Essential Oil of Two New Pigmented Citrus Hybrids, *Citrus clementina* × *Citrus sinensis*

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The chemical composition of the essential oils of two new Citrus hybrids, obtained by crossbreeding the diploid clementine *Citrus clementina* Hort. ex Tan. and the tetraploid orange *Citrus sinensis* (L.) Osbeck cv. Tarocco, was studied by GC/MS. In all, 42 components were fully characterized and grouped into four classes (monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpenes, and aliphatic aldehydes and alcohols) for an easier comparison with the oils of the parents. Since limonene is by far the main component of all the essential oils examined, representing generally ca. 95% of the total, significant hybrid−hybrid and parent−hybrid differences can be observed only within the remaining 5%. These new hybrids are the first example of a mandarin-like hybrid with a red pigmentation, a feature inherited from the Tarocco orange. These new fruits, therefore, add further to the very high production of pigmented citrus fruits, which are an almost exclusive patrimony of Sicilian citrus production. This pigmentation is due to the presence of anthocyanins, whose known antioxidant activity gives them an important biological role in the disease prevention of living systems.

Keywords: *Citrus clementina Hort. ex Tan.; Citrus sinensis (L.) Osbeck cv. Tarocco; Citrus breeding; Citrus triploid hybrid; essential oil composition; pigmented Citrus fruits*

INTRODUCTION

One of the most promising studies concerning the improvement of citrus fruit quality is the crossbreeding of extant species (Dugo et al., 1990; Moshonas et al., 1991; Soost, 1987; Soost and Cameron, 1980, 1985). The main aim of such a breeding program is to obtain seedless fruits with easily removable peel, optimal size, excellent organoleptic characteristics, and possibly precocious or late ripening, all properties widely appreciated for the fresh-fruit market (Starrantino, 1992). Furthermore, but no less important from the agronomic and economic point of view, the controlled pollination procedure is a way to obtain plants with high productivity and improved resistance against diseases and environmental stress (Ruberto et al., 1994).

In the course of our continued phytochemical studies of new Citrus hybrids, we have reported the chemical composition of the essential oils of new Citrus fruits (Ruberto et al., 1993, 1994). The present investigation deals with the chemical analysis of the oils of two new hybrids obtained by a cross between clementine (*Citrus clementina* Hort. ex Tan.) and orange cv. Tarocco (*Citrus sinensis* L. Osbeck). The new fruits are seedless and easy to peel, with size and shape similar to a big clementine, and with excellent organoleptic properties, therefore possessing features appreciated by the consumer. Further, a peculiar characteristic of these new mandarin-like hybrids is the strong pigmentation at the end of ripening, which is inherited from the male parent, Tarocco orange, and is due to anthocyanins. The pigmented citrus fruits represent an important sector of Sicilian production, amounting to ca. 70% of the total orange production (Rapisarda and Giuffrida, 1992; Spina and Di Martino, 1991). In recent years the pigmented fruits, mainly their juices, have experienced a significant increase in use; the original organoleptic features, a higher content of vitamins (20−25%), especially in the Tarocco cultivar, with respect to the blond orange (Spina and Di Martino, 1991), and a promotional campaign considerably raised consumption. To these important characteristics should be added the presence of anthocyanins, whose antioxidant activity can help to prevent the detrimental effects of free radicals and reactive oxygen species on living systems (Gordon, 1996; Saija et al., 1992; Tamura and Yamagami, 1994; Tsuda et al., 1994a,b).

EXPERIMENTAL PROCEDURES

Crosses. Controlled pollination using standard Citrus breeding methods between diploid clementine and tetraploid orange was carried out (Starrantino, 1993). Seeds from these crosses are generally incompletely developed and therefore unsuitable for natural germination (Esen and Soost, 1973). Therefore, immature embryos were taken from the seeds and grown on MS substrate (Murashige and Skoog, 1962) to give seedlings which were planted in peat mold and kept at 27 °C in a humidity saturated environment (Starrantino and Reforgiato, 1981). After 1 month the young plants were transferred to a greenhouse, where they were grown for 2 years. Scions were cut from the apical part of plants and grafted onto sour orange, Troyer and Carrizo citranges and entered in production after 4−5 years. The most promising hybrids were successively propagated to evaluate their behavior in the field.

