Essential oils of flowers of *Citrus sinensis* (L.) Osbeck and *Citrus clementina* Hort. Ex Tan. cultivated in Algarve (Portugal)

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Abstract

The essential oils, isolated by hydrodistillation, from the flowers of different cultivars of *Citrus sinensis* (L.) Osbeck (Cs) and *Citrus clementina* Hort. Ex Tan. (Cc) collected at different harvesting times, were analyzed by GC and GC-MS. All the samples studied afforded yellowish oil which yields ranged from 0.05 to 0.08 % (v/w). The monoterpene fraction dominated both oils (66-91%), being the monoterpene hydrocarbons the main components of this fraction (45-69%). Sabinene (31-48%), linalool (15-32%) and limonene (4-10%) dominated both Cs and Cc oils. *trans*-Nerolidol was the major sesquiterpene component, attaining 3-10%. Cluster analysis of the essential oil composition from the twelve samples studied, confirmed a major chemical homogeneity (S_{corr}>0.95) despite the fact of having used different periods of hydrodistillation, different harvesting times and being flowers of two different *Citrus* species.

Keywords: *Citrus sinensis* (L.) Osbeck (Cs), *Citrus clementina* Hort. Ex Tan., Rutaceae, flowers, essential oils, Valencia late, Newhall, Barnfield, Rohde.

Introduction

Citrus is a genus of evergreen, often spiny trees and shrubs. The gland-dotted leaves are alternate and simple, usually with winged stalks and the shallowly cup-shaped, 5-pettaled white fragrant flowers. The fruits in the form of hesperidium, take from five months to about one year to mature. Various species are useful, such as *C. limon* (lemon), *C. medica* (citron), *C. aurantium* (sour orange), *C. paradisi* (grapefruit), *C. sinensis* (sweet orange), *C. reticulata* (mandarin, tangerine) and *C. clementina* (Clementine). Although much research work has been done on the *Citrus* fruit essential oils, much less is found on the *Citrus* flower oils, even though this can be of high relevance due to the importance of the *Citrus* floral aroma in perfumery.

Previous studies on the floral aroma of *C. sinensis* addressed the relationship between the water status of the plant and the terpenoid emission, showing that the total terpenoid carbon emission of a flowering branch was 7.8 fold the emission of a non-flowering branch and that a considerable emission of linalool was linked to the presence of flowers (Hansen and Seufert, 1999). More recently, studies of the floral orange aroma have used solvent extracts, at 25ºC, that showed linalool to be the major component of orange (52%) and tangerine (75%) flowers, followed by sabinene (27% and 11% in orange and tangerine, respectively) (Alissandrakis *et al.* 2003). Sabinene (35%), myrcene (19%), linalool (19%) and *trans*-ocimene (10%) were the main components of the solid-phase micro-extraction (SPME) of the whole flowers of *Citrus deliciosa* (Flamini *et al.* 2003). The petals seemed to contribute largely to this composition since the petals volatiles included all these compounds in high amounts, whereas the other flower parts (gynoecia, stamens and pollen) showed a quite different blend of compounds.

Given the importance of *Citrus* flowers on orange blossom honey production and perfumery and since no previous study was made on their essential oil composition, the aim of this work was the detailed chemical characterization of the floral essential oils of *Citrus* species.

Materials and Methods

Plant material

Collective flower samples of *Citrus sinensis* and *C. clementina* were collected during May 2005 in Tavira and Silves (Algarve), respectively. *Citrus sinensis*, *C. clementina* common names, cultivars, harvesting moments and distillation times are listed in Table 1.

Essential oil isolation procedure

The essential oils of each collective sample were isolated from fresh plant material by

hydrodistillation, for different periods, Table 1, using a Clevenger-type apparatus (Anonymous, 1996).

Gas Chromatography

GC analyses were performed using a Perkin Elmer 8700 gas chromatograph equipped with two FIDs, a data handling system and a vaporizing injector port into which two columns of different polarities were installed: a DB-1 fused-silica column (30m x 0.25mm i.d., film thickness 0.25µm; J & W Scientific Inc., Rancho Cordova, CA, USA) and a DB-17HT fused-silica column (30m x 0.25mm i.d., film thickness 0.15µm; J & W Scientific Inc.). Oven temperature was programmed, 45-175°C, at 3°C/min, subsequently at 15°C/min up to 300°C, and then held isothermal for 10min; injector and detector temperatures, 280°C and 290°C, respectively; carrier gas, hydrogen, adjusted to a linear velocity of 30cm/s.

