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Essential Oil Composition of Green Peel of the Inter-Varietal Mandarin Hybrid, Kinnow Orange

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Abstract. The essential oil of green peel of large-sized Kinnow fruits was obtained by steam distillation, which recorded a yield of 0.34%. Kinow is a hybrid of inter-varietal cross of the mendarin orange cultivars, King x Mediterranean. The oil was analyzed by GC and MS procedures. Among a variety of the oil constituents, 24 compounds were identified by GC, which were further analyzed for their chemical nature through GC-MS. The major proportion of the indentified constituents comprised of 6-methyl-5-heptene-2-one (15.33%), carvone (13.8%), *cis*-carveol (10.04%) and thujanol (4.55%). Rest of the twenty identified compounds occurred in minor amounts, comprised of 35.84% of the total oil. Limonene, usually the major component of the citrus oils (35-85%), was present in rather low quantities (2.76%) in the Kinnow orange green peel oil.

Keywords: essential oils, limonene, Kinnow orange, Rutaceae, green orange peels, *Citrus reticulata* var. Kinnow

Introduction

Pakistan is rich in the production of citrus fruits, especially orange, the Kinnow orange, grapefruit and lemon. Citrus fruits and their by-products are used in the production of beverages, confectionery, ice-creams, flavours and pharmaceuticals. Essential oils are the major by-products obtained from citrus fruit peels, which find wide applications in food, flavour and pharmaceutical industries for the manufacture of a variety of valuable products (Mori, 2002; Lehner, 2000; Vargas-Arispuro *et al.,* 1998). Extensive research work has been done on the essential oils of various citrus species. These studies relate with the improvement of technologies involved in the production and consumption of citrus oils. Limonene, a monoterpene hydrocarbon, being the major constituent of citrus essential oils, has been separated using a process called deterpenation (Boelens and Jimenez, 1989; Ferrer and Matthews, 1987; Timelli, 1987; Owsusu-Yaw *et al.*, 1986; Uchida *et al.,* 1984). Using this process, terpeneless citrus oils are obtained, which are rich in oxygenated fractions comprising of aldehydes, alcohols and esters (Sugisawa *et al.,* 1989; Yamamoto *et al.,* 1989).

The present study reports the essential oil composition, obtained from green peels of *Citrus reticulata* var. Kinnow, which is a hybrid produced by the inter-varietal cross of two mandarin cultivars of *C. reticulata,* namely, King x Mediterranean (Nordby and Nagy, 1975). Constituents of the Kinnow orange green peel oil were examined, and a comparison was made with those of mature Kinnow orange peel oil reported earlier (Haque, 1989). Detection and identification of the green peel essential oils was carried out by GC-MS. Gas chromatogram provided the information of constituents present in the essential oils, while mass spectra of the compounds aided in the confirmation of chemical nature of these constituents.

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Materials and Methods

The peel material for essential oils extraction. The full-sized fruits of Kinnow orange fruit having green peel were obtained. Immature fruits were selected for the essential oil extraction. Due to the presence of chlorophyll, the fruit was green in colour and thus capable of photosynthetic fixation of CO_2, similar to the process occurring in leaves (Kefford and Chandler, 1970; Jhon and Sunday, 1965). When fruit ripens, the chlorophyll contents change to carotenoids, transforming the colour of the fruit from green to yellow. Green Kinnow orange fruit of full size was collected, and 4 kg peel was shredded. Hydrodistillation of fresh and finely divided green peels was carried out to obtain the green peel essential oil fraction (Gunther, 1948). The oil was extracted with ether : hexane (1: 4), the solvent mixture was dried over anhydrous Na_2SO_4, and removed under vacuum distillation. Vacuum distillation of the solvent extract gave essential oil fraction as the residue. The oil obtained was yellow in colour.

