International Journal of
AGRICULTURE AND BIOSCIENCES
www.ijagbio.com P-ISSN: 2305-6622 E-ISSN: 2306-3599 editor@ijagbio.com

RESEARCH ARTICLE

A Study on Peel Volatile Components and Juice Quality Parameters of Two Tangor (*Citrus reticulata* × *Citrus sinensis*) Scions

Babazadeh Darjazi B

Department of Horticulture, Faculty of Agriculture, Roudehen Branch, Islamic Azad University (IAU), Roudehen, Iran

ARTICLE INFO

Received: October 10, 2013
Revised: November 17, 2013
Accepted: December 15, 2013

Key words:
Flavor components
Juice quality
Mandarin hybrids
Peel oil
Tangor scions

***Corresponding Address:**
Babazadeh Darjazi B
babazadeh @riau.ac.ir

ABSTRACT

The peel components and juice quality of two tangor scions were investigated in this study. Peel components were extracted using the cold-press and eluted using n-hexane. Then all analyzed using GC-FID and GC-MS. Total soluble solids, total acid, pH value, ascorbic acid as well as density were determined in juice obtained from two tangor scions. Twenty and twenty-eight peel components were identified in Murcott and Temple scions respectively including: aldehydes, alcohols, esters, ketones, monoterpenes and sesquiterpenes. The major components were limonene, linalool, β-myrcene, (E)-β-ocimene, α-Pinene and sabinene. Between two scions examined, Murcott showed the highest content of aldehydes and TSS/TA. Since the aldehyde and TSS/TA content of Citrus are considered as two of the most important indicators of high quality, scion apparently has a profound influence on these factors.

Cite This Article as: Babazadeh Darjazi B, 2013. A study on peel volatile components and juice quality parameters of two tangor (*Citrus reticulate* × *Citrus sinensis*) scions. Inter J Agri Biosci, 2(6): 371-376. www.ijagbio.com

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). Mandarin hybrids are so variable as the result of hybridization between many fine-quality tangerines and Citrus species. Many of these varieties or cultivars are now being used successfully for juice production and as fresh fruit. Murcott and Temple resulted from a cross between the tangerine and the sweet orange. They have been regarded as two Citrus fruit with potential commercial value because of their attractive and pleasant aroma (Fotouhi and Fattahi, 2007). They are two of the most important cultivars used in world. Although they are as important cultivars, the peel components of Murcott and Temple have been investigated very little before.

Citrus oils occur naturally in special oil glands of 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 (Babazadeh Darjazi, 2011b), extraction method (Babazadeh 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 quantity 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 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 amount of TSS present in the juice is variable and depends upon a number of factors including: rootstock, scion or variety, degree of maturity, seasonal effects, climate, nutrition, tree age and etc (Hardy and Sanderson, 2010).

Several studies have shown that the cultivars used as scion 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 tangor juice. Therefore, it is very important to be able to assess the differences between tangors in terms of quantity of compositions (TSS, acids and vitamin C).

In this study, we compare the peel components isolated from two scions with the aim of determining whether the quantity of oxygenated compounds influenced by the scions. Also the present study reports the effects of scions on the juice quality parameters.

MATERIALS AND METHODS

Tangor scions

In 1989, tangor 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]. Murcott and Temple were used as scions in this experiment (Table 1).

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.

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.

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).

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^{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 (Adams, 2001; McLafferty and 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 and 6 juice characteristics. Variations between scions 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 Murcott peel

GC-MS analysis of the flavor compounds extracted from Murcott peel using cold-press allowed identification of 20 volatile components (Table 2, Fig 1): 10 oxygenated terpenes [5 aldehydes, 3alcohols, 2esters] and 10 non oxygenated terpenes [6 monoterpens, 4 sesqiterpens].

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

Common name	botanical name	Parents	category
Murcott(scion)	*Citrus sp. cv. Murcott*	*(C.reticulata× C.sinensis)*	Tangor*
Temple(scion)	*Citrus sp. cv. Temple*	*(C.reticulata× C.sinensis)*	Tangor
Sour orange (Rootstock)	*C. aurantium (L.)*	*Mandarin ×Pomelo*	

*The name tangor is a formation from the tang of tangerine and the or of orange.