Samples were injected using the split sampling technique, ratio 1:50. The percentage composition of the oils was computed by the normalization method from the GC peak areas, which were calculated as mean values of two injections of each oil sample, without using response factors.

Gas Chromatography-Mass Spectrometry

The GC-MS unit consisted on a Perkin Elmer Autosystem XL gas chromatograph, equipped with DB-1 fused-silica column (30mx0.25mm i.d., film thickness 0.25µm; J & W Scientific, Inc.), and interfaced with Perkin-Elmer Turbomass mass spectrometer (software version 4.1). Injector and oven temperatures were as above; transfer line temperature, 280°C; ion trap temperature, 220°C; carrier gas, helium, adjusted to a linear velocity of 30cm/s; split ratio, 1:40; ionization energy, 70eV; ionization current, 60µA; scan range, 40-300u; scan time, 1s.

The identity of the components was assigned by comparison of their retention indices, relative to C_8-C_{25} *n*-alkanes, and GC-MS data with corresponding data of components of reference oils, laboratory-synthesized components and commercially available standards from a home-made library.

Statistical analysis

The percentage composition of the essential oil samples was used to determine the relationship between the different samples of *Citrus* by cluster analysis using the NTSYS-pc software (version 2.02, Exeter Software, Setauket, New York) (Rohlf 1998). Correlation coefficient was selected as a measure of similarity among the twelve accessions, and the unweighted pair-group method with arithmetic average (UPGMA) was used for cluster definition. The degree of correlation was evaluated according to Pestana and Gageiro (2000) as very high if correlation ranged between 0.9 and 1, high between 0.7 and 0.89, moderate between 0.4 and 0.69, low between 0.2 and 0.3, and very low if <0.2.

Results and Discussion

All the flower oils studied from *Citrus sinensis* (L.) Osbeck (Cs) and *Citrus clementina* Hort. Ex Tan. (Cc), Table 1, afforded a yellowish oil in a yield ranging from 0.05 to 0.08% (v/w). The identified oil components are listed in Table 2 in order of their elution on the DB-1 column.

The monoterpene fraction dominated both the Cs and Cc oils (66-89% and 85-91%, respectively), the monoterpene hydrocarbons being the main components of this fraction (45-66% for Cs and 50-69% for Cc oils). Both Cs and Cc oils were dominated by sabinene (31-41% and 35-48%, respectively), linalool (15-32% and 17-29%, respectively) and limonene (4-10% and 6-10%, respectively). *trans*-Nerolidol was the major sesquiterpene component, attaining 3-10% in Cs and 4-7% in Cc oils.

Although the cluster analysis of the oils showed four groups, Fig. 1, they all revealed a very high correlation coefficient varying between 0.95 and 1. Two groups of samples showed a very high

degree of correlation in the oil composition (S_{corr}>0.99). One group was formed by six of the twelve oil samples studied (CsV3h, CsB1h, CsR1h, Cc1, Cc4 and CsR3h), Fig.1, being dominated by sabinene (31-41%), linalool (20-23%), limonene (6-10%) and *trans*-nerolidol (4-9%). The group of CsN3h, CsB3h and Cc3 oils (S_{corr}>0.99) showed slightly higher amounts of sabinene (39-48%), similar of limonene (9-10%) and lower of linalool (15-17%) and *trans*-nerolidol (3-4%). A third group was composed of just one oil, CsV1h, with a degree of correlation of 0.96 with the former two groups, showed an intermediate composition with the two previous groups and the highest amount of *trans*-nerolidol (10%). CsN1h and Cc2 oils, although showing a very high correlation between themselves (S_{corr}>0.99) were the less correlated with the remaining oils (S_{corr}=0.95). Even though sabinene was still the main component in these oils (32-35%), they showed the highest linalool content (29-32%), limonene (5-5%) and *trans*-nerolidol (6-7%) occurring in relative amounts similar to the first group.

