0.09			
2) α -pinene	1.94	0.24	0.55	0.03	F**
3) Sabinene	0.2	0.01	0.17	0.03	NS
4) β - pinene	1.42	0.31			
5) β -myrcene	1.49	0.13	1.75	0.05	F*
6) α -terpinene	0.24	0.09			
7) Limonene	67.6	4.28	94.72	1.14	F**
8) (Z)- β -ocimene	0.02	0			
9) (E)- β -ocimene			0.75	0.06	
10) γ -terpinene	17.7	0.88	0.11	0.03	F**
11) (E)-sabinene hydrate	0.39	0.08			
12) α -terpinolene	0.91	0.13			
total	92.5	6.24	98.05	1.34	
Sesquiterpenes					
1) α -copaene	0.01	0.002	0.03	0	
2) β -cubebene			0.02	0.006	
3) β -elemene	0.01	0.002			
4) (Z)- β -caryophyllene	0.08	0.01			
5) (Z)- β -farnesene	0.01	0.002	0.01	0	
6) α - humulene	0.01	0.002			
7) Germacrene D	0.01	0	0.01	0.006	
8) α - selinene	0.01	0.006			
9) Bicyclogermacrene			0.007	0.004	
10) E,E- α -farnesene	0.02	0.006			
11) δ -cadinene	0.003	0.001	0.02	0.01	
total	0.15	0.03	0.09	0.02	
Total oxygenated compounds	1.49	0.19	0.33	0.04	
Total	94.18	6.46	98.49	1.41	

Table 4. Statistical analysis of variation in juice quality parameters of mandarin cultivars. Mean is average parameter in % over the different cultivars 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.

cultivars	TSS (%)	Total Acids (%)	TSS /TA rate	Ascorbic acid (%)	PH	Juice (%)	Total dry matter (%)	Ash (%)
Willow leaf	9.8	0.51	19.21	20.06	3.79	62.53	14.01	2
King	7.6	2.03	3.74	28.34	2.88	61.27	16.59	4
	F**	F**	F**	F**	F**	F**		

Table 5. Correlation matrix (numbers in this table correspond with main components mentioned in Table 3).

	Octanal	Linalool	α -pinene	Sabinene	B-myrcene	Limonene
Linalool	0.40					
α -pinene	0.70	0.91*				
Sabinene	-0.22	0.70	0.49			
B-myrcene	-0.37	-0.87*	-0.73	-0.40		
Limonene	-0.54	-0.97**	-0.92**	-0.52	0.93**	
γ -terpinene	0.65	0.95**	0.99**	0.51	-0.82*	-0.97**

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

Table 6. Correlation matrix (numbers in this table correspond with juice quality parameters mentioned in Table 4).

	TSS (%)	TA (%)	TSS /TA	Ascorbic acid(%)	pH
TA (%)	-0.99**				
TSS /TA	0.99**	-0.99**			
Ascorbic acid(%)	-0.99**	1.00**	-0.99**		
pH	1.00**	-0.99**	0.99**	-0.99**	
Juice (%)	0.99**	-0.98**	0.98**	-0.98**	0.99**

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

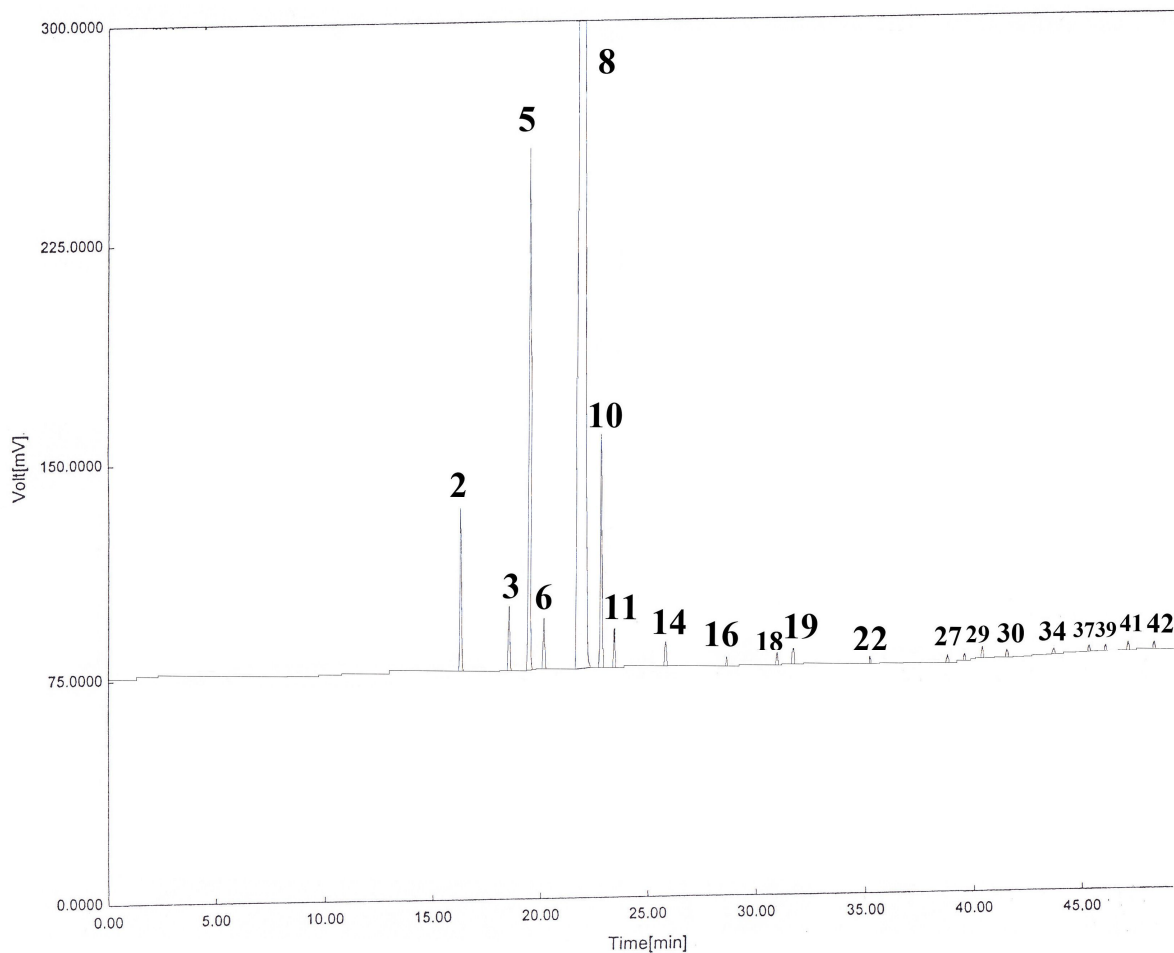


Fig1. HRGC chromatograms of King mandarin peel oil.