

Volatile Constituents of Redblush Grapefruit (*Citrus paradisi*) and Pummelo (*Citrus grandis*) Peel Essential Oils from Kenya

SIMON MUHOHO NJORGE,[†] HIROSHI KOAZE,[‡] PAUL NYOTA KARANJA,[†] AND MASAYOSHI SAWAMURA^{*,§}

Department of Food Science and Technology, Faculty of Agriculture, Jomo Kenyatta University of Agriculture and Technology, Post Office Box 62000-00200, Nairobi, Kenya, and Laboratory of Food Technology, Faculty of Agriculture, Obihiro University of Agriculture and Veterinary Medicine, Inada, Obihiro, Hokkaido, 080-8555, Japan, and Department of Bioresources Science, Faculty of Agriculture, Kochi University, B-200 Monobe, Nankoku, Kochi 783-8502, Japan

The volatile constituents of cold-pressed peel essential oils of redblush grapefruit (*Citrus paradisi* Macfadyen forma *Redblush*) and pummelo (*Citrus grandis* Osbeck) from the same locality in Kenya were determined by GC and GC–MS. A total of 67 and 52 compounds, amounting to 97.9 and 98.8% of the two oils, respectively, were identified. Monoterpene hydrocarbons constituted 93.3 and 97.5% in the oils, respectively, with limonene (91.1 and 94.8%), α -terpinene (1.3 and 1.8%), and α -pinene (0.5%) as the main compounds. Sesquiterpene hydrocarbons constituted 0.4% in each oil. The notable compounds were β -caryophyllene, α -cubebene, and (*E,E*)- α -farnesene. Oxygenated compounds constituted 4.2 and 2.0% of the redblush grapefruit and pummelo oils, respectively, out of which carbonyl compounds (2.0 and 1.3%), alcohols (1.4 and 0.3%), and esters (0.7 and 0.4%) were the major groups. Heptyl acetate, octanal, decanal, citronellal, and (*Z*)-carvone were the main constituents (0.1–0.5%). Perillene, (*E*)-carveol, and perillyl acetate occurred in the redblush grapefruit but were absent from the pummelo oil. Nootkatone, α - and β -sinensal, methyl-*N*-methylantranilate, and (*Z,E*)-farnesol were prominent in both oils.

KEYWORDS: Kenyan *Citrus* fruits; redblush grapefruit; *Citrus paradisi* Macfadyen forma *Redblush*; pummelo; *Citrus grandis* Osbeck; essential oil constituents

INTRODUCTION

Grapefruit (*Citrus paradisi* Macfadyen) and pummelo (*Citrus grandis* Osbeck) are major *Citrus* species in terms of cultivation and utilization in the world (1). The grapefruit is said to have developed by natural hybridization between pummelo and sweet orange (*Citrus sinensis*) and was first reported growing in Barbados in the West Indies around 1750 (1). Commercial grapefruit varieties were mainly developed in Florida, and several types are currently cultivated in many tropical and subtropical regions of warm and humid climates. The main producing countries are U.S.A. (Florida), Israel, Cuba, Argentina, and South Africa (1). Grapefruits are reputed for their pleasant and distinctive flavor, which makes them particularly popular as breakfast fruit when fresh, juice, salad fruit, or dessert. The juice is rich in potassium, and the seed extract is antimicrobial, particularly against molds. The peel oil has a strong and desirable aroma useful in industrial flavoring of foods, beverages, pharmaceutical products, perfumes, and

cosmetics, as is common with other *Citrus* oils (2). There are two natural groups of grapefruits depending upon the color of the flesh; the white (common) types, including the Duncan, Marsh seedless (White Marsh), and Triumph, and the pigmented types, including redblush, Thompson, and Foster pink (1). The redblush grapefruit was discovered in Texas, occurring as a limb sport of the Thompson (1). It is seedless and of good eating quality, containing abundant juice, strong aroma, and attractive red flesh color. While many studies on grapefruit oils have been published and most of the work reviewed (3–5), those concerning the redblush variety are scarce. Limonene occurs at between 85 and 96% in grapefruit oils while myrcene, sabinene, α -pinene, and γ -terpinene have been reported at less than 2.5% (4, 5). Nootkatone, octanal, nonanal, decanal dodecanal, octyl acetate, citronellyl acetate, citral, and carvone have been reported in the oil at low levels (<1.0%) and indicated as the major contributors to the aroma (3, 6, 7). Advanced chromatographic techniques for studies on compositions of *Citrus* essential oils, such as the interactive use of linear retention indices, MS spectra probability matching in GC–MS, and comprehensive GC and LC, have recently been reported (8).