As shown by the very high correlation coefficient of the isolated oils, although different periods of hydrodistillation were used and flowers of *C. sinensis* and *C. clementina* were studied, no particular correlation was found grouping the different flower species, different harvesting times, or the hydrodistillation times.

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Table 1. Scientific and common names of the studied species, their cultivars and distillation times.

Scientific / Common name	Cultivar / Observations	Distillation time (h)	Abbreviation
Citrus sinensis (L.) Osbeck	Valencia late	1	CsV1h
Sweet orange	Valencia late	3	CsV3h
	Newhall	1	CsN1h
	Newhall	3	CsN3h
	Barnfield	1	CsB1h
	Barnfield	3	CsB3h
	Rohde	1	CsR1h
	Rohde	3	CsR3h
Citrus clementina Hort. Ex Tan.		2	Cc1
Clementine	Collected at sequential weeks	2	Cc2
	of the same month	2	Cc3
		2	Cc4

Table 2. Composition of the essential oils, isolated by hydrodistillation, from the flowers of *Citrus sinensis* (L.) Osbeck and *Citrus clementina* Hort. Ex Tan. For abbreviations, see Table 1.

***Citrus* species**		***Citrus sinensis***								***Citrus clementina***			
Cultivars		**Valencia late**		**Newhall**		**Barnfield**		**Rohde**					
Distillation time		**1h**	**3h**	**1h**	**3h**	**1h**	**3h**	**1h**	**3h**	**2h**			
Components	**RI**	**CsV1h**	**CsV3h**	**CsN1h**	**CsN3h**	**CsB1h**	**CsB3h**	**CsR1h**	**CsR3h**	**Cc1**	**Cc2**	**Cc3**	**Cc4**
α-Thujene	924	t	t	t	t	t	t	t	t	t	t	t	t
Benzaldehyde	927	0.3	0.3	0.1	0.3	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.2
α-Pinene	930	0.9	1.2	0.6	1.4	1.0	1.3	0.9	1.0	1.2	0.8	1.2	0.9
Sabinene	958	**33.0**	**38.1**	**32.1**	**39.4**	**41.4**	**39.2**	**38.7**	**31.4**	**40.7**	**34.8**	**47.9**	**40.3**
β-Pinene	963	2.6	2.1	1.7	2.1	1.9	2.0	1.9	1.7	2.1	1.8	2.2	2.0
Myrcene	975	2.1	2.9	2.4	3.5	3.5	3.7	3.2	3.2	2.7	2.4	3.4	3.2
δ-3-Carene	1000	0.1	0.2	0.4	0.7	0.2	0.5	0.2	0.4	0.4	0.2	0.3	0.3
Benzene acetaldehyde	1002	t	0.1	0.1	0.3	0.3	0.6	0.2	0.3	0.1	0.1	0.1	t
p-Cymene	1003	1.6	1.9	2.0	2.0	1.5	1.5	1.5	2.6	1.7	2.3	2.4	2.0
β-Phellandrene	1005	0.3	0.4	0.4	0.5	0.5	0.6	0.5	0.5	0.7	0.5	0.5	0.6
Limonene	1009	**4.0**	**5.7**	**5.2**	**10.0**	**6.8**	**9.7**	**6.6**	**8.7**	**9.6**	**6.2**	**8.5**	**8.2**
cis-β-Ocimene	1017	t	0.1	0.2	0.2	0.1	0.2	0.1	0.1	0.1	t	0.1	t
trans-β-Ocimene	1027	t	2.3	1.0	4.6	2.4	4.8	1.9	3.5	1.1	0.5	1.4	1.0
γ-Terpinene	1035	0.3	0.8	0.2	1.3	1.2	1.6	1.0	1.3	0.2	0.1	0.6	0.4
trans-Sabinene hydrate	1037	0.4	0.2	0.2	0.3	0.3	0.4	0.2	0.3	0.2	0.3	0.3	0.1
n-Octanol	1045	t	t	0.1	t	t	t	t	t	t	0.1	t	0.1
Terpinolene	1064	t	0.2	0.1	0.5	0.4	0.6	0.3	0.4	0.1	0.2	0.2	0.2
cis-Sabinene hydrate	1066	t	0.2	0.2	0.2	0.2	0.3	0.2	0.3	0.1	0.2	0.2	0.1
Linalool	1074	**18.0**	**20.2**	**31.8**	**15.0**	**22.5**	**16.4**	**21.8**	**20.5**	**22.7**	**28.9**	**16.8**	**22.4**
trans-p-2-menthen-1-ol	1095	t	0.3	0.3	0.3	0.2	0.4	0.2	0.1	0.2	0.3	0.2	0.3
cis-p-2-menthen-1-ol	1110	t	0.3	0.1	0.2	0.1	0.3	0.3	0.4	0.3	0.3	0.2	0.1
Terpinen-4-ol	1148	2.3	3.8	3.4	4.2	3.4	4.4	3.7	5.6	3.0	3.9	3.5	3.8
α-Terpineol	1159	t	0.7	0.8	0.7	0.5	0.8	0.7	1.0	0.5	0.9	0.6	0.7
α-Ethyldiene benzene acetaldehyde*	1235	t	0.2	0.1	0.1	0.2	0.2	0.1	0.3	t	0.1	t	0.1
Geraniol	1236	t	t	t	t	t	t	t	t	t	t	t	t

