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Chemical Identifications of Citrus Peels Essential Oils

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ABSTRACT: Due to the importance of essential oils and their use in various industries, the essential oils of four types of citrus consisting of orange (*C. sinesis*), tangerine (*C. reticulate*), sweet lemon (*C. limetta*) and sour lemon (*C. limon*) were extracted and subjected to chemical analysis to identify and evaluate the chemical constituents of the oils. The average essential oils of orange, tangerine, sweet lemon and sour lemon peels were 1.48%, 0.90%, 0/54% and 0.46% (w/w) respectively. Seven compounds were identified in the orange peel essential oil where limonene (64.87%) followed by N-methyl-D3-aziridine (10.78%), cytidine (8.55%) and propane, 1,1, - oxybis (4.99) were the major components. Seventeen compounds were identified in tangerine peel essential oil where the main components were limonene (28.10%), xanthotoxin (18.40%), cytidine (8.34%) and 1,4-diethynylcyclobutene (4.73%). Eighteen compounds were identified in the essential oil of sweet lemon peel where limonene (49.79%), xanthotoxin (12.62%), 6,6-dideutero-nonen-1-ol-3 (10.20%) and 4-vinyl- 2-methoxy-phenol (5.20%) were the major components. Seven compounds consisting of limonene (83.03%), cytidine (4.16%), N-methyl-D3-aziridine (4.42%), 4- vinyl-2-methoxy-phenol (2.79%) were the major constituents of sour lemon peel essential oil in respective decreasing order. Limonene was the predominant compound in all the citrus fruit peels and showed the highest concentration of this compound among the samples examined.

Keywords: *Citrus Peel, Essential Oils.*

Introduction

The essential oils are aromatic compounds that are widely used in the perfume, pharmaceutical and food industries. Essential oils are mixtures of more than 200 different compounds. These compounds are mainly formed of monoterpene and sesquiterpene hydrocarbons and their oxygenated derivatives such as esters, alcohols and aliphatic aldehydes and ketones. Essential oils are generally created by aromatic plants. The specific gravity of essential oils is often less than water and only a small number of essential oils have a higher specific gravity than water. Essential oils are non-miscible with water but can transfer their odors to aqueous layer. These compounds are solved in most of organic solvents such as diethyl ether, hexane and ethyl acetate. Essential oils in the presence of air and heat are evaporated, therefore they might be called volatile oils or ethereal oils (Aberoomand *et al.*, 2011; Kamal *et al.*, 2011). Some physical and chemical properties of essential oils are different from fixed oils. The most important is that the essential oils are condensable and can be extracted by

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distillation methods and they are not glycerol esters. The essential oils do not produce soap with alkaline and do not make permanent stains on paper (Jaymand, 2001).

The essential oil of citrus genus belongs to the Rutaceae family. Several species of this genus are planted in Iran. The most important species found in Iran are; *C. aurantifolia, C. aurantium, C. grandis, C. llimon, C. medica, C. nobilis , C. paradise, C. sinensis* and *C.* maxima (Mozafarian, 1998).

The essential oils are complex natural mixtures that can be formed from different chemical compounds with various concentrations. Constituent compounds of essential oils are in two groups with two distinct biosynthetic pathways from each other. Terpenoids derivatives created of the intermediate acetate – malonic acid and aromatic compounds made from shikimic acid and phenylpropanoids. In addition to the above classification, components of essential oils might be classified based on the functional groups present in the structure that include hydrocarbons, alcohols, aldehydes, ketones, esters, phenols, phenolic ethers, oxides and peroxides. Terpenes and their oxygenated derivatives (Terpenoids) are the most important categories of the ingredients of essential oils. Depending on the number of isoprene units in the structure of terpenes, these compounds are classified to hemiterepens (C_5) menoterpenes (C_{10}), sesquiterpenes (C_{15}), diterpenes (C_{20}), sesterterpenes and, (C_{25}) triterpenes (C_{30}), tetra-terpenes (C_{40}) and poly-terpenes $(C_5)_n$, where n is greater than 8. Limonene is a single loop monoterpene that is present in citrus peel (Briitmaier, 2006).

