



The effect of extraction methods on Murcott mandarin (*Citrus reticulata*) peel components

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ABSTRACT

Studies had shown that oxygenated compounds are important in food products. It seems that extraction methods had a profound influence on this factor. The goal of the present study is to investigate on flavor components of Murcott mandarin 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 0.36% to 0.66%. Between two methods examined, cold-press showed the highest content of aldehydes. As a result of our study, can be concluded that the extraction methods used can influence the quantity of oxygenated compounds present in the oil. The application of Cold-press method can cause a lesser damage to thermal-sensitive molecules, so can be a good technique to recovery of Citrus compounds in large industrial scale.

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 tonnes (FAO, 2012). Murcott mandarin resulted from a cross between the tangerine and the sweet orange (Fotouhi and Fattahi, 2007). It is one of the most important mandarin cultivar used in world. Although it is as important cultivar, the peel components of Murcott mandarin have been investigated very little previously (Babazadeh- Darjazi, 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 aldehyd compounds present in the oil (Braddock, 1940). The quantity of aldehyd compounds present in the oil, is variable and depends upon a number of factors including: rootstock

(Verzera et al., 2003), scion (Babazadeh-Darjazi, 2013b), 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 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 to heating equipment and the operation is easy. Babazadeh-Darjazi (2014a; 2014b) compared the peel components of sweet orange and pummel with two methods. These studies showed the higher proportion of alcohol components in hydro distillation method and the higher proportion of aldehyd components in cold pressing. In this paper, we compared the peel compounds obtained using cold press (CP) with those obtained using hydro distillation (HD).

MATERIALS AND METHODS

Murcott scions

In 1989, Murcott 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 mandarin was 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 9 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

Flavor compounds of the Murcott mandarin obtained using cold-press (CP) and hydro distillation (HD) method

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

Aldehydes

Five aldehyde components that identified in this analysis were octanal, nonanal, citronellal, decanal and dodecanal (Table 3). In addition they were quantified from 0.36% 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 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 1.83 times (Table 3, Fig 2).

Alcohols

Three alcoholic components identified in this analysis were linalool, α -terpineol and β -citronellol (Table 3). The total amount of alcohols ranged from 0.59% to 1.15%. 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

methods examined, hydro distillation showed the highest content of alcohols. Compared with cold-pressing, hydro distillation improved and increased alcohol components about 1.94 times. (Table 3, Fig 2).

Esters

Two ester components identified in this analysis were citronellyl acetate and neryl acetate. The total amount of esters ranged from 0.01% to 0.03%. Between two methods examined, cold-pressing showed the highest content of esters (Table 3, Fig 2).

Monoterpene hydrocarbons

The total amount of monoterpene hydrocarbons ranged from 97.69 % to 97.71 %. 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, cold-pressing showed the highest content of monoterpenes (Table 3).

Sesquiterpene hydrocarbons

The total amount of sesquiterpene hydrocarbons ranged from 0.05 % to 0.10 %. (Z)- β -farnesene 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

Differences were considered to be significant at $P < 0.01$. These differences on the 1% level occurred in octanal, decanal, linalool and α -terpineol. These differences on the 5% level occurred in α -pinene and sabinene. The non affected oil components were β -myrcene, limonene and (E)- β -ocimene (Table 3).

3-8) Results of correlation

Not only α -terpineol showed a high positive correlation with linalool but also it showed a high positive correlation with sabinene. Octanal showed a high positive correlation with decanal whereas it showed a high negative correlation with linalool and α -terpineol (Table 4).

DISCUSSION

Our study showed that different methods had an effect on some of the components of citrus oil. It 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 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 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. The highest positive value (correlation) was observed between α -terpineol 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.

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

| Common name | botanical name | Parents | category |
|-------------------------|-------------------------------|---|-------------|
| Murcott(scion) | <i>Citrus sp. cv. Murcott</i> | (<i>C.reticulata</i> × <i>C.sinensis</i>) | Tangor* |
| Sour orange (Rootstock) | <i>C. aurantium (L.)</i> | <i>Mandarin</i> × <i>Pomelo</i> | Sour orange |

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

Table2. Peel components of Murcott mandarin obtained using cold-press and hydodistillation.

| | Component | Col d- pre ss | Hydro distillat ion | KI | Component | Cold- press | Hydro distillat ion | KI |
|----|------------------------|------------------------|---------------------------|------|-----------|---------------------------|---------------------------|------|
| 1 | α – Pinene | * | * | 935 | 11 | α – terpineol | * | 1195 |
| 2 | Sabinene | * | * | 975 | 12 | Decanal | * | 1205 |
| 3 | β –pinene | * | * | 979 | 13 | β –citronellol | * | 1229 |
| 4 | β –myrcene | * | * | 991 | 14 | Citronellyl acetate | * | 1350 |
| 5 | Octanal | * | * | 1003 | 15 | Neryl acetate | * | 1356 |
| 6 | Limonene | * | * | 1036 | 16 | α –copaene | * | 1373 |
| 7 | (E)- β – ocimene | * | * | 1049 | 17 | Dodecanal | * | 1409 |
| 8 | Linalool | * | * | 1100 | 18 | (Z)- β – farnesene | * | 1450 |
| 9 | Nonanal | * | * | 1109 | 19 | E,E, α – farnesene | * | 1513 |
| 10 | Citronellal | * | * | 1154 | 20 | δ -cadinene | * | 1530 |
| | | | | | | 20 | 20 | |