Table 2: Peel volatile components of tangor scions (*There is in oil)

	Component	Murcott	Temple	KI		Component	Murcott	Temple	KI
1	α - Pinene	*	*	935	18	Citronellyl acetate	*		1350
2	Sabinene	*	*	975	19	Neryl acetate	*		1356
3	β -pinene	*	*	979	20	α -copaene	*	*	1373
4	β -myrcene	*	*	991	21	Geranyl acetate		*	1389
5	octanal	*	*	1003	22	β -elemene		*	1399
6	Limonene	*	*	1036	23	Dodecanal	*	*	1409
7	(E)- β - ocimene	*	*	1049	24	(Z)- β -caryophyllene		*	1416
8	γ - terpinene		*	1061	25	(Z)- β - farnesene	*		1450
9	α -terpinolene		*	1091	26	α - humulene		*	1462
10	Linalool	*	*	1100	27	Germacrene D		*	1492
11	Nonanal	*	*	1109	28	Bicyclogermacrene		*	1504
12	Citronellal	*	*	1154	29	E,E, α - farnesene	*		1513
13	α - terpineol	*	*	1195	30	δ-cadinene	*	*	1530
14	Decanal	*	*	1205	31	Elemol		*	1558
15	β -citronellol	*		1229	32	(E)-Nerolidol		*	1567
16	Geranial		*	1275	33	Nootkatone		*	1811
17	δ - elemene		*	1344			20	28	

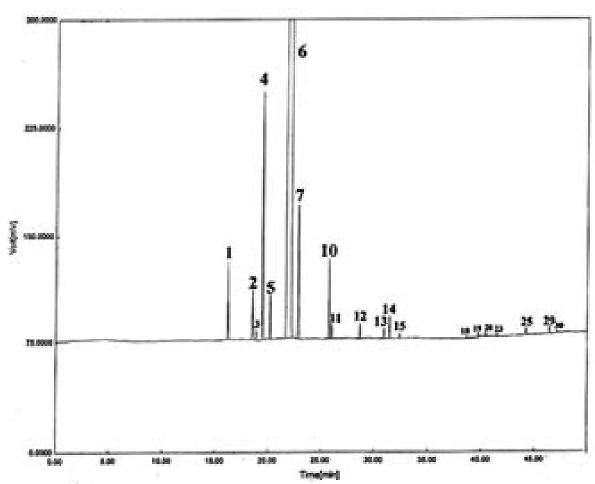

Fig. 1: HRGC chromatograms of Murcott peel oil.

Flavor compounds of the Temple peel

GC-MS analysis of the flavor compounds extracted from Temple peel using cold-press allowed identification of 28 volatile components (Table 2): 12 oxygenated terpenes [6 aldehydes, 4 alcohols, 1ester,1ketone] and16 non oxygenated terpenes [8 monoterpens, 8 sesqiterpens].

Aldehydes

Six aldehyde components that identified in this analysis were octanal, nonanal, citronellal, decanal, geranial and dodecanal (Table 3). In addition they were quantified from 0.32% to 0.66%. The concentrations of octanal and decanal were 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 scions examined, Murcott 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, scion apparently has a profound influence on this factor.

Murcott aldehydes were also compared to those of Temple in this study. Geranial was identified in Temple, while it was not detected in Murcott. Compared with Temple, the Murcott improved and increased aldehyde components about 2 times (Table 3).

Alcohols

Five alcoholic components identified in this analysis were linalool, α-terpineol, β-citronellol, elemol and (E)-nerolidol (Table 3). The total amount of alcohols ranged from 0.59% to 1.460%. 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 Citrus (Salem, 2003). Between two scions examined, Temple showed the highest content of alcohols (Table 3). Murcott alcohols were also compared to those of Temple in this study. Elemol and (E)-nerolidol were identified in Temple, while they were not detected in Murcott. Compared with Murcott, Temple improved and increased alcohol components about 2.47 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 0.03%. Between two scions examined, Murcott showed the highest content of esters (Table 3).