Gas chromatography-mass spectrometry. Jeol model JMS-AX505H mass spectrometer in combination with Hewlett Packard 5890 gas chromatograph was used for the GC-MS analysis. Oil sample was injected into a 25 m x 0.22 mm WCOT BP5 (5% phenyl, 95% dimethylsiloxane) fused silica column, using helium as the career gas with the split ratio of 1:100; EI positive mode, electron energy 70 ev, ionization current 300 μA, ionization source temperature 250 °C, interface temperature 230 °C, column temperature programmed at 60 °C for 4

min, with a 6 °C/min rise at 220 °C. Data acquisition and processing were performed by JEOL JMA-DA 5000 system. Various components were identified by their retention time and peak enhancement with standard samples in the gas chromatographic mode and MS library search from the derived fragmentation pattern of the various components of the essential oil.

Results and Discussion

Yield of the essential oil was 0.34%, which was comparatively lower than the peel oil obtained from matured fruits (0.41%). The physical characteristics were determined by following standard methods (Gunther, 1948), which included refractive index, specific gravity, and acid and ester values (Table 1). These are comparable to the values reported for other citrus oils (Sattar *et al*., 1986).

In order to determine the composition of the essential oils obtained from green Kinnow orange peels, gas chromatographic analysis was carried out. All EI mass spectra were scanned at 70 ev. The gas chromatogram of the Kinnow orange green peel oil is shown in Fig. 1. Each peak in the chromatogram was assigned to a specific compound according to the retention time. A total of 62 peaks were detected. Twenty four of which were identified, which constituted 77.64% of the essential oils analysed (Table 2).

The identified compounds were further classified in to two fractions, namely, the oxygenated fraction and the hydrocarbon fraction. The oxygenated fraction comprised of octanal, nonanal, hexadecanoic acid, *cis*-carvyl acetate, limonene oxide, citral, decanal, *trans*-carveol, carvone, undecenal, carvone oxide, thujanol, ascaridol, farnasol, 6-methyl-5-heptene-2-one, 3,7-dimethyl-1,7-octadiene-3,6-diol, *p*-mentha-2,8-dien-1-ol, 2,3-bornandiol, 3-(2-oxopropyl) cycloheptanone, and *p*-mentha-1(7),8-dien-2-ol, while limonene, ledane, 6-hexadecene-4-yne (E), and 12-methyl-1, 5,9,11-tridecatetraene were found as hydrocarbon fractions of the essential oils (Table 2).

Table 1. Physical characteristics of essential oils fraction of Kinnow orange green peel

Colour	light yellow
Smell	pleasant
Yield	0.34%
Specific gravity	0.8403
Refractive index	1.4127
Acid value	3.16
Aldehyde value	0.84

Limonene, which is a major monoterpene hydrocarbon of citrus oils (Shaw, 1979) occurring to the extent of (50-90%), was found only up to 2.76 %. The hydrocarbons portion of the oil was 4.34% and oxygenated hydrocarbons fraction was 68.8% in the total oil fraction.

The twenty four compounds given in Table 2 were identified and confirmed by mass spectral studies. The mass spectral data of these components is given in Table 3. Mass spectra were found more informative to confirm the structure by showing the molecular weight of each compound. Extensive mass cracking in the EI mode, analysis of base peak and stable fragments further aided in the confirmation of structure of each compound.

The essential oils of Kinnow orange peel were earlier studied by Haque (1989), reporting twenty three components. Limonene in that study was the major component (93.7%), while β-myrecene, ocimene, citral and decanal were present in minor amounts. The mature orange peel oil analysis has also been reported by Shaw *et al.* (1979), which revealed that limonene was present in large amounts (80-90%). The percent-

Table 2. Composition of the essential oil fraction of Kinnow orange green peels

Peak No.	Name of Compound	(%)
1	Octanal	0.193
2	*d*-Limonene	2.762
3	Nonanal	0.550
4	Hexadecanoic acid	3.531
5	*cis*-Carvyl acetate	1.55
6	Limonene oxide	0.60
7	Citral	0.53
8	Decanal	1.03
9	*trans*-carveol	10.04
10	Carvone	13.80
11	Undecenal	0.84
12	Carvone oxide	2.05
13	Thujanol	4.55
14	Ascaridol	2.19
15	Farnasol	1.26
16	6-Hexadecene-4-yne (E)	1.3
17	6-Methyl-5-heptene-2-one	15.33
18	12-Methyl (1,5,9,11-tridecatetraene)	3.30
19	Ledane	1.60
20	3,7-Dimethyl-1,7-octadiene-3,6-diol	3.271
21	*p*-Mentha-2,8-dien-1-ol	3.245
22	2,3-Bornandiol	0.47
23	3-(2-Oxopropylcycloheptanone)	1.08
24	*p*-Mentha-1(7),8-dien-2-ol	2.66

