

The Effects of Rootstock on the Volatile Flavour Components of Page Mandarin [(*C.Reticulata* var dancy × *C.Paradisi* var dancan) × *C.Clemantina*] Juice and Peel

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ABSTRACT : *The effects of rootstock on the volatile flavour components of page mandarin juice and peel were investigated in this study. Juice flavour components were extracted by using Poly dimethyl silaxane membranes(PDMS) and eluted by pentane dichloromethane and then analyzed by GC-FID and GC-MS. Peel flavour components were extracted by using cold-press and eluted by using n-hexane and then analyzed by GC-FID and GC-MS. Seventy-five juice components, sixty-eight green peel components and fourty- two red peel components including: aldehydes, alcohols, esters, ketons, monoterpens, sesquiterpens and acids were indentified and quantified. The major flavour components were linalool, limonene, octanal, decanal, α -pinene, sabinene, myrcene, δ -elemen and germacren-D. It is of interest to point out that juice and peel oil from fruit grown on Citrumelo Swingle and Yuzu showed the highest content of aldehydes. Since the aldehyde content of citrus oil is considered as one of the more important indicators of high quality, rootstock apparently has a profound influence on Page Mandarin juice and peel oil quality.*

KEYWORDS: *Page mandarin juice, Page mandarin peel, Flavor components, Rootstock, Pervaporation, Cold-Press.*

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INTRODUCTION

The essential oils of citrus are located in the spherically shaped ductless oil glands within the outer skin or flavedo of the fruit. These valuable essential oils are composed of many compounds including terpenes, sesquiterpenes, aldehydes, alcohols, esters and sterols. They may also be described as mixtures of hydrocarbons, oxygenated compounds and nonvolatile residue. Citrus flavors and odors, because of their freshness, lightness and fine fruity aroma, have found particularly wide acceptance with the consumer. The most important of these are the oils of orange and lemon and to a lesser extent lime, tangerine and grapefruit [1]. Peel oils of citrus can be used commercially in the flavouring of foods, beverages, perfumes, cosmetics, etc. [2]. Cold-pressed oils have general use in many food products including soft drinks, sherbet and ices, confectionery and bakery products, as well as household extracts [1]. The quality of an essential oil may be calculated from the quantity of oxygenated compounds present in the oil [1]. The quantity of oxygenated compounds present in the oil, is variable and depends upon a number of factors including : rootstock [3], variety [4], degree of maturity [5,6] and climat [4].

Various studies have shown that the rootstock used may influence the quantity of oxygenated compounds present in the oil. In 1970, *Hendrickson, et al.* [3] showed that rootstocks could have an influence on the aldehyde content of orange rind oil. In 2003, *Verzera, et al.* [7] found that the quality of bergamot peel oil was determined by a high amount of alcohols and esters, which in turn is related to the type of rootstock used. In 1994, *Usai, et al.* [8] showed that rootstock could have an influence on the content of aldehyds, alcohols and esters present in marsh seedless grapefruit peel oil. The greatest amounts of the better quality juice are consumed by the food and beverage industries [1]. The quality of a juice may be calculated not only with the amount of oxygenated components present in the juice but also with concentration of total soluble solids, relative amounts of sugar and acids.

Various studies have shown that rootstock used may influence the quality of volatile flavour components present in the juice and also the quality of chemical compositions (TSS, Sugar and Acid). *Marsh and Cameron* [9]; *Marsh* [10] pointed out the presence of a bitter substance,

limonene, which is found in the extracted juice of the Washington navel orange is markedly affected by rootstock. The present study reports the effects of rootstocks on the oxygenated compounds of Page mandarine juice and peel oil with the aim of verifying; if the quantity of oxygenated compounds present in the juice and peel oil was influenced by the rootstocks.

MATERIALS AND METHODS

Rootstocks

In 1989, rootstocks were planted at 8×4m² with three replication at Ramsar research station (latitude 36° 54' N, Longitude 50° 40' E; Caspian Sea climate, average rainfall 970 mm per year and average temperature 16.25 ° C ; soil was classified as loam-clay, pH range (6.9-7)). The following rootstocks were investigated (table 1).

Preparation of peel sample

in the last week of November 2007, at least 10 mature fruit were collected from many parts of the same trees located in Ramsar research station. About 50-150 g of Page mandarin peel of fresh mature fruit was cold-pressed and then the oil was separated from the crude extract by centrifugation (at 4000 R for 15 min at 4 °C). The supernatant was dehydrated with anhydrous sodium sulfate at 5 °C for 24h and then filtered. The oil was stored at -25 °C until analyzed. The yield of cold-pressed peel oil has been reported in tables 2 and 3.

