Progress in Essential Oils

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Orange Oil Chirality

Although α-copaene is present in cold-pressed orange oil in amounts exceeding 0.5%, Takeoka et al. (1990) determined that in orange oil its enantiomeric ratio was: (+)-α-copaene (16%):(-)-α-copaene (84%). Hara et al. (1999) determined that the enantiomeric distributions of some specific constituents of sweet orange oil were as follows:

(1R,5R)-(+)-α-pinene (99.6–99.7%):(1S,5S)-(-)-α-pinene (0.3–0.4%)
(1R,5R)-(+)-β-pinene (66.1–77.8%):(1S,5S)-(-)-β-pinene (22.2–33.9%)
(4R)-(+)-limonene (99.1–99.4%):(4S)-(-)-limonene (0.6–0.9%)
(3S)-(+)-linalool (82.5–96.3%):(3R)-(-)-linalool (3.7–17.5%)
(4R)-(+)-α-terpineol (97.0–98.4%):(4S)-(-)-α-terpineol (1.9–3.0%)
(3R)-(+)-citronellal (31.3–87.8%): (3S)-(-)-citronellal (12.2–68.7%)

The cold-pressed oils of the Japanese 'Hamlin,' 'Salustiana,' 'Valencia,' and 'Washington navel' cultivars of orange oil were analyzed by Mitiku et al. (2001) using chiral GC. The four major monoterpene hydrocarbons were found to have the following enantiomeric distribution:

(4R)-(+)-limonene (99.48–99.54%):(4S)-(-)-limonene (0.46–0.52%)
(1R,5R)-(+)-α-pinene (98.98–99.68%):(1S,5S)-(-)-α-pinene (0.32–1.02%)
(1R,5R)-(+)-sabinene (97.53–100.00%):(1S,5S)-(-)-sabinene (0.00–2.47%)
(1R,5R)-(+)-β-pinene (82.79–100.00%):(1S,5S)-(-)-β-pinene (0.00–17.21%)

Comparative composition of flavonoids in the peel extracts of three cultivars of orange grown in Algeria **T-1**

Flavonoid	'Hamlin'	'Valencia'	'Washington Navel'
hesperidin	0.12[a]	0.15	0.12
narirutin	0.04	0.05	0.04
demethyltangeretin	0.11	0.13	0.14
pentamethoxy flavone	0.38	0.41	0.36
sinesetin	1.40	1.37	0.54
hexamethoxy flavone	0.21	0.19	0.23
nobiletin	1.57	1.61	0.57
o-tetramethyl scutellain	0.16	0.44	0.24
heptamethoxy flavone	0.54	0.63	0.46
tangeretin	0.27	0.32	0.31

[a] g/100g of peel

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Orange Oil Heterocyclic Oxygenated Components

A technique known as over-pressured layer chromatography (OPLC) is a planar chromatographic method that has advantages of TLC and HPLC. In OPLC the procedure uses a high pressure TLC plate as stationary phase. This is held under pressure as the eluent is pumped onto the layer under controlled conditions with the layer covered completely by a flexible membrane. Using an aluminum-backed silica gel 60 F254 high pressure TLC plate with impregnated edges, a solvent system of butyl acetate: hexane (80:20) at a flow rate of 0.7 mL/min and UV detection at lλ= 254 nm and 366 nm. Dugo et al. (1996) determined the polymethoxyflavone content of Italian sweet orange oil. The polymethoxyflavones characterized were tangeretin, heptamethoxyflavone,

tetra-O-methylscutellarein, hexamethoxyflavone, nobiletin, sinesetin, epoxy-bergamottin hydrate and meranzin hydrate, though quantitative data were not reported.

