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Yield and chemical composition of *Citrus* essential oils as affected by drying pretreatment of peels

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Abstract: *Citrus* peel essential oils have an impressive range of food and medicinal uses. In the present study we investigated the variation in the yield and chemical composition of the essential oils isolated from fresh, ambient-, and oven-dried peels of three *Citrus* species namely *Citrus reticulata (C. reticulata)*, *Citrus sinensis (C. sinensis)* and *Citrus paradisii (C. paradisii)*. The hydro-distilled essential oil content from fresh-, ambient-, and oven-dried peels of *C. reticulata*, *C. sinensis* and *C. paradisii* ranged from 0.30-0.50, 0.24-1.07 and 0.20-0.40 g/100 g, respectively. The maximum amount of the oil was determined in oven-dried while the minimum in fresh peel samples. Using GC and GC/MS, a total of 16-27, 17-24 and 18-40 chemical constituents were identified in the peel essential oils of *C. reticulate*, *C. sinensis* and *C. paradisii*, respectively. The content of limonene, the most prevalent chemical constituent, detected in these essential oils, ranged from 64.1-71.1% *(C. reticulata)*, 66.8-80.9% *(C. sinensis)* and 50.8-65.5% *(C. paradisii)*. The yield and content of most of the chemical components including limonene (the principal chemical compound detected) of the tested essential oils varied significantly ($p < 0.05$) with respect to drying treatments and species employed.

Keywords: Limonene, drying conditions, peel oils, GC-MS, monoterpene hydrocarbons, Citrus

Introduction

The genus *Citrus*, belonging to the Rutaceae or Rue family, comprises of about 140 genera and 1,300 species. *Citrus sinensis* (Orange), *Citrus paradisi* (Grapefruit), *Citrus limon* (Lemon), *Citrus reticulata* (tangerine), *Citrus grandis* (shaddock), *Citrus aurantium* (sour orange), *Citrus medica* (Citron), and *Citrus aurantifolia* (lime) are some important fruits of genus *Citrus* (Singh *et al*., 1983; Anwar *et al*., 2008). *Citrus* are well known as one of the world's major fruit crops that are produced in many countries with tropical or subtropical climate. Brazil, USA, Japan, China, Mexico, Pakistan, and countries of the Mediterranean region, are the major *Citrus* producers. Worldwide, *Citrus* production is estimated to be at levels as high as 105 million metric tons (MMT) per annum, Brazil being the largest producer with contribution of 19.2 MMT followed by the United States. Pakistan with an annual production ca. 1.76 MMT of *Citrus* fruits stands among the ten top *Citrus* producing countries of the world (Mahmood, 2005 and Khan, 2005).

Citrus fruits and their by-products are of high economic and medicinal value because of their multiple uses, such as in the food industry, cosmetics and folk medicine (Silalahi, 2002; Saidani *et al*., 2004). In addition to large scale consumption as fresh fruits, the *Citrus* fruits are mainly processed to produce juice. The waste of *Citrus* processing industry, left after juice extraction, such as peels, seeds and pulps, corresponding to about 50% of the raw processed fruit, can be used as a potential source of valuable by-products (El-Adawy *et al*., 1999). Specifically, the *Citrus* peels, commonly treated as agro-industrial waste, are a potential source of valuable secondary plant metabolites and essential oils (Andrea *et al*., 2003).

Citrus peel essential oils are reported to be one of the rich sources of bioactive compounds namely coumarins, flavonoids, carotenes, terpenes and linalool etc. (Mondello *et al*., 2005). Recently, *Citrus* peel essential oils have also been searched for their natural antioxidant and antimicrobial properties (Tepe *et al*., 2005; Jayaprakasha *et al*., 2007; Viuda-Martos *et al*., 2008). It is widely accepted that biological activities of plant materials are strongly linked with their specific chemical composition, mainly the secondary metabolites such as plant phenolics and flavonoids (Jayaprakasha *et al*., 2007; Viuda-Martos *et al*., 2008). Furthermore, studies revealed that drying the plant materials under different conditions can exert significant effect on the chemical profile and biological attributes of the essential oils derived

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(Asekun *et al*., 2007a; Masotti *et al*., 2003; Angioni *et al*., 2006).

Although numerous studies have been reported in the literature showing the effect of drying methods on the yield and chemical quality and characteristics of essential oils from aromatic plants (Asekun *et al*., 2007a; Asekun *et al*., 2007b), however, to the best of our understanding, no such comparative study investigating the influence of drying pretreatment on the yield and chemical composition of essential oils, isolated from peels of different *Citrus* species, have yet been reported. The main objective of the present study was to assess the changes in the yield and chemical composition of essential oils of three *Citrus* (*C. reticulata*, *C. paradisii* and *C. sinensis*) species from Pakistan as affected by pretreatment conditions of peels.

