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GENETIC VARIABILITY TO ESSENTIAL OIL COMPOSITION IN FOUR CITRUS FRUIT SPECIES

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Abstract

Essential oils from the peels of Malta (*C. sinensis*), Mousami (*C. sinensis*), Grapefruit *(C. paradisi*) and Eureka lemon (*C. limon*) were extracted through cold pressing method. Highest oil yield (1.21%) was obtained from Malta peel followed by Eureka lemon (1.12%), Mousami (0.98%) and Grapefruit (0.73%). The extracted oils so obtained were investigated for composition by GC/FID on Carbowax 20 M packed glass column. Main constituents separated in Malta peel oil were limonene (61.08%), α-thujene (0.11%), α-pinene (0.84%), camphene (0.32%), citronellol (4.18%), citral (7.74%), capraldehyde (5.62%), caprinaldehyde (2.10%), borneol (7.63%), α-terpinolene (2.06%), linalool (1.28%) and citranelyl acetate (0.22%). In Mousami, the principal compounds were limonene (76.28%), α-pinene (1.26%), β-pinene (5.45%), α-terpinolene (1.56%), citral (1.74%), capraldehyde (0.35%), 2-hexene 1-ol (1.26%), decanol (0.35%) and linalool (2.32%). In Grapefruit peel oil, limonene (86.27%), α-thujene (0.15%), myrcene (6.28%), α-terpinene (2.11%), α-pinene (1.26%), citronellol (0.50%) and caprinaldehyde (0.31%) were among the principal components. Major constituents present in Eureka lemon oil were limonene (53.61%), α-thujene (0.45%), γ-terpinene (18.57%), camphene (0.13%), β-pinene (11.80%), sabinene (0.63%), α-terpinolene (0.25%), myrcene (11.16%), α-pinene (2.63%), citral (0.27%), citronellol (0.15%), caprinaldehyde (0.26%), borneol (0.16%), ∇^3-carene (0.45%) and p-cymene (0.12%). Chemical composition of essential oils of these species varied significantly, which may be due to the difference in their genetic make up.

Introduction

Essential oils are the complex mixture of terpenic hydrocarbons and oxygenated derivatives such as aldehydes, alcohols, ketones, organic acids and esters which can be obtained from fruit peel by cold press process (Clarke, 2002; Merle *et al*., 2004). The volatile compounds of oils are aromatic or odoriferous that occurs in plants as such or less frequently may result from the degradation of glycerides by enzyme action (Susan, 1996). Chemically, these oils are distinct from edible oils, because they are not esters of glycerides (Anon., 2005a).

The genus Citrus includes various species of oranges, mandarins, limes, lemons and grapefruit. The rapid growth of citrus fruit industry in the last three decades is due to improved economic conditions in consuming and also due to the natural distinct flavour of citrus. In Pakistan, the production of citrus fruits in the year 2002-03 was 1816,000 tones having an area of 197,000 hectares, which is increasing each year (Anon., 2004),

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Processing of citrus fruit results in peels and membrane residue from the juice extractor as a primary waste fraction; amounting approximately 40-50% of wet fruit mass. The peels are a potential source of essential oil present in balloon shaped oil sacs of flavedo (Braddock, 1999) and yield oil in the range of 0.5 to 3.0 kg/ tone of fruit (Sattar & Mahmud, 1986). Citrus oils contain large amounts of monoterpene hydrocarbons (70-95%) along with smaller amounts of sesquiterpene hydrocarbons which are responsible for a characteristic flavour. Aldehyde is the class of substances, which mainly contributes to the total content of oxygenated compounds and is an important indicator to establish the price of essential oils and to represent a reference of quality (Dugo, 1994; Braddock, 1995; Diaz *et al*., 2005).

The food and pharmaceutical industries in Pakistan are using citrus oils as flavouring and masking agents in abundant quantity. The present import of citrus peel oils is worth Rs.300 million (Saeed, 1989) which desires an intense need to develop a feasible technique for the production of essential oils from indigenous resources. There is a lack of information regarding volatile composition of essential oils extracted from Pakistani citrus fruits. In this study, variation in the content and composition of essential oils in the peels of four citrus cultivars were examined.

Materials and Methods

Four species of citrus fruits such as Malta (*C. sinensis*), Mousami (*C. sinensis*), Grape fruit (*C. paradisi*) and Eureka lemon (*C. limon*) were included in this study. These species were procured from the local orchards.

