Progress in Essential Oils

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Clementine Oil

According to Verzera et al. (2009), clementine was first discovered as an accidental hybrid in a mandarin plantation, where the trees were grown from seed in the vicinity of Oran, Algeria. It is known botanically as *Citrus clementina* Hort. ex Tan. [syn. *C. reticulata* Blanco (clementine cultivar)]. Because of the high quality of clementines, they have become very popular in Italy in Calabria and Sicily, where the main cultivars are 'Commune,' 'Monreal,' 'Oroval' and 'Nules.'

Five fresh clementines that were purchased from a local market in Spain were peeled, taking care not to injure the fruit segments. The peels were carefully sectioned and immediately frozen in liquid nitrogen, after which the solids were powdered using a blender. Extraction of this powder was performed immediately using methylene chloride (100 g powder and 5 x 100 mL solvent). After removing any traces of water with anhydrous Na_2SO_4, the extracts were combined and concentrated to 100 mL using a rotary film evaporator. The volatile fraction was then isolated using high-vacuum distillation. The volatile fraction was subjected to analysis by Buettner et al. (2003) using GC/MS.

It was also sensorially analyzed using GC-olfactometry. Although the authors did not report any qualitative data, they did characterize α-pinene, (Z)-3-hexenal, myrcene, limonene, octanal, 1-octen-3-one, (Z)-octa-1,5-dien-3-one, nonanal, (Z)-6-nonenal, citronellal, decanal, (E)-2-nonenal, (Z)-4-decenal, linalool, octanol, undecanal, (E,Z)-2,4-nonadienal, (E,E)-2,4-nonadienal, dodecanal, carvone, (E)-2-undecenal, (E,E)-2,4-decadienal, (E)-2-dodecenal, (E)-2-tridecenal, *trans*-4,5-epoxy-(E)-2-decenal, sotolone (3-hydroxy-4.5-dimethyl-2(5H) furanone), wine lactone (3,6-dimethyl-3,4,5,7-detrahydro-3H-1-benzofuran-2-one), β-sinensal, α-sinensal and vanillin as being components of this volatile fraction of clementine peel.

In addition, the authors measured the flavor dilution (FD) factor of the individual components that were characterized. The FD factor is a relative measure of impact effect of the individual component or the odor threshold of the component at its relative concentration. The higher the FD factor, the more important the contribution of the individual component to the overall aroma of the volatile fraction examined.

Buettner et al. found that the most important contributors to the aroma of clementines were (in order of importance) linalool, (E,E)-2,4-decadienal, wine lactone, α-pinene, myrcene, octanal, limonene, (E,E)-2,4-nonadienal, decanal, carvone, (Z)-3-hexenal, (E)-2-nonenal, (E)-2-dodecenal and *trans*-4,5-epoxy-(E)-2-decenal.

Chisholm et al. (2003) removed the outer peels of cleaned and washed clementines of Spanish origin with a zesting tool. The resulting zest was extracted (10 g peel, 100 mL of a 1:1 mixture of pentane and diethyl ether). After filtration and drying over anhydrous Na_2SO_4, the solvent was removed under vacuum using a rotary film evaporator. The authors noted that the quantitative amounts of some selected components were as follows:

limonene (52.3–87.4%)
linalool (0.78–1.04%)
decanal (0.46–0.59%)
neral (<0.01%)
α-sinensal (0.85–1.34%)