The pummelo is a native of southeastern Asia, where it is still much cultivated and utilized. The main areas of production

* To whom correspondence should be addressed. Telephone: +81-88-864-5184. Fax: +81-88-864-5200. E-mail: sawamura@cc.kochi-u.ac.jp.

[†] Jomo Kenyatta University of Agriculture and Technology.

[‡] Obihiro University of Agriculture and Veterinary Medicine.

[§] Kochi University.

in the Orient are southern China, southern Japan, Thailand, Vietnam, Malaysia and Indonesia. Pummelo is also cultivated in the U.S.A. (California and Florida), the Caribbean islands, and Africa (1). It is the largest of all *Citrus* fruits and contains a thick, spongy peel (1). The fruit is commonly eaten fresh or as juice and is also popular for jams, jellies, marmalades, and syrups. Pummelo is a good source of dietary vitamin C and potassium and also has a strongly aromatic peel essential oil. In some Oriental cultures, the decoction of the fruit peel has been employed for medicinal purposes, to alleviate coughs, ulcers, swellings, and epilepsy, because of the effectiveness of the volatiles (9). Pummelo oil has been reported to activate fat metabolism in the body, leading to anti-obesity (9). The oil has also been suggested to be an effective natural antioxidant (10). There are many cultivated varieties of the pummelo, such as the banpeiyu, hirado buntan, and Tosa buntan of Japan and the kao lang sat and kao phuang of Thailand. A *Citrus* hybrid, Sweetie, also known as Oroblanco, developed as a cross between pummelo and a white seeded grapefruit variety, is commercially produced in Israel (11). The volatile components of the peel essential oil of the *Citrus* hybrid have been reported to constitute an ideal basis for the creation of new taste nuances in the soft drink sector (11).

In Kenya, grapefruit and pummelo are the second major group of cultivated *Citrus* fruits, after sweet oranges, with an annual production of about 10 700 metric tons (12, 13). The fruits are mostly produced on the Indian ocean coastal belt, at an altitude less than 30 m above sea level. The region is hot and humid, with average annual temperatures of 22 to 30 °C and average annual rainfall of about 1100 mm. The production and utilization of *Citrus* fruits in the country are increasing rapidly (14). The peel essential oils could be utilized in flavor and fragrance industries but have not been commercially exploited. Previous studies on Kenyan *Citrus* oils are reported of sweet oranges (*Citrus sinensis*) and mandarins (*Citrus reticulata*) (14, 15). To our knowledge, there are no previous publications on the essential oils of redblush grapefruit and pummelo grown in Kenya. The understanding of the functional chemical constituents of valuable natural products is important for their utilization and quality standards. The aim of this study was to identify the volatile components of the peel essential oils of redblush grapefruit and pummelo from the Kenyan coast and make comparisons with other *Citrus* oils.

MATERIALS AND METHODS

Materials. The redblush grapefruit and pummelo fruits were obtained at harvest maturity in August 2004, from the same locality in the Kilifi district, about 50 km north of Mombasa City on the Indian Ocean coastal region of Kenya. The fruits were kept in a cold room in the course of oil extraction, which was completed within a week of harvest. Analytical reference compounds used in identification of the essential oil constituents were purchased from Fluka Fine Chemicals, Buchs, Switzerland; Wako Pure Chemical Industries, Osaka, Japan, and Aldrich Chemical Co., Milwaukee, WI. The peel essential oils were prepared in the Department of Food Science and Technology, Jomo Kenyatta University of Agriculture and Technology, by a cold-pressing method as described previously (16, 17). The fruit mesocarp and the albedo layers were peeled off using a sharp knife and discarded, to expose the flavedo and its oil ducts. The prepared flavedo was maintained on ice. It was pressed manually to crack the oil ducts and express the essential oil. The crude essential oil was collected on ice. It was saturated with brine and centrifuged at 2000g for 15 min at 4 °C. It was decanted, dried with anhydrous sodium sulfate for 24 h at 5 °C, and filtered to obtain the cold-pressed oil for the study. The essential oils were kept at -21 °C prior to analysis.