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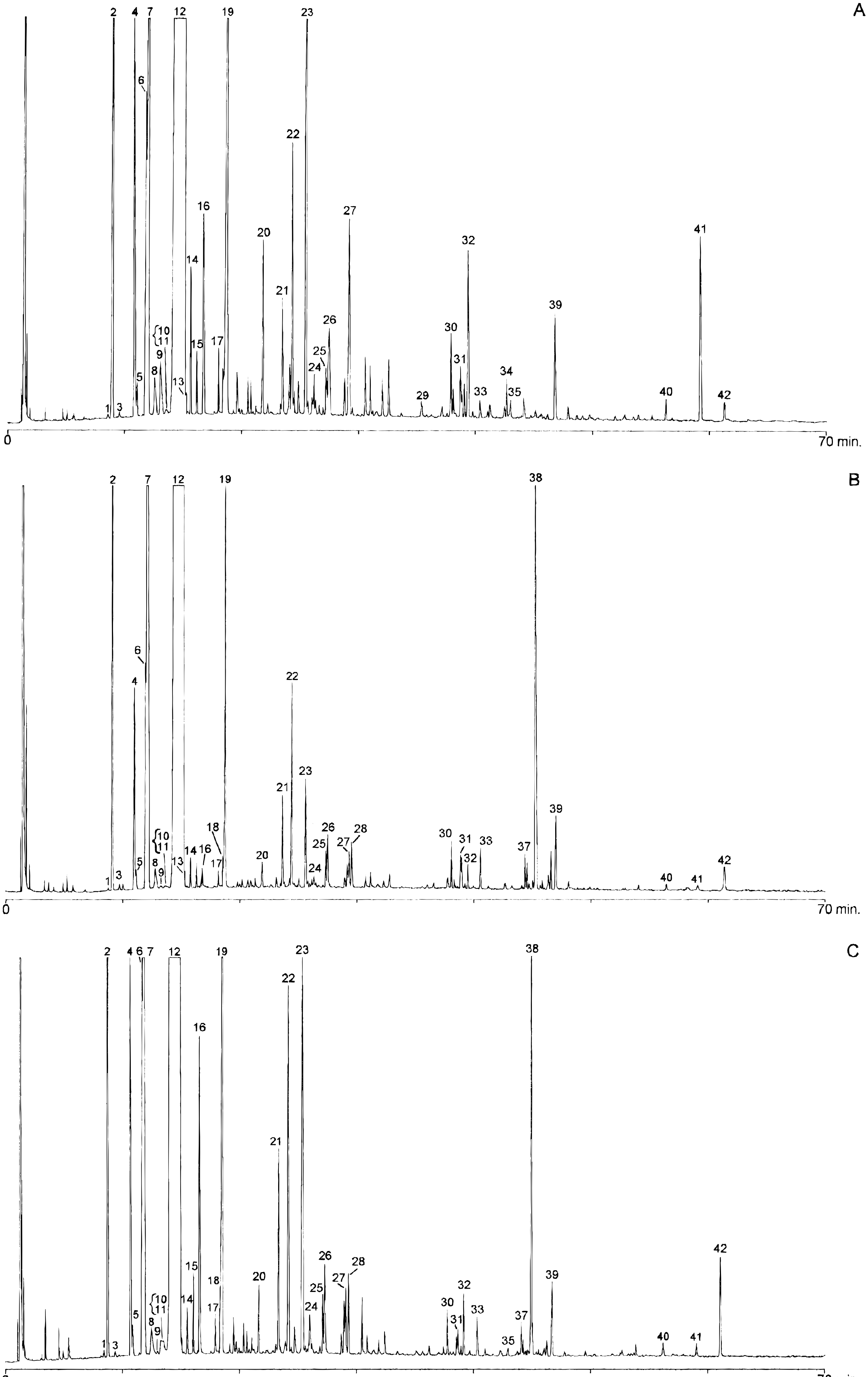
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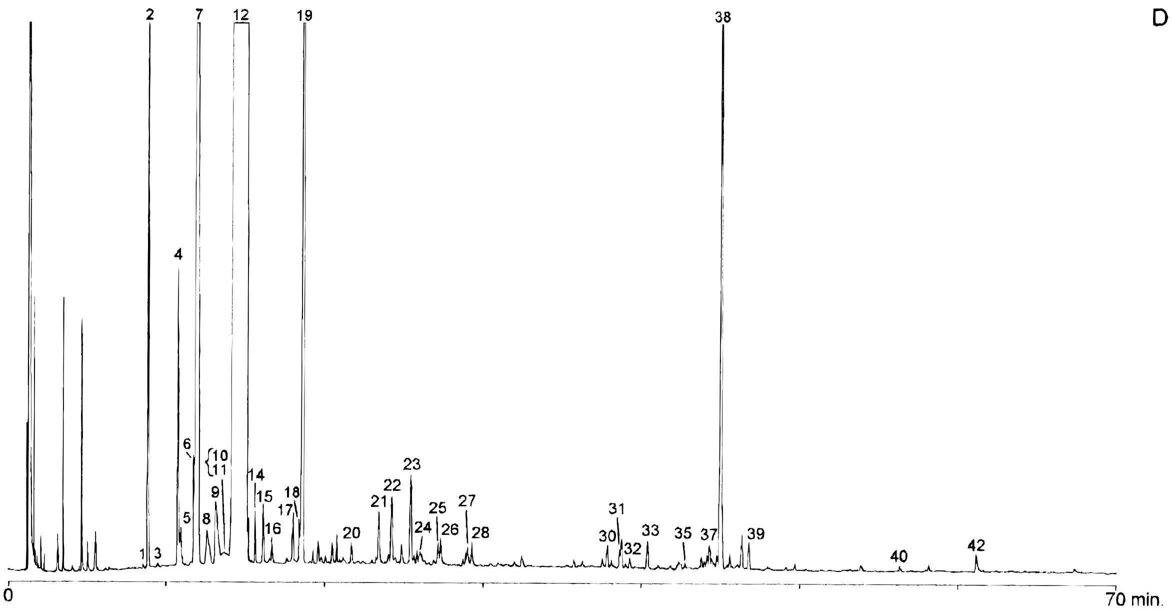