Ketones

One component identified in this analysis was Nootkatone. The total amount of ketones ranged from 0.00% to 0.01%. Between two scions examined, Temple showed the highest content of esters (Table 3).

Table 3: Statistical analysis of variation in peel flavor Components of tangor scions (see Materials and methods). Mean is average composition in% over the different scions 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	Murcott		Temple		F value
	Mean	St.err	Mean	St.err	
Oxygenated compounds					
a) Aldehyds					
1) Octanal	0.34	0.03	0.12	0.02	F**
2) Nonanal	0.08	0.006	0.04	0.006	
3) Citronellal	0.09	0.01	0.05	0.006	
4) Decanal	0.14	0.006	0.1	0.01	F**
5) Geranial			0.01	0	
6) Dodecanal	0.01	0	0.003	0.001	
Total	0.66	0.05	0.32	0.04	
b) Alcohols					
1) Linalool	0.5	0.03	1.36	0.2	F**
2) α-terpineol	0.07	0.02	0.03	0	
3) β-citronellol	0.02	0.01			
4) Elemol			0.07	0.01	
5) (E)-nerolidol			0.008	0.001	
Total	0.59	0.06	1.46	0.21	
c) Esters					
1) Citronellyl acetate	0.01	0.006			
2) Neryl acetate	0.02	0			
3) Geranyl acetate			0.02	0	
Total	0.03	0.006	0.02	0	
Ketones					
1) Nootkatone			0.01	0	
Monoterpenes					
1) α-pinene	0.49	0.05	0.43	0.06	NS
2) Sabinene	0.38	0.05	0.18	0.006	F**
3) β- pinene	0.07	0.02	0.1	0.02	
4) β-myrcene	1.54	0.09	1.4	0.33	NS
5) Limonene	94.4	0.56	86.58	4.24	F*
6) (E)-β-ocimene	0.83	0.14	2.44	0.35	F**
7) γ-terpinene			0.65	0.1	
8) α-terpinolene			0.02	0.006	
Total	97.74	0.91	91.8	5.11	
Sesquiterpenes					
1) δ-elemene			0.06	0.006	
2) α-copaene	0.02	0	0.01	0	
3) β-elemene			0.03	0.006	
4) (Z)-β-caryophyllene			0.02	0.006	
5) (Z)-β-farnesene	0.04	0.006			
6) α – humulene			0.02	0.01	
7) Germacrene D			0.24	0.006	
8) Bicyclogermacrene			0.008	0.002	
9) E,E-α-farnesene	0.03	0.01			
10) δ-cadinene	0.01	0.006	0.01	0	
Total	0.1	0.02	0.39	0.03	
Total oxygenated compounds	1.28	0.11	1.82	0.25	
Total	99.12	1.05	94.02	5.40	

Monoterpene hydrocarbons

The total amount of monoterpene hydrocarbons ranged from 91.8% to 97.74%. 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 scions examined, Murcott showed the highest content of monoterpenes (Table 3).

Sesquiterpene hydrocarbons

The total amount of sesquiterpene hydrocarbons ranged from 0.10% to 0.39%. Germacrene D was identified as the major component in this study and was the most abundant. Between two scions examined, Temple showed the highest content of sesquiterpenes (Table 3).

Juice quality parameters

Juice quality parameters are given in table 4. Brix (total soluble solids) ranged from 9% (Temple) to 9.6% (Murcott) and the content of total acidity ranged from 2.12% (Murcott) to 2.22% (Temple). TSS/TA rate ranged from 4.05 (Temple) to 4.52 (Murcott). Ascorbic acid ranged from 21.82% (Temple) to 51.57% (Murcott). The pH value ranged from 2.78 (Temple) to 2.94 (Murcott). The juice yield ranged from 50.28% (Temple) to 51.98% (Murcott). Total dry matter ranged from 15.44% (Temple) to 18.25% (Murcott). Ash ranged from 2% (Temple) to 3% (Murcott).