Gas Chromatography (GC) and Gas Chromatography–Mass Spectrometry (GC/MS). GC analysis was performed using a Shimadzu GC 14A gas chromatograph (Shimadzu Corp., Kyoto, Japan) fitted with DB-Wax fused silica capillary column (60 m × 0.25 mm i.d., 0.25 μm film thickness; J & W Scientific, Folsom, CA) and a flame ionization detector (FID). Polar capillary columns such as DB-Wax and Thermo 600T (18) provide high resolution of the complex mixture of volatile components of *Citrus* essential oils. The use of both polar and apolar columns is preferred. Polar and apolar capillary columns are commonly used to separate the complex mixture of volatile components of *Citrus* essential oils (17). The oven temperature was programmed from 70 °C (2 min) at an increasing rate of 2 °C/min to 230 °C (20 min). The injector and detector temperatures were 250 °C. Oil samples of 0.5 μL were injected. The split ratio was 1:50. The carrier gas was nitrogen at a flow rate of 2 mL/min. Relative peak areas were integrated using a Shimadzu C-R6A Chromatopac integrator (Shimadzu, Kyoto, Japan). The samples were analyzed in triplicates. The proportions of the volatile components were expressed as relative percentages obtained by peak area integration without using correcting factors. The analyses were done in triplicates. Retention indices (RIs) of the separated compounds and reference standards on the DB-Wax column were determined on the basis of a homologous series of *n*-alkanes (C₉–C₂₇).

GC–MS analysis was conducted on a Shimadzu GC 17A coupled with a Shimadzu QP 5000 MS (Shimadzu, Kyoto, Japan). Oil samples of 0.2 μL were injected. The GC conditions were similar to those indicated above. The mass spectra were obtained with an ionization voltage of 70 eV, ion source temperature of 250 °C, and a scanning time of 1 s over a range of 30–320 *m/z*. Identification of the separated volatile compounds was based on comparisons of their mass spectra similarities to those of registered compounds in the NIST 62 and 107 spectral libraries of the GC–MS. The RI of the separated compounds was compared to those of reference compounds in the laboratory of the authors, determined under similar analytical conditions in the laboratory of the authors. Tentative identification, where reference compounds were not available, was based on GC–MS databases in the laboratory of the authors.

RESULTS AND DISCUSSION

The redblush grapefruit and pummelo whole fruits had average weights of 278 and 633 g, respectively. The yields of essential oils relative to the whole fruits were 0.06 and 0.12% (w/w), respectively. The typical GC chromatogram of the redblush grapefruit oil is shown in **Figure 1**. The elution profile of the pummelo oil closely resembled that of the redblush grapefruit oil, except for some differences in peak areas of the few discriminating compounds between the two oils, as given in **Table 1**. The identified volatile constituents in the two oils and their relative concentrations, are shown in the **Table 1**, according to their GC elution order. A total of 67 constituents were found in the redblush grapefruit and 52 in the pummelo, accounting for 97.9 and 98.8% of the oils, respectively. Some peaks shown in **Figure 1** but are missing from **Table 1** were unidentified. The compounds were categorized into monoterpene hydrocarbons, sesquiterpene hydrocarbons, carbonyl compounds, alcohols, esters, oxides, and epoxides.