*There is in oil

Table 3. Statistical analysis of variation in peel components of Murcott mandarin obtained using cold-press and hydro distillation.

| Compounds | Cold-press | | Hydro distillation | | F value |
|-----------------------------------|------------|--------|--------------------|--------|---------|
| | Mean | St.err | Mean | St.err | |
| Oxygenated compounds | | | | | |
| a) Aldehyds | | | | | |
| 1) Octanal | 0.34 | 0.03 | 0.19 | 0.02 | F** |
| 2) Nonanal | 0.08 | 0.006 | 0.03 | 0.006 | |
| 3) Citronellal | 0.09 | 0.01 | 0.05 | 0.006 | |
| 4) Decanal | 0.14 | 0.006 | 0.09 | 0.01 | F** |
| 5) Dodecanal | 0.01 | 0 | 0.006 | 0.001 | |
| total | 0.66 | 0.05 | 0.36 | 0.04 | |
| b) Alcohols | | | | | |
| 1) Linalool | 0.5 | 0.03 | 0.94 | 0.06 | F** |
| 2) α -terpineol | 0.07 | 0.02 | 0.18 | 0.02 | F** |
| 3) β -citronellol | 0.02 | 0.01 | 0.03 | 0.006 | |
| total | 0.59 | 0.06 | 1.15 | 0.08 | |
| c) Esters | | | | | |
| 1) Citronellyl acetate | 0.01 | 0.006 | 0.004 | 0.001 | |
| 2) Neryl acetate | 0.02 | 0 | 0.01 | 0.00 | |
| total | 0.03 | 0.006 | 0.01 | 0.001 | |
| Monoterpenes | | | | | |
| 1) α -pinene | 0.49 | 0.05 | 0.64 | 0.05 | F* |
| 2) Sabinene | 0.38 | 0.05 | 0.51 | 0.04 | F* |
| 3) β - pinene | 0.07 | 0.02 | 0.13 | 0.02 | |
| 4) β -myrcene | 1.54 | 0.09 | 1.58 | 0.12 | NS |
| 5) Limonene | 94.4 | 0.56 | 93.82 | 0.21 | NS |
| 6) (E)- β -ocimene | 0.83 | 0.14 | 1.01 | 0.10 | NS |
| total | 97.71 | 0.91 | 97.69 | 0.54 | |
| Sesquiterpenes | | | | | |
| 1) α -copaene | 0.02 | 0 | 0.01 | 0.006 | |
| 2) (Z)- β -farnesene | 0.04 | 0.006 | 0.02 | 0.006 | |
| 3) E,E- α -farnesene | 0.03 | 0.01 | 0.02 | 0.00 | |
| 4) δ -cadinene | 0.01 | 0.006 | 0.007 | 0.001 | |
| total | 0.1 | 0.02 | 0.05 | 0.01 | |
| Total oxygenated compounds | 1.28 | 0.11 | 1.52 | 0.12 | |
| Total | 99.09 | 1.04 | 99.26 | 0.67 | |

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.

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

| | Octanal | Decanal | Linalool | α -terpineol | α -pinene | Sabinene | B-myrcene | Limonene |
|-----------------------|---------|---------|----------|---------------------|------------------|----------|-----------|----------|
| Decanal | 0.96** | | | | | | | |
| Linalool | -0.93** | -0.92** | | | | | | |
| α -terpineol | -0.93** | -0.89* | 0.99** | | | | | |
| α -pinene | -0.67 | -0.76 | 0.86* | 0.81* | | | | |
| Sabinene | -0.89* | -0.78 | 0.92** | 0.96** | 0.67 | | | |
| B-myrcene | 0.02 | -0.08 | 0.31 | 0.27 | 0.69 | 0.17 | | |
| Limonene | 0.49 | 0.69 | -0.55 | -0.44 | -0.75 | -0.21 | -0.37 | |
| (E)- β -ocimene | -0.40 | -0.55 | 0.66 | 0.58 | 0.93** | 0.40 | 0.84* | -0.76 |