Table 3. Mass spectral data of components of the essential oil fraction of Kinnow orange green peel

Components	Molecular formula, Molecular weight	Mass spectral data
Octanal	$C_8H_{16}O$, **128**	M^+ very weak, 111 (4%), 95 (10%), 69 (22%), 56 (53%), 43 (100%), 41 (75%), 29 (68%)
d-Limonene	$C_{10}H_{16}$, **136**	M^+ 20%, 121 (18%), 93 (50%), 79 (22%), 68 (100%), 67 (45%), 65 (9%), 53 (22%), 41 (25%)
Nonanal	$C_9H_{18}O$, **142**	M^+ 2%, 124 (5%), 109 (1%), 95 (20%), 81 (25%), 70 (45%), 57 (100%), 41 (98%), 29 (80%)
Hexadecanoic acid	$C_{16}H_{32}O_2$, **256**	M^+ 3%, 125 (5%), 112 (35%), 98 (100%), 84 (30%), 69 (25%), 55 (98%), 41 (75%)
cis-Carvyl acetate	$C_{12}H_{18}O_2$, **194**	M^+ very weak, 152 (45%), 135 (5%), 134 (30%), 119 (85%), 84 (80%), 67 (20%), 55 (25%), 43 (98%), 28 (100%)
Limonene oxide	$C_{10}H_{16}O$, **152**	M^+ 10%, 137 (20%), 119 (15%), 108 (22%), 93 (40%), 81 (60%), 67 (65%), 55 (45%), 43 (100%), 41 (85%)
Citral	$C_{10}H_{16}O$, **152**	M^+ 10%, 137 (12%), 123 (10%), 109 (15%), 94 (20%), 83 (13%), 69 (100%), 41 (80%), 29 (10%)
Decanal	$C_{10}H_{18}O$, **154**	M^+ very weak, 147 (2%), 138 (9%), 128 (5%), 112 (25%), 95 (20%), 82 (30%), 57 (65%), 43 (98%), 41 (100%)
trans-Carveol	$C_{10}H_{16}O$, **152**	M^+ 28, 134 (20%), 119 (35%), 109 (100%), 93 (22%), 91 (40%), 84 (75%), 41 (52%)
Carvone	$C_{10}H_{14}O$, **150**	M^+ 20, 135 (10%), 109 (12%), 108 (65%), 93 (40%), 82 (100%), 79 (14%), 54 (50%)
Undecenal	$C_{11}H_{22}O$, **170**	M^+ very weak, 109 (12%), 110 (5%), 95 (20%), 81 (45%), 79 (80%), 67 (65%), 55 (70%), 41 (100%), 29 (40%)
Carvone oxide	$C_{10}H_{14}O_2$, **166**	M^+ 20, 123 (35%), 109 (25%), 91 (15%), 81 (50%), 67 (30%), 43 (100%)