Indole	1255	0.1	0.4	t	0.5	0.2	0.1	0.5	0.1	0.2	0.2	0.1	0.1
Methyl anthranilate	1300	t	0.3	0.5	0.3	0.3	0.2	0.3	0.3	0.4	0.3	0.1	t
trans-Jasmone*	1368	t	0.2	t	t	0.1	t	t	0.1	0.1	t	t	t
Ethyl anthranilate*	1376	1.0	0.1	t	0.1	t	0.1	0.2	0.1	0.1	0.1	t	t
β-Elemene	1388	1.0	0.8	t	0.6	0.3	0.7	0.5	0.7	0.2	0.3	0.2	0.7
β-Caryophyllene	1414	4.9	0.6	t	0.3	0.3	0.4	0.3	0.5	0.2	0.4	0.6	1.3
Geranyl acetone	1434	t	0.1	t	t	t	t	t	t	0.1	t	t	t
α-Humulene	1447	t	0.2	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.3	t	t
trans-β-Farnesene	1455	2.8	0.6	0.4	0.3	0.3	0.3	0.3	0.4	t	0.1	0.4	t
α-*trans*,*trans*-Farnesene	1500	t	0.2	0.5	0.1	0.3	0.1	0.2	0.2	0.2	t	t	t
***trans*-Nerolidol**	1549	**10.0**	**8.6**	**5.8**	**3.6**	**3.8**	**2.9**	**5.4**	**5.4**	**4.2**	**7.1**	**3.5**	**4.4**
β-Caryophyllene oxide	1561	3.9	0.2	t	0.1	t	0.1	t	0.1	0.3	0.5	0.5	0.9
Tetradecanal	1596	t	0.3	t	0.1	t	0.4	t	0.1	0.1	t	t	t
Heptadecene	1668	t	0.5	0.9	0.3	0.9	0.5	1.5	1.2	0.2	0.6	0.4	0.5
Pentadecanal	1688	t	0.2	t	0.1	t	t	t	0.1	t	0.1	t	t
trans,*cis*-Farnesol	1693	t	1.9	4.6	1.5	2.0	1.2	3.8	2.7	1.3	1.7	1.1	0.7
% Identification		89.6	97.4	96.3	95.8	97.4	96.8	97.5	96.0	95.7	96.8	97.8	95.6
Grouped components													
Monoterpene hydrocarbons		44.9	55.9	46.3	66.2	60.9	65.7	56.8	54.8	60.6	49.8	68.7	59.1
Oxygen containing monoterpenes		20.8	26.4	36.8	21.4	27.5	23.1	27.6	28.4	27.4	35.0	21.9	27.6
Sesquiterpene hydrocarbons		8.7	2.2	0.5	1.3	1.0	1.5	1.2	1.8	0.6	1.1	1.2	2.0
Oxygen containing sesquiterpenes		13.9	10.9	10.9	5.3	6.1	4.3	9.4	8.4	6.0	9.3	5.1	6.0
Others		1.3	2.0	1.8	1.6	1.9	2.2	2.5	2.6	1.1	1.6	0.9	0.9

RI = Retention index relative to C_8-C_{17} *n*-alkanes on the DB-1 column

t = trace (<0.05 %)