Manthey and Grohmann (1996) determined the heterocyclic oxygenated components in cold-pressed orange oil produced in the United States. The flavonoids characterized were:

sinensetin (399 ppm)
hexa-O-methylquercetagetin + hexa-O-methylgossypetin (3578 ppm)
nobiletin + tetra-O-methylscutellarein (2710 ppm)
3,4,5,6,7,3a,4a-heptamethoxyflavone (4473 ppm)
tangeretin (812 ppm)
5-hydroxy-3,7,8,3a,4a-pentamethoxyflavone (419 ppm)

Del Rio et al. (1998a) determined that the highest level of polymethoxyflavones accumulated during the developments of young citrus fruits. The same authors (1998b) observed that polymethoxyflavones might help the citrus fruit combat various fungal diseases. Del Rio et al. (2004) further studied the effect of hesperidin, isonaringin and the polymethoxyflavones (sinensetin, nobiletin, tangeretin, and heptamethoxyflavone) on resistance to brown rot lesion caused by *Phytophthora citrophthora*. This was demonstrated by the fact that when the citrus fruit ('Valencia late' in this case) were infected with *P. citrophthora* the hesperidin and naringenin contents dropped by 13% and 67%, respectively, while the hesperetin and naringenin (the corresponding aglycones) increased. It was postulated that the fungus partially hydrolyzed the glycosylated flavanones while at the same time the levels of heptamethoxyflavone, nobiletin, sinensetin and tangeretin increased by 48%, 28%, 26% and 24%, respectively. This shows that the fruit possesses some naturally occurring antifungal agents, in particular naringenin and hesperitin.

Ortuno et al. (1999) suggested that the accumulation of polymethoxyflavones may be related to the maturation of citrus fruits.

Bonaccorsi et al. (1999) showed that HPLC of the oxygen heterocyclic compounds in sweet orange oil could be completed in 7 min using the fast HPCL methodology. The constituents characterized were sinensetin, hexamethoxyflavone, nobiletin tetra-O-methylscutellarein and tangeretin. The same results were also published in a follow-up study in which two fast HPLC analyses of heterocyclic oxygenated compounds of orange oil were reported by Bonaccorsi et al. (2000).

The potential mutagenicity of a mixture of citrus polymethoxyflavones such as nobiletin (32.5%), 3,3',4,5,6,7,8-heptamethoxyflavone (25.0%), tangeretin (14.0%), trimethyscutellarein (9.1%), sinensetin (3.9%), 5-demethylnobiletin (2.8%), hexa-O-methylquercetagetin (3.3%), 5-demethyl-tetramethylscutellarein (0.7%), 5-hydroxy- 3,3',4',6,7,8-hexamethoxyflavone (0.7%) and a small quantity of unidentified flavonoids (3.9%) was evaluated by Delaney et al. (2002) using the Ames test (Ames et al. 1975). They found that the mixture was not genotoxic in in vitro assay systems.

Dugo and McHale (2002) summarized the findings on the non-volatile oxygen heterocyclic compounds found in sweet orange oil. The components listed were aurapten (syn. 7-geranoxycoumarin), citropten (syn. 5,7-dimethoxycoumarin), bergaptol (syn. 5-methoxypsoralen), auranetin (syn. 3, 4',6,7,8-pentamethoxyflavone), tangeretin (syn. 4',5,6,7,8-pentamethoxyflavone), nobiletin (syn. 3',4', 5,6,7,8-hexamethoxyflavone), 3,3',4',5,7,8-heptamethoxyflavone, sinesetin (syn. 3',4',5,6,7-pentamethoxyflavone),3,3',4',5,6,7-hexamethoxyflavone, tetra-O-methylscutellarein (4',5,6,7-tetramethoxyflavone), 5,8-dihydroxy-3,3',4',7-tetramethoxyflavone, 3,3',4',5,7,8-hexamethoxyflavone, 4',5,7,8-tetramethoxyflavone, 3',3',5,7,8-pentamethoxyflavone, 5-hydroxy-3,3',4',7,8-pentamethoxyflavone and 5-hydroxy-3,3',4',6,7,8-hexamethoxyflavone.

The flavonoid content of three orange cultivars ('Hamlin,' 'Valencia' and 'Washington Navel') was the subject of study by Hadj-Mahammed and Meklat (2003). The results of this study are presented in **T-1**.