Preparation of juice sample

In the last week of December 2007, at least 10 mature fruit were collected from many parts of the same trees located in Ramsar research station. Page mandarin juice was obtained by using the Indelicato Super Automatic, Type A2 104 extractor. After extraction, juice is screened to remove peel, membrane, pulp and seed pieces according to the standard operating procedure. Each juice replicate was made with 10 page mandarin. Three replicates were used for the quantitative analysis (n=3).

Chemical methods

The total titratable acidity was assessed by titration with sodium hydroxide (0.1 N) and expressed as % citric acid. Total soluble solids, expressed as Brix, was determined using a Carl Zeiss, Jena (Germany) refractometer.

Table 1: Common and botanical names for citrus taxa used as scions and rootstocks.

Bakraei (Rootstock)	C.reticulata × C. aurantifolia
Changsha (Rootstock)	C.reticulata Blanco
Citrumelo 'Swingle' (Rootstock)	C. paradisi var dancan × P.trifoliata (L.) Raf .
Cleopatra Mandarin (Rootstock)	C. reticulata Blanco (C. reshni Hort.ex.Tan)
Page (scion)	[(C.reticulata var dancy × C. paradisi var dancan) × C. Clemantina]
Sour orange (Rootstock)	C. aurantium (L.)
Trifoliolate orange (Rootstock)	Poncirus trifoliata (L.) Raf .
Troyer citrange (Rootstock)	C. sinensis (L.) osbeck× P. trifoliata (L.) Raf .
Yuzu (Rootstock)	C. junos Sieb. ex Tan. (C.Ichangensis swing. × C.reticulata Blanco)

Table 2: Average values of fruit weight, peel weight, peel thickness and oil yield for the samples analyzed (green peel). (Each Sample: Ten Fruits)

Rootstock	Production year (kg/ha) (2007-2008)	*Fruit weight (g)	*Peel weight (g)	*Peel thickness(mm)	† Oil (for 50 g peel) (%)
Bakraei (Rootstock)	109.5	125.2	35.81	3.68	11.31
Changsha (Rootstock)	161.7	126.2	28.81	3.22	6.05
Citrumelo 'Swingle' (Rootstock)	207.3	137	33.51	3.30	10.12
Cleopatra Mandarin (Rootstock)	150.0	124.9	31.77	3.43	1.99
Sour orange (Rootstock)	124.9	125.2	26.29	3.57	5.28
Trifoliolate orange (Rootstock)	167.1	129.1	31.32	3.67	10.58
Troyer citrange (Rootstock)	149.2	121.8	30.69	3.33	5.40
Yuzu (Rootstock)	141.6	136.1	31.12	3.49	2.23

* For each fruit. † yield (%) of oil was for 50g flavedo by weight.

Table 3: Effects of rootstocks on fruit length, fruit diameter, peel thickness and oil yield for the samples analyzed (red peel).

Rootstocks	Fruit* Length (mm)	Fruit* Diameter (mm)	Peel* Thickness (mm)	Oil † (for 150 g peel) %
Bakraei	64.77	53.49	3.68	15.18
Changsha	64.53	53.35	3.22	15.25
Citrumelo 'Swingle'	66.45	54.23	3.30	15.25
Cleopatra Mandarin	65.32	53.18	3.43	10.49
Sour orange	65.22	52.69	3.57	10.32
Trifoliolate orange	64.90	52.13	3.67	15.85
Troyer citrange	65.53	52.23	3.33	8.84
Yuzu	67.30	54.64	3.49	10.65

* For each fruit † yield (%) of oil was for 150 g flavedo by weight.

Ascorbic acid was determined by titration with a coloured oxidation / reduction indicator, 2,6- dichloro-indophenol.

Juice extraction technique

The samples were passed through a PDMS membranes (Poly dimethyl silaxane) in the plate and frame geometry with a total area of 0.257 m², namely pervap 1060 which contains in corporated silicates to isolate flavour compounds (table 4). Extraction was done in triplicate. The flavour compounds were eluted successively with 50 mL From pentane dichloromethane (2:1, v/v). The eluate was dried over anhydrous sodium sulfate. A volume of 1 µL of the pervaporated sample was injected in to GC-MS for analysis.