* = significant at 0.05

** = significant at 0.01

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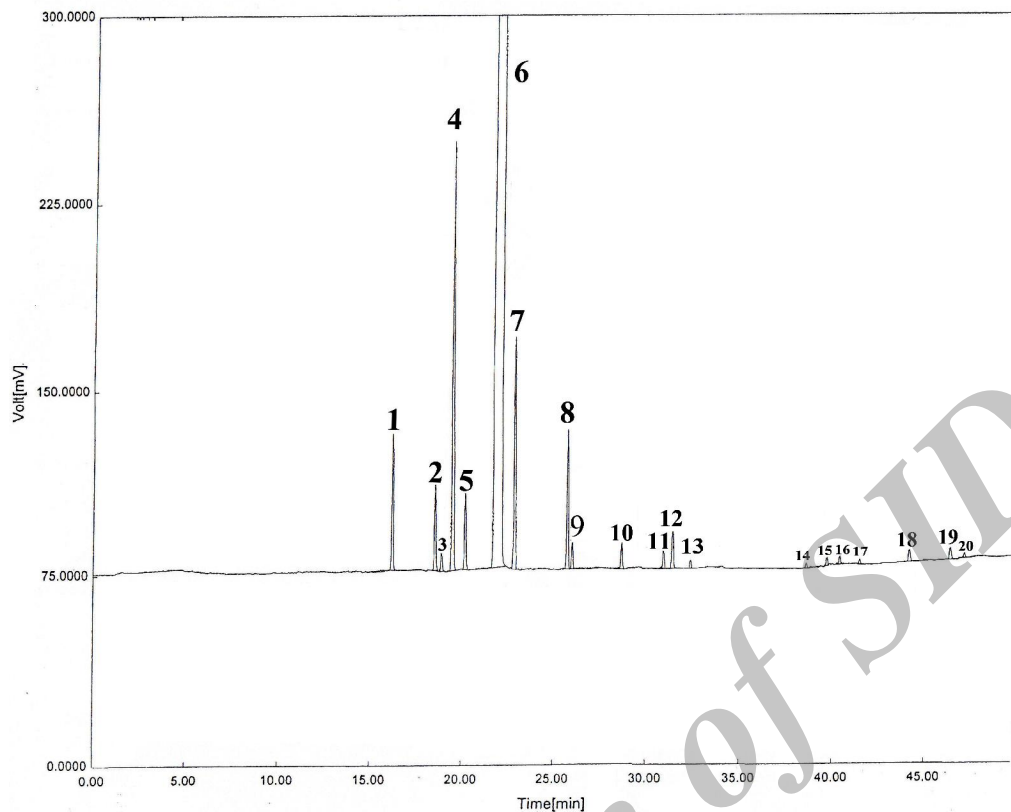


Fig1. HRGC chromatograms of Murcott mandarin peel oil obtained using cold-press.

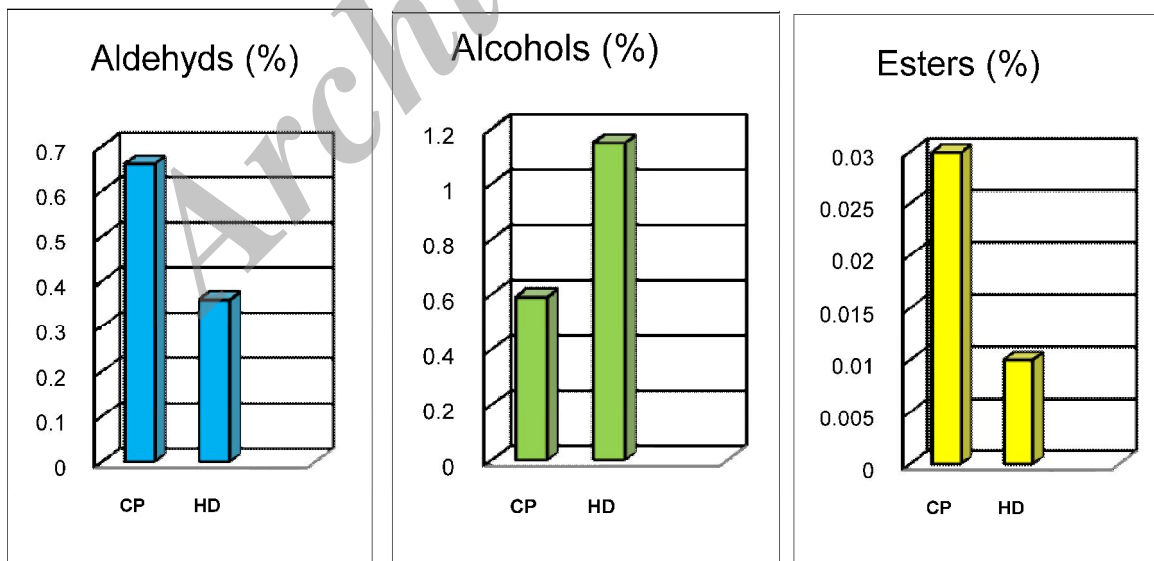


Fig2. Comparison of Peel Components of Murcott mandarin (*Citrus reticulata*) obtained using cold-press (CP) and hydro distillation (HD) method.