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Analytical Methods

Volatile composition of hybrids *Citrus* juices by headspace solid-phase micro extraction/gas chromatography/mass spectrometry

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ABSTRACT

The volatile compounds of *Citrus* juices have been extracted by headspace solid-phase microextraction (HS-SPME) and analysed by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS). This work deals with the analysis of 65 cross pollinated hybrid fruits and their parents: mandarin (*Citrus reticulata Blanco var. Willow Leaf*) and clementine (*Citrus reticulata* × *Citrus sinensis* var. *Commune*). Among the 44 components identified which accounted for 90.2 to 99.8% of the volatile fraction, limonene (56.8–93.3%) and γ-terpinene (0.1–36.4%) were the major components in all samples. The clementine juice was characterised by the pre-eminence of limonene (90.0%) and a minor amount of γ-terpinene (1.2%) while the mandarin juice exhibited high amount of limonene (66.3%) and γ-terpinene (21.1%). All hybrid juices showed qualitatively similar composition but differing in the quantitative profile of the couple limonene/γ-terpinene. The principal component analysis (PCA) and the discriminant analysis indicated that hybrids samples were symmetrically distributed around the both parents. If some studies were found in the literature about mandarin juice, to our knowledge, this work is the first study on the volatile compounds of clementine juice and a large amount of hybrids.

1. Introduction

The worldwide production of *Citrus* fruits was estimated at over 95 millions tons in 2006, with more than half of this being oranges (FAO, 2006). In Corsica, clementine fruits have a seal called "Indication Géographique Protégée" (IGP) and their production reaches 99% of the French production (over 20,000 tons per year) (La clémentine Corse, 2007). Mandarins (*Citrus reticulata blanco*) and especially clementines (*Citrus reticulata* × *Citrus sinensis*) considered as a variety of mandarin (Swingle & Reece, 1967) or as a specie on its own (Tanaka, 1961) called *Citrus clementina Hort. Ex Tan.*, are become very famous in Europe and have of great importance in the fresh fruit market (Verzera, Trozzi, Modello, Dellacassa, & Lorenzo, 1998). The mandarin and clementine productions are estimated at about 15.2 millions tons in the world (FAO, 2006). A constant progression in the demand of small fruits obliged the farmers to increase the production of them. The strategy selected is the sexual hybridisation between sire diploid and tetraploid. Since 20 years, the research center in agronomy of the French group INRA-CIRAD of San Giuliano (Corsica, France) develops a hybrid between mandarin and clementine trees. The aim of the hybridisation is twofold: (i) increase the resistance of trees against diseases and climatic conditions, (ii) produce fruits with flavors in adequacy with the consumer's taste and fresh juices which preserve the organoleptic properties of the fruits. The main characteristics of fresh fruit are the odor and the taste (sugar and acidity). Fruit juices are the product of processed fruits which commercialisation requires simple and rapid analytical procedures. The analysis allowed the identification and the determination of abundance flavors and off-flavors which affect directly the sensorial quality of these products. It remains difficult to establish a link between sensory characteristics and chemical composition of volatile compounds. The sensory characteristics of a compound fragrant and in particular the threshold of perception can change depending on whether one is alone or in combination with other volatile compounds (Buettner & Schieberle, 2000). Several techniques were used for the sampling of flavors in foods products (Wilkes et al., 2000), among them, the pre-concentration of volatile compounds by solid-phase microextraction in headspace mode (HS-SPME) was a solvent free, simple, rapid and reliable method that does not induce modifications of volatile compounds due to temperature or solvent effect (Holt, 2001; Kataoka, Lord, & Pawlisyn, 2000; Zhang, Yang, & Pawlisyn, 1994). SPME directly coupled with GC and/or GC/MS was used for the analysis of odours, such as tomato, apricot, pear, peach, strawberry and apple (Riu-Aumatell

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et al., 1994; Servili, Selvaggini, Begliomini, & Montedoro, 1998; Song, Fon, & Beaudry, 1998; Song, Gardner, Holland, & Beaudry, 1997), as well as orange (Jia, Zhangn, & Min, 1998; Jordan, Tillman, Mucci, & Laenana, 2001; Steffen & Pawlisyn, 1996; Yang & Peppard, 1994) and mandarin (Dharmawan, Kasapis, Curran & Johnson, 2007; Peréz, Luaces, Oliva, Rios, & Sanz, 2005). The sampling of volatile constituents from fruit juices require optimisation of HS-SPME parameters including fibers, salt addition, volume of sample, extraction and desorption temperatures, equilibrium and extraction times. According to the literature data on orange and mandarin juices (Dharmawan et al., 2007; Jia, Zhangn, & Min, 1998; Peréz, Luaces, Oliva, Rios, & Sanz, 2005; Steffen & Pawlisyn, 1996; Yang & Peppard, 1994), the SPME device coated with a single polymer or with a mixture of polymers was used for the sampling of the volatile compounds in the juices. The extraction was typically carried out under agitation, with addition of salt (NaCl or $CaCl_2$) and a ratio (1/1) for sample/headspace volumes. Due to the occurrence of off-flavors in commercial fruit juices during heat treatment and storage, the volatile composition of orange juices were well studied (Jia et al., 1998; Jordan et al., 2001; Moshonas & Shaw, 1997; Nisperos-Carriedo & Shaw, 1990; Steffen & Pawlisyn, 1996; Yang & Peppard, 1994). Conversely, only four studies described the volatiles compounds identified from mandarin juices (Dharmawan et al., 2007; Peréz et al., 2005; Pérez-López & Carbonell-Barachina, 2006; Pérez-López, Saura, Lorente, & Carbonell-Barachina, 2006). Ethanol (94110 ng/mL), followed by limonene (34516 ng/mL), were always the major components identified by HS-SPME/GC/MS from the Spanish *Hernandina* mandarin variety (Peréz et al., 2005). Limonene (always more than 87%) was also the major volatile compound of mandarin juices obtained from Philippine *Dalandan* (Dharmawan, Kasapis, Curran, & Johnson, 2005), *Clemenules* and *Fortuna* varieties (Pérez-López & Carbonell-Barachina, 2006; Pérez-López et al., 2006). Lopez et al. (2006) showed that limonene, linalool, α-terpineol and terpinen-4-ol, were considered as quality control parameters in juices processing. It is noticeable that limonene and γ-terpinene, were the two major compounds of the peel essential oils of *C. reticulata Blanco* var. *Willow Leaf* and *C. clementina* var. *Commune* and were considered as statistical marker (Lota, De Rocca Serra, Tomi, & Casanova, 2001). The aim of this work was to characterise the volatile compounds of 67 fresh fruit juices obtained from clementine and mandarin hybrids and from their parents, using the HS-SPME coupling with GC for the determination of relative abundances and with GC/MS for the identification. To our knowledge, the volatile composition of clementine juices and hybrid clementine–mandarin juices were reported for the first time.

