

THE EFFECT OF GEOGRAPHICAL LOCATION ON SOUR ORANGE (*CITRUS AURANTIUM L.*) PEEL COMPONENTS

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ABSTRACT: Studies have shown that oxygenated compounds are important in food products. It seems that geographical location has a profound influence on this factor. The goal of the present study is to investigate on peel components of sour orange from two different locations. In the last week of January 2012, at least 10 mature fruit were collected and peel components were extracted using cold-press method, then 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 oxygenated compounds ranged from 1.96% to 2.63%. Between two locations examined, Jahrom showed the highest content of oxygenated compounds. As a result of our study, we can conclude that the geographical location can influence the quantity of oxygenated compounds present in the oil.

KEYWORDS: Cold-Press, Flavor Components, Geographical Location, 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 tonnes (FAO, 2012). The sour orange (*Citrus aurantium L.*) is a hybrid of the lime and citron that is extensively cultivated in Iran (Fotouhi and Fattahi, 2007). It is one of the most important Citrus used in world. Although it is as important Citrus, the peel components of sour 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. Citrus oils are commercially used for flavoring foods, beverages, perfumes, cosmetics, medicines and etc (Salem, 2003). In addition, recent studies have identified insecticidal, antimicrobial, antioxidative and antitumor properties for Citrus oils (Shahidi and Zhong, 2012).

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: geographical location (Boussaada et al., 2007; Njoroge et al., 2009; Staroscik and Wilson, 1982), rootstock (Babazadeh et al., 2006),

cultivars (Lota et al., 2001), organ, extraction method (Babazadeh, 2014) and etc.

Branched aldehydes and alcohols are important flavor compounds extensively used in food products (Salem, 2003). Several studies have shown that oxygenated terpenoids such as linalool, Linalyl acetate and α -terpineol are important in sour orange flavor (Azadi, 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).

In this paper, we compared the peel compounds isolated from sour orange with the aim of determining whether the quantity of oxygenated compounds influenced by the location.

MATERIALS AND METHODS

2.1. Sour Orange Trees

In 1989, sour orange trees 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)]. Also, trees were planted at 8 × 4 m with three replication at an orchard around the Jahrom in 1989 (latitude 28°

50' N, longitude 53° 33' E; dry climate, average rainfall 200 mm per year and average temperature 21.0°C; soil was classified as loam-clay with lime and gypsum in some parts; pH=8.5). Sour orange was used as plant material in this experiment (Table 1, Figure 1).



Figure 1: Fruit were collected from two geographical locations in Iran

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 orchards of the Ramsar and Jahrom. 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. GC and GC-MS

An Agilent 6890N gas chromatograph (USA) equipped with a DB-5 (30 m × 0.25 mm i.d; film thickness=0.25µm) 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.

2.4. 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).

2.5. Data Analysis

SPSS 18 was used for analysis of the data obtained from the experiments. Analysis of variations was based on the measurements of 9 peel components. 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 components was evaluated using Pearson's correlation coefficient.

RESULTS

3.1. Peel Compounds of the Sour Orange

GC-MS analysis of the compounds extracted from sour orange peel using cold-press allowed identification of 38 volatile components (Table 2, Figure 2), 21 oxygenated terpenes [8 aldehydes, 7 alcohols, 6 esters] and 17 non oxygenated terpenes [11 monoterpenes, 6 sesquiterpenes].

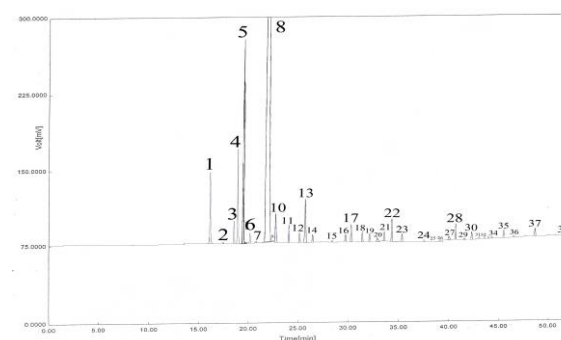


Figure 2: HRGC chromatogram peel oil of sour orange from Ramsar

3.2. Aldehydes

Eight aldehyde components that identified in this analysis were octanal, nonanal, citronellal, decanal, neral, geranial, undecanal and dodecanal (Table 3). In addition they were quantified from 0.47% to 0.65%. The concentration of octanal and decanal was higher in our samples. Octanal has a citrus-like aroma and is considered as one of the major contributors to citrus flavor (Buettner *et al.*, 2003). Between two locations examined, Jahrom 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, location apparently has a profound influence on this factor (Table 3).