GC and GC-MS

An Agilent 6890 N gas chromatograph equipped with a DB-5 (30 m × 0.25 mm i.d ; film thickness = 0.25 µm) fused silica capillary column (J & W Scientific) and a flame ionization detector (FID) was used. The column temperature was programmed from 50 °C (2min) to 188 °C (20 min) at a program rate of 3 °C/ min. The injector and detector temperatures were 220 °C. Helium was the carrier gas at a flow rate of 0.8 ml/min and a linear velocity of 22 cm/s. The linear retention indices (LRIs) were calculated for all volatile components using a homologous series of n-alkanes (C9-C16) under the same GC conditions. The weight percent of each peak was calculated according to the correlation factor to the FID. Gas chromatography combined mass spectrometry was used for identifying the volatile components that had been detected. The analysis was carried out with a Varian Saturn 2000R. 3800 GC (wainut Creek, CA) linked with a varian Saturn 2000R MS.

The oven condition, injector and detector temperatures, and column (DB-5) were the same as those given above for the Agilent 6890 N GC. Helium was the carrier gas at a flow rate of 1.1 mL/min and a linear velocity of 38.7 cm/s.

Identification of Components

Components were identified by comparing their LRIs and matching their mass spectra with those of reference compounds in the data system of the Wiley library and NIST Mass Spectral Search program (Chem SW. Inc ; NIST 98 version database) connected to a Varian Saturn

Table 4: Characteristics of membrane.

System	Binary, ternary and multicomponent solutions (Me, Et, Pr, EtAc, EtBu) industrial condensate from orange juice concentration
Membrane	PDMS-1060 ^c
Module	PF
Operating range	0.002 ≤ c ≤ 13.65% ^b 5 ≤ T ≤ 45°C P _r < 10 mmHg

e) General electric Co, b) Permeant-dependent value

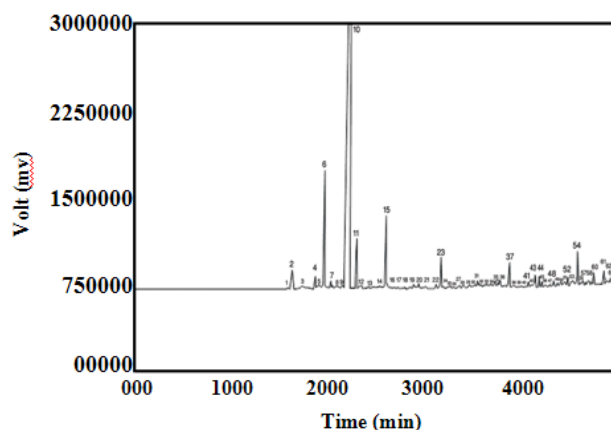


Fig. 1: HRGC chromatogram of page mandarin green peel oil.

2000R MS. The identification GC-MS was also based on the Match of the Most abundant peaks with published data [11,12].

RESULTS AND DISCUSSION

Composition of the Page mandarin juice

The chemical composition of the Page mandarin juice is given in table 5. The juice yield was from 25 % (Trifoliolate orange) to 39 % (Citrumelo Swingle and Cleopatra mandarin). The content of total acidity was from 0.61 % (Bakraei) to 0.74 % (sour Orange), and Brix (total soluble solids) was from 9.5 % (Bakraei) to 11.2 % (Citrumelo Swingle) (table 5).

Flavour compounds of the Page mandarin green peel

GC-MS analysis of the flavour compounds extracted from Page mandarin green peel by using cold-press allowed identification of sixty-eight volatile components (table 6): twelve aldehydes, thirteen alcohols, five esters, three ketones, fourteen monoterpenes, twenty sesquiterpenes, and one acid. Several aldehydes and alcohols are known to contribute to the desirable flavour of Page mandarin green peel [13] (Fig. 1).

Table 5: Composition of page mandarin juice on various rootstocks.

Rootstock	*Fruit weight (g)	Juice (%)	TSS (%)	Acids (%)	TSS/Acid rate	Ascorbic acid (mg) %
Bakraei (Rootstock)	908	33	9.5	0.61	15.57	63.53
Changsha (Rootstock)	980	31	10.4	0.69	15.07	63.35
Citrumelo 'Swingle' (Rootstock)	916	39	11.2	0.72	15.55	65.2
Cleopatra Mandarin (Rootstock)	896	39	10.8	0.72	15.08	62.72
Sour orange (Rootstock)	898	38	10.9	0.74	14.82	60.37
Trifoliolate orange (Rootstock)	944	25	10.2	0.73	14.72	59.02
Troyer citrange (Rootstock)	766	32	10.04	0.71	14.14	58.03
Yuzu (Rootstock)	868	35	10.9	0.72	15.15	63.62

*For ten fruit.