The peel oil isolated from the zest was subjected to GC-olfactometry and GC/MS analysis for component identification, combined with retention indices. The non-aldehydic components characterized in the oil were: myrcene, *cis*-linalool oxide (furanoid form), octanol, limonene, 1,8-cineole, furaneol (2,5-dimethyl-4-hydroxy-3(2H)furanone), linalool, citronellol, *cis*-limonene oxide, carvone, a β-ionone isomer, sotolone and neryl acetate. The aldehydes found in the oil were hexanal, heptanal, octanal, nonanal, decanal, undecanal, dodecanal, tridecanal, tetradecanal, pentadecanal, hexadecanal, heptadecanal, octadecanal, (E)-2-hexenal, (E)-2-heptenal, (E)-2-octenal, (E)-2-decenal, (E)-2-undecenal, (E)-2-dodecenal, (E)-2-tridecenal, (E)-2-tetradecenal, (E)-2-hexadecenal, (Z)-4-decenal, (Z)-4-undecenal, (Z)-5-dodecenal, (Z)-6-dodecenal, (Z)-2-dodecenal, (Z)-6-tridecenal, (Z)-5-tetraddecenal, (Z)-6-tetradecenal, (Z)-2-tetradecenal, (E,Z)-2,6-nonadienal, (E,Z)-2,4-decadienal, (E,E)-2,4-decadienal, (E,Z)-2,6-dodecadienal, (E,E)-2,4-dodecadienal, *trans*-4,5-epoxy-(E)-2-nonenal, *trans*-4,5-epoxy-(E)-2-decenal, *trans*-4,5-epoxy-(E)-2-dodecenal, citronellal, neral, geranial, perillaldehyde, β-sinensal and α-sinensal. Finally, the authors determined that α-sinensal, β-sinensal, *trans*-4,5-epoxy-(E)-2-decenal, (E,Z)-2,6-dodecadienal and linalool were found to be dominant in the peel oil aroma of clementine.

Verzera et al.'s (2009) analysis of the cold-pressed peel oil of the 'Nules' clementine cultivar revealed that it contained:

α-pinene (0.6%)
sabinene (0.2%)
myrcene (2.5%)
octanal (0.4%)
α-phellandrene (0.2%)
δ-3-carene (0.1%)
limonene (92.9%)
terpinolene (0.3%)
linalool (0.4%)

- citronellal (0.5%)
- decanal (0.4%)
- geranial (0.1%)
- neryl acetate (0.1%)
- α-copaene (0.1%)
- β-cubebene + β-elemene (0.1%)
- dodecanal (0.1%)
- (E,E)-α-farnesene (0.1%)
- β-bisabolene (0.1%)
- β-sinensal (0.1%)
- α-sinensal (0.2%)

In addition, trace amounts (<0.05%) of α-thujene, α-terpinene, (Z)-β-ocimene, (E)-β-ocimene, *trans*-sabinene hydrate, γ-terpinene, *cis*-sabinene hydrate, octanol, *cis*-limonene oxide, *trans*-limonene oxide, octanol, nonanal, terpinen-4-ol, α-terpineol, octyl acetate, nerol, citronellol, piperitone, neral, undecanal, geraniol, methyl geranate, α-terpinyl acetate, citronellyl acetate, geranyl acetate, β-caryophyllene, *trans*-α-bergamotene, β-gurjunene, (Z)-β-farnesene, β-santalene, α-humulene, germacrene D, bicyclogermacrene, valencene, γ-elemene, tetradecanal, norbornanol, campherenol, α-bisabolol and nootkatone were also characterized in this oil.

An oil of 'Oroval' clementine cultivar was analyzed by Fabroni et al. (2012) using a combination of gas chromatographic techniques. The constituents characterized in this oil were:

- α-pinene (0.3%)
- sabinene (0.1%)
- myrcene (1.7%)
- limonene (94.0%)
- octanal (0.6%)
- decanal (0.5%)
- dodecanal (0.1%)
- octanol (0.1%)
- linalool (0.9%)
- citronellal (0.1%)
- terpinen-4-ol (0.1%)
- α-terpineol (0.1%)

Trace amounts (<0.05%) of α-terpinene, γ-terpinene, citronellol, geranial, decanol, valencene, β-sinensal and α-sinensal were also identified in this oil.

Kirbaşlar et al. (2012) produced both a cold-pressed oil and a supercritical CO_2 extract of the peels of clementines grown in Anatalya (Turkey). The results of the analysis of the oil and extract using GC-FID and GC/MS can be seen in **T-1**. In addition, trace amounts (<0.05%) of α-thujene, camphene, (Z)-β-ocimene, *cis*-sabinene hydrate, nonanal, nonanol, terpinen-4-ol, octyl acetate, citronellol, *cis*-carveol, neral, geranial, perillyl alcohol, *cis*-p-menth-1-en-9-ol, (E,E)-2,4-decadienal, α-terpinyl acetate, citronellyl acetate, *cis*-carvyl acetate, α-humulene, γ-muurolene, germacrene D, valencene, bicyclogermacrene, elemol, dodecanoic acid, tetradecanal and tetradecanol were characterized in both the oil and extract.