2. Materials and methods

2.1. Fruit

Mature fruits were randomly collected from the trees of clementine (*C. reticulata* × *C. sinensis var. Commune*), mandarin (*C. reticulata Blanco var. Willow Leaf*) and their 65 cross pollinated hybrids. Sanitation and physiological development of these trees have been controlled every year. All the hybrid plants were grafted on Carrizo citrange (*Poncirus trifoliate* × *C. sinensis*) rootstock, planted in the same orchard and submitted to homogenous cultural conditions, in order to reduce an environmental effect on the phenotype. The experimental orchard is located at the "Station de Recherches Agronomiques – INRA-CIRAD", San Giuliano, Corsica, France (latitude 42° 17′N, longitude 9° 32′E; Mediterranean climate – averages: rainfall and temperature, 840 mm and 15.2 °C per annum respectively, soil derived from alluvial deposits and classified as fersiallitic, pH range (6.0–6.6). The trees are planted according to an experimental device of square type Latin and were obtained by pollination. The Latin square is made of 12 lines of 12 trees (67 samples were taken among the 144 trees, including one sample from each genitor). In total, 201 fruits (67 * 3 fruits taken at random from the tree) have been collected (mandarin, clementine and 65 hybrids) on the periphery of the canopy and the juice extracted with a rotor machine. All fruits were harvest at commercial maturity stage (i.e. with ratio [Sugars]/[Acid]: 7–7.5). The fresh fruits were pressed with peel according to the industrial process and then was placed in a freezer (−80 °C) for the conservation and then defrosted before the analysis.

2.2. HS-SPME conditions

The juices were subjected directly after thawing to headspace solid-phase microextraction (HS-SPME). Optimisation of conditions was carried out using 2.5, 5, 10, 12.5, 15 mL of sample into a 20 mL vial; it was based on the sum of total peak areas. Volatiles from clementine, mandarin juices were extracted using five fibers: polydimethylsiloxane (PDMS), carbowax/divinylbenzene (CW/DVB), carboxen/polydimethylsiloxane (CAR/PDMS), polydimethylsiloxane/divinylbenzene (PDMS/DVB) and divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS). The fibers were conditioned in a GC injector port as indicated by manufacturing (Supelco, France). The choice of the optimised fiber was carried out at ambient temperature with a ratio (1/1) for sample/headspace volumes and both equilibrium and extraction times were fixed at 1 h. The equilibrium time was selected by variation of time (30 min to 24 h) at ambient temperature. The time and temperature extraction were determined by experimenting combining four different temperatures: 20, 30, 40, 50 °C and seven different times: 10, 20, 30, 60, 120, 180 and 240 min. The salt concentration for extraction was selected by variation of concentration of Na_2SO_4 (0–50% of total mass in juice). After sampling, SPME fiber was inserted into the GC injector at 260 °C for desorption of volatile components (5 min). All juices were analysed three times following the procedure described above.

2.3. Gas chromatography (GC)

GC analysis were carried out using a Perkin–Elmer Autosystem XL GC apparatus (Waltham, MA, USA) equipped with dual flame ionization detector (FID) system and two fused-silica capillary columns (60 m, 0.22 mm i.d., film thickness 0.25 μm, Restek, France), Rtx-1 (polydimethylsiloxane) and Rtx-wax (polyethylene glycol). The use of both capillary column with two different stationary phases allowed: (i) to resolve overlapping of compounds which have unfortunately the same retention times, and (ii) a better identification of components by the calculation of two RI for some compounds.

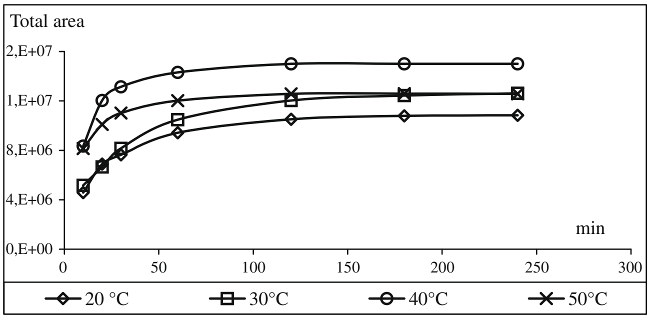

Fig. 1. Total areas of the GC-FID-signals measured under times and temperatures conditions of extraction.

Table 1
Volatile chemical compositions of mandarin and hybrids juices.