Table 6: Retention indices on mega 5 MS for the components identified in page mandarin green peel oils.

Component		Mega 5 Ms		Component		Mega 5 Ms
1	α - thujene	928	36	(E) 2,4-decadieneal		1322
2	α - Pinene	935	37	δ - elemene		1344
3	camphene	951	38	1-P-menthen-8-yl acetate		1353
4	sabinene	975	39	cis-carvyl acetate		1365
5	β - Pinene	979	40	β - patchulene		1377
6	myrcene	991	41	α - copaene		1385
7	octanal	1003	42	5-dodecen-1-al		1392
8	α - phellandrene	1006	43	β - elemene		1399
9	δ - 3 - carene	1012	44	dodecanal		1409
10	limonene	1036	45	limonene-10-yl acetate		1414
11	(Z) β - ocimene	1049	46	P-Menth-1-en-9-yl acetate		1425
12	γ - terpinene	1061	47	(Z)- β -caryophyllene		1431
13	1,8 - cineole	1070	48	γ - elemene		1440
14	terpinolene	1091	49	α - guaiene		1447
15	linalool	1100	50	aromadendrene		1456
16	nonanal	1109	51	(Z)- β - farnesene		1458
17	P-Mentha - trans - 2,8 - dien-1-ol	1124	52	α - humulene		1466
18	cis - limonene oxide	1137	53	α - amorphene		1486
19	trans-limonene oxide	1141	54	germacrene D		1493
20	citronellal	1154	55	bicyclogermacrene		1499
21	terpinen-4-ol	1182	56	valencene		1504
22	α - terpineol	1195	57	α - muurolene		1508
23	decanal	1205	58	δ - guaiene		1516
24	3-cyclohexene - 1 acetaldehyde	1223	59	γ - cadinene		1527
25	citronellol	1229	60	δ - cadinene		1532
26	cis-carveol	1236	61	elemol		1559
27	neral	1244	62	(E) - nerolidol		1567
28	carvone	1250	63	germacrene B		1572
29	linalyl acetate	1255	64	germacrene D - 4 - ol		1588
30	geranial	1275	65	1,2 - benzendicarboxylic acide		1601
31	3,5,5 - trimethyl cyclohex-2-en-1-one	1278	66	13-tetradecen-2-yn-1-ol		1634
32	perillaldehyde	1282	67	tetrahydroxy cyclopentadienone		1641
33	perillalcohol	1296	68	alloaromadendren		1650
34	P-Menth-1-en-9-ol	1301				
35	undecanal	1307				

Table 7: Retention indices on mega 5 MS for the components identified in page mandarin red peel.

Component	Mega 5 Ms	Component	Mega 5 Ms
1) α - Pinene	935	22) 1-P-menthen-8-yl acetate	1353
2) sabinene	975	23) α - copaene	1385
3) myrcene	991	24) 5- dodecen-1-al	1392
4) octanal	1003	25) β - elemene	1399
5) α - phellandrene	1006	26) dodecanal	1409
6) δ - 3 – carene	1012	27) limonene-10-yl acetate	1414
7) limonene	1036	28) P-Menth-1-en-9-yl acetate	1425
8) (Z) β - ocimene	1049	29) (Z)- β - caryophyllene	1431
9) 1,8 – cineole	1070	30) γ - elemene	1440
10) terpinolene	1091	31) (Z)- β - farnesene	1458
11) linalool	1100	32) α – humulene	1466
12) trans-limonene oxide	1141	33) germacrene D	1493
13) citronellal	1154	34) valencene	1504
14) α - terpineol	1195	35) α - muurolene	1508
15) decanal	1205	36) δ - guaiene	1516
16) 3-cyclohexene – 1 acetaldehyde	1223	37) δ - cadinene	1532
17) carvone	1250	38) elemol	1559
18) perillaldehyde	1282	39) (E) – nerolidol	1567
19) perillalcohol	1296	40) germacrene B	1572
20) P-Menth-1-en-9-ol	1301	41) germacrene D – 4 – ol	1588
21) δ - elemene	1344	42) 1,2 – benzenedicarboxylic acide	1601

Flavour compounds of the page mandarin red peel

GC-MS analysis of the flavour compounds extracted from Page mandarin red peel by using cold-press allowed identification of forty-two volatile components (table 7) seven aldehydes, eight alcohols, three esters, one ketone, nine monoterpenes, thirteen sesquiterpenes, and one acid. Several aldehydes, and alcohols are known to contribute to the desirable flavour of Page mandarin red peel [13].