Volatile Constituents of the Redblush Grapefruit Oil. The oil contained eight monoterpene hydrocarbons amounting to a relative 93.3% of the total volatiles. Limonene was the most abundant (91.1%), followed by α -terpinene (1.3%), α -pinene (0.5%), and sabinene (0.4%). α -Thujene (19), α -pinene, γ -terpinene, and terpinolene occurred at <0.05%. The relative amount of the monoterpene hydrocarbons was close to those reported for similar oils (6, 7, 20) and to those of sweet orange oils from the same origin (14). The oil was notably devoid of myrcene, a compound that occurs widely among several grapefruit varieties in Japan (17–1.8%) (5). In a study on the chemotaxonomic value of essential oil compounds in *Citrus*

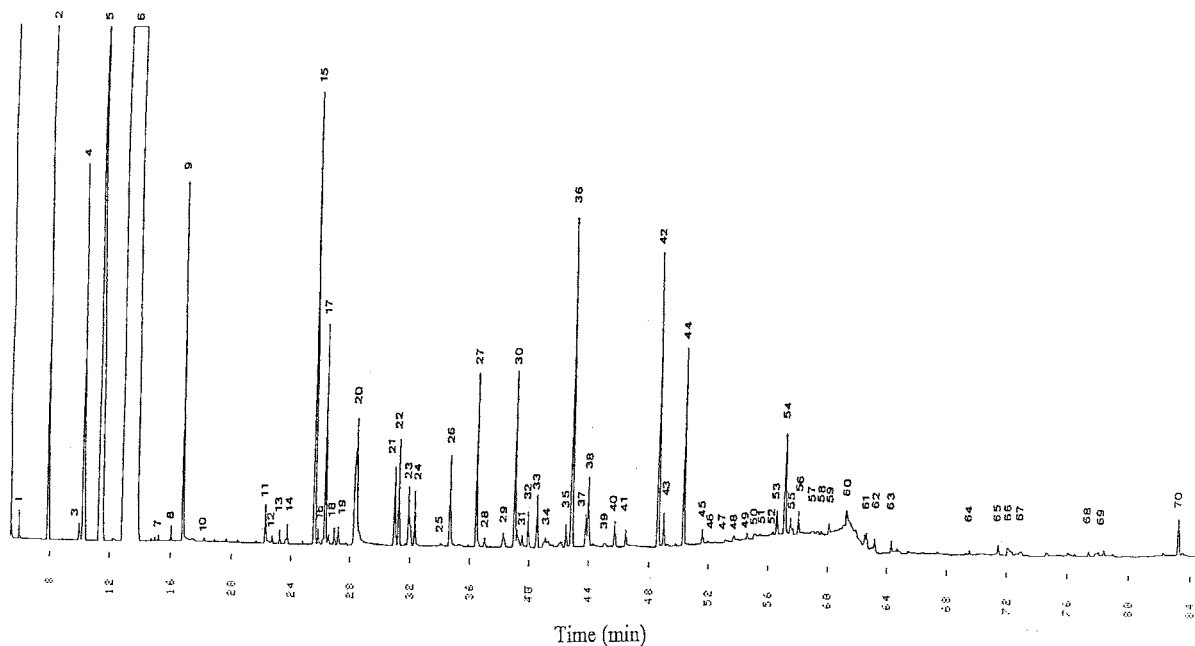


Figure 1. Capillary gas chromatogram of cold-pressed peel essential oil of redblush grapefruit from Kenya.