No.[a]	Compounds	RIl[b]	RIa[c]	RIp[d]	SPME (%) H_M														
					M	H28	H32	H33	H34	H35	H36	H37	H39	H40	H41	H42	H44	H45	H46
1	α-Thujene	932	922	1003	tr	tr	0.2	0.2	0.1	0.1	0.1	0.1	0.2	0.1	0.2	0.3	0.3	0.5	0.6
2	α-Pinene	936	930	1012	2.2	1.0	1.3	1.1	1.1	1.1	1.2	1.3	1.3	1.1	1.5	1.5	1.7	1.5	3.1
3	Camphene	950	943	1058	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	0.2	tr	tr
4	β-Pinene	978	970	1096	tr	0.4	0.6	0.6	0.6	0.6	0.3	0.5	1.0	0.4	0.8	0.9	0.8	1.1	0.7
5	Myrcene	987	981	1134	1.7	2.0	3.7	2.9	2.9	2.9	2.8	2.7	2.7	2.0	3.4	2.2	3.5	2.4	0.5
6	α-Phellandrene	1002	997	991	tr	tr	tr	tr	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1	tr	tr	tr
7	δ-3-Carene	1010	1004	1132	tr	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.1	0.1	0.1	0.3	0.1	0.2	0.3
8	α-Terpinene	1013	1008	1163	0.2	0.1	0.5	0.4	1.0	1.0	0.6	0.7	0.1	0.4	0.4	0.4	0.1	0.4	0.3
9	*para*-Cymene	1015	1012	1245	0.2	0.1	0.3	0.2	0.6	0.6	0.6	0.5	0.5	0.1	0.5	0.8	0.1	0.5	0.2
10	Limonene	1025	1026	1142	66.3	78.4	77.8	75.8	72.6	72.6	72.1	72.0	71.9	71.9	70.9	69.9	68.1	68.1	66.0
11	(*E*)-β-Ocimene	1041	1039	1227	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	0.1	tr	tr
12	γ-Terpinene	1051	1061	1201	21.1	13.4	12.1	13.2	14.1	14.1	14.6	16.7	14.5	15.0	18.1	19.1	20.9	17.8	23.4
13	*para*-Cymenene[e]	1075	1071	1107	1.7	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.9	0.2	0.1	0.1	tr	0.1
14	α-Terpinolene	1082	1085	1264	tr	0.8	0.6	1.0	0.8	0.8	0.8	0.2	1.5	0.4	1.0	1.1	1.4	0.9	1.9
15	Linalool[f]	1086	1091	1522	tr	0.3	0.1	0.6	1.2	1.2	0.9	0.2	1.0	0.6	0.9	0.1	tr	tr	tr
16	Nonanal[f]	1087	1091	1362	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	0.2	0.3	0.1
17	Terpinen-4-ol	1164	1163	1569	0.2	0.2	0.1	0.3	0.6	0.6	0.5	0.2	1.0	0.4	0.2	0.1	0.2	0.5	0.2
18	α-Terpineol	1176	1174	1688	0.4	0.2	0.1	0.2	0.7	0.7	0.6	0.2	0.2	0.3	0.2	0.1	0.2	0.6	0.2
19	Decanal	1185	1184	1471	tr	0.1	tr	tr	0.1	0.1	0.2	tr	tr	tr	tr	tr	0.2	0.2	0.3
20	Octyl acetate	1188	1192	1458	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
21	Citronellol	1213	1208	1748	tr	0.2	tr	0.1	0.2	0.2	0.2	0.2	0.3	tr	tr	tr	0.1	0.1	tr
22	Carvone	1214	1214	1719	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	0.1	tr
23	Linalyl acetate	1239	1240	1534	-	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	-	-	-
24	Perillaldehyde	1260	1246	1764	tr	tr	tr	0.1	0.1	0.1	tr	tr	tr	tr	tr	tr	tr	tr	0.1
25	Decanol[e]	1263	1254	1607	tr	tr	0.1	0.2	0.1	0.1	0.1	tr	0.3	tr	tr	tr	tr	tr	tr
26	Thymol	1267	1267	2155	tr	tr	tr	tr	0.1	0.1	tr	tr	0.3	tr	tr	tr	tr	tr	tr
27	Citronellyl acetate	1333	1331	1634	tr	tr	tr	0.1	tr	tr	tr	tr	tr	tr	tr	tr	0.1	0.1	0.2
28	α-Terpinyl acetate	1335	1336	1669	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	0.3	tr
29	Neryl acetate	1342	1340	1702	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
30	Geranyl acetate	1362	1350	1737	tr	–	–	–	-	-	-	-	-	-	-	-	tr	tr	tr
31	α-Copaene	1379	1373	1468	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	0.1	tr	tr
32	Methyl-*N*-methylanthranilate	1385	1380	2052	0.6	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.8	0.1	0.6	0.1
33	Dodecanal	1389	1389	1688	tr	0.1	0.4	0.1	tr	tr	tr	tr	tr	tr	tr	tr	0.1	tr	tr
34	Decyl acetate[e]	1393	1390	1575	–	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
35	(*E*)-β-Caryophyllene	1421	1414	1561	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	0.1	0.2	0.1
36	Geranyl acetone	1430	1428	1831	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
37	(*E*)-β-farnesene[e]	1446	1442	1309	tr	–	–	–	–	–	–	–	–	–	–	–	tr	tr	tr
38	β-Ionone	1468	1461	1906	tr	tr	tr	tr	–	–	–	–	–	–	–	–	–	–	–
39	(*Z*,*E*)-α-Farnesene[e]	1480	1478	1802	0.1	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	0.1	tr	0.1
40	(*E*,*E*)-α-Farnesene	1498	1498	1714	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
41	δ-Cadinene	1520	1515	1719	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
42	(*E*)-Nerolidol	1553	1546	2007	–	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
43	β-Sinensal[e]	1675	1667	2120	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	0.1
44	α-Sinensal[e]	1726	1721	2122	tr	tr	tr	0.1	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
	Monoterpene hydrocarbons				93.6	96.4	97.4	95.7	94.2	94.2	93.7	95.3	94.8	92.7	97.0	96.7	97.3	93.5	96.9
	Oxygenated monoterpenes				0.9	1.1	0.3	1.7	3.2	1.0	2.4	0.9	2.9	1.3	1.5	0.5	0.6	2.1	0.8
	Sesquiterpene hydrocarbons				0.2	0.0	0.0	0.0	0.1	0.2	0.3	0.2	0.3	0.1	0.1	0.2	0.2	0.2	0.1
	Oxygenated sesquiterpenes				0.1	0.0	0.0	0.2	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.1
	Others				0.9	0.4	0.9	0.5	0.5	0.5	0.6	0.4	0.5	0.2	0.1	0.9	0.6	0.8	0.3
	Total identified				95.8	97.9	98.7	98.1	98.0	98.0	97.0	96.9	99.5	94.3	98.7	98.3	98.8	97.4	98.2
						H47	H48	H50	H51	H52	H55	H56	H57	H58	H59	H61	H62	H64	H65
1	α-Thujene	932	922	1003		0.2	0.5	0.2	0.2	0.5	0.5	0.1	0.4	0.2	0.8	0.7	0.5	0.4	0.1
2	α-Pinene	936	930	1012		1.5	1.4	1.9	2.0	1.5	2.0	1.6	1.2	1.2	2.5	2.2	2.2	2.0	0.5
3	Camphene	950	943	1058		tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
4	β-Pinene	978	970	1096		0.7	1.2	0.9	0.8	1.5	1.3	0.5	1.3	0.6	1.8	1.8	1.7	1.4	0.1
5	Myrcene	987	981	1134		2.0	2.3	2.6	2.8	2.7	3.0	2.8	2.4	2.8	0.6	2.8	2.6	2.9	2.3
6	α-Phellandrene	1002	997	991		0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	tr	tr
7	δ-3-Carene	1010	1004	1132		0.1	0.2	0.1	0.1	0.1	0.1	0.1	1.5	0.3	0.1	0.5	0.4	0.1	0.1
8	α-Terpinene	1013	1008	1163		0.3	0.8	0.2	0.1	0.1	0.2	0.3	0.6	0.3	0.6	0.1	0.1	0.1	0.1
9	*para*-Cymene	1015	1012	1245		0.1	0.7	0.4	0.1	0.7	1.0	0.4	0.9	0.4	0.2	0.5	0.1	0.3	0.1
10	Limonene	1025	1026	1142		66.1	65.5	65.1	64.7	64.4	63.8	63.6	62.6	62.3	61.7	59.3	59.3	57.8	56.8
11	(*E*)-β-Ocimene	1041	1039	1227		tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
12	γ-Terpinene	1051	1061	1201		20.3	21.2	21.3	24.0	22.6	21.1	18.6	19.0	14.7	26.6	27.5	25.4	27.2	36.4
13	*para*-Cymenene[e]	1075	1071	1107		0.1	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
14	α-Terpinolene	1082	1085	1264		1.1	1.0	1.5	1.5	1.4	1.4	1.2	1.0	1.2	2.0	1.5	1.5	1.9	0.3
15	Linalool[f]	1086	1091	1522		tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
16	Nonanal[f]	1087	1091	1362		0.5	0.6	0.4	0.4	0.4	1.0	0.6	0.2	0.8	0.1	0.2	0.1	0.2	0.2
17	Terpinen-4-ol	1164	1163	1569		0.5	0.6	0.4	0.3	0.3	0.3	0.6	0.3	0.8	0.2	0.3	0.2	0.2	0.1
18	α-Terpineol	1176	1174	1688		0.5	0.6	0.2	0.3	0.3	0.3	0.3	0.2	0.4	0.1	0.2	0.1	0.5	0.1
19	Decanal	1185	1184	1471		0.1	0.1	0.1	0.1	0.1	0.3	0.2	1.1	0.9	0.1	0.1	0.1	0.1	0.1

(*continued on next page*)

Table 1 (*continued*)