Figure 1. GC-FID profiles of the essential oils of *C. clementina* (A), *C. sinensis* (B), A146 hybrid (C), and C1867 hybrid (D).

Plant Material. Fruits of hybrids (A146 and C1867) and parents were collected in January 1995. All trees were cultivated in the experimental field Palazzelli of the Citrus Experimental Institute (Istituto Sperimentale per l'Agrumicoltura), Lentini, Sicily.

Isolation and Analysis of Essential Oils. Fresh rind tissue (flavedo, 10 g) of each sample was subjected to simultaneous steam distillation–extraction (SDE) for 3 h with a modified Likens–Nickerson apparatus using a 1:1 mixture of pentane:diethyl ether as the solvent. The essential oil solutions were immediately analyzed on a Hewlett-Packard gas chromatograph model 5890 equipped with a flame ionization detector and coupled to an electronic integrator. Analytical conditions: HP-1 dimethylpolysiloxane capillary column (25 m × 0.2 mm), helium as carrier gas, injector and detector temperatures 250 and 270 °C, respectively. The oven temperature was held at 60 °C for 6 min, then programmed from 60 to 250 °C at 3 °C/min. GC/MS analyses were carried out on the same chromatograph equipped with a Hewlett-Packard MS computerized system, model 5971A, ionization voltage 70 eV, electron multiplier 1700 V, ion source temperature 180 °C, GC conditions same as above.

Identification of components was based on GC retention times (Jennings and Shibamoto, 1980), computer matching with the NBS library, comparison of the fragmentation patterns with those reported in the literature (Jennings and Shibamoto, 1980; Eight Peak Index, 1974), and, whenever possible, co-injection with authentic samples.