Weber et al. (2006) demonstrated that liquid chromatography combined with ^{13}C-NMR spectroscopy should be used for the unambiguous identification of polymethoxyflavones in orange oil. Li et al. (2006) used liquid chromatography-electrospray ionization mass spectrometry, electron impact high-resolution mass spectrometry, UV spectroscopy and ^{1}H- and ^{13}C-NMR spectroscopy to characterize the hydroxylated polymethoxyflavone and methylated flavones in a peel extract of Floridian cold-pressed orange oil. The compounds characterized in this extract were 5-hydroxy-6,7,4'-trimethoxyflavone, 5-hydroxy-6,7,8,4'-tetramethoxyflavone, 3-hydroxy-5,6,7,4'-tetramethoxyflavone, 3-hydroxy-5,6,7,8,4'-pentamethoxyflavone, 5-hydroxy-3,6,7,8,3',4'-hexamethoxyflavone, 5-hydroxy-3,7,3',4'-tetramethoxyflavone, 5-hydroxy- 3,7,8,3',4'-pentamethoxyflavone, sinesetin, pentamethoxyflavone, 3,5,6,7,3',4'-hexamethoxyflavone, nobiletin (3',4',5,6,7,8-hexamethoxyflavone), 5,6,7,4'-tetramethoxyflavone, 3,5,6,7,8,3',4'-heptamethoxyflavone, tangeretin (4',5,6,7,8-pentamethoxyflavone), 4,5,6,4'-tetramethoxyflavone, 5,6,7,4'-tetramethoxyflavanone,5-hydroxy-6,7,8,3',4'-pentamethoxyflavanone,

Pesticide residues of orange oils (ppm) **T-2**

Pesticide	Italian orange oil		California orange oil	
	Neat	Deterpenated	Neat	Deterpenated
diazonon	0.07	0.03	0.12	0.07
methyl parathion	1.36	0.10	1.76	0.21
parathion	2.10	0.12	-	-
quinalphos	0.82	0.08	-	-
methdiathion	1.59	0.29	20.63	2.00

2'-hydroxy-3,4,4',5',6-pentamethoxychalcone and 2'-hydroxy-3,4,3',4',5',6'-hexamethoxychalcone. The reason for this study by Li et al. (2006) was attributed to the wide spectrum of biological activities associated with polymethoxyflavones.

Finally, Benavente-Garcia et al. (1997) published an interesting review on the uses and biological properties, and in particular the antioxidant activity, of the flavonoids found as minor constituents of citrus oils, including orange oil.

Pesticide residues in Italian sweet orange oil (ppm) **T-3**

Pesticide	Sicilian oils	Calabrian oils
Organophosphorus		
sulphotep	0–0.90	–
dimetoate	0–0.94	0–0.45
diazinon	0–4.28	0–3.12
methyl parathion	0–77.2	0.04–13.40
fenitrothion	0–0.31	–
methyl pirimiphos	0–0.72	0–0.12
malathion	0–1.17	0–2.19
ethyl parathion	0.01–51.40	0.05–32.10
chlorfenvinphos	0–0.10	–
quinalphos	0–11.18	0.01–2.48
methidathion	0–97.20	0.04–5.83
ethyl bromophos	0–12.20	–
ethion	0–0.01	–
methyl azinphos	0–5.95	0–2.01
ethyl azinphos	0–0.03	–
Organchlorine		
p,p-dichlorobenzophenone	0.51–5.48	0–4.01
dicofol	0.28–5.02	0–3.33
tetradiphon	0.14–2.15	0–1.25

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Orange Oil Agrochemical Residues

Leoni and D'Alessandro DeLuca (1978) reported that the organophosphorus pesticide residues found in Italian orange oil were as follows:

- methyl parathion (0–1.91 ppm)
- parathion (0.51–11.52 ppm)
- paraoxon (0.73–15.73 ppm)
- phentoate (0–0.26 ppm)
- biomophos (1.81–2.65 ppm)
- fenitrothion (0–0.71 ppm)

Dugo et al. (1987, 1990 and 1992) and DiBella et al. (1991 and 1995; cited in Dugo and DiBella 2002) determined that the organophosphorus pesticide residues found in Sicilian orange oils produced between 1989–1992 were as follows:

- sulphotep (0–0.90 ppm)
- dimetoate (0–0.94 ppm)
- diazinon (0–4.28 ppm)
- methyl parathion (0–77.20 ppm)
- fenitrothion (0–0.31 ppm)
- methyl pirimiphos (0–0.72 ppm)
- malathion (0–1.17 ppm)
- ethyl parathion (0.01–51.40 ppm)
- clorfenvinphos (0–0.10 ppm)
- quinalphos (0–11.18 ppm)
- methidathion (0–97.20 ppm)
- ethyl bromophos (0–12.0 ppm)
- ethion (0–0.01 ppm)
- methyl aginphos (0–5.95 ppm)

In contrast the organophosphours pesticide residues found in Calabian orange oils between 1989–1992 (cited in Dugo and DiBella 2002) were found to be as follows:

- dimetoate (0–0.45 ppm)
- diazinon (0–3.12 ppm)
- methyl parathion (0.04–13.40 ppm)
- methyl pirimiphos (0–0.12 ppm)
- malathion (0–2.19 ppm)
- ethyl parathion (0.05–32.10 ppm)
- quinalphos (0.01–2.46 ppm)
- methidiathion (0.04–5.83 ppm)
- methyl azinphos (0–2.01 ppm)

Tateo and Crivelli (1994) examined some orange oils for their pesticide residues. The results of this study can be found in **T-2**.

Calvarano and Gazea (1996) determined that the organophosphous pesticide residues found in Italian orange oil were as follows:

dimethoate (0.025–0.063 ppm)
diazonon (0.037–0.046 ppm)
methyl parathion (0.021–0.023 ppm)
methdiathion (0.049–0.054 ppm)
methyl azinphos (0.012–0.031 ppm)

Dugo et al. (1997) determined the organophosphorus and organochlorine pesticide residues in Italian citrus oils among which was sweet orange oil. The results obtained from this study are reported in **T-3**.

Verzera et al. (2004) examined the organophosphorus pesticide residues in Italian orange oils produced from oranges grown under standard conditions and organically grown oranges. The results of these analyses are shown in **T-4**.

DiBella et al. (2006) determined that the organophosphorus pesticide residues in Italian orange oil samples were as follows:

methyl chlorpyriphos (0.02–0.10 mg/L)
methyl parathion (0.02–0.16 mg/L)
ethyl chlorpyriphos (0.03–2.18 mg/L)
ethyl parathion (0.02–0.06 mg/L)
quinalphos (0.01–0.24 mg/L)
methidation (0.03–2.62 mg/L)
methyl azinphos (0.02–0.16 mg/L)

DiBella et al. (2006) also determined the organochlorine pesticide residues in Italian orange oil samples were as follows:

dicofol (2.27–7.92 mg/L)
tetradifon (0.03–1.71 mg/L)

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Orange Oil Contaminants

Cold pressed citrus oils produced in the United States by either the FMC method or the Brown Peel Shaver system use water as the carrier of the peel oil. The process water used is chlorinated to ensure sanitary conditions during idle time. Weiss et al. (2003a) found that limonene chlorohydrin [(1R,2R,4R)-2-chloro-p-menth-8-en-1-ol] was formed from the contact of hypochlorous acid (HOCI) and limonene. The quantity of limonene chlorohydrin (detection threshold: 0.5 ppm) found in a variety of Midseason, Valencia and orange essence oils ranged from 1–140 ppm. The authors determined that if the portable water used were kept in the range 0.5–4 ppm residual chlorine, no chlorohydrins were formed. If levels of residual chlorine were kept higher (30 ppm) as was found in a citrus pilot plant, limonene chlorohydrin would form. In a follow-up study, Weiss et al. (2003b) determined that in addition to (1R,2R,4R)-2-chloro-p-menth-8-en-1-ol, (1S,2S,4R)-2-chloro-p-menth-8-en-1-ol was also formed from the reaction between limonene and hypochlorous acid.

Braddock and Goodrich (2005) determined that through the use of a continuous mixing of chlorohydrins-containing orange oil with 0.5 NKOH for a period of hours, the chlorohydrin levels were reduced below detection limits. Furthermore, it was found that the sensory characteristics of the treated orange oil were not affected.

