

## Composition and Seasonal Variation of the Essential Oils from Two Mandarin Cultivars of Southern Brazil

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Cold-pressing and hydrodistilled peel oils of two Brazilian mandarin cultivars, commonly called Cai and Montenegrina (*Citrus deliciosa* Tenore), were obtained from fruits collected on mandarin trees submitted to the same pedoclimatic and cultural conditions. Their chemical composition and seasonal variation of the main volatile constituents were investigated by capillary GC and GC-MS, and the results were submitted to statistical analysis. To better characterize the oils, the enantiomeric distribution ratio of seven components ( $\alpha$ -pinene, sabinene,  $\beta$ -pinene, limonene, linalool, terpinen-4-ol, and  $\alpha$ -terpineol) was determined by multidimensional gas chromatography (MDGC). The similarities found between both *C. deliciosa* cultivars are strong evidence for the hypothesis that Montenegrina originated from Cai as previously reported from agronomic studies.

**KEYWORDS:** Rutaceae; *Citrus deliciosa*; mandarin; Cai; Montenegrina; peel oil; GC; GC/MS; multidimensional gas chromatography (MDGC); statistical analysis

### INTRODUCTION

Oranges, limes, lemons, grapefruits, and pummelos are classified in three genera, *Citrus*, *Poncirus*, and *Fortunella* (Rutaceae, Aurantoidea) (1). Various *Citrus* fruits comprise one of the most important tree crops in Brazil, which is the world's largest producer of oranges and ranks fourth in the production of mandarins, after China, Spain, and Japan, with an annual production estimated at 770,000 tons in 2000 and a planted area of 31000 ha (2, 3). However, despite the high diversity presented by this citrus fruit, only a small number of cultivars are used commercially (4). Mandarins are cultivated mainly in the southern region of Brazil (Rio Grande do Sul State), where they represent an important and strategical economic resource with strong social implications, associated with a small-property culture system (an average of 2 ha), covering an area estimated as a whole in 12000 ha (2, 3).

The mandarin is the largest and most varied group of edible citrus (5, 6), being classified according to Tanaka into more than 30 species, comprising from one to several tens of varieties. Cultivars of mandarin present a great diversity of morphological and horticultural characters. Furthermore, mandarins are the most phenotypically heterogeneous group in *Citrus*; both monoembryonic and polyembryonic clones exist, as do self-fertile and self-incompatible types (7). Sweet mandarin types have been used for dessert fruit since ancient times, and sour types have been used as rootstocks and for flavorings and medicine. Thus, it is difficult to assess the relative importance

of genetic versus mutational variation in the complex history of this species.

The study of the dependence of citrus oil composition on variables that affect the raw plant material, such as freshness, climate, location, and harvest time, is a necessary step in the development of their production on a large scale. Furthermore, only a detailed knowledge of the composition of the oils obtained by different cultivars of mandarin can provide the basis on which to choose the cultivars that will produce the best oils (8–13). With this in mind, the joint research of our group concerns the genetic improvements of high-quality cultivars and the production of new citrus fruits. In particular, our interest has been focused on the study of the chemical composition of new mandarin varieties with the aim of phytochemical characterization. To exemplify this topic, we report the composition and seasonal variation of the main volatile constituents of the most important cultivars of mandarin, commonly called Cai and Montenegrina, growing in southern Brazil (*Citrus deliciosa* Tenore) (14) in order to elucidate their aroma profiles.

In our opinion, the oils of the Cai and Montenegrina mandarins currently hold promise as a trend-setting innovation as these cultivars are only rarely known on the fresh fruit market outside Brazil. In addition, no previous reports were found in the literature for the oils of these cultivars, so the compositions of these oils may be useful in taxonomic studies as well as in the identification of aromatic compounds of mandarin oils obtained commercially by both cold-pressing and steam distillation processes.

### MATERIALS AND METHODS

**Taxonomy of the Variety.** The mandarin plants used in the present study were of the cultivars Montenegrina and Cai (*Citrus deliciosa*

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Tenore) cultivated in a small scale experimental area located at Aripê Citrus, Montenegro (Brazil). Clonal propagated trees, grafted on *Poncirus trifoliata* rootstock, were 12 years old and grown in the same pedoclimatic and cultural conditions.