Flavour compounds of the page mandarin juice

GC-MS analysis of the flavour compounds extracted from Page mandarin juice by using PDMS membranes

allowed identification of seventy-five volatile components (table 8) fourteen aldehydes, fourteen alcohols, seven esters, three ketones, fifteen monoterpenes, twenty-one sesquiterpenes and one acid. Several aldehydes and alcohols are known to contribute to the desirable flavour of Page mandarin juice, [14,15,16].

Aldehydes

Aldehydes are formed from linoleic and linolenic acids via the lipoxygenase pathway and they are important to the flavor of citrus. They also play a less significant but important role in fruit flavours [17].

Table 8: Retention indices on mega 5 MS for the components identified in page mandarin juice.

Component	Mega 5 Ms	Component	Mega 5 Ms
1) α - thujene	928	39) (E) 2,4-decadienal	1319
2) α - Pinene	937	40) δ - elemene	1344
3) camphene	951	41) 1-P-menthen-8-yl acetate	1353
4) sabinene	978	42) Neryl acetate	1356
5) myrcene	993	43) cis-carvyl acetate	1365
6) octanal	1002	44) β - patchulene	1377
7) α - phellandrene	1007	45) α - copaene	1385
8) δ - 3 - carene	1013	46) 5-dodecen-1-al	1390
9)limonene	1040	47) 7-dodecen-1-al	1395
10)(Z) β -ocimene	1053	48) β - elemene	1398
11) cis- β - ocimene	1055	49) dodecanal	1409
12) γ - terpinene	1059	50) limonene-10-yl acetate	1414
13) 1,8-cineole	1075	51) P-Menth-1-en-9-yl acetate	1424
14) terpinolene	1093	52) (Z)- β -caryophyllene	1431
15) linalool	1105	53) γ -elemene	1440
16) P-Mentha - trans - 2,8 - dien-1-ol	1127	54) α - guaiene	1447
17) Allo ocimene	1130	55) aromadendrene	1450
18) cis - limonene oxide	1138	56) (Z)- β -farnesene	1457
19) trans-limonene oxide	1143	57) α - humulene	1467
20) citronellal	1155	58) α - amorphene	1486
21) P-mentha - 1 , 5 - dien - 8 - ol	1172	59) Germacrene D	1493
22) terpinen-4-ol	1182	60) bicyclogermacrene	1499
23) α - terpineol	1195	61) valencene	1504
24) decanal	1207	62) α - muurolene	1509
25) Octyl acetate	1210	63) δ - guaiene	1516
26) 3-cyclohexene-1 acetaldehyde	1224	64) E,E- α -Farnesene	1523
27) citronellol	1228	65) γ -cadinene	1527
28) cis-carveol	1236	66) δ -cadinene	1532
29) neral	1244	67) elemol	1559
30) carvone	1250	68) (E) - nerolidol	1566
31) linalyl acetate	1255	69) Germacrene B	1571
32) (E)-2-decanal	1263	70) germacrene D - 4 - ol	1585
33) geranial	1272	71) 1,2 - benzendicarboxylic acide	1597
34) 3,5,5 - trimethyl cyclohex-2-en-1-one	1278	72) tetradecanal	1612
35) perillaldehyde	1281	73) 13-tetradecen-2-yn-1-ol	1634
36) perillalcohol	1295	74) tetra hydroxycyclopentadienone	1641
37) P-Menth-1-en-9-ol	1300	75) alloaromadendren	1649
38) undecanal	1307		

Recent investigations which have been focused on the identification of saturated and unsaturated aldehydes in mandarin and tangerine peels, have led to the identification of 29 aldehydes (mainly unsaturated ones) and several other compounds [13]. Fifteen aldehyde components identified in this analysis were octanal, nonanal, citronellal, decanal, 3-cyclohexen-1-acetaldehyde, neral, geranial, perill aldehyde, undecanal, (E), 2,4-decadieneal, 5-dodecen-1-al, 7-dodecen-1-al, dodecanal, (E)-2-decenal and tetradecanal.

It was also suggested that the tangerine - like smell was mainly due to the presence of carbonyl compounds, such as decanal, octanal, geranial, citronellal, and perillaldehyde which were quantified from 0.39 % to 0.85 % (green peel) in this study. These findings were similar to the previous study undertaken by *Buettner et al.* [13]. Tangerine oil is easily distinguished from other citrus oils by its content of various aliphatic aldehydes. Two main aliphatic aldehydes were octanal and decanal. In addition, tangerine oil also contained undecanal [18].