Materials and Methods

Plant materials

Fully ripened fresh fruits of three *Citrus* species i.e. *C. reticulata* (Kinnow), *C. sinensis* (Mussami) and *C. paradisii* (Grape fruit) were obtained from *Citrus* orchards of Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan during 2010. The specimens were further identified and authenticated by Department of Botany, University of Agriculture Faisalabad, Pakistan. The fruits were then peeled off carefully with the help of a sharp knife to avoid any damage of oil glands. Due to practical reasons, the *Citrus* peels under testing were processed under three categories: one portion used as fresh, the other dried at ambient temperature (30 ^{0}C) and the third portion used after drying in oven at 45^{0}C. Drying process was simultaneously coupled with hot air-circulation to facilitate the drying and to avoiding expected fungal growth.

Isolation of the essential oil

The samples of fresh-, ambient -and oven-dried *Citrus* peels were subjected to hydro-distillation for 3 h using a Clevenger-type apparatus. Distillates of essential oils were dried over anhydrous sodium sulfate, filtered and stored at -4°C until analyzed (Hussain *et al*., 2008).

Analysis of the essential oil

Physical analysis

The refractive index and density of the Citrus peel essential oils were determined following standard methods (Guenther, 1964). A digital refractometer RX-7000α (Atago Co. Ltd., Tokyo, Japan) was used for the determination of refractive index of the oils.

Gas chromatography

A Perkin-Elmer gas chromatograph (model 8700), equipped with a flame ionization detector (FID) and HP-5MS capillary column (30 m x 0.25 mm, film thickness 0.25 µm) was used for the chemical analysis of the essential oils. Injector and detector temperatures were set at 220 and 290^{o}C, respectively. The column oven temperature was programmed from 80^{o}C to 220^{o}C at the rate of 4^{o}C min^{-1}; initial and final temperatures were held for 3 and 10 minutes, respectively. Helium was used as a carrier gas with a flow of 1.5 mL min^{-1}. A sample of 1.0 µL was injected, using slit mode (split ratio, 1:100). A built-in data-handling program provided by the manufacturer of the gas chromatograph (Perkin-Elmer, Norwalk, CT, USA) was used for quantification purposes. The composition was reported as a relative percentage of the total peak area.

Gas chromatography/mass spectrometry analysis

For GC–MS analysis of the essential oils an Agilent-Technologies (Little Falls, California, USA) 6890N Network gas chromatographic (GC) system, equipped with an Agilent-Technologies 5975 inert XL Mass selective detector and Agilent-Technologies 7683B series auto injector was used. Compounds were separated on HP-5 MS capillary column (30 m x 0.25 mm, film thickness 0.25 µm; Little Falls, CA, USA). A sample of 1.0 µL was injected in the split mode with split ratio 100:1. An electron ionization system, with ionization energy of 70 eV, was used for GC/MS detection. Column oven temperature program was the same as selected in GC analysis. Helium was used as a carrier gas at a flow rate of 1.5 mL min^{-1}. Mass scanning range was varied over 50 –550 m/z while injector and MS transfer line temperatures were set at 220 and 290ºC, respectively.

Compounds identification

The identification of the oil constituents was based on the comparison of their retention indices relative to (C_9-C_{24}) *n*-alkanes either with those of published data or with authentic compounds. Compounds were also identified using their MS data compared to those from the NIST mass spectral library and published mass spectra (Adam, 2001).

Statistical Analysis

All determinations were made in triplicates and the data is reported as mean ± SD for (n = 1 x 3). Analysis of Variance (ANOVA) was carried out using software STATISTICA 5.5 (Stat Soft Inc, Tulsa, Ok,

USA). A probability value of $p \leq 0.05$ was considered to denote the statistical significance difference.

Results and Discussion

Yield and physical analysis of citrus peel essential oils

The essential oil contents from peels of *Citrus sinensis*, *Citrus reticulata* and *Citrus paradisii* are presented in Table 1. The yields of essential oils of *Citrus* species were significantly ($p<0.05$) affected by drying treatments. The highest amount of the essential oil was obtained from oven-dried sample of *C. sinensis* peel (1.07%) while minimum from fresh sample of *C. paradisii* peel (0.20%). Major effect of drying on essential oil percentage was noted in *C. sinensis* (0.24-1.07%) followed by *C. reticulata* (0.30-0.50%) and *C. paradisii* (0.20-0.40%). In general, oven-dried *Citrus* peels had higher oil yield followed by the ambient-dried and fresh samples. The results of our present analysis regarding the effects of drying conditions on peel essential oil yield are in agreement with the findings of Asekun *et al*. (2007 b) who also investigated higher oil contents from oven-dried samples of *Helichrysum odoratissimum* in comparison with fresh material. Asekun *et al*. (2006) studied the effects of drying on the yield and chemical composition of essential oil from the aerial parts of *Leonotis leonurus* and found that the oils derived from sun-dried plant material had better yield than those from the air and oven-dried materials. Some other reports in the literature also revealed considerable effects of drying on the yield and characteristics of the essential oils (Rahula *et al*., 1973; Laranja *et al*., 2003).