* Identification based on mass spectra only

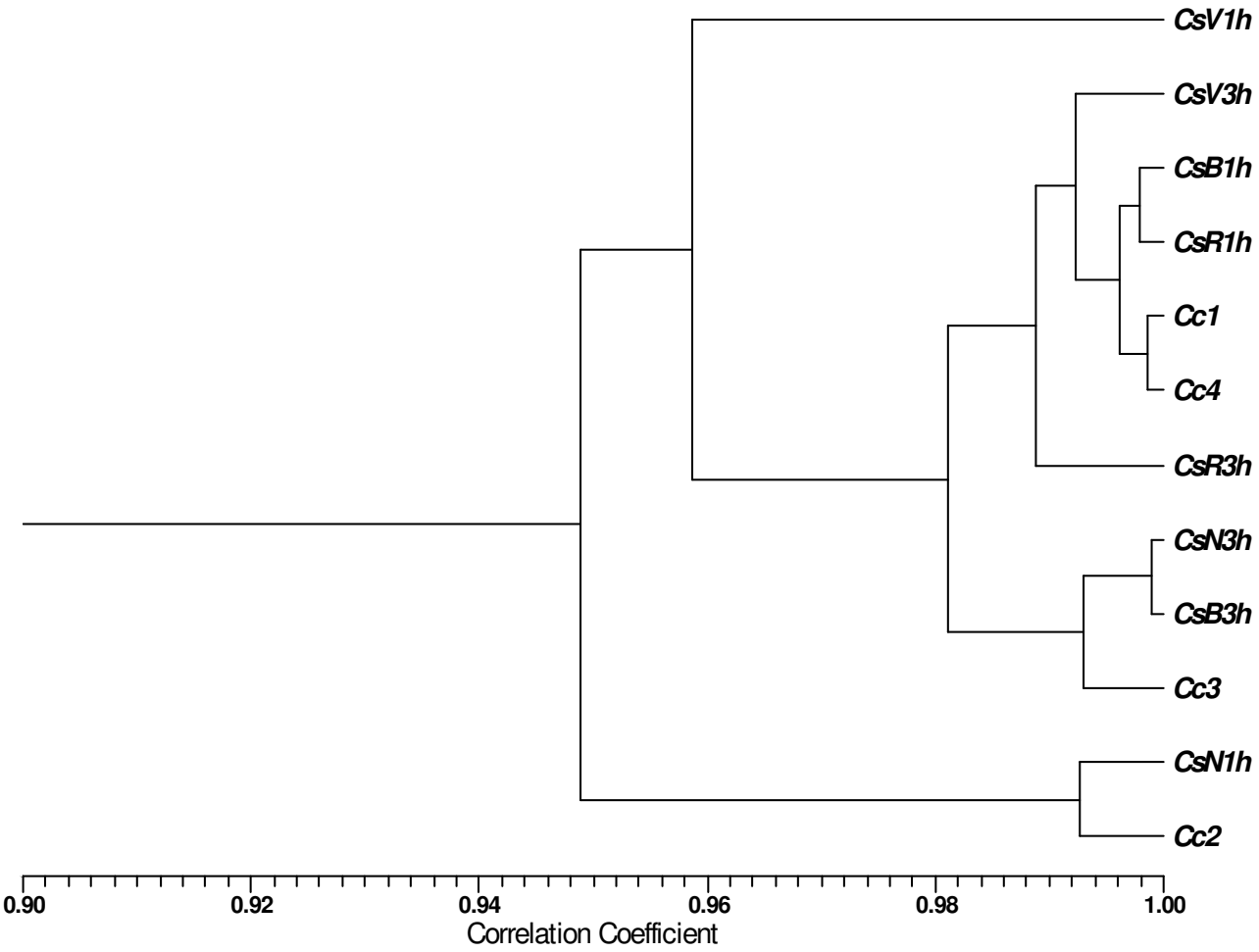

Figure 1. Dendrogram obtained by cluster analysis of the percentage composition of essential oils from *Citrus sinensis* (Cs) and *Citrus clementina* (Cc) samples examined, based on correlation and using the unweighted pair-group method with arithmetic average (UPGMA). For abbreviations, see Table 1.