Decanal has a green - fresh aroma, and is considered one of the major contributors to mandarin flavour [13]. Since the aldehyde content of citrus oil is considered one of the more important indicators of high quality, rootstock apparently has a profound influence on Page mandarin oil quality [3]. Among the eight rootstocks examined, Yuzu (table 9), Bakraei and citrumello swingle (table 11) Showed the highest content of aldehydes.

Alcohols

Alcohols are formed from C1 to C20 fatty acids via the Lox pathway [17]. The alcohol is an important contributor to the mandarin flavour [2]. fourteen alcohol components identified in the analysis were 1.8 - cineol, linalool, p-mentha-trans-2,8 dien-1-ol, terpinene -4- ol, α -terpineol, citronellol, cis-carveol, peril alcohol, p-menth-1-en-9-ol, elemol, (E) nerolidol, germacrene D-4-ol, 13-tetradecen-2-yn-1-ol and p-mentha-1,5-dien-8-ol.

The total amount of alcohols was from 0.81 % to 1.87 % (green peel) and linalool was the most abundant. Linalool, the most significant alcohol compound of mandarin peel oil and juice, is recognized as being very important to good mandarin flavor. Linalool has a flowery aroma and its level (in mandarin peel and juice) is important to flavor character [13]. Citrus fruit contain enzymes for conversion of mevalonic acid to isopentenyl

pyrophosphate and dimethylallyl pyrophosphate as well as for the synthesis of linalool and its cyclization to 2.8- menthadiene 1-ol, α -terpineol and D-limonene. Linalool may be a key intermediate in terpenoid metabolism of citrus. [17]. among the eight rootstocks examined, Yuzu and cleopatra showed the highest content of alcohols in green Peel oil.

Esters

Alcohols are then converted to esters using acetyl CoA and alcohol acyltransferase (AAT) [17]. Seven ester components identified in the analysis were octyl acetate, linalyl acetate, 1-p-menthen-8-yl acetate, neryl acetate, cis-carvyl acetate, limonene -10 yl acetate and p-menth -1-en-9-yl acetate. The total amount of esters was from 0.008 % to 0.17 % (green peel) and the limonene -10 yl acetate was the most abundant. Among the eight rootstocks examined, Yuzu and Cleopatra, showed the highest content of esters in green Peel oil.

Ketone

Three ketone compounds identified in the analysis were carvone, 3,5,5-trimethyl cyclohex-2-en-1-one and tetra hydroxy cyclopentadienone. Carvone has mint-like flavor aroma [13]. Among the eight rootstocks examined Yuzu and sour orange showed the highest content of ketones in green Peel oil.

Monoterpenes

The total amount of monoterpene hydrocarbons was from 88.16 % to 97.17 % (green peel). Limonene, myrcene, α - pinene and sabinene were the major components among the monoterpene hydrocarbons of Page mandarin juice and peel oil. Limonene has a weak citrus-like aroma and is considered one of the major contributors to mandarin flavour [13]. Myrcene is the second most abundant monoterpene in Page mandarin juice and peel oil. It has a geranium leaf-like aroma [13]. Among the eight rootstocks examined, Trifoliolate orange had the highest monoterpenes content in green Peel oil.

Sesquiterpenes

The total amount of sesquiterpen hydrocarbons was from 0.8 % to 2.81 % (green peel). Germacren D and δ - elemen were the major components among the sesquiterpen hydrocarbons of Page mandarin juice and peel oil. Among the eight rootstocks examined, Yuzu had the highest sesquiterpenes content in green Peel oil.

Table 10: Statistical analysis of variation in volatile flavour components of page mandarin red peel budded on eight different rootstocks (see materials and methods). Mean is average composition in % over the different rootstocks used with three replicates. St. err. = standard error. F value is accompanied by its significance, indicated by :

NS = not significant, * = significant at P = 0.05, ** = significant at P = 0.01.