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T-1. Percentage composition of the cold-pressed oil and supercritical CO_2 extract of *Citrus clementine* peel

Compound	Oil	Extract
α-pinene	1.3	0.9
sabinene	0.8	1.0
β-pinene	0.1	t
myrcene	4.6	3.8
octanal	0.4	0.3
α-phellandrene	0.1	0.1
δ-3-carene	0.2	0.1
α-terpinene	0.1	t
p-cymene	0.1	t
limonene	88.1	89.3
(E)-β-ocimene	t	0.1
γ-terpinene	0.1	t
octanol	0.1	0.1
terpinolene	0.1	t
linalool	1.0	1.2
trans-p-mentha-2,8-dien-1-ol	0.1	t
cis-limonene oxide	0.1	0.1
trans-limonene oxide	0.1	0.1
citronellal	0.1	0.1
α-terpineol	0.2	0.2
decanal	0.7	0.7
carvone	0.1	0.1
linalyl acetate	t	0.1
perillaldehyde	0.1	0.1
piperitenone	0.1	0.1
neryl acetate	0.1	0.1
α-copaene	0.1	0.1
geranyl acetate	0.1	0.1
β-cubebene	0.1	0.1
β-elemene	0.1	t
dodecanal	0.2	0.1
β-caryophyllene	0.1	t
β-gurjunene	0.1	0.1
(E)-β-farnesene	0.1	0.1
(E)-2-dodecenal	0.1	t
(E,E)-α-farnesene	0.2	0.1
δ-cadinene	0.1	0.1
(E)-nerolidol	0.1	0.1
β-sinensal	0.1	0.1
α-sinensal	0.3	0.6
nootkatone	0.1	0.1

t=trace (<0.05%)

S. Fabroni, G. Ruberto and P. Rapisarda, *Essential oil profiles of new* Citrus *hybrids, a tool for genetic* Citrus *improvement*. J. Essent. Oil Res., **24**, 159–169 (2012).

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Clove Oils

The clove tree is indigenous to the wettest of the Maluku (Moluccas) islands of Ambon, Buru and Ceram, as well as West Papua. Currently, it is cultivated in the Maluku Islands, West Java, and Sulawesi and Sumatra in Indonesia. The other main clove-growing areas outside Indonesia are the Comoro Islands, Madagascar and Tanzania (Zanzibar and Pemba).

The botanical origin of the clove tree, which yields leaf, stem and bud oils, is *Syzygium aromaticum* (L.) Merr. et Perry (syn. *Eugenia aromatica* (L.) Baill., *E. caryophyllus* (Spreng.) Bullock et Harrison). The clove bud is extremely important both as a spice and an integral ingredient of the Indonesian cigarette known as 'kretek.' It is estimated that the world production of clove bud is in excess of 140,000 mt, with a large portion of the Indonesian production being used in the manufacture of kreteks. Because the leaves, stems and buds are all aromatic, they possess oil contents of 2–3%, 5–6% and 14–19%, respectively. It is estimated that the worldwide production of the leaf, stem and bud oils is ca. 3,000 mt, 12 mt and 125 mt, respectively. In addition, the production of clove oleoresin is estimated to be 10–20 mt.

Oils of clove buds and fruits were produced by the Chinese Pharmacopoeia method (type of hydrodistillation) from material purchased from a pharmacy in Changsha (Hunan, China) by Zhao et al. (2006). Analysis of the bud and fruit oils that were produced in 16.2% and 2.4%, respectively, was by GC/MS only. A majority of the correctly identified components characterized in the bud oil were:

methyl salicylate (0.1%)
bornyl acetate (0.1%)
α-cubebene (0.6%)
eugenol (57.1%)
α-copaene (1.0%)
β-caryophyllene (14.4%)
α-humulene (2.3%)
allo-aromadendrene (0.1%)
epi-bicyclosesquiphellandrene (0.2%)
γ-muurolene (0.1%)
germacrene D (0.2%)
α-muurolene (0.1%)
α-farnesene* (0.3%)
cadina-3,9-diene† (1.6%)
eugenyl acetate (17.9%)
caryophyllene oxide (0.3%)
caryophylla-4(12),8(13)-dien-5β-ol (0.2%)
2,3,4-trimethoxyacetophenone† (1.4%)