The mandarin cultivars were characterized for seed number per fruit and polyembryony according to the method of Rodrigues et al. (14). The plantation was managed under an organic approach with no additional levels of fertilization.

**Sampling.** Previous results have shown no significant difference of peel oil composition from different trees of the same cultivar for both mandarin cultivars (15). Moreover, the composition of peel oil of the same cultivar obtained from material collected from the same tree, each month, during the year was not significantly influenced by the vegetative stage of the tree. Consequently, for each cultivar of mandarin, at least 30 ripe fruits were collected twice a month from various positions on the same tree, early in the morning and in dry weather during the 2002 season (March–July).

**Essential Oil Extraction.** Essential oils from mandarin peels were isolated by both steam distillation and cold pressing. In the first case, fresh peels were subjected to hydrodistillation for 1 h using a Clevenger-type apparatus (16) according to the following procedure: the fruits were cleaned, and the peels were cut and distilled separately for 1 h. Cold-pressing essential oils were obtained from the peels of 2.0–2.5 kg of mandarins, and then the oil was separated from the crude extract by centrifugation (10 min at 15000 rpm). In addition, in both cases, oil samples were collected, dried over anhydrous sodium sulfate, and stored under nitrogen in vials at  $-18\text{ }^{\circ}\text{C}$  until their analysis. Two extractions were performed for each type of oil extraction.

**HRGC Analysis.** The composition of the oil was carried out by GC on a Shimadzu 14 B gas chromatograph equipped with a FID and a Shimadzu data processor software EZ-Chrom, using two capillary columns. The first was an SE-52 (Mega, Legnano, Italy) cross-linked fused-silica capillary column (25 m  $\times$  0.32 mm i.d.), coated with 5% phenyl-polymethylsiloxane (0.40–0.45  $\mu\text{m}$  phase thickness); column temperature, 60  $^{\circ}\text{C}$  for 8 min, rising to 180  $^{\circ}\text{C}$  at 3  $^{\circ}\text{C}/\text{min}$  and to 250  $^{\circ}\text{C}$  at 20  $^{\circ}\text{C}/\text{min}$ , then held at 250  $^{\circ}\text{C}$  for 10 min; injector temperature, 250  $^{\circ}\text{C}$ ; detector temperature, 280  $^{\circ}\text{C}$ ; injection mode, split; split ratio, 1:30; carrier gas, hydrogen at 55 kPa (30.4 cm/s). The second was a Carbowax 20M (Ohio Valley Specialty Chemicals, Marietta, OH) bonded fused-silica capillary column (25 m  $\times$  0.32 mm i.d.), coated with poly(ethylene glycol) (0.25  $\mu\text{m}$  phase thickness); column temperature, 40  $^{\circ}\text{C}$  for 8 min, rising to 180  $^{\circ}\text{C}$  at 3  $^{\circ}\text{C}/\text{min}$ , then to 230  $^{\circ}\text{C}$  at 20  $^{\circ}\text{C}/\text{min}$ ; injector temperature, 250  $^{\circ}\text{C}$ ; detector temperature, 250  $^{\circ}\text{C}$ ; injection mode, split; split ratio, 1:30; carrier gas, hydrogen at 30 kPa (32.3 cm/s). The injection volume was 0.5  $\mu\text{L}$  of a 20% (v/v) solution of mandarin oil in dichloromethane (chromatography grade reagent, Aldrich, Milwaukee, WI), using *n*-tetradecane (reference substance for gas chromatography, Aldrich) as an internal standard. Peak areas from different chromatograms were compared after they had been normalized with this standard.