species produced in Venezuela (21), limonene was the most dominant component of the essential oils from red and white varieties of *Citrus paradisi*. The limonene content of the Kenyan redblush grapefruit oil was also closely similar to those reported for Sweetie peel oils from Israel (11). The sabinene content of the Kenyan redblush grapefruit oil was comparatively less than reported for the Sweetie oil (0.8–1.0%). The aliphatic hydrocarbon, 4-methyl-1-octene, was detected at <0.05%. Sesquiterpene hydrocarbons amounted to 0.4% of the total volatiles. Among the six constituents detected in the oil, α -cubebene, α -cedrene, β -caryophyllene, and (*E,E*)- α -farnesene each occurred at 0.1%. δ -Elemene and α -copaene were found at <0.05%. According to reported studies, the peel oils of grapefruits, oranges, and mandarins generally contain low amounts of sesquiterpene hydrocarbons (<1.0%) (4, 5, 13, 15, 22, 23). Oxygenated compounds constituted 4.2% of the total oil volatiles, where carbonyl compounds (2.0%) were the major group. Aliphatic aldehydes accounted for 0.7%, with octanal (0.3%), decanal (0.3%), and nonanal (0.1) as the main constituents. The level of octanal was much less than reported for Venezuelan grapefruit oils (2.2–3.6%) (21). Dodecanal, tetradecanal, and tetradecenal occurred at trace levels. Terpene aldehydes constituted 0.4%, where citronellal (0.2%), cumin, and cinnamic aldehydes at 0.1% each were relatively prominent. Neral, geranial, perilla aldehyde, and α - and β -sinensal occurred at <0.05%. Ketones accounted for 0.9%. The constituents included (*Z*)-carvone (0.4%), nootkatone (0.2%), sabina ketone (0.2%), and (*E*)-carvone (0.1%). The Kenyan redblush grapefruit oil had a higher level of nootkatone than reported for Sweetie oil (11). Sabina ketone, a bicyclic monoterpene ketone (24), was a prominent component of the oil. The ketone has been reported in other cold-pressed *Citrus* peel oils (14). Nootkatone is the characteristic aroma compound of grapefruit oil and has been thoroughly studied (3). Alcohols amounted to 1.4%. The main constituents were (*Z*)-carveol (0.3%), (*E*)-*p*-mentha-2,8-dienol (0.2%), and (*E*)-carveol (0.2%). Linalool, α -terpineol, *p*-mentha-1-en-9-ol, (*Z*)-nerolidol, elemol, and cedrol occurred at 0.1% levels. The other alcohols, including (*E*)-sabinene hydrate, isothujol, (*E,Z*)-2,6-nonadien-1-ol, (*Z,E*)-farnesol, di-

hydrocarveol, and (*E*)-*p*-2,8-menthadien-1-ol occurred at <0.05%. (*E*)-Sabinene hydrate occurs widely among *Citrus* species (5). Esters accounted for 0.7%, where the main constituents were heptyl acetate (0.5%), linalyl acetate (0.1%), and dodecyl acetate (0.1%). Perillyl acetate, nerolidyl acetate, and geranyl propionate occurred at <0.05%. Except for linalyl acetate, which has been reported at appreciable levels in bergamot (*C. bergamia* Risso) oil (30–36%) (5), the other esters are usually absent or at low levels in most *Citrus* oils (4, 5). Octyl formate, reported at appreciable levels in Venezuelan *Citrus paradisi* peel oils (21), was not found in the Kenyan sample. Perillene was detected at <0.05%. The compound has been reported in sweet orange oils (14). Methyl-*N*-methylantranilate, a nitrogen-containing compound, was detected at a trace quantity. It is known to be the character-impact aroma compound of concord grapes, and is a major contributor to tangerine aroma (25). Oxides and epoxides amounted to 0.1%, comprised of caryophyllene oxide (0.1%), caryophyllene epoxide, and myrcene epoxide. Studies on the inheritance mechanisms with regards to leaf and peel volatile compounds of an interspecific *Citrus* somatic hybrid from Mexican lime [*Citrus aurantifolia* (Christm.) Swingle] and Star Ruby grapefruit (*Citrus paradisi* Macfadyen) showed that the hybrid retained the capacity of the parents to produce β -sinensal in the leaves and nootkatone in the peel (26). Such knowledge would be valuable for the improvement of *Citrus* cultivars and aroma quality of *Citrus* essential oils.

Volatile Constituents of the Pummelo Oil. A total of six monoterpene hydrocarbons amounting to 97.5% of the oil volatiles were identified. Limonene was the most abundant (94.8%), as reported for other pummelo oils (5), followed by α -terpinene (1.8%), α -pinene (0.5%), and sabinene (0.4%). β -Pinene and γ -terpinene occurred at <0.05%. α -Thujene and terpinolene were not detected, unlike in the redblush grapefruit oil. Myrcene was virtually absent, as in most other pummelo oils (5). The limonene content of the Kenyan pummelo peel oil was closely similar to that of an Italian pummelo cultivar (cv. Chandler), extracted by cold-pressing manual pressure and analyzed by GC/MS using a fused silica capillary column (27). Sesquiterpene hydrocarbons constituted 0.3%. The main con-