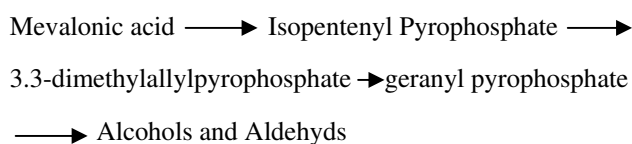
Component	Sour orange		Yuzu		Citrumelo Swingle		Bakraei		Changsha		Cleopatra Mandarin		Troyer Citrange		Trifoliolate Orange		F value
	X	St. err.	X	St. err.	X	St. err.	X	St. err.	X	St. err.	X	St. err.	X	St. err.	X	St. err.	
Oxygenated compounds																	
a) Aldehyds																	
1) octanal	0	0	0.06	0.01	0	0	0	0	0.06	0.005	0	0	0.04	0.005	0	0	
2) citronellal	0.02	0	0.02	0	0.02	0	0.02	0	0.02	0	0.02	0	0.02	0	0.02	0	
3) decanal	0.25	0.005	0.28	0.01	0.34	0	0.30	0.005	0.28	0.02	0.25	0.01	0.28	0.005	0.27	0.005	F**
4) 3-cyclohexen-1- acetaldehyde	0.03	0	0.03	0	0.02	0.005	0.03	0	0.02	0.005	0.02	0	0.02	0	0.02	0	
5) perill aldehyde	0.02	0	0.02	0	0.02	0	0.02	0	0.02	0	0.02	0	0.02	0	0.02	0	
6) 5-dodecen-1-al	0	0	0	0	0	0	0.01	0	0.01	0	0	0	0	0	0	0	
7) dodecanal	0.05	0.005	0.05	0.005	0.06	0	0.05	0.005	0.05	0.01	0.05	0	0.05	0.005	0.05	0.005	
Total	0.37	0.01	0.46	0.02	0.46	0.005	0.43	0.01	0.46	0.04	0.36	0.01	0.43	0.01	0.38	0.01	
b)Alcohols																	
1) 1,8 -cineol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.04	0.02	
2) linalool	0.84	0.01	0.92	0.01	0.86	0	0.97	0.005	0.93	0.05	0.78	0.03	0.84	0.005	0.90	0.01	F**
3) α - terpineol	0.06	0	0.07	0.005	0.08	0	0.08	0	0.07	0.005	0.06	0	0.06	0.005	0.07	0	
4) perill alcohol	0.02	0.005	0	0	0	0	0	0	0.02	0.005	0	0	0.03	0	0	0	
5) p-menth-1-en-9-ol	0	0	0	0	0	0	0	0	0	0	0	0	0.01	0	0	0	
6) elemol	0.06	0.005	0.06	0	0.05	0	0.05	0.005	0.05	0.01	0.04	0.005	0.03	0	0.05	0	
7) (E) nerolidol	0	0	0	0	0	0	0	0	0	0	0.001	0	0	0	0	0	
8) Germacrene D-4 -ol	0.01	0	0.01	0	0.01	0	0.01	0	0.008	0.001	0.01	0	0	0	0.009	0.001	
Total	0.99	0.02	1.06	0.01	1.00	0	1.11	0.01	1.07	0.02	0.89	0.03	0.97	0.01	1.06	0.03	
c) Esters																	
1) 1-p-menthen-8-yl acetate	0.007	0.001	0.008	0.0005	0	0	0.009	0.001	0	0	0.009	0.0005	0	0	0.008	0.001	
2) Limonene-10-yl acetate	0.03	0.005	0.03	0.005	0	0	0.04	0	0.02	0	0.03	0.005	0	0	0.03	0	
3) p-menth-1-en-9-yl acetate	0.01	0	0.009	0.0005	0	0	0	0	0	0	0.009	0.0005	0	0	0	0	
total	0.04	0.006	0.04	0.006	0	0	0.04	0.001	0.02	0	0.04	0.006	0	0	0.03	0.001	
d) ketone																	
1) carvone	0	0	0	0	0	0	0	0	0	0	0	0	0.02	0	0	0	
Monoterpenes																	
1) α -pinene	0.53	0.01	0.52	0.02	0.57	0	0.50	0	0.52	0.02	0.54	0	0.46	0.01	0.55	0.005	F**
2)sabinene	0.28	0.01	0.26	0.01	0.29	0	0.28	0	0.23	0.005	0.25	0.005	0.18	0.005	0.31	0.005	F**
3) myrcene	2.11	0.04	2.12	0.01	2.14	0.005	2.08	0.01	2.08	0.07	2.09	0.005	2.06	0.02	2.11	0.02	NS
4) α - phellandrene	0.03	0.005	0	0	0.04	0	0.04	0	0	0	0.04	0.005	0.04	0	0.04	0.005	
5) δ -3-carene	0.08	0.005	0.04	0.005	0.03	0.005	0.02	0	0.04	0	0.04	0.005	0.02	0	0.05	0	
6) limonene	93.56	0.11	93.48	0.09	93.53	0.01	93.59	0.04	93.65	0.06	93.14	0.53	93.96	0.03	92.84	0.16	F**
7) (Z)- β -ocimene	0.58	0.01	0.63	0.05	0.48	0.005	0.50	0	0.47	0.005	1.48	0	0.71	0.02	1.12	0.15	F**
8) α - terpinolene	0.02	0	0.01	0	0.01	0	0.01	0	0.01	0	0.01	0	0.01	0	0.01	0	