					H47	H48	H50	H51	H52	H55	H56	H57	H58	H59	H61	H62	H64	H65
20	Octyl acetate	1188	1192	1458	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
21	Citronellol	1213	1208	1748	0.1	0.1	tr	tr	0.1	tr	tr	tr	0.1	tr	tr	tr	tr	tr
22	Carvone	1214	1214	1719	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
23	Linalyl acetate	1239	1240	1534	–	–	–	–	–	–	–	–	–	–	–	–	–	–
24	Perillaldehyde	1260	1246	1764	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.3
25	Decanol[e]	1263	1254	1607	0.2	0.1	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
26	Thymol	1267	1267	2155	tr	0.1	tr	tr	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
27	Citronellyl acetate	1333	1331	1634	0. 1	0.1	0.4	0.1	0.1	0.1	0.8	0.1	2.0	0.2	0.1	0.1	0.1	0.1
28	α-Terpinyl acetate	1335	1336	1669	tr	tr	tr	tr	tr	tr	0.5	0.1	1.3	0.2	0.1	0.1	0.2	0.1
29	Neryl acetate	1342	1340	1702	tr	tr	0.1	tr	tr	tr	0.3	tr	0.7	0.1	tr	tr	tr	tr
30	Geranyl acetate	1362	1350	1737	tr	tr	tr	tr	tr	tr	0.5	tr	1.2	tr	tr	tr	0.1	tr
31	α-Copaene	1379	1373	1468	tr	tr	tr	tr	tr	tr	tr	tr	0.2	tr	tr	tr	tr	tr
32	Methyl-*N*-methylanthranilate	1385	1380	2052	0.8	0.1	0.1	0.2	1.5	0.1	0.2	0.7	0.1	0.1	0.3	1.2	2.1	1.4
33	Dodecanal	1389	1389	1688	tr	tr	tr	tr	tr	0.1	0.1	0.1	0.1	tr	tr	0.1	tr	tr
34	Decyl acetate[e]	1393	1390	1575	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
35	(*E*)-β-Caryophyllene	1421	1414	1561	0.1	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	0.3	tr	tr
36	Geranyl acetone	1430	1428	1831	tr	tr	0.2	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
37	(*E*)-β-Farnesene[e]	1446	1442	1309	tr	tr	0.5	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
38	β-Ionone	1468	1461	1906	–	–	–	–	–	–	–	–	–	–	–	–	–	–
39	(*Z*,*E*)-α-Farnesene[e]	1480	1478	1802	0.1	tr	tr	0.1	tr	0.1	tr	tr	tr	tr	0.1	0.1	tr	tr
40	(*E*,*E*)-α-Farnesene	1498	1498	1714	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
41	δ-Cadinene	1520	1515	1719	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
42	(*E*)-Nerolidol	1553	1546	2007	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
43	β-Sinensal[e]	1675	1667	2120	tr	tr	tr	tr	0.1	tr	tr	0.1	0.1	tr	tr	tr	tr	tr
44	α-Sinensal[e]	1726	1721	2122	tr	tr	tr	tr	0.1	0.1	tr	0.1	0.1	tr	tr	0.1	tr	tr
	Monoterpene hydrocarbons				92.5	95.0	94.3	96.6	95.4	95.3	89.4	91.0	84.3	97.1	97.1	93.7	94.3	96.5
	Oxygenated monoterpenes				1.6	2.3	2.8	1.1	1.3	1.7	3.7	1.0	7.3	0.8	0.8	0.6	1.3	0.4
	Sesquiterpene hydrocarbons				0.2	0.1	1.6	0.1	0.1	0.2	0.1	0.2	0.3	0.0	0.1	0.7	0.0	0.0
	Oxygenated sesquiterpenes				0.0	0.1	0.0	0.0	0.2	0.1	0	0.2	0.1	0.1	0.1	0.1	0.1	0.0
	Others				1.4	0.5	0.5	0.5	1.6	0.7	0.4	2.2	2.8	0.3	0.6	1.5	2.3	2.0
	Total identified				95.7	98.0	99.2	98.3	98.6	98.0	93.6	94.6	94.8	98.3	98.7	96.6	98.0	99.1

% Mean percentage of individual fruit juice constituents from triplicate experiments; tr: trace (<0.05%); – not detected.

Standard deviations for: limonene ($4.2 < SD < 7.4$), γ-terpinene ($0.008 < SD < 2.8$), others compounds $\geqslant 1\%$ ($0.015 < SD < 0.615$) and others compounds <1% ($0.015 < SD < 0.137$).

[a] Order of elution are given on apolar column (Rtx-1).

[b] Retention indices of literature on the apolar column (RIl) König et al. (2001) and NIST WebBook (2005).

[c] Retention indices on the apolar Rtx-1 column (RIa).

[d] Retention indices on the polar Rtx-Wax column (RIp).

[e] Compounds identified from literature mass spectra libraries Adams (2001), König et al. (2001) and NIST/EPA/NIH (1999).

[f] Co elution, percentage given on polar column.

The oven temperature was programmed from 60 °C to 230 °C at 2 °C/min and then held isothermally at 230 °C for 35 min. Injector and detector temperatures were maintained at 280 °C. Samples were injected in the splitless mode, using helium as carrier gas (1 mL/min). Volatiles components were desorbed in a GC injector with a specific SPME inlet liner (0.75 mm. i.d., Supelco Co.). Retention indices (RI) of compounds were determined relative to the retention times of series of *n*-alkanes (C_5–C_{30}) with linear interpolation, using Van den Dool and Kratz (1963) equation and software from Perkin–Elmer. Relative amounts of components were calculated based on GC peak areas without using correction factors.

2.4. Gas chromatography/mass spectrometry (GC/MS)

Samples were analysed with a Perkin–Elmer (Waltham, MA, USA) Turbo mass detector (quadrupole), coupled to a Perkin–Elmer Autosystem XL, equipped with fused-silica capillary columns Rtx-1 and Rtx-Wax. Carrier gas: helium (1 mL/mim), ion source temperature was set at 150 °C, oven temperature programmed from 60 °C to 230 °C at 2 °C/min and then held isothermally 230 °C (35 min), injector temperature: 280 °C, splitless, energy ionization: 70 eV, electron ionization mass spectra were acquired over the mass range 35–350 Da. Volatile components were desorbed in a GC injector with a SPME intel liner (0.75 mm. i.d., Supelco Co.).

2.5. Component identifications

Identification of the components was based: (i) on the comparison of their GC retention indices (RI) on non polar and polar columns, determined relative to the retention time of a series of *n*-alkanes with linear interpolation, with those of authentic compounds or literature data (König, Hochmuth, & Joulain, 2001; NIST WebBook, 2005); and (ii) on computer matching with commercial mass spectral libraries (Adams 2001; König et al., 2001; NIST/EPA/NIH, 1999) and comparison of spectra with those of our laboratory-made library. The majority of these compounds were commercial standard components and the few others, were previously identified at large amounts in essential oils or fractions obtained by CC, by comparison with literature spectral data and retention indices and ensured by ^{13}C NMR. Relative amount of individual components were performed on the basis of their GC peak areas on the two capillary Rtx-1 and Rtx-Wax columns, without FID response factor correction.

2.6. Statistical analysis

Principal component analysis (PCA), correlation analysis and dendogram (using Ward's technique with a Euclidean distance measure) were reported. The Statistical Graphics Corporation®'s "Statgraphics for Windows" software was used for these various tests.

3. Results and discussion

3.1. Optimization of SPME parameters

The optimisation of HS-SPME sampling parameters was carried out using the clementine and mandarin juices and it was based on

Table 2
Volatile chemical compositions of clementine and hybrids juices.