Pure standards were purchased from Aldrich Chemicals Co., U.S., Extrasynthese, France, and Fluka Chemie AG, Switzerland.

RESULTS AND DISCUSSION

The GC-FID profiles of the essential oils from fruits of the two hybrids *C. clementina* × *C. sinensis* (A146 e C1867) and those of the parents are shown in Figure 1. Table 1 lists the 42 components identified, grouped into four classes (monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpenes, and aliphatic aldehydes/alcohols).

As previously observed (Dugo et al., 1990; Moshonas et al., 1991; Ruberto et al., 1993, 1994; Scora et al., 1970; Soost, 1987; Soost and Cameron, 1980, 1985), the chemical composition of the essential oils of hybrids appears as the superimposition of those of the parent species, the composition of an individual hybrid being closer to one or the other parent. Limonene is the main component, representing about 95% of the total oils of both hybrids and parents. Among the additional components of the monoterpene hydrocarbon class, no significant hybrid–hybrid or hybrid–parent difference is observed. Only β-pinene, α-terpinene, β-phellandrene, and γ-terpinene are present in slightly higher amounts in both hybrids. The same holds true for α-pinene, myrcene, and 3-carene of C1867 hybrid.

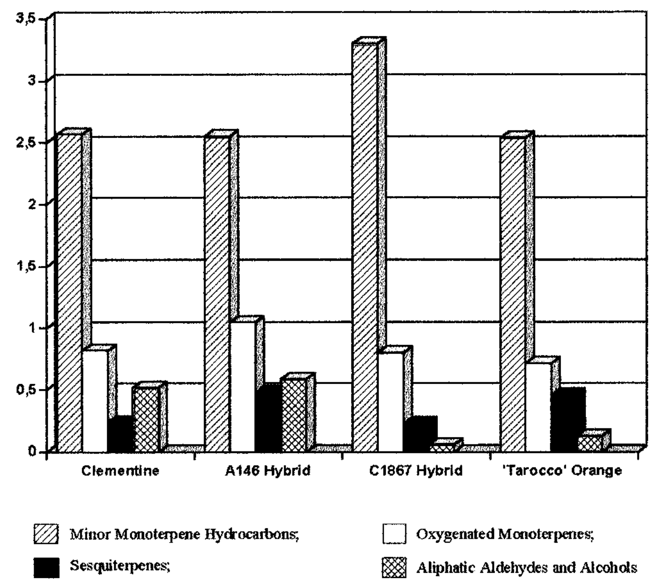

Figure 2. Histograms of the percentage composition of four classes of components of the essential oils of clementine, orange, and the two hybrids. Limonene has been omitted. Bars from left to right: minor monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpenes, and aliphatic aldehydes and alcohols.

The relevant differences between the oils are concentrated in the remaining classes of substances, particularly the oxygenated compounds (terpenoids and aliphatic compounds). The histograms in Figure 2 illustrate the different content of oxygenated monoterpenes, sesquiterpenes, and aliphatic aldehydes and alcohols in the four fruits.

Concerning the oxygenated monoterpenes, the A146 hybrid shows a higher content of these compounds than

Table 1. Chemical Composition of Clementine, Orange and New Hybrid Essential Oils[a]