**HRGC-MS Analysis.** GC-MS analyses were conducted using a Shimadzu QP 5050 apparatus equipped with reference libraries (17, 18) using two capillary columns. The first was an SE-52 (Mega) cross-linked fused-silica capillary column (25 m  $\times$  0.25 mm i.d.), coated with 5% phenyl-polymethylsiloxane (0.25  $\mu\text{m}$  phase thickness); column temperature, 60  $^{\circ}\text{C}$  for 8 min, rising to 180  $^{\circ}\text{C}$  at 3  $^{\circ}\text{C}/\text{min}$ , then to 230  $^{\circ}\text{C}$  at 20  $^{\circ}\text{C}/\text{min}$ ; injector temperature, 250  $^{\circ}\text{C}$ ; injection mode, split; split ratio, 1:40; volume injected, 0.2  $\mu\text{L}$  of a 20% (v/v) solution of mandarin oil in dichloromethane (chromatography grade reagent, Aldrich). carrier gas, helium at 122.2 kPa (51.6 cm/s); interface temperature, 250  $^{\circ}\text{C}$ ; acquisition mass range, *m/z* 40–400. The second was a BP 20 (SGE, Ringwood, Australia) bonded fused-silica capillary column (25 m  $\times$  0.25 mm i.d.), coated with poly(ethylene glycol) (0.25  $\mu\text{m}$  phase thickness); column temperature, 40  $^{\circ}\text{C}$  for 8 min, rising to 180  $^{\circ}\text{C}$  at 3  $^{\circ}\text{C}/\text{min}$ , then to 230  $^{\circ}\text{C}$  at 20  $^{\circ}\text{C}/\text{min}$ ; injector temperature, 250  $^{\circ}\text{C}$ ; injection mode, split; split ratio, 1:40; volume injected, 0.2  $\mu\text{L}$  of a 20% (v/v) solution of mandarin oil in dichloromethane (chromatography grade reagent, Aldrich); carrier gas, helium at 92.6 kPa (55.9 cm/s); interface temperature, 250  $^{\circ}\text{C}$ ; acquisition mass range, *m/z* 40–400.

**Identification and Quantification.** The components of the oil were identified by comparison of their linear retention indices (LRIs) on the two columns, determined in relation to a homologous series of *n*-alkanes, with those from pure standards or reported in the literature (18, 19). Pure standards were purchased from Sigma-Aldrich Corp., Milwaukee, WI (compounds for peaks 2, 3, 5–9, 11, 13, 16, 17, 20, 22, 24–28, 30, 32–34, 36–39, 41, and 43 as listed in **Tables 1** and **2**); Extrasynthese, Genay Cedex, France (compounds for peaks 4, 14, 21, 31, 40, and 47–48); and International Flavors & Fragrances Inc., New York (compound for peak 55). Comparison of fragmentation patterns in the mass spectra with those stored on the GC-MS database (17, 18) and reported in the literature (18) was also performed. For quantitative evaluation, all GC peak areas were compared relative to the internal standard (*n*-tetradecane). Repeatability of the measuring system showed variation coefficients under 5% for all of the components reported in **Tables 1** and **2**.

**Chiral Analysis.** Enantiomeric ratios of  $\alpha$ -pinene, sabinene,  $\beta$ -pinene, limonene, linalool, 4-terpineol, and  $\alpha$ -terpineol were obtained by multidimensional GC, using a developmental model (20) set up with two GC ovens. The first oven was equipped with a column coated with SE-52 and the second with a chiral column coated with a derivatized  $\beta$ -cyclodextrin, a hot interface, a rotary switching valve, and a system to maintain a constant flow during the transfer. With this system a heart-cut of the relevant fractions can be made and transferred from the nonchiral column to the chiral one in the following experimental conditions: precolumn, SE-52 (Mega) cross-linked fused-silica capillary column (30 m  $\times$  0.32 mm i.d.), coated with 5% phenyl-polymethylsiloxane (0.40–0.45  $\mu\text{m}$  phase thickness); injection temperature, 250  $^{\circ}\text{C}$ ; column temperature, 45  $^{\circ}\text{C}$  for 6 min, rising to 280  $^{\circ}\text{C}$  at 2  $^{\circ}\text{C}/\text{min}$ , held at 280  $^{\circ}\text{C}$  for 15 min; analytical column, fused-silica capillary column (25 m  $\times$  0.25 mm i.d., 0.25  $\mu\text{m}$  phase thickness), coated with 2,3-di-*O*-ethyl-6-*O*-*tert*-butyldimethylsilyl- $\beta$ -cyclodextrin in PS 086 (13% phenylmethyl-polysiloxane) (Mega); column temperature, 50  $^{\circ}\text{C}$  for 6 min, rising to 90  $^{\circ}\text{C}$  at 2  $^{\circ}\text{C}/\text{min}$ , held at 90  $^{\circ}\text{C}$  for 20 min, rising from 90 to 180  $^{\circ}\text{C}$  at 2  $^{\circ}\text{C}/\text{min}$ , held at 180  $^{\circ}\text{C}$  for 10 min; interface temperature, 200  $^{\circ}\text{C}$ ; detector FID, 280  $^{\circ}\text{C}$  (for both chromatographs); volume injected, 1  $\mu\text{L}$  of an oil dilution 1:10 in *n*-hexane; injection mode, split; split ratio, 1:15; carrier gas, helium at 90 kPa (precolumn) or 110 kPa (analytical column). The enantiomers of  $\alpha$ -pinene,  $\beta$ -pinene, limonene, linalool, and  $\alpha$ -terpineol were assigned by injection of enantiomerically pure standards purchased from Sigma-Aldrich (Sigma-Aldrich Corp.). The order of elution of all the enantiomers, including sabinene and 4-terpineol, was checked—under the same chromatographic conditions—as previously described (21).