Table 1. Volatile Constituents of Redblush Grapefruit and Pummelo Peel Essential Oils from Kenya

number	RI	compound	concentration (%) ^a			number	RI	compound	concentration (%) ^a		
			redblush	pummelo	identification				redblush	pummelo	identification
1	900	α -thujene	b	c	MS ^d	35	1737	geranial	b	0.1	MS, RI ^e
2	1021	α -pinene	0.5	0.5	MS, RI ^e	36	1744	(Z)-carvone	0.4	0.1	MS, RI ^e
3	1121	β -pinene	b	b	MS, RI ^e	37	1764	(E,E)- α -farnesene	0.1	0.1	MS, RI ^e
4	1131	sabinene	0.4	0.4	MS, RI ^e	38	1794	cumin aldehyde	0.1	b	MS, RI ^e
5	1193	α -terpinene	1.3	1.8	MS, RI ^e	39	1814	geranyl propionate	tr ^f	tr ^f	MS, RI ^e
6	1231	limonene	91.1	94.8	MS, RI ^e	40	1817	perilla aldehyde	b	tr ^f	MS ^d
7	1266	γ -terpinene	b	b	MS, RI ^e	41	1835	nerol	b	tr ^f	MS, RI ^e
8	1287	terpinolene	b	c	MS, RI ^e	42	1842	(Z)-carveol	0.3	b	MS, RI ^e
9	1303	octanal	0.3	0.4	MS, RI ^e	43	1849	(E)-carvone	0.1	b	MS, RI ^e
10	1315	4-methyl-1-octene	b	c	MS ^d	44	1884	(E)-carveol	0.2	c	MS, RI ^e
11	1407	nonanal	0.1	b	MS, RI ^e	45	1895	dodecyl acetate	0.1	c	MS, RI ^e
12	1415	myrcene epoxide	b	c	MS ^d	46	1902	perillyl acetate	tr ^f	c	MS, RI ^e
13	1423	(E,Z)-2,6-nonadien-1-ol	b	c	MS ^d	47	1921	tetradecanal	tr ^f	c	MS, RI ^e
14	1431	perillene	b	c	MS ^d	48	1948	p-mentha-1-en-9-ol	0.1	tr ^f	MS ^d
15	1451	heptyl acetate	0.5	0.3	MS, RI ^e	49	1967	tetradecenal	tr ^f	tr ^f	MS, RI ^e
16	1469	(E)-sabinene hydrate	b	c	MS, RI ^e	50	1976	globulol	tr ^f	c	MS ^d
17	1481	citronellal	0.2	0.1	MS, RI ^e	51	1979	limonen-10-ol	tr ^f	tr ^f	MS ^d
18	1484	δ -elemene	b	tr ^f	MS, RI ^e	52	1984	dihydrocarveol	0.1	tr ^f	MS ^d
19	1492	α -copaene	b	b	MS, RI ^e	53	2001	caryophyllene oxide	0.1	tr ^f	MS ^d
20	1511	decanal	0.3	0.3	MS, RI ^e	54	2005	(Z)-nerolidol	0.1	c	MS, RI ^e
21	1553	α -cubebene	0.1	0.1	MS, RI ^e	55	2010	(E)-caryophyllene epoxide	tr ^f	tr ^f	MS ^d
22	1558	linalool	0.1	0.1	MS, RI ^e	56	2021	perillyl alcohol	tr ^f	b	MS, RI ^e
23	1568	linalyl acetate	0.1	0.1	MS, RI ^e	57	2026	nonanoic acid	b	tr	MS, RI ^e
24	1571	α -Cedrene	0.1	tr ^f	MS, RI ^e	58	2037	cinnamic aldehyde	0.1	c	MS, RI ^e
25	1575	isothujol	tr ^f	b	MS, RI ^e	59	2072	methyl-N-methylantranilate	tr ^f	b	MS ^d
26	1614	β -caryophyllene	0.1	0.1	MS, RI ^e	60	2086	elemol	0.1	tr ^f	MS, RI ^e
27	1640	(E)-p-mentha-2,8-dienol	0.2	0.1	MS ^d	61	2127	cedrol	0.1	tr ^f	MS, RI ^e
28	1650	dihydrocarveol	b	tr ^f	MS ^d	62	2249	β -sinensal	b	tr ^f	MS, RI ^e
29	1670	(E)-p-2,8-menthadien-1-ol	b	tr ^f	MS ^d	63	2271	nerolidyl acetate	b	tr ^f	MS ^d
30	1683	sabina ketone	0.2	0.1	MS ^d	64	2291	(Z,E)-farnesol	b	*	MS, RI ^e
31	1686	neral	b	tr ^f	MS, RI ^e	65	2304	α -sinensal	b	tr ^f	MS ^d
32	1691	(Z)-piperitol	b	b	MS, RI ^e	66	2419	undecanoic acid	tr ^f	c	MS, RI ^e
33	1707	α -terpineol	0.1	0.1	MS, RI ^e	67	2563	nootkatone	0.2	0.1	MS, RI ^e
34	1716	dodecanal	b	0.1	MS, RI ^e						