Many studies on the measurement of the essential oils of different types of citrus fruits have been carried out. The essential oils of some varieties of citrus fruits were investigated by GC /MS and found that the essential oils were predominantly formed of β-pinene, sabinene, limonene, sytronelal, linalool, neral, geranial and acetate neryl at different concentrations depending on the type of cultivar (Samdja *et al*., 2005).

The essential oils of three varieties of C.sinensis in Kenya (washington, valencia and salutiana – navel) were evaluated by GC / MS. Among the 56 identified compounds in salutianat, 72 identified compounds in the valencia and 73 identified compounds in the washington, limonene, α terpinene, α-pinene and sabinene had the highest concentrations (Njoroge *et al*., 2005).

Due to the importance of the essential oils in the peels, pulps and even leaves of citrus fruits, four types of citrus fruits consisting of orange (C. sinesis, thomson species), tangerine (C. reticulate, kinnow species), sweet lemon (C.limetta, dulsis species) and sour lemon (C. limon, Shiraz species) were selected and then the essential oils of their peels were evaluated and identified.

Materials and Methods

- Preparation of citrus fruits

Four types of citrus fruits consisting of orange, tangerine, sweet lemon and sour lemon were purchased from Tehran market, the species were determined and the essential oils were extracted after the removal of the skins.

- Evaluation of the essential oils concentrations

The concentrations of the essential oils in the orange, tangerine, sweet lemon and sour lemon peels were determined by extraction method using Clevenger apparatus. In order to extract the essential oils, 50 g of citrus peel were separately weighed and placed in one liter conical flask and connected to the Clevenger apparatus. 500 ml of distilled water was added to the flask and heated to the boiling point. The steam in combination with the essential oils were distilled into a graduated cylinder for 5 hours and then separated from aqueous layer. The essential oils were diluted by n-hexane at the ratio of

1 to 10 and kept in the refrigerator until required for further analysis.

- *Identification of the essential oils*

Due to the fact that the constituents of the essential oils are volatile, therefore their identification requires the application of gas chromatography-mass spectrometry (GC-MS). The mass spectra of the samples are compared to the mass spectra of the standards and firm identifications are made. A Hewlett Packard GC model HP-6890 equipped with a 30 meter of 5% phenyl dimethyl siloxan (HP-SMS) capillary column with a Hewlett Packard MS model HP-5973 was employed to carry out the identification of the chemical constituents.

Results and Discussion

The essential oils of citrus peels consisting of orange, tangerine, sweet lemon and sour lemon were isolated and the concentrations were determined using Clevenger apparatus. The average yields for orange, tangerine, sweet lemon and sour lemon peels were 1.48%, 0.90%, 0/54% and 0.46% (w/w) respectively as shown in Figure 1.

Firm identification of the chemical composition of the essential oil of each citrus peel was performed. The results of the analysis for orange, tangerine, sweet lemon and sour lemon are presented in Figures 2 to 5 and Tables 1 to 4 respectively. By studying the mass spectra and retention times, seven compounds were identified in the orange peel essential oil that are the predominant components. Limonene with the concentration of 64.87% followed by N-methyl-D3-aziridine (10.78%) and cytidine (8.55%) were the major peaks obtained. Seventeen compounds were identified in tangerine peel essential oil and limonene with the concentration of 28.10% followed by xanthotoxin (18.40%) were the predominant compounds identified. Eighteen compounds were identified in the essential oil of sweet lemon peel and limonene with 49.79% concentration was identified as the predominant compound. Limonene with the concentration of 83.03% as the predominant and major peak with some of the minor compounds were identified in sour lemon peel essential oil.