**Statistical Analysis.** For the statistical analysis, an analysis of variance (ANOVA) was used. All statistical analyses were performed using the Statistica 5.1 software (StatSoft, Inc., 1998).

## RESULTS AND DISCUSSION

**Extraction Process.** A real comparative study of the composition of peel by the two oil extraction processes proposed for two cultivars of *C. deliciosa* could be carried out because all trees are grown in the same pedoclimatic and cultural conditions. Similarly, extraction conditions were identical for all samples (see Materials and Methods). Therefore, we attempted to minimize the influence of environmental and technical parameters on the chemical composition of the essential oils under the reported experimental conditions.

To minimize variations from the native composition, one of the preferable methods for preliminary separation of citrus peel oil would be a cold-pressing preparation, rather than separation by means of distillation and liquid extraction. However, this study was designed to investigate the volatile constituents of the oils obtained through hydrodistillation by comparison with the cold-pressed oils, keeping in mind the commercial importance of the distilled mandarin oil production in southern Brazil. The reported data are averages of two extractions for each type of oil extraction. The average yields for mandarin oils obtained by steam distillation and cold pressing were 0.45

**Table 1.** Percentage Composition as Classes of Substances and as Single Components of Cai Mandarin Oils and Linear Retention Index (LRI) of Its Components