Among the *Citrus* species tested, *C. sinensis* exhibited the maximum oil yield (0.24-1.07%) followed by *C. reticulata* (0.30-0.50%) and *C. paradisii* (0.20-0.40%). These results are in agreement with the findings of Tue *et al*. (2002), who reported that yield of *Citrus* essential oils differed with individual plant species ranging in most of the cases from 0.2-2.0%. According to Weiss (1997), the total oil yields from sweet orange, eureka lemon and mandarin were 0.80, 0.90 and 0.8%, respectively.

The values determined for two physical parameters namely density and refractive indices (25^{0}C) of the peel essential oils of the *Citrus* species tested are presented in Table 1. The values of density and refractive indices for the *Citrus* essential oils tested varied from 0.815 to 0.836 and 1.4596 to 1.4637, respectively, revealing no significant difference ($p>0.05$) with respect to the species and drying conditions selected. Both of these physical parameters are useful in the identification and authenticity of the oils. Guenther (1964), studied the physical properties of essential oils of Brazillian mandarin peel, Palestinian sweet orange peel and California lemon peel and found the specific gravities at 15^{0}C to be varying between 0.854 and 0.858 g/cm^{-3}.

Chemical analysis of the essential oils

GC-MS chromatogram for typical *Citrus* oil analyzed presently is shown in Fig 1. The chemical composition of *C. reticulata* peel essential oil is shown in Table 2. The total numbers of compounds identified in the essential oils from fresh, ambient-dried and oven-dried peels of *C. reticulata* were 27, 27 and 16, representing 99.98, 99.50 and 97.25% of the total oil, respectively. Limonene (69.9, 64.1 and 71.1%), *β*-myrcene (3.27, 4.05 and 4.02%) and decanal (2.33, 7.71 and 5.80%) were the main constituents of essential oil from fresh, ambient-dried and oven-dried peels of *C. reticulata*, respectively. In addition, the fresh peel oil sample also contained considerable amount of nootkatone (3.95%).

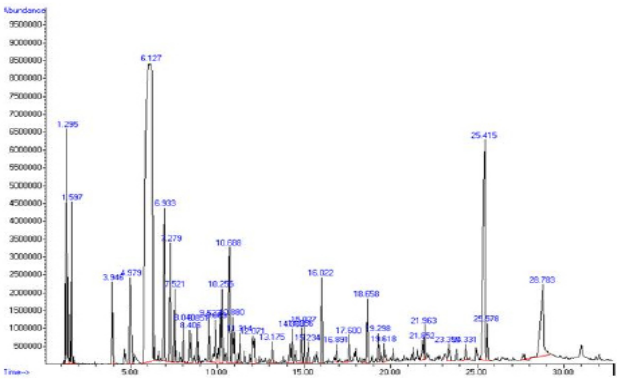

Figure 1. A typical GC-MS chromatogram showing the chemical analysis of essential oil from air-dried peels of *C. paradisii*

As far as the groups of chemical constituents are concerned, the fresh, ambient-dried and oven-dried peel essential oils of *C. reticulata* mainly consisted of monoterpene hydrocarbons, 74.80, 70.78 and 76.79%, respectively with limonene and *β*-myrcene as the major components. The oxygenated monoterpenes hydrocarbons with contribution of 11.53, 18.22 and 15.93% constituted the second major portion of the essential oils from fresh, ambient-dried and oven-dried peels of *C. reticulata*. Linalool (1.10, 2.00 and 2.56%) and decanal (2.33, 7.71 and 5.80%) were the main oxygenated monoterpenes hydrocarbons detected in these oils, respectively. Sesquiterpene hydrocarbons contributed 6.19 and 2.39% of the oils of fresh and ambient-dried *C. reticulata* peels, respectively with germacarene D as the major component. On the other hand, no sesquiterpene hydrocarbon was detected in the essential oil from oven-dried peel of *C. reticulata*. Oxygenated sesquiterpnes were present at levels