After peeling manually, the peels were shredded to a size of 2×0.3 cm, by using citrus peel shredder (Turnsbull's Model # 266069, UK). Cold pressing was done at 15000 psi under ambient conditions, using locally made hydraulic press. The extracted mixture so obtained was centrifuged for 45 min., at 15000 rpm by using refrigerated centrifuge (Model # ALC-4227 R, Germany). After centrifugation, each oil was separated by separating funnel, passed through Sodium sulfate (Sigma-Aldrich, USA) in order to remove traces of moisture and then stored in amber colour bottle at 4 °C.

GC analysis was carried out on Perkin-Elmer Gas Chromatograph, Model # 3920, equipped with flame ionization detector (FID). The column used was 2m x 2 mm id glass, packed with 15% Carbowax 20 M on Chromosorb WAW (Dickes & Nicholas, 1976). Operating conditions for the detection of volatile components of these essential oils were: N_2 flow rate 25 ml/min; H_2 pressure 22 psi; air pressure 50 psi; injector temperature 150^{o}C; detector temperature 250^{o}C; injection volume 0.06 µl**. C**olumn temperature programming: 90^{o}C for 4 min., 90-220^{o}C at the rate of 32^{o}C/min, stay at 220^{o}C for 16 min. Quantitative data were obtained by comparing the retention time of the identified components with that of the standards (Sigma-Aldrich, USA).

Results and Discussion

Percent peel portion and yield of essential oils: The results indicated that percent peel portion of Eureka lemon was the highest and ranked at top with 45.0% followed by Mousami 41.0%, Grapefruit 38.0% and Malta 37.0% (Table 1). The highest oil yield was observed in Malta peel (1.21%), followed by Eureka lemon (1.12%), Mousami (0.98%) and Grapefruit (0.73%) peels.

Table1 Percent peel portion and yield of oils of various Pakistani citrus fruits.

Citrus fruit	Peel portion (%)	Oil yield (%)
Malta	37	1.21
Mousami	41	0.98
Grapefruit	38	0.73
Eureka lemon	45	1.12

Table 2 Componential concentration (%) of volatiles of various citrus peel essential oils.

Volatile compound	Retention time	Malta	Mousami	Grape Fruit	Eureka lemon
Monoterpenes					
α-thujene	0.675	0.11	-	0.15	0.45
α-Pinene	2.659	0.84	1.26	1.26	2.63
Camphene	2.981	0.32	-	-	0.13
β-Pinene	3.503	-	5.45	-	10.80
Sabinene	3.632	-	-	-	0.43
Myrcene	3.703	-	-	6.28	10.16
Limonene	4.403	61.08	76.28	86.27	53.61
∇^3-carene	5.645	-	-	-	0.45
ρ-cymene	6.541	-	-	-	0.12
α-terpinolene	7.101	2.06	1.56	-	0.25
Sesquiterpenes					
α-Terpinene	4.255	-	-	2.11	-
ϒ-Terpinene	5.149	-	-	0.07	18.57
Monoterpenole					
Citronellol	7.306	4.18	-	0.50	0.15
Capraldehyde	3.462	5.62	0.35	-	-
Aldehydes					
Caprinaldehyde	7.050	2.10	-	0.31	0.26
Citral	7.730	7.74	1.74	-	0.27
Benzaldehyde	9.734	-	-	0.07	0.11
2-hexene 1-ol	2.742	-	1.26	-	-
Decanol	3.288	-	0.35	-	-
Borneol	6.276	7.63	-	-	0.16
Linalool	11.087	1.28	2.32	0.02	0.71
Ester					
Citranelyl acetate	13.116	0.22	-	-	-
Total (%)		93.17	90.57	97.05	99.26

Table 3 Percent portions of Various classes of volatile compounds in citrus peel oils.

Classes	Malta	Mousami	Grapefruit	Eureka lemon
Monoterpenes	64.41	84.55	93.98	80.03
Sesquiterpenes	-	-	2.17	18.57
Monoterpenole	4.18	-	0.50	0.15
Aldehydes	15.46	2.09	0.38	0.49
Alcohols	8.90	3.93	0.02	0.72
Ester	0.22	-	-	-

These results are in agreement with the reports of Weiss (1997) who found that peel portions of sweet orange, lemon and mandarin were 25.0, 40.0 and 28.0%, respectively. Similarly, the peels of mandarin, orange, grapefruit and lemon were in the range from 25.0-45.0% (Anon., 2005b) and tangerine, orange, grapefruit and lemon in the range from 25.6-33.0, 21.5-38.1, 33.7-36.4 and 32.0-46.6%, respectively (Ohloff, 1991).