N[a]	Compounds	RIl[b]	RIa[c]	RIp[d]	SPME (%)																		
					H_C																		
					C	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12	H13	H14	H15	H16	H17	H18
1	α-Thujene	932	922	1003	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	0.6	tr	tr	tr	tr	tr	tr	tr	tr
2	α-Pinene	936	930	1012	0.4	1.2	0.7	0.4	0.6	1.2	0.6	0.9	0.3	0.7	0.2	0.8	0.7	0.8	0.6	0.7	0.4	0.6	0.7
3	Camphene	950	943	1058	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
4	β-Pinene	978	970	1096	tr	0.5	tr	tr	tr	tr	tr	tr	tr	tr	0.1	tr	tr	tr	0.6	tr	tr	tr	tr
5	Myrcene	987	981	1134	3.4	0.5	0.4	3.7	4.0	0.7	3.5	3.8	2.8	3.9	4.1	3.7	3.7	3.4	3.4	0.7	2.3	3.4	3.4
6	α-Phellandrene	1002	997	991	tr	0.1	tr	tr	tr	tr	0.1	tr	tr	tr	tr	tr	tr	0.2	tr	tr	tr	0.1	tr
7	δ-3-Carene	1010	1004	1132	tr	tr	tr	tr	tr	tr	tr	tr	0.2	0.5	tr	tr	tr	tr	tr	tr	tr	0.2	tr
8	α-Terpinene	1013	1008	1163	0.1	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	0.2	tr	tr	tr	tr	tr	tr	tr
9	*para*-Cymene	1015	1012	1245	0.3	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.3
10	Limonene	1025	1026	1142	90.0	93.3	93.0	93.0	92.8	92.7	92.5	91.9	91.3	91.0	90.6	90.5	90.3	90.2	90.2	89.6	89.4	89.0	88.7
11	(*E*)-β-Ocimene	1041	1039	1227	tr	tr	0.1	tr	tr	tr	tr	tr	tr	0.2	0.1	0.1	tr	tr	0.1	tr	tr	tr	tr
12	γ-Terpinene	1051	1061	1201	1.2	0.7	0.6	0.3	0.1	0.1	0.2	0.3	0.5	0.4	0.2	0.5	0.9	1.0	1.4	2.4	0.6	0.9	2.5
13	*para*-Cymenene[e]	1075	1071	1107	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	0.3	tr	tr	tr	tr
14	α-Terpinolene	1082	1085	1264	0.1	tr	tr	0.2	0.2	0.2	0.2	0.1	0.1	0.2	0.2	1.0	0.2	0.2	0.1	0.2	0.2	0.1	0.2
15	Linalool[f]	1086	1091	1522	0.3	0.3	1.5	0.1	0.2	0.2	0.3	0.3	1.8	1.4	0.2	0.2	0.2	0.2	0.4	0.8	1.6	0.2	0.2
16	Nonanal[f]	1087	1091	1362	0.2	–	–	–	–	–	–	–	–	–	0.1	–	–	–	–	–	–	–	–
17	Terpinen-4-ol	1164	1163	1569	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.2	0.1	0.2	0.2	0.1	0.1
18	α-Terpineol	1176	1174	1688	0.2	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.4	0.4	0.1	0.1
19	Decanal	1185	1184	1471	0.1	tr	tr	tr	–	tr	–	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
20	Octyl acetate	1188	1192	1458	tr	tr	-	–	tr	tr	–	tr	–	–	–	–	–	tr	–	tr	–	–	–
21	Citronellol	1213	1208	1748	tr	–	tr	–	–	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
22	Carvone	1214	1214	1719	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
23	Linalyl acetate	1239	1240	1534	tr	–	–	–	–	–	–	–	tr	tr	–	–	–	–	–	–	–	–	tr
24	Perillaldehyde	1260	1246	1764	–	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	0.7	0.3	tr	tr
25	Decanol[e]	1263	1254	1607	–	–	tr	tr	tr	–	–	tr	0.1	tr	–	–	–	–	–	–	–	tr	–
26	Thymol	1267	1267	2155	tr	–	–	–	–	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
27	Citronellyl acetate	1333	1331	1634	0.2	tr	tr	tr	tr	0.1	tr	tr	tr	tr	tr	tr	tr	0.1	tr	tr	tr	0.4	tr
28	α–Terpinyl acetate	1335	1336	1669	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
29	Neryl acetate	1342	1340	1702	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
30	Geranyl acetate	1362	1350	1737	–	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
31	α-Copaene	1379	1373	1468	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
32	Methyl-*N*-methylanthranilate	1385	1380	2052	0.1	0.1	0.1	0.3	0.6	0.5	0.1	0.3	0.1	0.1	0.1	0.1	0.9	0.2	0.3	1.0	1.0	0.3	0.9
33	Dodecanal	1389	1389	1688	tr	tr	tr	tr	tr	0.1	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	0.1	tr
34	Decyl acetate[e]	1393	1390	1575	tr	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
35	(*E*)-β-Caryophyllene	1421	1414	1561	0.1	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
36	Geranyl acetone	1430	1428	1831	–	–	–	tr	–	tr	tr	tr	tr	–	tr	–	–	–	–	tr	tr	–	–
37	(*E*)-β-Farnesene[e]	1446	1442	1309	–	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
38	β-Ionone	1468	1461	1906	–	tr	tr	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
39	(*Z*,*E*)-α-Farnesene[e]	1480	1478	1802	–	0.1	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
40	(*E*,*E*)-α-Farnesene	1498	1498	1714	–	–	–	–	–	–	–	–	–	–	–	tr	tr	tr	tr	–	–	–	–
41	δ-Cadinene	1520	1515	1719	–	–	–	–	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	0.1	tr	tr	tr	tr
42	(*E*)-Nerolidol	1553	1546	2007	tr	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
43	β-Sinensal[e]	1675	1667	2120	tr	–	–	–	–	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
44	α,α-Sinensal[e]	1726	1721	2122	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
	Monoterpene hydrocarbons				95.8	95.8	95.0	97.8	97.8	93.8	97.2	97.3	95.5	97.0	96.1	97.0	96.1	96.2	96.8	93.8	94.2	94.9	96.0
	Oxygenated monoterpenes				0.9	0.6	1.9	0.4	0.3	0.9	0.7	0.6	2.2	1.8	0.5	0.6	0.6	0.8	0.7	2.5	1.2	0.9	0.7
	Sesquiterpene hydrocarbons				0.1	0.1	0.2	0.0	0.0	0.1	0.0	0.1	0.0	0.0	0.1	0.1	0.2	0.0	0.2	0.0	0.0	0.2	0.0
	Oxygenated sesquiterpenes				0.0	0.1	0.0	0.0	0.0	0.1	0.1	0.0	0.1	0.0	0.1	0.2	0.0	0.0	0.0	0.1	0.1	0.1	0.0
	Others				0.6	0.2	0.1	0.6	0.6	0.9	0.2	0.4	0.3	0.3	0.3	0.3	0.9	0.5	0.6	1.3	1.2	0.7	1.0
	Total identified				97.4	97.3	97.2	98.8	99.0	96.8	98.2	98.5	98.6	99.1	97.2	98.2	97.8	97.5	98.3	98.1	96.8	96.8	97.7
					H_C													H_a					
					H19	H20	H21	H22	H23	H24	H25	H26	H27	H29	H30	H31	H38	H49	H53	H54	H60	H63	
1	α-Thujene	932	922	1003	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	0.1	0.1	0.1	0.3	0.5	0.3	
2	α-Pinene	936	930	1012	0.5	0.5	0.6	0.4	1.3	0.4	0.4	0.6	0.5	0.9	0.8	0.3	0.8	1.2	0.7	0.8	1.3	1.2	
3	Camphene	950	943	1058	tr	tr	tr	tr	tr	tr	tr	tr	tr	0.1	0.1	tr	tr	tr	tr	tr	tr	tr	
4	β-Pinene	978	970	1096	tr	tr	tr	0.1	0.6	0.1	0.1	0.1	0.1	0.2	0.1	0.2	0.4	0.3	0.1	0.9	1.0	1.1	
5	Myrcene	987	981	1134	3.5	2.6	3.2	2.7	4.0	2.4	1.2	3.1	3.2	1.3	1.1	1.7	1.8	3.0	2.1	2.2	1.1	1.8	
6	α-Phellandrene	1002	997	991	tr	tr	0.1	tr	tr	tr	tr	tr	tr	tr	0.1	tr	0.2	tr	0.1	0.1	0.7	0.1	
7	δ-3-Carene	1010	1004	1132	tr	tr	tr	0.1	0.1	0.1	0.1	0.1	0.4	0.1	0.1	0.1	0.1	0.1	1.9	0.3	0.3	1.2	
8	α–Terpinene	1013	1008	1163	tr	0.4	tr	0.1	0.1	0.1	0.1	0.1	1.0	0.5	0.1	0.1	0.3	0.8	2.2	0.5	0.5	1.8	
9	*para*-Cymene	1015	1012	1245	0.2	0.2	0.2	0.1	0.1	0.4	0.3	0.1	0.3	0.1	0.4	1.0	1.5	1.9	3.3	0.8	2.9	1.5	
10	Limonene	1025	1026	1142	88.6	88.0	87.6	87.3	85.6	84.7	84.2	88.5	82.6	80.2	79.0	78.5	77.0	65.3	64.2	64.1	61.0	58.9	
11	(*E*)-β-Ocimene	1041	1039	1227	tr	tr	tr	0.1	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	
12	γ-Terpinene	1051	1061	1201	3.1	2.6	1.8	0.4	1.3	3.3	3.2	1.7	4.3	1.7	1.7	3.0	4.8	23.8	10.6	15.3	21.8	13.3	
13	*para*-Cymenene[e]	1075	1071	1107	0.3	tr	tr	0.1	0.1	0.2	0.9	0.1	0.1	0.1	0.8	0.6	0.1	0.1	tr	tr	tr	tr	
14	α-Terpinolene	1082	1085	1264	0.2	0.3	0.2	0.2	0.2	0.7	1.4	0.4	0.3	0.1	0.1	1.1	0.6	1.3	2.0	0.8	0.7	1.6	
15	Linalool[f]	1086	1091	1522	0.5	0.7	0.2	0.8	0.6	0.2	0.2	0.1	0.3	0.9	2.7	0.8	3.1	tr	tr	tr	tr	tr	
16	Nonanal[f]	1087	1091	1362	–	–	–	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	2.0	1.4	2.1	3.0	1.5	
17	Terpinen-4-ol	1164	1163	1569	0.2	0.1	0.2	0.1	0.4	0.3	0.4	0.2	0.1	1.2	0.7	0.2	0.8	1.8	0.3	3.0	0.6	0.9	