Table 1. Yield and physical properties of three *Citrus* essential oils

Parameters	*Citrus reticulate*			*Citrus sinensis*			*Citrus paradisii*		
	Fresh	**Air-dried**	**Oven-dried**	**Fresh**	**Air-dried**	**Oven-dried**	**Fresh**	**Air-dried**	**Oven-dried**
Yield (g $100g^{-1}$)	0.30 ± 0.01[a]	0.48 ± 0.02[b]	0.50 ± 0.02[b]	0.24 ± 0.01[a]	0.50 ± 0.02[b]	1.07 ± 0.04[c]	0.20 ± 0.01[a]	0.30 ± 0.01b	0.40 ± 0.01[c]
Density (g/cm^{-3}) (25 °C)	0.834 ± 0.02[a]	0.835 ± 0.02[a]	0.836 ± 0.02[a]	0.815 ± 0.02[a]	0.816 ± 0.02[a]	0.816 ± 0.02[a]	0.831 ± 0.02[a]	0.833 ± 0.02[a]	0.834 ± 0.03[a]
Refractive Index (25 °C)	1.4596 ± 0.03[a]	1.4622 ± 0.04[a]	1.4622 ± 0.03b[a]	1.4631 ± 0.03[a]	1.4636 ± 0.04b[a]	1.4637 ± 0.03[a]	1.4634 ± 0.03[a]	1.4627 ± 0.04[a]	1.4635 ± 0.04[a]

Results are mean ± S.D. of three samples of each species analyzed individually in triplicate.
Different letters in superscripts indicate a significant ($P < 0.05$) difference among drying conditions.

Table 2. Chemical composition of essential oil from peels of *Citrus reticulate*

Components	RI	% composition			Mode of identification
		Fresh	Air dried	Oven dried	
Monoterpene Hydrocarbons					
α-pinene	939	1.27 ± 0.03[a]	1.62 ± 0.03[b]	1.67 ± 0.03[b]	a, b, c
Sabinene	976	-	0.49 ± 0.01	-	a, b, c
β-pinene	980	0.40 ± 0.02	-	-	a, b, c
β-myrcene	991	3.27 ± 0.04[a]	4.05 ± 0.05[b]	4.02 ± 0.01[b]	a, b, c
Limonene	1031	69.9 ± 1.30[b]	64.1 ±1.20[a]	71.1 ±1.42[c]	a, b, c
Z-β-ocimene	1040	-	0.28 ± 0.04	-	a, b
γ-terpinene	1062	-	0.23 ± 0.006	-	a, b, c
Oxygenated Monoterpene Hydrocarbons					
Octanal	1005	-	1.06 ± 0.03[a]	1.15 ± 0.19[a]	a, b
1-octanol	---	-	0.95 ± 0.19[b]	0.55 ± 0.01[a]	b
Linalool oxide	1088	0.60 ± 0.02	-	-	a, b
Linalool	1097	1.10 ± 0.08[a]	2.00 ± 0.05[b]	2.56 ± 0.08[c]	a, b, c
Menthadien-1-ol	---	0.42 ± 0.01	-	-	b
trans-*p*-1,8-dienol	1123	-	0.52 ± 0.01	-	a, b
Citronellal	1153	0.78 ± 0.02[c]	0.45 ± 0.01[a]	0.58 ± 0.01[b]	a, b, c
α -terpineol	1191	-	1.10 ± 0.02	-	a, b, c
4-carvon menthenol	---	-		0.95 ± 0.02	b
α-terpineneol	1195	1.51 ± 0.02	-	-	a, b, c
Decanal	1207	2.33 ± 0.18[a]	7.71 ± 0.2[c]	5.80 ± 0.19[b]	a, b
Z-carveol	1229	1.29 ± 0.04	-	-	a, b, c
Citronellol	1228	0.80 ± 0.04[a]	-	1.20 ± 0.02[b]	a, b, c
Carvone	1243	-	0.47 ± 0.04[a]	0.62 ± 0.04[b]	a, b, c
Perillaldehyde	---	-	1.64 ± 0.2[a]	1.65 ± 0.08[a]	b
Isopropyl cresol	---	1.36 ± 0.02	-	-	b
4-vinyl guaiacol	---	-	2.32 ± 0.2[b]	0.87 ± 0.09[a]	b
Sesquiterpene Hydrocarbons					
α-cubebene	1347	-	0.48 ± 0.03	-	a, b, c
Copaene	1369	0.89 ± 0.06	-	-	a, b, c
Allyl isovalerate	---	0.36 ± 0.04	-	-	b
β-cubebene	1389	0.84 ± 0.04[b]	0.37 ± 0.03[a]	-	a, b, c
β-caryophyllene	1418	1.39 ± 0.08	-	-	a, b, c
Germacarene	1489	1.07 ± 0.06[b]	0.30 ± 0.03[a]	-	a, b, c
α-farnesene	1491	0.48 ± 0.02[b]	0.36 ± 0.02[a]	-	a, b, c
γ-munrolene	1483	1.16 ± 0.08	-	-	a, b, c
δ-cadinene	1524	-	0.88 ± 0.06	-	a, b, c
Oxygenated Sesquiterpene Hydrocarbons					
Dodecanal	1411	0.42 ± 0.04[a]	0.54 ± 0.03[b]	0.65 ± 0.04[c]	a, b, c
Elemol	1549	0.35 ± 0.03[a]	0.73 ± 0.08[b]	0.89 ± 0.06[c]	a, b
γ-eudesmol	1630	-	1.08 ± 0.04	-	a, b, c
α-cadinol	1658	-	0.46 ± 0.04	-	a, b, c
β-sinensal	1695	0.67 ± 0.05	-	-	a, b
Farnesol	1713	1.14 ± 0.08	-	-	a, b, c
α-sinensal	---	0.93 ± 0.33[a]	5.00 ± 0.25[c]	2.76 ± 0.14[b]	b
Nootkatone	---	3.95 ± 0.15[b]	0.30 ± 0.11[a]	-	b
Total		99.98	99.50	97.25	