9)trans limonene oxide	0	0	0	0	0	0	0.01	0	0	0	0	0	0	0	0	0	
total	97.19	0.19	97.06	0.18	97.09	0.02	97.03	0.05	97	0.16	97.59	0.55	97.44	0.08	97.03	0.34	
sesquiterpenes																	
1) δ - elemene	0.09	0	0.14	0.01	0.11	0.005	0.10	0.005	0.14	0.02	0.13	0.005	0.12	0.005	0.15	0	F**
2) α -copaene	0.04	0	0.04	0	0.03	0	0.04	0	0.03	0.005	0.03	0	0.02	0	0.03	0	
3) β -elemene	0.08	0.005	0.09	0	0.08	0	0.08	0.005	0.08	0.01	0.07	0.005	0.06	0	0.08	0	
4)(Z)- β -caryophyllene	0.01	0	0.01	0.005	0.01	0	0.01	0	0.01	0	0.02	0	0	0	0.01	0.005	
5) γ -elemene	0.03	0	0.03	0	0.03	0	0.03	0.005	0.02	0.005	0.02	0	0.02	0	0.02	0	
6)(Z)- β -farnesene	0.02	0.01	0.03	0.01	0.02	0.005	0.02	0	0.01	0	0.01	0	0.01	0	0.01	0.005	
7) α -humulene	0.05	0	0.05	0	0.04	0	0.05	0	0.04	0.005	0.04	0	0.03	0.005	0.04	0	
8) Germacrene D	0.13	0.005	0.18	0.01	0.16	0	0.15	0.01	0.17	0.01	0.17	0.01	0.16	0.005	0.18	0.005	F**
9) valencene	0	0	0.008	0.001	0.01	0	0.006	0	0.006	0.0005	0.005	0.002	0.01	0	0	0	
10) α -muurolene	0.03	0.005	0.02	0.005	0.02	0	0.03	0	0.01	0.005	0.02	0	0.01	0.005	0.02	0.005	
11) δ -guaiene	0.01	0.01	0.01	0.005	0	0	0.01	0	0	0	0.01	0	0	0	0.01	0	
12) δ -cadinene	0.07	0.005	0.06	0.005	0.05	0	0.06	0.005	0.06	0.01	0.05	0.005	0.03	0.005	0.06	0	
13) germacrene B	0.01	0.006	0.01	0.004	0	0	0.01	0.006	0	0	0.02	0	0	0	0.02	0.01	
Total	0.57	0.04	0.67	0.05	0.56	0.01	0.59	0.03	0.57	0.07	0.59	0.02	0.47	0.02	0.63	0.03	
Acids																	
1,2-Benzendicarboxylic acide	0.004	0.001	0	0	0.003	0	0.002	0.001			0.01	0	0.001	0.0005	0.002	0.001	

Statistical Analyses

The Duncan's Multiple Range test was used to separate the significant rootstocks. Of the 31 individual oil components analyzed, only 30 showed statistically significant differences due to the influence of individual rootstocks and even here differences were small. These differences on the 1 % level occurred in decanal, Octanal, dodecanal, linalool, elemol, α -pinene, sabinene, myrcene, limonenes, (Z) - β - Farnesene, δ -elemene, β -elemene, α -humulene, Germacren D, δ cadinene and (Z) β -Ocimene. The non affected oil component was myrcen (red peel) and it is provided only for convenience of the reader (tables 9-11).

CONCLUSION

The discovery of geranyl pyrophosphate (GPP), as an intermediate between mevalonic acid and oxygenated compounds (Alcohols and aldehyds), led to a rapid description of the oxygenated compounds biosynthetic pathway. The major pathway of oxygenated compounds biosynthesis in higher plants is as below:



The steps in the pathway are catalyzed by isopentenyl pyrophosphate isomerase and geranyl pyrophosphate synthase, respectively [19]. The pronounced enhancement in the amount of oxygenated compounds, when yuzu was used as the rootstock, showed that either the synthesis of oxygenated compounds is enhanced or activities of both enzymes increased.

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