*correct isomer not identified
†incorrect identification in the bud oil as is a component of the fruit oil

Trace amounts (<0.05%) of limonene, camphor, benzyl acetate, ethyl benzoate, p-cresol, epi-globulol, cedran-8β-ol (doubtful identification), ledol, α-cadinol, benzyl benzoate and 2,4,6-trimethoxyacetophenone were also characterized in the bud oil. In contrast the fruit oil was found to contain:

limonene (0.1%)
camphor (0.3%)
borneol (0.1%)
methyl salicylate (0.1%)
bornyl acetate (0.5%)
α-cubebene (0.7%)
eugenol (22.7%)
α-ylangene (0.1%)

α-copaene (1.7%)
β-elemene (0.1%)
methyl eugenol (0.2%)
α-cedrene (0.1%)
β-caryophyllene (7.2%)
α-humulene (1.3%)
allo-aromadendrene (0.2%)
epi-bicyclosesquiphellandrene (0.5%)
γ-muurolene (0.4%)
α-muurolene (0.4%)
α-farnesene* (0.1%)
cadina-1(10),4-diene (0.5%)
cadina-3,9-diene† (2.9%)
epi-zonarene (0.4%)
caryophyllene oxide (0.5%)
epi-globulol (0.1%)
T-cadinol (0.8%)
α-cadinol (0.5%)
2,3,4-trimethoxyacetophenone (49.6%)
2,4,6-trimethoxyacetophenone (1.6%)

†incorrect identification; *correct isomer not identified

Trace amounts (<0.05%) of 2-nonanone, linalool, 2-nonanol, terpinen-4-ol, α-terpineol, cedran-8β-ol (doubtful identification), ledol and benzyl benzoate were also identified in this clove fruit oil.

Fricke et al. (1995) used chiral GC analysis to separate (+)-β-caryophyllene from (-)-caryophyllene. They used a 25 m 20% heptakis (2,6-di-O-methyl-3-O-pentyl)-β-cyclodextrin in polysiloxane OV 1701 (w/w) column at 100°C and a carrier gas flow of 50 kPa to show that (+)-β-caryophyllene was the exclusive enantiomer found in clove oil.

An oil of clove that was screened for its fungicidal properties by Pawar and Thaker (2007) was an adulterated oil, and therefore the results of their study are meaningless. These authors reported that the clove oil used in this study, which should possess more than 80% eugenol, was found to possess the following major constituents:

benzyl alcohol (34.1%)
eugenol (47.6%)
vanillin (0.9%)
β-caryophyllene (1.0%)
chavibetol† (5.0%)
caryophyllene oxide (1.4%)

†incorrect identification

This composition is included only for completeness of a literature review.

Pal et al. (2008) used GC-FID and GC/MS to analyze a hydrodistilled oil (23% yield) obtained from clove buds collected from Shimla (Himachal Pradesh, India). The components characterized in the oil were:

methyl salicylate (0.1%)
isochavicol* (0.1%)
eugenol (89.5%)
α-copaene (0.2%)
β-caryophyllene (2.0%)
α-humulene (0.2%)
eugenyl acetate (7.0%)
caryophyllene oxide (0.2%)

*correct isomer not identified

Trace amounts (<0.1%) of heptyl acetate, 2-nonanone, benzyl acetate, δ-cadinene and β-copaen-4α-ol were also determined to be components of this oil.

Williams (2008) reported that a commercial sample of clove bud oil contained the following constituents:

chavicol (0.2%)
eugenol (82.9%)
β-caryophyllene (5.0%)
α-humulene (0.6%)
eugenyl acetate (9.0%)
δ-cadinene (0.2%)
caryophyllene oxide (0.1%)

He also reported that trace amounts of methyl salicylate, benzyl benzoate and a calamenene isomer were found in this oil.