*Results are ± standard deviation of three different samples of each species analyzed individually in triplicate. Different letters in superscripts indicate a significant ($P < 0.05$) difference among drying conditions.
a= identification based on retention indices; b= identification based on comparison of mass spectra; c= identification based on co-injection with authentic compounds
*compounds are listed in order of retention times on HP-5MS column

of 7.46, 7.61 and 4.3% in the essential oils from fresh, ambient-dried and oven-dried peel samples, respectively. *α*-Sinensal (0.93-2.76%) was established to be the main oxygenated sesquiterpnes component.

A total of 18-22 compounds were identified in the *C. sinensis* peel essential oils, representing 98-100% of the oil (Table 3). Limonene (80.9, 72.7 and 66.8%), *β*-myrcene (4.19, 3.76 and 4.41%), were the main constituents in the oils from fresh, ambient-dried and oven-dried peels of *C. sinensis*. A considerable amount of *α*-pinene, in the range of 1.65- 2.48% was also determined. Among groups of the chemical compounds, monoterpene hydrocarbons mainly comprising of limonene (66.75-80.88%) constituted a major portion (75.78-91.00%) of the fresh, ambient-dried and oven-dried *C. sinensis* peel essential oils. Oxygenated monoterpenes, mainly comprising of linalool and isophorone with amounts varying from 1.52-2.10% and 1.09-2.92%, respectively constituted 5.78-18.49% of the oils. Sesquiterpenoides and oxygenated sesquiterpenes constituted 0.41-1.90% and 1.32-5.70% of the oils of *C. sinensis*. *β*-Sinensal (0.83 and 0.33) and α-sinensal (0.49 and 0.35) were the two major components in the fresh and oven-dried *C. sinensis* peel essential oils, respectively while these compounds were not detected in the essential oil from ambient-dried peel sample.

In case of *C. paradisii*, the total number

Table 3. Chemical composition of essential oil from peels of *Citrus sinensis*

Components	RI	% composition			Mode of identification
		Fresh	Air dried	Oven dried	
Monoterpene Hydrocarbons					
α-pinene	939	1.65 ± 0.12[a]	1.77 ± 0.13[a]	2.48 ± 0.14[b]	a, b, c
Sabinene	976	0.37 ± 0.03[a]	-	0.62 ± 0.09[b]	a, b, c
α -phyllandrene	1003	-	0.46 ± 0.05	-	a, b, c
β-myrcene	991	4.19 ± 0.24[b]	3.76 ± 0.13[a]	4.41 ± 0.14[b]	a, b, c
Δ[3]-carene	1011	1.03 ± 0.09[b]	0.78 ± 0.04[a]	0.82 ± 0.05[a]	a, b, c
Limonene	1031	80.9±1.50[c]	72.7 ± 2.40[b]	66.8±2.60[a]	a, b, c
Z-*β*-ocimene	1040	0.34 ± 0.02	-	-	a, b, c
4-carene	1022	0.85 ± 0.04[b]	0.61 ± 0.05[a]	0.70 ± 0.05[a]	a, b, c
1,3,8-*p*-menthatriene	---	1.69 ± 0.09[b]	1.21 ± 0.05[a]		b
Oxygenated Monoterpene Hydrocarbons					
Linalool Oxide	1088	-	0.48 ± 0.04[a]	1.12 ± 0.09[b]	a, b, c
Linalool	1097	1.52 ± 0.08[a]	2.10 ± 0.12[b]	1.60 ± 0.08[a]	a, b, c
tras-*p*-2,8-menthadien-1-ol	1123	-	0.79 ± 0.05[a]	1.07 ± 0.09[b]	a, b, c
Limonene oxide	1136	0.76 ± 0.08	-	-	a, b, c
α-terpinenol	1195	-	2.34 ± 0.14[a]	3.10 ± 0.13[b]	a, b
Decanal	1207	1.02 ± 0.08[b]	0.71 ± 0.04[a]	-	a, b, c
Z-carveol	1229	0.68 ± 0.06[a]	2.38 ± 0.13[b]	4.53 ± 0.21[c]	a, b, c
Citronellol	1228	0.71 ± 0.05[a]	1.24 ± 0.06[b]	1.60 ± 0.09[c]	a, b, c
d-carvone	1243	-	-	0.39 ± 0.02	a, b
Isophorone	---	1.09 ± 0.07[a]	1.31 ± 0.08[b]	2.92 ± 0.12[c]	b
4-vinyl guaiacol	---	-	1.27 ± 0.08[a]	1.21 ± 0.09[a]	b
Piperitenone	---	-	-	0.42 ± 0.04	b
Eugenol	1358	-	-	0.53 ± 0.03	a, b, c
Sesquiterpene Hydrocarbons					
α-cubebene	1347	0.70 ± 0.06	-	-	a, b, c
Valencene	1491	1.20 ± 0.09[c]	0.41 ± 0.04[a]	0.63 ± 0.07[b]	a, b
δ-cadinene	1524	-	-	0.32 ± 0.02	a, b, c
Oxygenated Sesquiterpene Hydrocarbons					
3-furanacetic acid		-	-	3.00 ± 0.13	a, b
β-sinensal	1695	0.83 ± 0.07[b]	-	0.33± 0.03[a]	a, b, c
α-sinensal	---	0.49 ± 0.04[b]	-	0.35 ± 0.04[a]	b
Nikkol	---	-	5.70 ± 0.23	-	b
Total		100.00	100.00	99.99	