peak	compound	LRI <sup>a</sup>		identity assignment <sup>b</sup>	hydrodistilled, <sup>c</sup> %				cold-pressed, <sup>c</sup> %			
		SE-52	Carbowax 20M		March	April	May	June	March	April	May	June
1	$\alpha$ -thujene	921	1021	B	0.38	0.47	0.50	0.48	0.25	0.53	0.19	0.10
2	$\alpha$ -pinene	927	1015	A	1.04	1.34	1.44	1.35	0.71	1.53	0.57	0.26
3	camphene	940	1054	A	0.01	0.01	0.01	0.01	tr	0.01	tr	nd
4	sabinene	967	1118	A	0.17	0.19	0.19	0.18	0.14	0.17	0.12	0.11
5	$\beta$ -pinene	970	1102	A	1.01	1.07	1.11	1.07	0.85	1.17	0.70	0.50
6	$\beta$ -myrcene	989	1167	A	1.53	1.69	1.71	1.61	1.53	1.78	1.49	1.33
7	octanal	1003	nd <sup>d</sup>	A	0.35	0.31	0.25	0.38	0.19	0.19	0.19	0.06
8	$\alpha$ -terpinene	1015	1195	A	0.38	0.33	0.32	0.37	0.23	0.32	0.27	0.32
9	limonene	1034	1255	A	70.54	75.35	76.41	74.80	72.84	75.04	72.88	69.29
10	( <i>E</i> )- $\beta$ -ocimene	1049	1244	A	0.02	0.01	0.01	0.02	0.02	0.02	0.02	0.03
11	$\gamma$ -terpinene	1061	1472	A	15.80	13.76	13.76	14.16	18.03	15.41	17.55	19.52
12	<i>cis</i> -sabinene hydrate	1066	1568	A	0.04	0.06	0.03	0.04	0.07	0.05	0.07	0.09
13	1-octanol	1072	1278	A	0.21	0.11	0.07	0.06	0.01	tr	0.01	nd
14	$\alpha$ -terpinolene	1087	1554	A	0.83	0.67	0.66	0.69	0.84	0.73	0.89	1.11
15	<i>trans</i> -sabinene hydrate	1095	1560	B	0.11	0.14	0.07	0.07	0.17	0.12	0.19	0.16
16	linalool	1100	1394	A	0.69	0.47	0.34	0.52	0.23	0.12	0.22	0.26
17	nonanal	1105	nd	A	0.04	0.04	0.04	0.06	0.06	0.04	0.06	0.08
18	<i>cis</i> -pinene hydrate	1119	nd	B	0.05	0.04	0.03	0.04	tr	tr	0.01	nd
19	<i>p</i> -menth-2-en-1-ol	1133	1506	B	0.04	0.02	0.01	0.02	nd	nd	nd	nd
20	camphor	1140	1483	A	0.03	0.02	0.01	0.02	tr	tr	tr	nd
21	citronellal	1154	nd	A	0.04	0.04	0.04	0.07	0.04	0.05	0.08	0.10
22	1-borneol	1162	1604	A	0.02	0.01	0.01	0.01	tr	tr	tr	nd
23	4-terpineol	1174	1705	B	0.75	0.42	0.32	0.47	0.08	0.06	0.10	0.13
24	$\alpha$ -terpineol	1188	1501	A	1.46	0.91	0.61	0.80	0.41	0.29	0.53	0.52
25	decanal	1205	1846	A	0.11	0.11	0.11	0.15	0.14	0.12	0.22	0.30
26	<i>trans</i> -carveol	1216	nd	A	0.03	0.03	0.02	0.03	0.02	0.01	0.02	0.09
27	octyl acetate	1219	1782	A	0.02	0.04	0.02	0.02	tr	tr	tr	nd
28	$\beta$ -citronellol	1229	1597	A	0.20	0.13	0.10	0.12	0.12	0.03	0.07	0.04
29	thymol methyl ether	1234	1685	B	0.03	0.02	0.01	0.01	nd	nd	nd	nd
30	neral	1240	1722	A	0.06	0.04	0.03	0.05	0.02	0.01	0.02	nd
31	piperitone	1251	1863	A	0.02	0.01	0.01	0.01	tr	tr	tr	nd
32	geraniol	1255	1782	A	0.04	0.03	0.02	0.02	tr	tr	tr	nd
33	perilla aldehyde	1270	1737	A	0.27	0.21	0.16	0.25	0.10	0.08	0.14	0.16
34	geranial	1273	nd	A	0.04	0.02	0.01	0.02	tr	tr	tr	nd
35	<i>p</i> -cymen-7-ol	1287	nd	B	0.06	0.04	0.03	0.05	tr	0.02	0.04	0.05
36	thymol	1294	2242	A	0.67	0.31	0.20	0.27	0.17	0.16	0.23	0.32
37	carvacrol	1300	nd	A	0.01	nd	nd	nd	0.02	tr	0.02	0.05
38	undecanal	1307	1765	A	0.01	0.01	0.01	0.01	0.03	0.01	0.03	0.04
39	( <i>E,E</i> )-2,4-decadienal	1308	1667	B	tr	0.01	0.01	0.01	tr	tr	0.01	nd
40	citronellyl acetate	1358	1731	A	0.01	nd	nd	0.01	tr	tr	tr	nd
41	neryl acetate	1370	1480	A	tr	nd	0.01	0.01	tr	tr	tr	nd
42	$\alpha$ -copaene	1371	1764	B	0.02	0.01	0.01	nd	tr	tr	0.03	0.05
43	geranyl acetate	1386	1530	A	0.01	0.01	0.01	0.01	0.01	0.02	0.03	0.04
44	$\beta$ -cubebene	1388	nd	B	tr	nd	0.01	0.01	0.01	tr	0.01	nd
45	methyl <i>N</i> -methylanthranilate	1404	nd	B	1.45	0.82	0.59	0.89	0.71	0.41	0.89	1.05
46	dodecanal	1408	1583	B	0.02	0.02	0.03	0.04	0.03	0.04	0.06	0.16
47	$\beta$ -caryophyllene	1413	1658	A	0.10	0.06	0.05	0.05	0.13	0.11	0.17	0.27
48	$\alpha$ -humulene	1445	1863	A	0.01	0.01	0.01	0.01	0.02	0.01	0.05	0.06
49	3-dodecen-1-al	1465	nd	B	0.02	0.01	0.01	0.02	0.02	0.03	0.05	0.12
50	germacrene D	1470	1713	B	0.01	0.01	nd	nd	tr	0.02	0.02	0.03
51	$\alpha$ -selinene	1490	1753	B	0.05	0.03	0.03	0.03	tr	0.06	0.10	0.18
52	( <i>E,E</i> )- $\alpha$ -farnesene	1508	1743	B	0.22	0.14	0.15	0.18	0.51	0.31	0.51	0.84
53	$\delta$ -cadinene	1520	1927	B	0.01	0.01	0.01	0.01	0.01	0.02	0.03	0.05
54	tetradecanal	1600	nd	B	0.01	nd	0.01	nd	0.01	tr	0.01	0.03
55	$\alpha$ -sinensal	1752	nd	A	0.05	0.27	0.25	0.28	0.61	0.55	0.63	1.05
identified components					99.55	99.80	99.84	99.74	99.39	99.65	99.52	98.96
grouped components												
monoterpene hydrocarbons					91.71	94.87	96.12	94.72	95.44	96.71	94.68	92.57
O-containing monoterpenes					4.68	2.95	2.07	2.85	1.46	1.02	1.77	2.02
sesquiterpene hydrocarbons					0.92	0.53	0.51	0.56	1.29	1.08	1.55	2.53
O-containing sesquiterpenes					0.50	0.27	0.25	0.28	0.61	0.55	0.63	1.05
others					0.79	0.65	0.54	0.73	0.49	0.43	0.63	0.79
aldehydes					1.47	1.08	0.96	1.31	1.25	1.12	1.50	2.10
alcohols					4.34	2.63	1.82	2.44	1.23	0.81	1.44	1.62
esters					1.52	0.89	0.64	0.94	0.72	0.43	0.92	1.09
methyl <i>N</i> -methylanthranilate/ $\alpha$ -sinensal					2.90	3.04	2.35	3.27	1.16	0.75	1.41	1.00