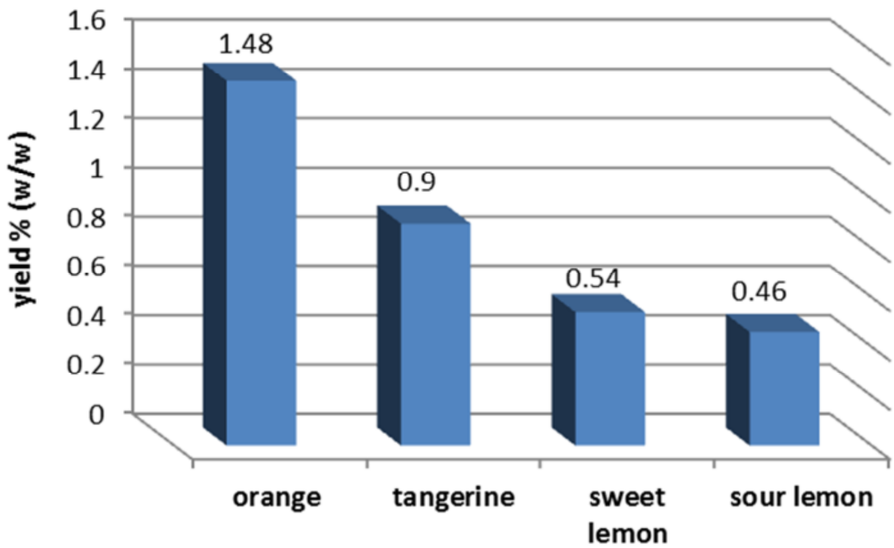

Fig. 1. The average yields of essential oils in citrus peel samples

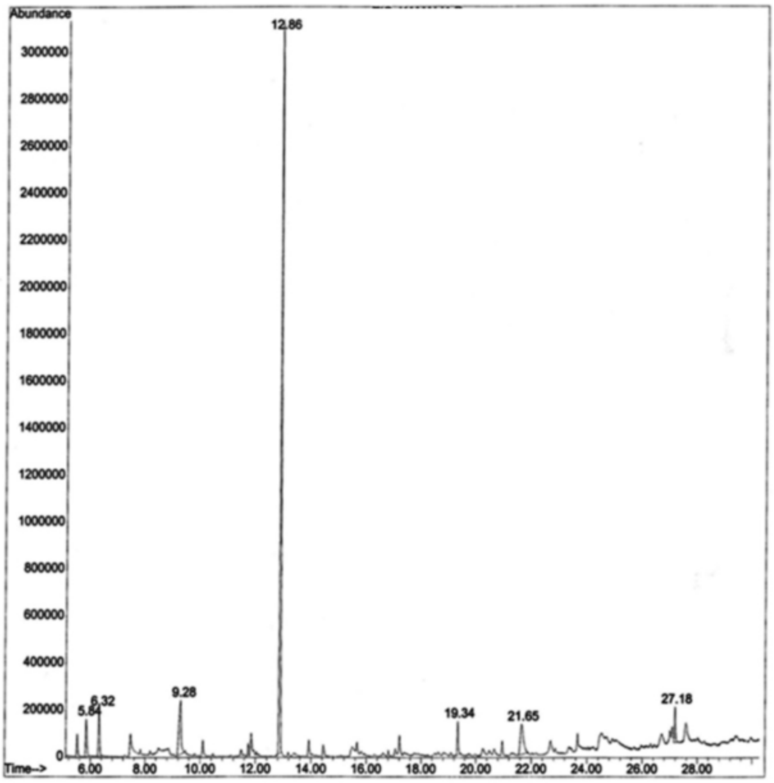

Fig. 2. GC-MS Chromatogram of orange peel essential oil

Table 1. Chemical composition of orange peel essential oil

Components	Concentration (%)	Retention Time (min)
Oxiranemethanol	4.19	5.84
Propane,1,1,- Oxybis	4.99	6.32
N-Methyl-D_3-Aziridine	10.78	9.28
Limonene	64.87	12.86
4-Vinyl-2-Methoxy-Phenol	3.28	19.34
Cytidine	8.55	21.65
Alpha-Sinensal	3.34	27.18