(*continued on next page*)

Table 2 (*continued*)

					H_C													H_a				
					H19	H20	H21	H22	H23	H24	H25	H26	H27	H29	H30	H31	H38	H49	H53	H54	H60	H63
18	α-Terpineol	1176	1174	1688	0.2	0.2	0.1	0.2	0.1	0.3	0.5	0.2	0.2	1.4	1.9	0.4	0.9	0.3	0.2	3.2	0.9	0.5
19	Decanal	1185	1184	1471	tr	_	_	0.3	0.1	tr	tr	0.1	0.9	0.5	0.2	0.1	0.3	0.2	0.3	0.4	0.1	0.2
20	Octyl acetate	1188	1192	1458	–	–	–	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
21	Citronellol	1213	1208	1748	tr	tr	tr	tr	0.2	tr	tr	tr	tr	0.1	0.2	0.2	0.2	tr	0.3	0.2	tr	0.2
22	Carvone	1214	1214	1719	–	–	–	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	0.2	tr	tr
23	Linalyl acetate	1239	1240	1534	tr	tr	tr	tr	tr	tr	tr	tr	tr	0.1	0.1	tr	0.2	–	–	–	–	–
24	Perillaldehyde	1260	1246	1764	tr	tr	tr	tr	0.3	tr	tr	tr	tr	0.4	0.1	0.5	tr	0.1	0.1	0.1	0.1	0.1
25	Decanol[e]	1263	1254	1607	0.1	tr	tr	0.1	tr	tr	tr	0.1	0.1	0.6	tr	tr	0.2	tr	tr	tr	tr	tr
26	Thymol	1267	1267	2155	tr	tr	tr	tr	0.1	tr	tr	tr	tr	0.1	0.4	tr	tr	tr	0.1	0.2	0.2	0.2
27	Citronellyl acetate	1333	1331	1634	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	0.1	1.1	0.1	0.1	1.7
28	α-Terpinyl acetate	1335	1336	1669	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	0.6	0.4	tr	0.1	0.9
29	Neryl acetate	1342	1340	1702	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	0.7	0.8	tr	tr	0.1
30	Geranyl acetate	1362	1350	1737	tr	tr	tr	–	–	–	–	–	–	–	–	–	–	1.3	0.1	tr	tr	1.3
31	α-Copaene	1379	1373	1468	tr	tr	tr	tr	tr	tr	tr	0.2	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
32	Methyl-*N*-methylanthranilate	1385	1380	2052	0.1	0.2	0.5	0.3	2.0	0.1	0.2	0.2	0.3	0.4	0.1	0.2	0.2	0.2	0.6	0.1	0.2	0.1
33	Dodecanal	1389	1389	1688	tr	tr	tr	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.3	tr	tr	tr	0.1	tr	tr
34	Decyl acetate[e]	1393	1390	1575	–	–	–	0.1	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
35	(*E*)-β-Caryophyllene	1421	1414	1561	tr	tr	tr	tr	tr	tr	tr	tr	0.1	tr	tr	tr	tr	tr	tr	tr	tr	0.1
36	Geranyl acetone	1430	1428	1831	tr	–	–	tr	tr	tr	0.4	tr	0.5	tr	tr	tr	0.1	0.5	tr	tr	tr	tr
37	(*E*)-β-Farnesene[e]	1446	1442	1309	tr	tr	tr	tr	tr	–	–	–	–	–	–	–	–	0.7	tr	tr	tr	tr
38	β-Ionone	1468	1461	1906	–	–	–	tr	tr	tr	tr	tr	tr	tr	tr	tr	–	–	–	–	–	–
39	(*Z*,*E*)-α-Farnesene[e]	1480	1478	1802	tr	tr	tr	0.1	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	0.1	tr
40	(*E*,*E*)-α-Farnesene	1498	1498	1714	–	–	–	0.1	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
41	δ-Cadinene	1520	1515	1719	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	0.3	tr	tr	tr	tr	tr	tr
42	(*E*)-Nerolidol	1553	1546	2007	–	–	–	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
43	β-Sinensal[e]	1675	1667	2120	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	0.1	tr	tr
44	α-Sinensal[e]	1726	1721	2122	tr	tr	tr	tr	tr	tr	tr	0.1	tr	tr	tr	tr	0.2	0.1	tr	0.1	tr	tr
	Monoterpene hydrocarbons				96.6	94.9	93.8	96.9	93.3	92.4	92.0	94.7	93.2	85.7	84.5	86.6	87.9	76.8	83.9	85.7	91.9	83.1
	Oxygenated monoterpenes				1.0	1.1	0.4	0.8	2.1	0.9	1.9	0.5	1.0	4.4	6.3	2.3	5.4	13.1	7.7	9.7	4.5	7.3
	Sesquiterpene hydrocarbons				0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.4	0.1	0.1	0.1	0.1	0.2	5.7	0.1	0.1	0.1	0.2
	Oxygenated sesquiterpenes				0.1	0.1	0.0	0.1	0.1	0.0	0.0	0.2	0.1	0.1	0.1	0.1	0.3	0.1	0.1	0.1	0.1	0.1
	Others				0.4	0.3	0.5	0.7	2.2	0.1	0.4	0.6	1.4	1.7	0.4	1.1	1.0	1.4	1.2	0.6	0.7	0.2
	Total identified				98.2	96.5	94.8	94.2	97.8	93.4	94.6	96.2	95.8	92.0	91.4	90.2	94.8	94.1	93.0	96.2	97.4	90.9