3.3. Alcohols

Seven alcoholic components identified in this analysis were linalool, terpinene-4-ol, α -terpineol, nerol, geraniol, (E)-nerolidol and (Z,E)-farnesol (Table 3). The total amount of alcohols ranged from 1.04% to 1.41%. 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 sour orange flavor ([Azadi, 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 locations examined, Jahrom showed the highest content of alcohols (Table 3).

3.4. Esters

Sex ester components identified in this analysis were linalyl acetate, nonanyl acetate, terpinyl acetate, neryl acetate, geranyl acetate and linalyl butanoate. The total amount of esters ranged from 0.45% to 0.57%. Linalyl acetate was identified as the major component in this study and was the most abundant. Linalyl acetate has been recognized as one of the most important components for sour orange flavor ([Azadi, 2003](#)). Linalyl acetate has a lemon like smell ([Miller and Miller, 1990](#)) and its level is important to the characteristic favor of Citrus. Between two locations examined, Jahrom showed the highest content of esters (Table 3).

3.5. Monoterpene Hydrocarbons

The total amount of monoterpene hydrocarbons ranged from 95.64% to 97.11%. 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 sour orange flavor. Between two locations examined, Ramsar showed the highest content of monoterpenes (Table 3).

3.6. Sesquiterpene Hydrocarbons

The total amount of sesquiterpene hydrocarbons ranged from 0.18% to 0.23%. Germacrene D was identified as the major component in this study and was the most abundant. Between two locations examined, Ramsar showed the highest content of sesquiterpenes (Table 3).

3.7. Results of Statistical Analyses

Differences were considered to be significant at $P < 0.01$. These differences on the 1% level

occurred in octanal, linalool and (E)- β -ocimene. This difference on the 5% level occurred in limonene. The non affected oil components were α -terpineol, linalyl acetate, α -pinene, β -Pinene and β -myrcene (Table 3).

3.8. Results of Correlation

Simple intercorrelations between 9 components are presented in a correlation matrix (Table 4). The highest positive values or r (correlation coefficient) were observed between linalool and octanal (100%); linalyl acetate and α -terpineol (99%); (E)- β -ocimene and limonene (97%). The highest significant negative correlations were observed between (E)- β -ocimene and octanal (76%); (E)- β -ocimene and linalool (76%) (Table 4).

DISCUSSION

Our observation that geographical locations had an effect on some of the components of sour orange oil was in accordance with previous findings ([Boussaada et al., 2007](#); [Njoroge et al., 2009](#); [Staroscik and Wilson, 1982](#)). The compositions of the peel oils obtained by cold-press from different locations were very similar. However, the relative concentration of compounds was different according to the type of location.

Comparison of our data with those in the literatures revealed some inconsistencies with previous studies ([Boussaada et al., 2007](#)). It may be related to environmental factors such as altitude, insolation (solar radiation), average temperature, humidity and chemical composition of soil that can influence the oil compositions ([Vidic et al., 2010](#)). However, it should be kept in mind that the extraction methods also may influence the results. Fertilizer and irrigation affects the content of oil present in Citrus ([Kesterson et al., 1974](#)). Fertilization, irrigation and other operations were carried out uniform in this study so we did not believe that this variability was 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 Jahrom used as the location, showed that either the synthesis of geranyl pyrophosphate was enhanced or activities of both enzymes increased.

Also, the higher proportion of the detected oxygenated compounds in peel was probably due to seasonal temperature (Sekiya *et al.*, 1984), which is the most important environmental factor in the control of endogenous enzymes. Solar radiations can also be involved in activation or inactivation of certain enzymatic groups, leading to the predominance of a particular biosynthetic pathway (Khadhri *et al.*, 2011).

High positive correlations between pairs of terpenes suggest a genetic control (Scora *et al.*, 1976) and such dependence between pairs of terpenes was due to derivation of one from another that was not known. Similarly, high negative correlations between pairs of terpenes indicated that one of the two compounds had been 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. 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 Jahrom.