Larijani (2004), evaluated the essential oils of four species of orange and sour lemon. The results indicated the presence of fourteen compounds in the *C.sinesis (Thomson*) essential oil where limonene constituted 90.4% of the total chemical composition. The essential oil of *C.sinesis (Valencia*) peel consisted of 22 compounds that constituted 99.2% of the total oil and the major component of the oil was limonene (90.5%). In the essential oil of *Citrus limon* peel 21compounds were identified that constituted 100% of the total oil and the main component of the oil was limonene with 61.4% concentration.

Mirza and Bahernik, (2006) identified the chemical composition of the essential oil of orange peel by gas chromatography and mass spectrometry GC / MS. There were 21 compounds in citrus essential oil where limonene (94.3%) followed by myrcene (1.5%), linalool (0.9%), decanal (0.5%), alpha-pinene (0.4%) and actonel (0.3%) constituted the composition of the oil.

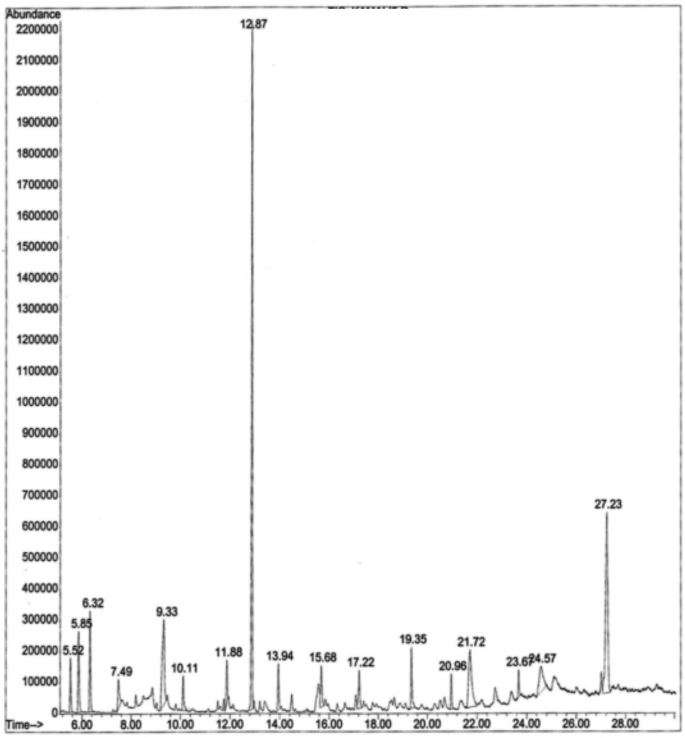

Fig. 3. GC-MS Chromatogram of tangerine peel essential oil

Table 2. Chemical composition of tangerine peel essential oil

Components	Concentration (%)	Retention Time (min)
Butanal,3-Methyl	2.65	5.52
Oxiranemethanol	4.35	5.85
Propanoic Acid, 2-Methyl-,Methyle	4.99	6.32
Acetic acid	2.03	7.49
n-Methyl-D_3-Aziridine	9.07	9.33
4-Hydroxy Cyclopent	1.93	10.11
4-Methyl-1-D_1-Aziridine	2.18	11.88
Limonene	28.10	12.87
1,3,5-Triazine-2,4,6-Triamine	2.36	13.94
4-H-Pyran-4-one,2,3-Dihydro-3,5-Dih	2.65	15.68
4-Vinylphenol	2.50	17.22
4-Vinyl-2-Methoxy-Phenol	2.98	19.35
Benzene,1-Chloro-4-Methoxy	1.65	20.97
Cytidine	8.34	21.72
Dodecanoic acid	1.09	23.67
1,4-Diethynylcyclobutene	4.73	24.58
Xanthotoxin	18.40	27.23