Thujanol	$C_{10}H_{18}O$, **154**	M^+ very weak, 136 (15%), 121 (40%), 95 (70%), 81 (65%), 79 (50%), 67 (55%), 55 (90%), 43 (100%), 41 (88%)
Ascaridol	$C_{10}H_{16}O_2$, **168**	M^+ very weak, 152 (5%), 136 (15%), 121 (10%), 109 (70%), 79 (68%), 67 (30%), 55 (20%), 43 (100%)
Farnasol	$C_{15}H_{26}O$, **222**	M^+ 5, 179 (3%), 161 (4%), 136 (8%), 121 (6%), 107 (12%), 93 (20%), 81 (22%), 69 (100%), 41 (50%)
6-Hexadecene -4-yne (E)	$C_{16}H_{28}$, **220**	M^+ 8, 177 (5%), 163 (5%), 149 (9%), 135 (10%), 121 (15%), 107 (20%), 93 (32%), 79 (100%), 65 (12%), 51 (10%), 43 (35%), 41 (45%)
6-Methyl-5-heptene-2-one	$C_8H_{14}O$, **126**	M^+ 18, 111 (15%), 108 (50%), 93 (12%), 69 (40%), 55 (45%), 43 (100%), 41 (50%)
12-Methyl-1,5, 9,11-tridecatetraene	$C_{14}H_{22}$, **190**	M^+ 10, 175 (4%), 147 (10%), 133 (12%), 121 (28%), 107 (35%), 93 (100%), 79 (70%), 65 (12%), 55 (50%), 41 (90%)
Ledane	$C_{15}H_{26}$, **206**	M^+ 8, 163 (20%), 135 (15%), 121 (25%), 107 (38%), 93 (40%), 79 (30%), 67 (45%), 55 (40%), 41 (100%)
3,7-Dimethyl-1,7-octadiene-3,6-diol	$C_{10}H_{18}O_2$, **170**	M^+ very weak, 152 (5%), 137 (10%), 119 (15%), 109 (12%), 91 (10%), 82 (60%), 71 (95%), 67 (100%), 55 (60%), 43 (90%), 41 (70%)
p-Mentha-2,8 -dien-1-ol	$C_{10}H_{16}O$, **152**	M^+ 5, 138 (10%), 137 (55%), 109 (72%), 95 (30%), 79 (75%), 67 (35%), 43 (100%)
2,3-Bornandiol	$C_{10}H_{18}O_2$, **170**	M^+ very weak, 152 (15%), 134 (10%), 121 (12%), 111 (30%), 95 (100%), 81 (40%), 69 (42%), 55 (38%), 43 (60%), 41 (55%)
3-(2-Oxopropyl) cycloheptanone	$C_{10}H_{16}O_2$, **168**	M^+ 10, 150 (3%), 126 (2%), 111 (68%), 97 (12%), 83 (25%), 67 (13%), 55 (20%), 43 (100%)
p-Mentha-1(7), 8-dien-2-ol	$C_{10}H_{16}O$, **152**	M^+ 10, 137 (15%), 134 (98%), 119 (55%), 109 (100%), 91 (50%), 67 (55%), 55 (60%), 41 (80%)