Values are ± standard deviation of two different experiments. Different letters in superscripts indicate a significant ($P < 0.05$) difference among drying conditions.
a= identification based on retention indices; b= identification based on comparison of mass spectra; c= identification based on co-injection with authentic compounds
*compounds are listed in order of retention times on HP-5MS column

of compounds identified were 40, 27 and 17, representing 99.56, 99.69 and 99.28 % of the fresh, ambient-dried and oven-dried peels essential oil (Table 4 and Figure 1). Limonene (50.8, 53.8 and 65.5%), *β*-myrcene (3.51, 3.57 and 3.60%), linalool oxide (2.29, 6.52 and 4.03%) and nootkatone (25.40, 8.47 and 10.90%) were the main constituents of the essential oils from fresh, ambient-dried and oven-dried peels of *C. paradisii*, respectively. Among groups of the chemical compounds, monoterpene hydrocarbons constituted the major portion (56.92-70.58%) of these oils, with limonene and *β*-myrcene as the main components. Oxygenated monoterpenes hydrocarbons represented 17.67, 21.85 and 21.74% of the fresh, ambient-dried and oven-dried *C. paradisii* peel essential oils, respectively. Decanal and linalool oxides were the two major oxygenated monoterpenes hydrocarbons detected. Another chemical group, sequiterpenoides comprised 9.83, 6.50 and 2.59% of the essential oils from fresh, ambient -dried and oven-dried peels of *Citrus paradisii*, respectively. In this category, *β*-caryophyllene and *δ*-cadinene were the two major components found.

Oxygenated sesquiterpenes constituted 15.14, 12.70 and 4.37% of the essential oils from fresh, ambient-dried and oven-dried peels of *C. paradisii*, respectively. Nootkatone with contribution of 4.37-10.90% was the major component. A considerable amount, 3.84 and 0.70% of farnesol was also determined in the fresh and ambient-dried peel essential oil, respectively, however essential oil from oven-dried peel samples was devoid of this compound.

Overall, the analyzed *C. reticulata*, *C. paradisii* and *C. sinensis* essential oils isolated from fresh, ambient-dried and oven-dried peels mainly contained monoterpenes with contribution of 74.80, 70.78 and 76.79; 56.92, 58.63 and 70.58; 89.91, 81.27 and 76.87%, respectively. *α*-Terpinene, limonene, *α*-pinene, *β*-pinene and *β*-myrcene were the main monoterpenes determined in these oils. *Citrus reticulata*, *C. paradisii* and *C. sinensis* also contained considerable quantity of sesquiterpenes oxygenated monoterpenes and sesquiterpenes (Figure 2).