Mushtaq Ahmad *et al*. (2006) analyzed the essential oils of orange (malta), grapefruit and sour lemon peels. The essential oils obtained from peels of three fruits were 0.98 %, 0.73% and 1.12% respectively. The analyses for orange peel oil indicated limonene (61.08%), citral (7.74%), borneol (7.63%), capraldehyde (5.62%), citronellol (4.18%), caprinaldehyde (2.10%), α- terpinolene (2.06%), linalool (1.28%), α-pinene (0.84%), citranelyl acetate (0.22%), camphene (0.32%) and thujene (0.11%), and for grapefruit peel oil showed limonene (86.27%), myrcene

(6.28%), α terpinene (2.11%), α-pinene (1.26%), citronellol (0.50%), caprinaldehyde (0.31%) and α-thujene (0.15%) and for sour lemon peel oil indicated limonene (53.61%), γ-terpinene (18.57%), β-pinene (11.80%), myrcene (11.16%), α-pinene (2.63%), sabinene (0.63%), α-thujene (0.45%), carene (0.45%), citral (0.27%), caprinaldehyde (0.26%), α-terpinolene (0.25%), citronellol (0.15%), camphene (0.13%), borneol (0.16%), and p-cymene (0.12%).

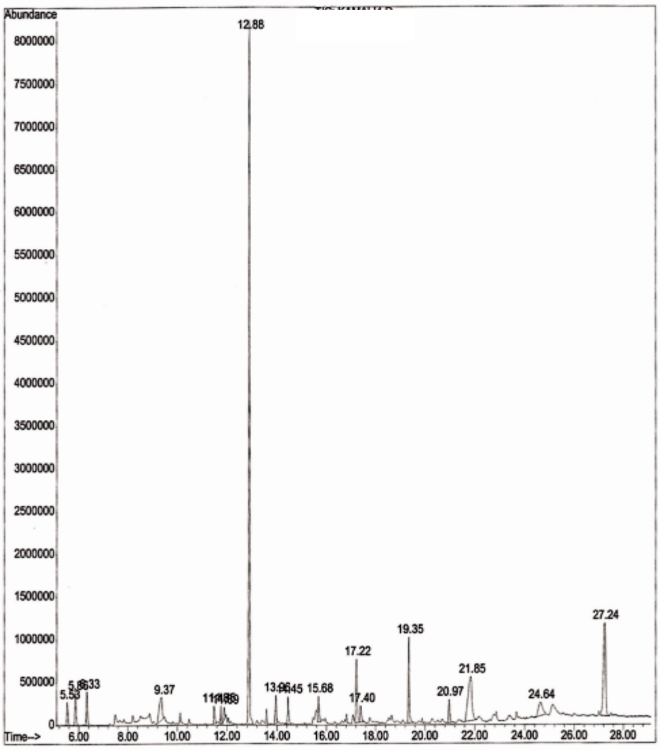

Fig. 4. GC-MS Chromatogram of sweet lemon peel essential oil

Table 3. Chemical composition of sweet lemon peel essential oil

Components	Concentration (%)	Retention Time (min)
Butanal,3-Methyl	1.62	5.53
Oxiranemethanol	2.34	5.86
Propanoic Acid, 2-Methyl-,Methyle	2.27	6.33
N-Methyl-D_3-Aziridine	4.13	9.37
Subianene	1.20	11.48
Myrcene	0.88	11.76
Butanedial	0.79	11.89
Limonene	41.79	12.88
2,4-Pyrimidinedione, 5 Methyle	1.89	13.96
L-Linalool	1.80	14.45
4-h-Pyran-4-one,2,3-Dihydro-3,5-Dih	1.99	15.68
4-Vinylphenol	4.85	17.22
2-Furancarboxaldehyde	1.30	17.40
4-Vinyl-2-Methoxy-Phenol	5.20	19.35
Naphthalene,1-Methyl	1.72	20.97
6,6-Dideutero-Nonen-1-ol-3	10.20	21.85
1,4-Diethynylcyclobutene	3.41	24.64
Xanthotoxin	12.62	27.24