peak no.	compounds	*C. clementina*	*C. clementina* × *C. sinensis* A 146	*C. clementina* × *C. sinensis* C 1867	*C. sinensis*
	monoterpene hydrocarbons	**98.03**	**97.30**	**98.46**	**98.19**
	minor monoterpene hydrocarbons	**2.57**	**2.55**	**3.30**	**2.54**
1	α-thujene	t	t	t	t
2	α-pinene[b]	0.43	0.39	0.59	0.44
3	camphene[b]	t	t	t	t
4	sabinene[b]	0.15	0.20	0.25	0.13
5	β-pinene[b]	0.02	0.02	0.02	0.01
7	myrcene	1.82	1.81	2.19	1.86
8	α-phellandrene[b]	0.03	0.04	0.06	0.04
9	δ-3-carene[b]	0.05	t	0.09	t
10	α-terpinene[b]				
11	β-phellandrene[b]	t	0.02	0.02	0.01
12	limonene[b]	95.46	94.75	95.16	95.65
13	(*Z*)-β-ocimene	t	t	t	t
14	(*E*)-β-ocimene	0.03	0.02	0.02	0.02
15	γ-terpinene[b]	0.02	0.03	0.03	0.02
17	terpinolene[b]	0.02	0.02	0.03	0.01
	oxygenated monoterpenes	**0.82**	**1.05**	**0.80**	**0.72**
19	linalol[b]	0.53	0.58	0.69	0.44
20	citronellal[b]	0.05	0.04	0.01	0.01
21	terpinen-4-ol[b]	0.04	0.09	0.02	0.04
22	α-terpineol[b]	0.07	0.14	0.03	0.12
24	carveol[b]	0.01	0.02	t	0.01
25	carvone[b]	0.01	0.03	0.01	0.02
26	neral[b]	0.03	0.07	0.02	0.03
27	perillaldehyde[b]	0.07	0.04	0.01	0.02
28	geranial[b]	—	0.04	0.01	0.03
29	α-terpinyl acetate	0.01	—	—	—
	sesquiterpenes	**0.24**	**0.48**	**0.23**	**0.46**
30	α-copaene[b]	0.03	0.02	0.01	0.02
31	α-cubebene[b]	0.02	0.01	t	0.01
33	β-caryophyllene[b]	0.01	0.02	0.01	0.02
35	α-humulene[b]	0.01	0.01	t	t
36	β-farnesene	0.02	t	—	—
37	β-cubebene	0.02	0.02	t	0.02
38	valencene[b]	—	0.28	0.19	0.35
39	γ-cadinene	0.04	0.04	0.01	0.03
40	β-sinensal	0.01	0.01	t	t
41	α-sinensal	0.07	0.01	—	t
42	nootkatone	0.01	0.06	0.01	0.01
	aliphatic aldehydes and alcohols	**0.52**	**0.59**	**0.06**	**0.13**
6	octanal[b]	0.13	0.24	t	0.03
18	nonanal[b]	t	t	0.01	0.01
23	decanal[b]	0.27	0.22	0.04	0.06
32	dodecanal[b]	0.05	0.03	t	0.01
34	2-dodecenal[c]	0.01	—	—	—
16	1-octanol[b]	0.06	0.10	0.01	0.02

[a] The numbering refers to elution order (Figure 1), and values (area percent) represent averages of three determinations (t = trace < 0.01). [b] Co-injection with authentic sample. [c] Correct isomer not identified.

the parents, with a pattern similar to that of orange, but the amount of each compound is higher. The C1867 hybrid has a composition qualitatively similar, apart from an "anomalous" amount of linalol that alone represents the 85% of the oxygenated monoterpenes class (Table 1).

Regarding the sesquiterpenes, the greater influence of the orange parent is evident (Table 1). α-Sinensal, the main and characteristic sesquiterpene of clementine oil, is present only in the A146 hybrids' oil, whereas valencene, the most abundant component of orange oil (83% ca. of sesquiterpenes), is the main sesquiterpene in both hybrids (75% and 58% of C1867 and A146 hybrids, respectively). A further significant and differentiating aspect between the hybrids' oils is the presence, in the oil of A146 hybrid, of nootkatone, 6-fold greater than parents and C1867 hybrid.

The last class of compounds here analyzed, aliphatic aldehydes and alcohols, shows a very different behavior in the hybrids' oils, contributing to the differences in their aroma. 2-Dodecenal is present only in clementine oil, while the remaining components are the same in all the oils, but whereas the A146 hybrid has a quantitative composition similar to that of clementine, the C1867 hybrid shows a total amount of these compounds lower than that of orange oil.

The introduction of new Citrus hybrids together with the genetic improvement of extant cultivars is, in our opinion, among the most innovative developments in this important agronomic field. By focusing the attention on the high fruit quality, it is possible to satisfy consumer requirements and to obtain a high qualitative standard for industrial processing. In this context the hybrids here analyzed, which can be considered as two new orange-like pigmented species, despite being relatively recent, are experiencing a good reception both by producers and in the market.

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