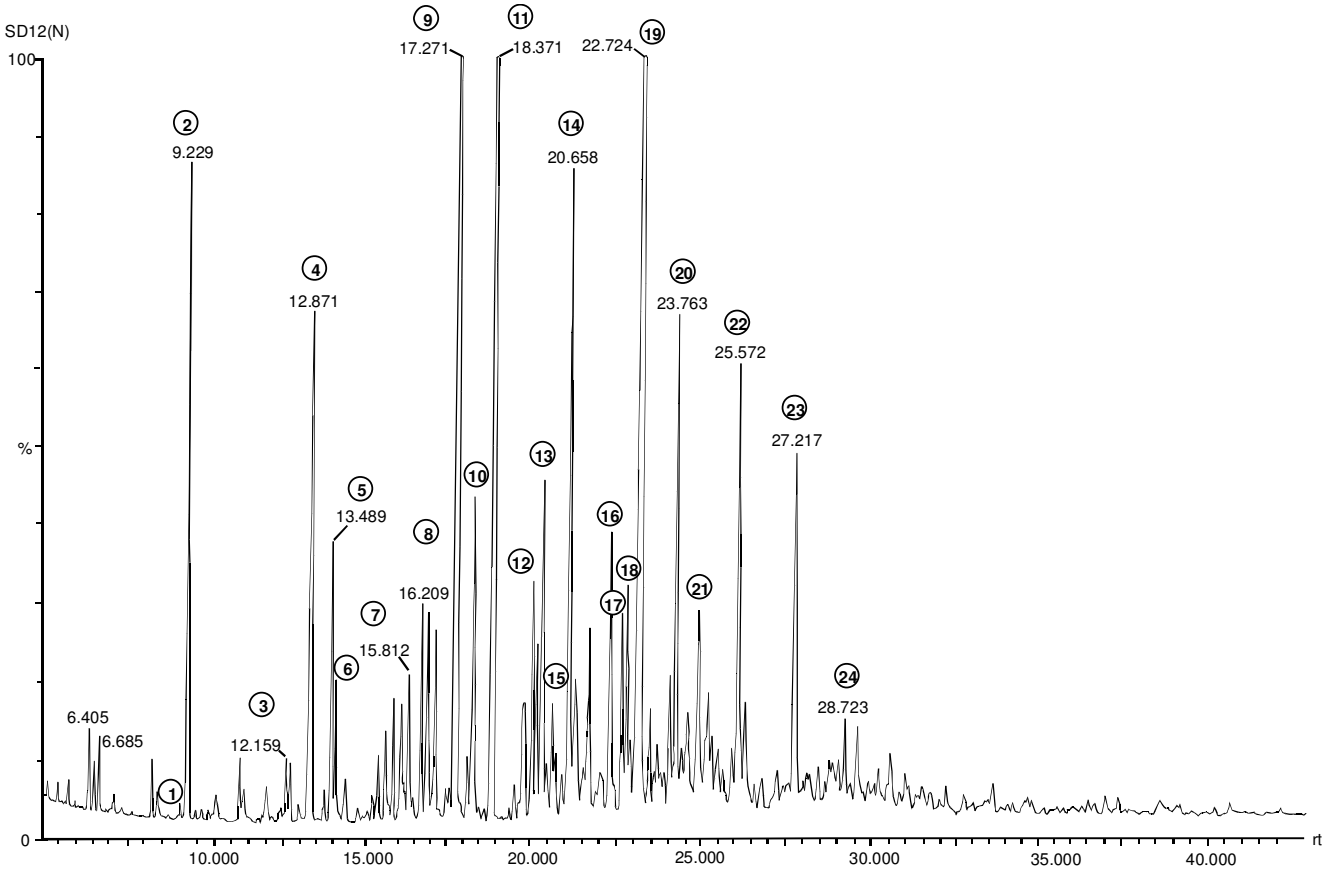

Fig. 1. Gas chromatogram of the essential oil fraction of Kinnow orange green peels; the peak numbers correspond to the peak numbers and the compounds listed in Table 2.

ages of terpene hydrocarbons and oxygenated fractions in the mature peel oils of citrus species has been reported to be 85-93% and 5-7%, respectively (Sattar *et al.,* 1986; Kefford and Chandler, 1970). From the present studies it is concluded that in the essential oil of green peels, the percentage of terpene hydrocarbon (2.76 %), limonene, was low, while the percentage of oxygenated compounds (68.8 %) was very high, as compared to the essential oils extracted from peels of mature Kinnow orange (Haque, 1989), mature orange (Shaw *et al.,* 1979), and mature citrus fruits (Kefford and Chandler, 1970). It was observed that among the total essential oil composition, 6-methyl-5-heptene-2-one (15.33%), carvone (13.8%), *cis*-carveol (10.04%), and thujanol (4.55%) were found as the major components, with higher percentages. Aldeyhdic and alcoholic contents of the essential oils of Kinnow orange green peel were 3.143% and 26.42%, respectively.

The results obtained from the analysis of green peel essential oil fraction indicate that the biogenetic pathway leading to the synthesis of terpenes is incomplete in the immature state. With the maturation of the fruit, some of the intermediate molecules, such as 6-methyl-5-heptene-2-one, 3,7-dimethyl-1,7-octadiene-3,6-diol, 3-(2-oxopropyl) cycloheptanone, 3,7-dimethyl-1,7-octadiene-3,6-diol, 6-hexadecene-4-yne (E), and 12-methyl-1,5,9,11-tridecatetraene and others are transformed through biosynthetic routes to the monoterpenes normally found in the essential oil of mature Kinnow orange and other citrus fruits (Kefford and Chandler, 1970).

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