Between two scions examined, Murcott showed the highest content of TSS, TSS /TA and pH (Table 4).

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 octanal, decanal, linalool, sabinene, (E)-β-ocimene, TSS, TA, TSS /TA, ascorbic acid, pH and juice**.** These differences on the 5% level occurred in limonene. The non affected oil components were α-Pinene and β-myrcene (Table 3 and 4).

Results of correlation

Simple inter correlations between 8 components are presented in a correlation matrix (Table 5). The highest positive values or r (correlation coefficient) were observed between decanal and octanal (96%); limonene and decanal (93%); sabinene and decanal (92%). The highest significant negative correlations were observed between E)-β-ocimene and decanal (98%), (Table 5).

Also simple inter correlations between 6 juices characteristics are presented in a correlation matrix (Table 6). The highest positive values or r (correlation coefficient) were observed between pH and TSS (99%); Ascorbic acid and TSS /TA (99%); Juice and TSS /TA (99%); Juice and Ascorbic acid (99%). The highest significant negative correlations were observed between TSS /TA and TA (91%), (Table 6).

DISCUSSION

Our observation that Citrus scions have an effect on some of the components of 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 two scions of tangor were very similar. However, the relative concentration of compounds was different according to the type of scion.

Table 4: Statistical analysis of variation in juice quality parameters of tangor scions. Mean is average parameter in % over the different scions 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.

scions	TSS (%)	Total Acids (%)	TSS /TA rate	Ascorbic acid (%)	PH	Juice (%)	Total dry matter (%)	Ash (%)
Murcott (scion)	9.6	2.12	4.52	51.57	2.94	51.98	18.25	3
Temple (scion)	9	2.22	4.05	21.82	2.78	50.28	15.44	2
	F**	F**	F**	F**	F**	F**		

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

	Octanal	Decanal	Linalool	α-pinene	Sabinene	B-myrcene	Limonene
Decanal	0.96**						
Linalool	-0.92**	-0.86*					
α-pinene	0.67	0.65	-0.35				
Sabinene	0.88*	0.92**	-0.90*	0.34			
B-myrcene	0.45	0.52	-0.08	0.89*	0.25		
Limonene	0.86*	0.93**	-0.67	0.74	0.84*	0.73	
(E)- β-ocimene	-0.95**	-0.98**	0.86*	-0.60	-0.95**	-0.51	-0.95**

*=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 (%)	-0.75				
TSS /TA	0.95**	-0.91*			
Ascorbic acid (%)	0.96**	-0.89*	0.99**		
pH	0.99**	-0.66	0.90*	0.92**	
Juice (%)	0.98**	-0.85*	0.99**	0.99**	0.95*

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

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 → Isopentenyl Pyrophosphate → 3.3-dimethylallylpyrophosphate→ geranyl pyrophosphate → 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 Temple used as the scion, Indicate that either the synthesis of geranyl pyrophosphate is enhanced or activities of both enzymes increased.

High positive correlations between pairs of terpenes such as decanal and octanal (96%); limonene and decanal (93%); sabinene and decanal (92%) suggest the presence of a genetic control (Scora *et al*., 1976) and such dependence between pairs of terpenes is due to their derivation of one from another that is not known. Similarly, high negative correlations observed between E)-β-ocimene and decanal (98%) 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 decanal and octanal (96%). 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 Temple. Our results showed that there was a positive correlation between pH and TSS. This finding was similar to previous studies (Baldwin, 2002).

Conclusion

Present study showed that there was a great variation in most of the measured characters between two scions. Also the present study demonstrates that volatile compounds in peel and quality parameters in juice can vary when different scions are utilized. Between two scions examined, Murcott showed the highest content of TSS, TSS /TA and pH. The lowest of TSS, TSS /TA, pH content were produced by Temple. These results show that there is a positive correlation between pH and TSS. Studies like this is very important to determine the amount of chemical compositions existing in the scions 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 scions and quality parameters is necessary.

Acknowledgment

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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