In case of yield, Weiss (1997) also delineated that sweet orange, lemon and mandarin had 0.80, 0.90 and 0.80% and bergamot orange 0.45-0.65% oil yields depending mainly on climacteric conditions. There are reports that yield of cold pressed peel oils of orange, bergamot and petitgrain was 0.5% each whereas, mandarin oil had 0.2% yield (Anon., 2005c). Yield of citrus essential oils differed with individual plant species ranging in most of the cases from 0.2 to 2.0% (Tu *et al*., 2002).

Chemical composition of citrus peel oils: The results of gas chromatographic analysis of these oils are given in Table 2. These components belong to hydrocarbon and oxygenated classes such as monoterpene, sesquiterpene, monoterpenole, aldehyde, alcohol and ester (Table 3). Quantitatively, the most representative monoterpene was limonene separated as 61.08, 76.28, 86.27 and 53.61% in Malta, Mousami, Grapefruit and Eureka lemon, respectively. Other monoterpenes identified in these oils were α-thujene, α-pinene, camphene, β-pinene, sabinene, myrcene, ∇^3-carene, α-terpinolene and p-cymene, in different concentrations. One monoterpenole; citranellol in concentration of 4.18%, 0.50% and 0.15% was found present in Malta, Grapefruit and Eureka lemon peel oils, respectively.

Two sesquiterpenes such as α-terpinene and γ-terpinene in Grapefruit and γ-terpinene in Eureka lemon were quantified in appreciable amounts. Aldehydes as principal derivatives were isolated as capraldehyde, caprinaldehyde, citral and benzaldelyde. Capraldehyde was separated as 5.62% and 0.35% in both Malta and Mousumi whereas, caprinaldehyde was found in Malta, Grapefruit and Eureka lemon as 2.10, 0.31 and 0.26, respectively. Benzaldehyde was isolated in Grapefruit (0.07%) and Eureka lemon (0.11%) oils only. Among alcohols, linalool was also separated in all the oils as 1.28%, 2.32%, 0.02% and 0.71% in Malta, Mousami, Grapefruit and Eureka lemon, respectively. Other alcohols included borneol which was found in Malta (7.63%) and Eureka lemon (0.16%) oils whereas 2-hexene 1-ol (1.26%) and decanol (0.35%) were isolated from Mousami peel oils only. One ester, citranelyl acetate (0.22%) was also identified in Malta peel oil.

These results are in conformity with the previous findings regarding GC separation of essential oils from Vietnames pummelo, sweet orange, tangerine, bergamote, grapefruit peel and mousami. Terpenes and oxygenated compounds such as limonene, γ-terpinene, β-pinene, α-pinene, myrcene, valencene, linalool, octanal, decanal, and butyle butyrate has been found as the major constituents through GC separation (Gancel *et al*., 2002; Hognadottir & Russell, 2003; Khanum *et al.,* 2004; Mahalwal & Ali, 2001). Steam-distilled volatile peel oil of Indian orange when analysed through GC and GC-MS, limonene was found more dominant followed by myrcene, α-terpinolene and β-pinene (Kirbaslar & Kirbaslar, 2003). Almost similar results were reported by Feger *et al*., (2003) and Tu *et al*., (2003) while working on orange and tangerine essential oils.

The componential separation of Eureka lemon peel oil in this study are also in agreement with previous findings (Rodriguez *et al*., 1998; Sawamura *et al*., 1999; Lota *et al.,* 1999; Vikiari *et al.,* 2003; Mahalwal & Ali, 2003). Volatile components identified

and quantified were monoterpenes as limonene, β-pinene, myrcene, camphene, α-phellendrene, sabinene, ρ-cymene, β-caryophyllene and ∇-carene; monoterpenoles as α-terpinol, β-terpinol; aldehydes as citral, geranial and neral; ester as neryle acetate and the chemotypes as limonene, limonene/α-pinene/γ-terpinenes and limonene/linayl acetate / linalool, in the peel and leaves of lemon by using HRGC and GC–MS.

Comparatively, however, the GC separation of the citrus peel oils of Pakistani origin showed both quantitative and qualitative variation in volatiles composition. This variation could be due to the difference in variety and genetic make up (Huet, 1999).

Conclusion

The components identified in these oils related to both hydrocarbon and oxygenated classes such as monoterpene, sesquiterpene, monoterpenole, aldehyde, alcohol and ester. The components identified in Eureka lemon peel oil were more than other oils. All these oils showed different volatile composition due to some difference in genetic make up, however, limonene and α-pinene and linalool were the three common volatiles present in all the four oils in varying amounts.

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