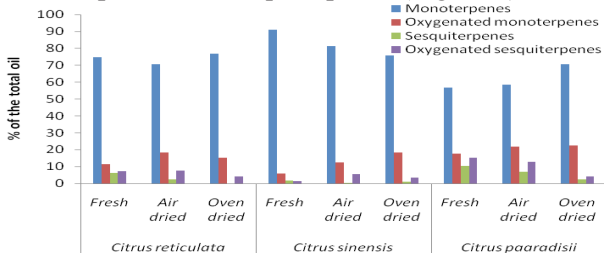

Figure 2. Proportion of different classes of compounds in *Citrus* essential oils

The quantitative data, especially on the main chemical components, of *Citrus* essential oils tested from Pakistan were quite comparable with those of *Citrus* oils reported in the literature from other regions of the world, however in some cases a notable variation in the composition of oils was also observed. This is in agreement with the earlier literature studies

Table 4. Chemical composition of essential oil from peels of *Citrus paradisii*

Components	RI	% composition			Mode of identification
		Fresh	Air dried	Oven dried	
Monoterpene Hydrocarbons					
α-pinene	939	1.19 ± 0.06[a]	1.26 ± 0.05[a]	1.50 ± 0.08[b]	a, b, c
Sabinene	976	0.52 ± 0.04	-	-	a, b, c
β-myrcene	991	3.51 ± 0.13[a]	3.57 ± 0.10[a]	3.60 ± 0.11[a]	a, b, c
γ-terpinene	1062	0.91 ± 0.02	-	-	a, b, c
Limonene	1031	50.8± 1.02[a]	53.8 ± 1.25[b]	65.5 ± 1.45[c]	a, b, c
Oxygenated Monoterpene Hydrocarbons					
Linalool oxide	1088	2.29 ± 0.12[a]	6.52 ± 0.36[c]	4.03 ± 0.25[b]	a, b, c
Linalool	1097	1.07 ± 0.07[a]	1.55 ± 0.08[b]	1.04 ± 0.05[a]	a, b, c
trans-*p*-2,8-menthadien-1-ol	1123	0.63 ± 0.03[a]	0.67 ± 0.05[a]	-	a, b
Limonene oxide	1136	0.87 ± 0.06	-	-	a, b, c
Citronellal	1153	0.83 ± 0.24[b]	0.80 ± 0.21[a]	-	a, b, c
4-terpineol	1179	0.78 ± 0.05[a]	-	1.18 ± 0.08[b]	a, b,
4-carvon menthenol	---	0.88 ± 0.06	-	-	b
α-terpineol	1191	0.75 ± 0.06	-	-	a, b, c
α-terpinenol	1195	-	1.33 ± 0.07[a]	2.68 ± 0.13[b]	a, b
Decanal	1207	2.29 ± 0.13[a]	2.50 ± 0.11[a]	-	a, b, c
Z-carveol	1229	1.50 ± 0.14[a]	3.65 ± 0.25[b]	7.87 ± 0.26[c]	a, b, c
Citronellol	1228	1.78 ± 0.07[b]	1.48 ± 0.06[a]	-	a, b, c
E-carveol	1207	-	-	1.87 ± 0.08	a, b, c
Isophorone	---	-	-	1.29 ± 0.09	b
d-carvone	1243	0.64 ± 0.07[a]	0.65 ± 0.04[a]	-	a, b, c
Geraniol	1256	0.44 ± 0.04	-	-	a, b, c
Citral	---	1.22 ± 0.08	-	-	b
4-vinyl guaiacol	---	0.33 ± 0.05[a]	0.67 ± 0.04[b]	0.59 ± 0.03[a]	a, b
Carvyl acetate	1368	0.66 ± 0.03	-	-	a, b
Eugenol	---	-	1.34 ± 0.9[b]	0.79 ± 0..04[a]	b
Geranyl butyrate	---	0.16 ± 0.07	-	-	b
Geranyl acetate	1380	0.55 ± 0.04[b]	0.69 ± 0.06[c]	0.40 ± 0.04[a]	a, b
Sesquiterpene Hydrocarbons					
α-copaene	1376	0.79 ± 0.04[b]	0.79 ± 0.03[b]	0.39 ± 0.02[a]	a, b, c
β-cubebene	1389	0.81 ± 0.04[b]	0.56 ± 0.04[a]	-	a, b, c
β-caryophyllene	1418	1.73 ± 0.06[b]	1.66 ± 0.05[b]	1.37 ± 0.01[a]	a, b, c
Germacarene D	1489	0.83 ± 0.06[a]	0.79 ± 0.04[a]	-	a, b, c
Valencene	---	3.36 ± 0.18	-	-	b
α-panasinsen	---	0.39 ± 0.02	-	-	b
δ-cadinene	1518	1.25 ± 0.08[b]	1.34 ± 0.09[b]	0.83 ± 0.06[a]	a, b, c
β-gurjunene	---	-	0.65 ± 0.06	-	b
γ-gurjunene	1472	0.67 ± 0.05[a]	0.71 ± 0.06[a]	-	a, b, c
Oxygenated Sesquiterpene Hydrocarbons					
Dodecanal	1412	0.31 ± 0.02	-	-	a, b, c
Elemol	1549	0.52 ± 0.04[a]	0.56 ± 0.06[a]	-	a, b
Nerolidol	1565	0.32 ± 0.03[a]	0.55 ± 0.04[b]	-	a, b
β-caryophyllene oxide	1583	0.56 ± 0.04	-	-	a, b, c
β-sinensal	1695	0.51 ± 0.04	-	-	a, b, c
Farnesol	1697	3.84 ± 0.11[b]	0.70 ± 0.09[a]	-	a, b, c
α-sinensal	---	0.19 ±0.01	-	-	b
Santolina epoxide	---	0.42 ± 0.03	-	-	b
Nootkatone	---	8.47 ± 0.25[b]	10.90 ± 0.24[c]	4.37 ± 0.23[a]	b
Total		99.56	99.69	99.28	