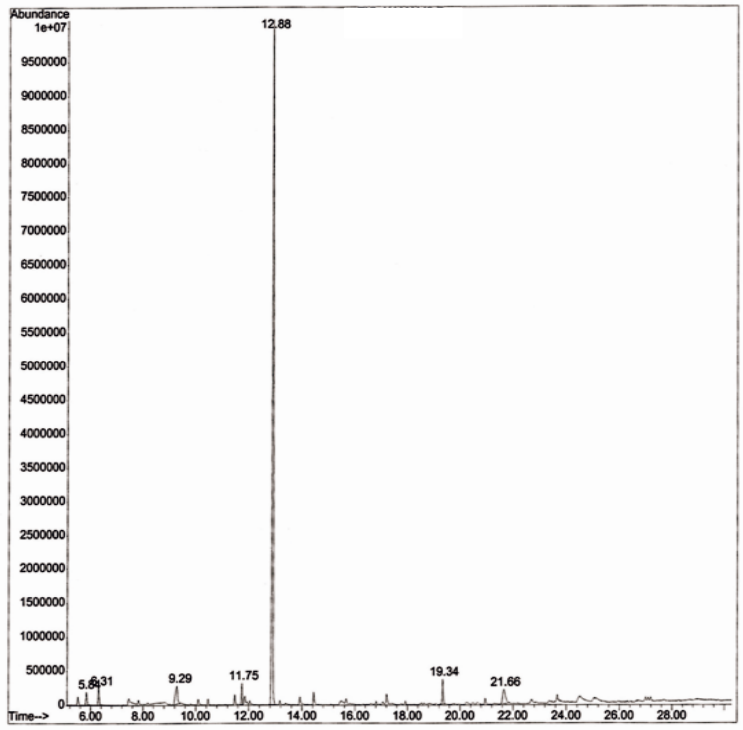

Fig. 5. GC-MS Chromatogram of sour lemon peel essential oil

Table 4. Chemical composition of sour lemon peel essential oil

Components	Concentration (%)	Retention Time (min)
Oxiranemethanol	1.65	5.84
Propane,1,1,- Oxybis	2.00	6.31
N-Methyl-D_3-Aziridine	4.42	9.29
Myrcene	1.95	11.75
Limonene	83.03	12.88
4-Vinyl-2-Methoxy-Phenol	2.79	19.34
Cytidine	4.16	21.66

Bourgou *et al.* (2012) studied the essential oils of orange, tangerine and sour lemon peels. According to the results, the most important components of the orange peel oil were limonene (67.90-90.95%) and 1,8-cineole (14.72%). The most important components of the sour lemon peel essential oil were limonene (37.63-69.71%) followed by β-pinene (0.63-31.49%), γ-terpinene (0.04-9.96%) and p-cymene (0.23-9.84%) and the most important components of the tangerine peel oil were limonene (51.81-69.00%), 1,8-cineole (0.01-26.43%), γ-terpinene (2.53- 14.06%).

Kostadinov *et al.,* (2012) examined the composition of several varieties of tangerine peel essential oils using GC/ MS and concluded that different classes of compounds were present particularly limonene as the major constituent.

Conclusion

The results of this study were similar and in agreement to the results obtained by other researchers. The varieties, different planting and harvesting conditions as well as weather conditions and degree of fruit ripening might have affected the chemical composition of essential oils. In general the essential oils of citrus peels are made of different classes of chemical compounds that have some contribution to aroma, taste and other characteristics of the fruit and might be used in food formulations and other industries. These essential oils apart from their flavor contribution might have effective role in the preservation and stability of some classes of food compounds and further investigation is required to establish such effects.

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