compound	concentration (%) ^a		compound	concentration (%) ^a	
	redblush	pummelo		redblush	pummelo
monoterpene hydrocarbons	93.3	97.5	alcohols	1.4	0.3
sesquiterpene hydrocarbons	0.4	0.3	esters	0.7	0.4
aliphatic hydrocarbons	b	c	oxides and epoxides	0.1	tr ^f
carbonyl compounds	2.0	1.3			

^a Relative concentration, mean of triplicate determinations. ^b Peak area more than 0.005% but less than 0.05%. ^c Not detected. ^d Tentatively identified on the basis of MS databases. ^e Identified on the basis of RI and MS of reference compounds. ^f tr = detected but not quantitated.

stituents were α -cubebene, β -caryophyllene, and (E,E)- α -farnesene, each at 0.1%. The others were δ -elemene, α -cedrene, and α -copaene at <0.05% levels. Several oxygenated compounds amounting to 2.0% occurred in the oil, as indicated in **Table 1**. The main groups were carbonyl compounds (1.3%), alcohols (0.3%), and esters (0.4%). Aliphatic aldehydes (0.7%) consisted of octanal (0.4%), decanal (0.3%), dodecanal (0.1%), and tetradecanal (trace amount). Tetradecanal was not detected in the pummelo oil, unlike in the redblush grapefruit oil. The other aliphatic aldehydes present in the pummelo oil were nonanal, dodecanal, and tetradecanal. The main terpene aldehydes were citronellal and geranial. Neral, cumin aldehyde, perilla aldehyde, and α - and β -sinensal occurred at trace levels. Cinnamic aldehyde was not detected, unlike in the redblush grapefruit oil. Nootkatone, sabina ketone, and (Z)-carvone occurred at 0.1% each. Nootkatone has been reported in all types of pummelos and grapefruits growing in the Orient, at <0.05% (5). Alcohols constituted 0.3% of the oil volatiles. The prominent constituents were linalool, (E)-p-menthadien-1-ol, and α -terpineol at 0.1% each. The linalool content of the Kenyan pummelo oil was much less reported for *Citrus grandis* grown in Venezuela (50.3%) (21). The other alcohols in Kenyan pummelo

oil occurred at <0.05% and were similar to those in the redblush grapefruit oil, except for (Z)-nerolidol, globulol, (E)-carveol, and sabinene hydrate, which were absent in the former but present in the later oil. Perillene was not detected, unlike in the redblush grapefruit oil. Methyl-N-methylantranilate occurred at <0.05%. Esters amounted to 0.4%, out of which heptyl acetate (0.3%) and linalyl acetate were the main constituents. The level of heptyl acetate was higher than reported for other pummelo oils (5, 23). Geranyl acetate and nerolidyl acetate occurred at trace quantities. Perillyl and dodecyl acetates were not detected in the oil, unlike in the redblush grapefruit oil. The compounds are rare in pummelo oils (5). Caryophyllene oxide and epoxide occurred at trace quantities, but myrcene epoxide was not detected.

As a conclusion, this study provided qualitative information about the volatile constituents of the peel essential oils of Kenyan redblush grapefruit and pummelo, which had not previously been reported. There were many similarities of the components of the essential oils to those of other related *Citrus* oils. The redblush grapefruit and pummelo oils were clearly distinct in the profiles of their oxygenated compounds. The former oil possessed twice as much of the total oxygenated

compounds and (*E*)-carveol, (*Z*)-nerolidol, cinnamic aldehyde, and dodecyl acetate, which were not detected in the pummelo oil.

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