Phthalates are the largest group of plasticizers that are encountered with all plastic materials. As all citrus oils and orange oils specifically come into contact with a number of plastic materials during processing and storage, it is not unexpected that low levels of phthalates become solubilized in the oils. Because phthalates have been implicated in processing hepatotoxic and other potentially damaging biological activities, it is becoming more important for users of essential oils to monitor the levels of phthalates in their oils. DiBella et al. (1999) examined the phthalate content of orange oil produced in the crop gears in Sicily during the period 1994–1996. They found that the residue concentrations of di-isobutyl phthalate and bis(2-ethylhexyl)phthalate in orange oil were below the level of detection in only 6% and 3% of oil samples analyzed, respectively. The oils were found to possess a range of concentration of phthalates as follows:

di-isobutyl phthalate (0.03–26.0 mg/kg)
bis(2-ethylhexyl)phthalate (0.12–29.9 mg/kg)
dibutyl phthalate (0.14–0.53 mg/kg)

DiBella et al. (2001) determined that phthalate contamination of orange oil resulted from (a) contact of the fresh fruit with fruit transport conveyors, (b) the tubing used for pouring and blending, (c) the tubing used for emulsion recycling and (d)

Organophosphorus pesticide residues found in orange oils produced in Sicily **T-4**

Pesticide	Amount found in ppm	
	Organic oils	Standard oils
methyl parathion	–	0.89–0.97
ethyl parathion	–	0.45–0.53
methidathion	t–0.18	1.15–1.21
chloropyriphos	t–0.09	0.05–0.15

the pump gasket—all used during the processing of fresh fruit to oil.

DiBella et al. (2006) analyzed Italian orange oil samples for their phthalate and adipate ester residues. The residues that were determined were as follows:

diethyl phthalate (0.03–0.38 mg/L)
dibutyl adipate (0.10–0.25 mg/L)
di-isobutyl phthalate (0.11–1.69 mg/L)
dibutyl phthalate (0.01–02.05 mg/L)
bis(2-ethyl hexyl)phthalate (0.10–0.12 mg/L)
di-octyl phthalate (0.05–2.27 mg/L)

Spinosad, which is a mixture of compounds known as spinosyns that are derived from a *Saccharopolyspora spinosa* bacterium, is used as an insect control agent in citrus crops. West and Turner (2000) used a combination of column chromatographic separations followed by revised phase HPLC to determine that the limits of quantitative detection for spinosyn A, B, D, K and N-dimethylspinosyn were 0.001–0.005 mg/g; however, no data was reported on the actual levels that may or many not have been found in commercially available orange oil.

It is well known that organophosphorus insecticides have been found as common contaminants of citrus oils; however, low levels of triaryl phosphates (TAPS) have been found in a number of citrus oils (Saitta et al. 1997). The authors postulated that contamination of citrus oils with TAPS was probably due to the storage of oils in polyvinyl chloride plastic container where TAPS are used as both plasticizers and flame retardants. Saitta et al. (1997) determined that the level of TAPS in Italian orange oil were less than the detection level (20.01 ppm).

DiBella et al. (2001) determined that contaminations of citrus oils with TAPS appeared to have resulted from citrus fruits coming in contact with one type of new conveyer used to transport fruit.

Using derivative potentiometric analysis with both acid treatment and alcohol/acid dissolution the Cadmium (Cd11), Copper (Cu11), Lead (Pb11) and Zinc (Zn11) contents of Italian orange oil were determined by La Pera et al. (2003) to be as follows:

Cd: 7.80–8.96 ng/g
Cu: 78.05–83.06 ng/g
Pb: 70.40–76.53 ng/g
Zn: 1605.30–1640.30 ng/g

La Pera et al. (2005) analyzed the heavy metal and selenium content of blood and blood orange oils of Italian origin using derivative stripping potentiometry. Their results were as follows:

Cu: 77–203 mg/kg
Pb: 65.1–196 mg/kg
Zn: 550–1235 mg/kg
Manganese (Mn11): 123–1401 mg/kg
Nickel (Ni11): 28.2–768 mg/kg
Selenium (SeIV): 8.1–13.6 mg/kg

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