CONCLUSION

In the present study we found that the amount of peel compositions was significantly affected by locations and there was a great variation in most of the measured characters between two locations. The present study demonstrated that volatile compounds in peel can vary when different locations are utilized. Between two locations examined, Jahrom showed the highest content of oxygenated compounds. The lowest of oxygenated compounds content were produced by Ramsar. Studies like this are very important to determine the amount of chemical compositions existing in different locations. Further research on the relationship between geographical locations and oxygenated compounds is necessary.

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

| Common name | botanical name | Parents | category |
|-------------|--|-------------------|-------------|
| Sour orange | <i>C. aurantium</i> (L.) var. <i>amara</i> | Mandarin × Pomelo | Sour orange |

Table 2: Peel components of sour orange from two geographical locations (*There is in oil)

| No. | Component | Ramsar | Jahrom | KI | No. | Component | Ramsar | Jahrom | KI |
|-----|------------------|--------|--------|------|-----|---------------------|--------|--------|------|
| 1 | α - Pinene | * | * | 935 | 20 | Neral | * | * | 1244 |
| 2 | Camphene | * | * | 951 | 21 | Geraniol | * | * | 1259 |
| 3 | Sabinene | * | * | 975 | 22 | Linalyl acetate | * | * | 1262 |
| 4 | β - Pinene | * | * | 979 | 23 | geranial | * | * | 1275 |
| 5 | β - myrcene | * | * | 991 | 24 | undecanal | * | * | 1313 |
| 6 | octanal | * | * | 1003 | 25 | Nonanyl acetate | * | * | 1315 |
| 7 | α - phellandrene | * | * | 1006 | 26 | α-Terpinyl acetate | * | * | 1351 |
| 8 | Limonene | * | * | 1036 | 27 | Neryl acetate | * | * | 1356 |
| 9 | (Z)-β-ocimene | * | * | 1044 | 28 | Geranyl acetate | * | * | 1389 |
| 10 | (E)-β-ocimene | * | * | 1049 | 29 | dodecanal | * | * | 1409 |
| 11 | γ-terpinene | * | * | 1061 | 30 | (Z)-β-caryophyllene | * | * | 1416 |
| 12 | α-terpinolene | * | * | 1091 | 31 | Linalyl butanoate | * | * | 1423 |
| 13 | Linalool | * | * | 1100 | 32 | (Z)-β-farnesene | * | * | 1450 |
| 14 | nonanal | * | * | 1109 | 33 | (E)-β-farnesene | * | * | 1457 |
| 15 | Citronellal | * | * | 1154 | 34 | α-humulene | * | * | 1462 |
| 16 | Terpinen-4-ol | * | * | 1182 | 35 | germacrene D | * | * | 1492 |
| 17 | α-terpineol | * | * | 1195 | 36 | bicyclogermacrene | * | * | 1504 |
| 18 | decanal | * | * | 1205 | 37 | (E)-nerolidol | * | * | 1567 |
| 19 | Nerol | * | * | 1236 | 38 | (Z,E)-farnesol | * | * | 1701 |

Table 3: Statistical analysis of variation in peel flavor Components from different locations.