% Mean percentage of individual fruit juice constituents from triplicate experiments; tr: trace (<0.05%); – not detected.Standard deviations for: limonene ($4.2 < SD < 7.4$), γ-terpinene ($0.008 < SD < 2.8$), others compounds ⩾1% ($0.015 < SD < 0.615$) and others compounds <1% ($0.015 < SD < 0.137$).

[a] Order of elution are given on apolar column (Rtx-1).

[b] Retention indices of literature on the apolar column (RIl) König et al. (2001) and NIST WebBook (2005).

[c] Retention indices on the apolar Rtx-1 column (RIa).

[d] Retention indices on the polar Rtx-Wax column (RIp).

[e] Compounds identified from literature mass spectra libraries Adams (2001), König et al. (2001) and NIST/EPA/NIH (1999).

[f] Co elution, percentage given on polar column.

the sum of total GC peak areas. The triple fibre (DVB/CAR/PDMS) was chosen for the analysis of the volatile compounds of all juice samples because of its quantitative as well as qualitative capacity of extraction. The optimisation of the equilibrium time was made at ambient temperature and the maximal FID- response was measured after an equilibrium time of 120 min. Combining time and temperature variations, the optima extraction conditions were established for an extraction time of 120 min at 40 °C (Fig. 1). Conversely to the literature report (Yang & Peppard, 1994), in our experiments, better extractions of the volatile compounds of juices were performed without salt addition. Indeed, the use of various quantities of sodium sulfate (0–50% of mass juice) involved the decrease of volatile amounts. Moreover, with out salt, no new compounds due to enzymatic degradation were observed. The coefficient of variation calculated on the basis of total area obtained from the FID-signal for the 67 samples (9.8% < CV < 15.2%) induced that our equipments and the method were reliable. In the same way, the CV of the major compounds was always less than 8%. The standard deviations were reported in Table 1.

3.2. Volatile chemical compositions

HS-SPME/GC and GC/MS analysis of the juices obtained from both parents (M: mandarin and C: clementine) and 65 hybrids led to the identification of 44 compounds which represented 90.2–99.8% of the total volatile compounds (Tables 1 and 2). Among them, 25 components were monoterpenes (94.5–98.8%), nine were sesquiterpenes (0–5.8%) and 10 were non terpenic compounds (0.1–2.8%). Among the 44 individual compounds identified, 37 were performed by comparing their EI-mass spectra and their retention indices with those of our own library and the similarity with the retention indicers of literature (RIl) has been audited. In the present study, *para*-cymenene, decanol, decyl acetate, (*E*)-β-farnesene, (*Z,E*)-α-farnesene, β-sinensal and α-sinensal were identified by comparison of their EI-mass spectra and their retention indices with those of commercial libraries and/or literature data. All samples were characterised by the high content of hydrocarbon compounds (82.5–97.8%) represented by 14 monoterpenes (76.8–97.8% of hydrocarbon amount) and six sesquiterpenes (0–5.7%).

In all samples the main compounds were limonene (56.8–93.3%) and γ-terpinene (0.1–36.4%; Tables 1 and 2). The other monoterpene hydrocarbons identified at appreciable abundances were myrcene (0.4–4.1%) and α-pinene (0.4–3.1%). The oxygenated compounds amounted to 0.9–14.6% and the main polar compounds were linalool (traces to 3.0%) and α-terpineol (0.1–3.2%). Methyl-*N*-methylanthranilate, thymol, β-pinene, γ-terpinene and α-sinensal were the main contributors to mandarine flavours

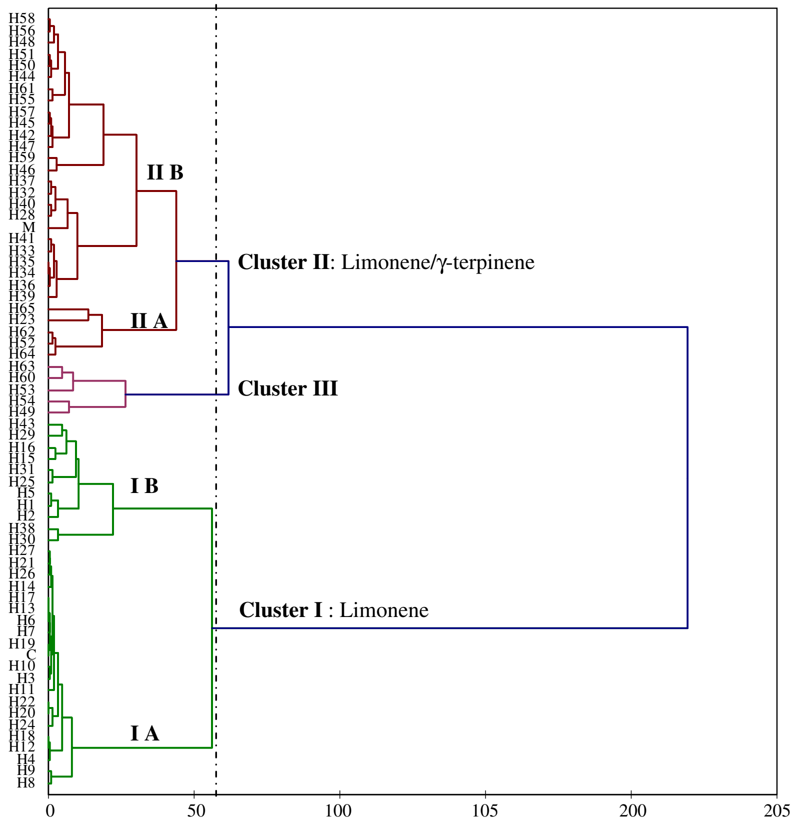

Fig. 2. Dendrogram obtained from the cluster analysis of 67 sample juices. Samples are clustered using Ward's technique with a Euclidean distance measure.

(Shaw, 1991). These compounds were identified in appreciable abundance in the citrus juices investigated in this study.