The main constituents of a commercially available (in India) clove bud oil was reported by Khan et al. (2009) to be:

geraniol (1.3%)
eugenol (74.3%)
isocaryophyllene (6.0%)
β-caryophyllene (4.9%)
α-humulene (4.1%)
δ-cadinene (7.0%)
caryophyllene oxide (2.4%)

A laboratory prepared steam-distilled oil of clove buds was screened against Varroa destructor, one of the scourges of bees, by Maggi et al. (2010). The oil used in this study was determined to contain the following major components:

eugenol (86.7%)
isocaryophyllene (0.5%)
β-caryophyllene (3.2%)
allo-aromadendrene (1.3%)
α-humulene (0.9%)
eugenyl acetate (1.9%)
caryophyllene oxide (0.4%)

Dried clove buds that were obtained from traditional herb supplies in Chang Mai (Thailand) were subjected to water distillation to produce an oil in 1.50% yield. Analysis of this oil was performed by Sutthanont et al. (2010) using GC/MS only. The components characterized in the oil were:

citronellol (0.7%)
chavicol (0.3%)
eugenol (77.4%)
α-copaene (0.4%)
β-caryophyllene (13.7%)
α-humulene (1.5%)
α-farnesene* (0.2%)
δ-cadinene (0.1%)
calamenene* (0.2%)
eugenyl acetate (4.6%)
β-cadinene (0.8%)
cadina-1,4-diene (0.2%)

*correct isomer not identified

Baruah et al. (2010) reported that a leaf oil of *S. aromaticum* that was produced by hydrodistillation from leaves collected from a spice garden in Diphu (Karbi Anglong District, Assam, India) was found to contain:

1,8-cineole (4.6%)
linalool (0.4%)
eugenol (84.9%)
β-caryophyllene (6.0%)
isoeugenol* (1.5%)
eugenyl acetate (0.4%)
ethyl cinnamate* (0.5%)

*correct isomer not identified

A trace amount (<0.05%) of a methyl cinnamate isomer was also characterized in this leaf oil.

El-Mesallamy et al. (2012) examined the antimicrobial and antioxidant activity of an oil of clove buds that were obtained commercially in Egypt. The oil, which was analyzed by both GC-FID and GC/MS, was found to contain the following major constituents:

methyl salicylate (0.1%)
eugenol (75.8%)
β-caryophyllene (4.2%)
α-humulene (0.1%)
γ-cadinene (0.3%)
eugenyl acetate (18.9%)
caryophyllene oxide (0.3%)

Trace amounts (<0.05%) of chavicol, α-copaene and 2-methoxy-3-(2-propenyl) phenol were also found as components of this oil.

An oil produced in the laboratory from a commercial sample of clove buds (of

T-2. Comparative percentage composition of selected components of clove bud, stem and leaf oils

Compound	Bud oil	Bud oil (cold-pressed)	Stem oil	Leaf oil
α-cubebene	t–0.7	0.1	t–0.5	t–0.2
eugenol	**69.8–84.4**	**46.5**	**76.4–90.0**	**77.0–91.0**
α-copaene	t–0.8	t	t–0.6	t–0.8
methyl eugenol	t–0.1	t–0.1	t–0.1	t–0.1
β-caryophyllene	4.0–19.5	17.1	6.6–12.4	2.5–17.4
α-humulene	0.6–2.1	1.7	0.3–1.5	0.4–2.5
cis-calamenene	t–0.1	t	t–0.1	t–0.3
γ-cadinene	t–0.8	0.3	t–0.1	t–0.4
δ-cadinene	t–0.6	t	t–0.6	t–0.6
eugenyl acetate	**4.5–22.1**	**30.2**	**1.5–8.0**	**0.8–1.6**
caryophyllene oxide	0.1–1.8	0.5	0.1–0.5	0.1–0.9

t=trace (<0.05%)

unknown origin) was analyzed by Fathi et al. (2012) using GC-FID and GC/MS. The composition of this oil was determined to be as follows:

chavicol (0.3%)
thymol (0.3%)
eugenol (48.8%)
β-caryophyllene (16.8%)
α-humulene (2.4%)
(Z,E)-α-farnesene (0.2%)
eugenyl acetate (27.8%)
caryophyllene oxide (1.7%)

Finally, a comparative percentage composition range of the bud, stem and leaf oils can be seen in **T-2**.

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