| Compounds | Ramsar | | Jahrom | | F-value |
|--------------------------------|--------|--------|--------|--------|---------|
| | Mean | St.err | Mean | St.err | |
| Oxygenated compounds | | | | | |
| a) Aldehyds | | | | | |
| 1) Octanal | 0.13 | 0.01 | 0.17 | 0.01 | F** |
| 2) Nonanal | 0.08 | 0.006 | 0.12 | 0.01 | |
| 3) Citronellal | 0.01 | 0.00 | 0.02 | 0.006 | |
| 4) Decanal | 0.12 | 0.014 | 0.15 | 0.01 | |
| 5) Neral | 0.04 | 0.006 | 0.06 | 0.006 | |
| 6) Geranial | 0.07 | 0.006 | 0.09 | 0.01 | |
| 7) Undecanal | 0.01 | 0.00 | 0.02 | 0.006 | |
| 8) Dodecanal | 0.01 | 0.006 | 0.02 | 0.006 | |
| Total | 0.47 | 0.04 | 0.65 | 0.06 | |
| b) Alcohols | | | | | |
| 1) Linalool | 0.5 | 0.05 | 0.7 | 0.05 | F** |
| 2) Terpinen-4-ol | 0.07 | 0.006 | 0.11 | 0.01 | |
| 3) α -terpineol | 0.19 | 0.02 | 0.22 | 0.02 | NS |
| 4) Nerol | 0.09 | 0.01 | 0.12 | 0.01 | |
| 5) Geraniol | 0.11 | 0.01 | 0.15 | 0.02 | |
| 6) (E)-nerolidol | 0.07 | 0.006 | 0.09 | 0.01 | |
| 7) (Z,E)-farnesol | 0.01 | 0.00 | 0.02 | 0.006 | |
| Total | 1.04 | 0.1 | 1.41 | 0.12 | |
| d) Esteres | | | | | |
| 1) Linalyl acetate | 0.23 | 0.02 | 0.27 | 0.02 | NS |
| 2) Nonanyl acetate | 0.007 | 0.001 | 0.01 | 0.00 | |
| 3) α -terpinyl acetate | 0.02 | 0.006 | 0.03 | 0.006 | |
| 4) Neryl acetate | 0.05 | 0.006 | 0.07 | 0.01 | |
| 5) Geranyl acetate | 0.14 | 0.01 | 0.17 | 0.02 | |
| 6) Linalyl butanoate | 0.01 | 0.00 | 0.02 | 0.006 | |
| Total | 0.45 | 0.04 | 0.57 | 0.06 | |
| Monoterpenes | | | | | |
| 1) α -pinene | 0.75 | 0.06 | 0.71 | 0.04 | NS |
| 2) Camphene | 0.01 | 0.00 | 0.00 | 0.001 | |
| 3) Sabinene | 0.23 | 0.02 | 0.20 | 0.02 | |
| 4) β -Pinene | 1.11 | 0.06 | 1.02 | 0.08 | NS |
| 5) β -myrcene | 2.01 | 0.21 | 1.98 | 0.21 | NS |
| 6) α -phellandrene | 0.02 | 0.006 | 0.01 | 0.00 | |
| 7) Limonene | 92.37 | 0.38 | 91.28 | 0.34 | F* |
| 8) (Z)- β -ocimene | 0.03 | 0.006 | 0.02 | 0.006 | |
| 9) (E)- β -ocimene | 0.30 | 0.02 | 0.20 | 0.02 | F** |
| 10) γ -terpinene | 0.16 | 0.02 | 0.12 | 0.01 | |
| 11) α -terpinolene | 0.12 | 0.01 | 0.10 | 0.01 | |
| Total | 97.11 | 0.79 | 95.64 | 0.73 | |
| Sesquiterpenes | | | | | |
| 1) (Z)- β -caryophyllene | 0.07 | 0.006 | 0.06 | 0.006 | |
| 2) (Z)- β -farnesene | 0.02 | 0.006 | 0.01 | 0.00 | |
| 3) (E)- β -farnesene | 0.01 | 0.006 | 0.01 | 0.00 | |
| 4) α -humulene | 0.02 | 0.00 | 0.01 | 0.00 | |
| 5) Germacrene D | 0.1 | 0.01 | 0.08 | 0.01 | |
| 6) Bicyclgermacrene | 0.01 | 0.006 | 0.01 | 0.00 | |
| Total | 0.23 | 0.03 | 0.18 | 0.01 | |
| Total oxygenated compounds | 1.96 | 0.18 | 2.63 | 0.24 | |
| Total | 99.30 | 1.00 | 98.45 | 0.98 | |

Mean is average composition in % over the different locations 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.

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

| | Octanal | Linalool | α -terpineol | Linalyl acetate | α -pinene | β -Pinene | β -myrcene | Limonene |
|-----------------------|---------|----------|---------------------|-----------------|------------------|-----------------|------------------|----------|
| Linalool | 1.00** | | | | | | | |
| α -terpineol | 0.90* | 0.90* | | | | | | |
| Linalyl acetate | 0.95** | 0.95** | 0.99** | | | | | |
| α -pinene | -0.01 | -0.01 | 0.40 | 0.27 | | | | |
| β -Pinene | -0.29 | -0.29 | 0.14 | 0.00 | 0.93** | | | |
| β -myrcene | 0.28 | 0.28 | 0.66 | 0.55 | 0.94** | 0.82* | | |
| Limonene | -0.64 | -0.64 | -0.26 | -0.40 | 0.74 | 0.89* | 0.52 | |
| (E)- β -ocimene | -0.76 | -0.76 | -0.41 | -0.54 | 0.65 | 0.83* | 0.40 | 0.97** |

*=significant at 0.05

**=significant at 0.01