Values are ± standard deviation of two different experiments. Different letters in superscripts indicate a significant ($P < 0.05$).
a= identification based on retention indices; b= identification based on comparison of mass spectra; c= identification based on co-injection with authentic compounds
*compounds are listed in order of retention times on HP-5MS column

which revealed considerable variation in the chemical composition of peel essential oils with respect to varieties and drying conditions (Rahula *et al*., 1973; Laranja *et al*., 2003; Asekun *et al*., 2006; Asekun *et al*., 2007 a; Asekun *et al*., 2007b). Lota *et al*. (2000) examined limonene and *γ*-terpinene as the two major monoterpines in the peel essential oil of *Citrus reticulata* (mandarin). In our analysis, limonene and *β*-myrcene were determined to be the two major components in the *C. reticulata* peel essential oil. Similar to our findings, Feger *et al*. (2003) investigated that limonene was the major component in peel oils of commercial Brazillian Murcot Tangerines. Anon. (2004) investigated that limonene and citral were the main chemical components in tangerine peel essential oil. Choi and Sawanura (2000b) investigated limonene (80.35-82.39%), *α*-terpinene (7.71-9.03%), myrcene (2.11-2.28%), linalool (1.37-2.01%), and *α*-pinene (1.17-1.43%) as the most prevalent components in Hyuganatsu oils. Our results are also in agreement with the findings of Gancel *et al*. (2003) who worked on the chemical composition of *Citrus paradisii* oil. Vekiari *et al*. (2002) reported that the main components of *Citrus* essential oils were limonene, *β*-pinene, myrcene, neral, geranial, neryl acetate and *β*-caryophyllene. Lota *et al*., (2001) found limonene and *α*-pinene as the main compounds in the peel oils of sour orange. Ahmad *et al*. (2006) extracted essential oils from the peels of Malta (*C. sinensis*), Mousami (*C. sinensis*), Grapefruit (*C. paradisi*) and Eureka lemon (*C. limon*) through cold pressing method. According to them the main constituents detected in Malta peel oil were limonene (61.08%), citronellol (4.18%), citral (7.74%), borneol (7.63%), *α*-terpinolene (2.06%) and linalool (1.28%). The principal compounds in Mousami essential oils were

limonene (76.28%), α-pinene (1.26%), β-pinene (5.45%), citral (1.74%), and linalool (2.32%) while limonene (86.27%), myrcene (6.28%), γ-terpinene (2.11%) and α-pinene (1.26%) in Grapefruit essential oils (Ahmad *et al*., 2006). According to the investigation of Choi and Sawamure (2000a), the essential oil of *Citrus tamurana* contained hydrocarbons (95.95-96.95%), aldehydes (0.33-0.62%), alcohols (1.91%-2.64%), ketones (0.40-0.62%), esters (0.28-0.39%), oxides (0.04-0.06%), acids (0.01%) and trace amounts of eugenol methyl ether. In our analysis the analyzed oils also mainly contained monoterpene hydrocarbons, although their concentration varied significantly in relation to *Citrus* species and drying regimes employed. The significant variation in the chemical composition of essential oils of the tested *Citrus* species may be linked to the differences in their genetic makeup as well as to the peels drying conditions.

Conclusions

Overall, the peel essential oils from the selected *Citrus* species native to Pakistan were rich in limonene with maximum amount detected in *C. sinensis* followed by *C. reticulata* and *C. paradisii*. *Citrus* essential oils tested mainly consisted of monoterpene hydrocarbons. The yield and content of most of the chemical components including limonene, the most prevalent chemical constituent, of the tested essential oils varied significantly ($p \leq 0.05$) with respect to drying treatments and species used. Further research to evaluate effects of peel treatments on the biological activities and antioxidant principles of essential oils isolated is recommended to explore their potential uses for functional food and pharmaceutical applications.

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