The mandarin juice (sample M) obtained from a *Willow Leaf* variety (Table 1), contained large amounts of limonene (66.3%) and γ-terpinene (21.1%); the other components identified at appreciable amounts were α-pinene (2.2%), myrcene (1.7%) and *para*-cymenene (1.7%). These results differed from those reported in the literature for the *Clemenules*, *Fortuna* and *Dalandan* varieties mandarin juices (Jia et al., 1998; Peréz et al., 2005) in which amount of γ-terpinene never exceeded 0.1% while limonene was always the main component (86.9–97.1%). Conversely, the volatile chemical composition of the sample M was similar to those of the essential oils obtained from *Willow Leaf* mandarin peel in which the couple limonene/γ-terpinene accounted for 52.2–81.3% and 11.2–36.7%, respectively (Lota et al., 2001). It is noticeable that the chemical composition of the clementine juice (C), characterised by the pre-eminence of limonene (90.0%) and a low abundance of myrcene (3.4%) and γ-terpinene (1.2%; Table 2), was similar to those reported for one of groups of mandarin peel essential oils (Lota et al., 2001) in which limonene was the ultra major compound (83.8–96.2%). Moreover, the chemical composition of the clementine juice (C) was similar of those of the peel essential oil obtained from different varieties of clementine (Lota et al., 2001). Finally, hybrid juices showed qualitative close chemical compositions but differed by quantitative variations in the amount of the couple limonene/γ-terpinene.

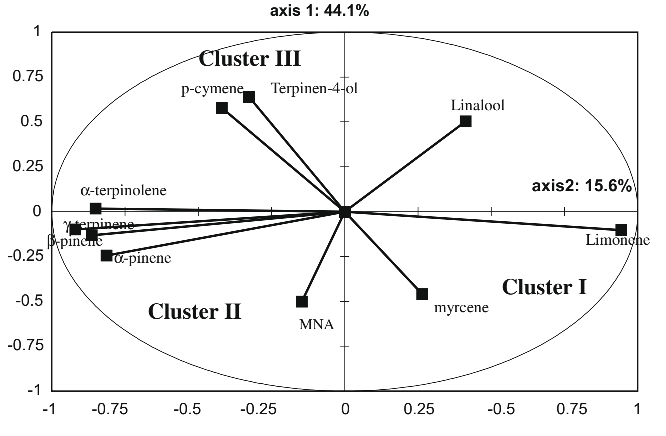

Fig. 3. Discriminant analysis of mandarin, clementine and hybrid juices constituents.

3.3. Statiscal analysis

The dendrogram, obtained from cluster analysis and reported in Fig. 2, suggested the existence of three principal clusters within the juices of the individuals of mandarin, clementine and hybrids. Cluster I was characterised by the clementine group and exhibited the limonene chemotype (H_C: 31 hybrid samples; Table 2). These samples were characterised by large amount of limonene (77.0–93.3%) and low percentages of γ-terpinene (less than 3.2%). This cluster could be divided into two subgroups (IA and IB) on the basis of the content of myrcene which was high (2.7–4.1%) in the subgroup IA (20 samples) and always lower than 1.7% in the subgroup IB (11 samples). Cluster II was characterised by the mandarin group and contained the samples with limonene/γ-terpinene chemotype (H_M: 29 hybrid samples; Table 1) in which limonene (56.8–80.4%) and γ-terpinene (11.4–36.4%) were identified as major components. Cluster II could be divided into two subgroups IIA and IIB. The subgroup IIA (5 samples) was characterised by large amount of methyl-*N*-methylanthranilate (MNA; 1.2–2.1%) and

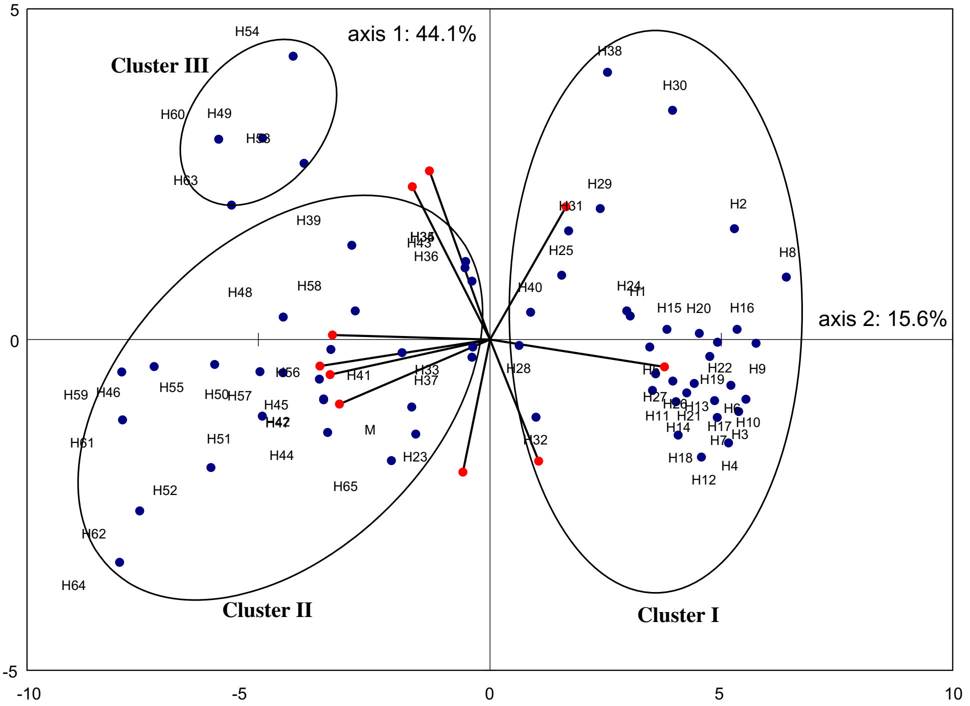

Fig. 4. Principal component analysis of both parental and hybrid *Citrus* juices.

the subgroup IIB (24 samples) was characterised by large amount of γ-terpinene (11.4–36.4%). Cluster III exhibited five atypical samples of juices (H49, H53, H54, H60 and H63; Table 2) which differed to the parental odor profile by high contents of *para*-cymene (0.8–3.3%) and/or terpinen-4-ol (0.3–3.0%).

Discriminant analysis (Fig. 3) and principal component analysis (Fig. 4) performed for the 10 major components, confirmed this clustering with respect to the contents of limonene and γ-terpinene. Both factors represented here, collectively take in 59.7% of the total variability of the dataset (Fig. 3). The first axis (explains 44.1% of total variance) was positively correlated with linalool, limonene and myrcene and opposed with terpinen-4-ol, *para*-cymene, α-terpinolene, γ-terpinene, β-pinene, α-pinene and MNA. The second axis (explains 15.6% of total variance) was positively correlated with *para*-cymene, terpinen-4-ol, α-terpinolene and linalool and negatively with γ-terpinene, α- and β-pinene, MNA, myrcene and limonene. Discriminant analysis offered a representation of the distribution of volatile compound families from mandarin, clementine and hybrid juices based on the 10 majors compounds.

4. Conclusion

The analysis by HS-SPME/GC and/or GC/MS of the volatile compositions of 67 juices obtained from mandarin, clementine and hybrids fresh fruits, allowed the identification of 44 compounds. The clementine juice was characterised by the pre-eminence of limonene (90.0%) and a low abundance in γ-terpinene (1.2%) while the mandarin juice exhibited high amount of limonene (66.3%) and γ-terpinene (21.1%). All hybrid juices showed qualitative close composition but differed by quantitative variation in the amount of the couple limonene/γ-terpinene. The statically analysis indicated that the hybrid samples were symmetrically distributed around the both parents, 48% of the hybrid samples exhibited an odor profile close to those of the clementine parental juice and 45% exhibited an odor profile close to the mandarin parental juice. Finally, 7% of the hybrid samples exhibited atypical profiles on the basis of the amounts of *para*-cymene and/or terpinen-4-ol.

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