<sup>a</sup> LRI based on a series of *n*-hydrocarbons reported according to their elution order on SE-52. <sup>b</sup> A, identities confirmed by comparing mass spectra and retention time with those of authentic standards supplied by Aldrich (Milwaukee, WI) and Extrasynthese (Genay Cedex, France);  $\alpha$ -sinensal (Sinensals, Nat.) was supplied by International Flavors & Fragrances (New York). B, identities tentatively assigned by comparing mass spectra with those obtained from the following literature: Adams, R. P. *Identification of Essential Oil Components by Gas Chromatography/Quadrupole Mass Spectroscopy*; Allured: Carol Stream, IL, 2001. McLafferty, F. W.; Stauffer, D. B. *The Wiley/NBS Registry of Mass Spectral Data*, 5th ed.; Wiley: New York, 1991. Verzera, A.; Trozzi, A.; Cotroneo, A.; Lorenzo, D.; Dellacassa, E. *Uruguayan Essential Oil*. 12. Composition of Nova and Satsuma Mandarin Oils. *J. Agric. Food Chem.* **2000**, *48*, 2903–2909. Dellacassa, E.; Rossini, C.; Lorenzo, D.; Moyna, P.; Verzera, A.; Trozzi, A.; Dugo, G. *Uruguayan essential oils. Part IV. Composition of lemon oil. Flavour Fragrance J.* **1997**, *12*, 247–255 (28). <sup>c</sup> For quantitative evaluation, all GC peak areas were compared relative to the internal standard (*n*-tetradecane). Repeatability of the measuring system showed variation coefficients under 5% for all of the components. <sup>d</sup> Not determined.

**Table 2.** Percentage Composition as Classes of Substances and as Single Components of Montenegrina Mandarin Oils and Linear Retention Index (LRI) of Its Components

peak	compound	LRI <sup>a</sup>		identity assignment <sup>b</sup>	hydrodistilled, <sup>c</sup> %				cold-pressed, <sup>c</sup> %			
		SE-52	Carbowax 20M		March	April	May	June	March	April	May	June
1	$\alpha$ -thujene	921	1021	B	0.45	0.48	0.44	0.48	0.21	0.54	0.23	0.13
2	$\alpha$ -pinene	927	1015	A	1.21	1.36	1.24	1.36	0.60	1.49	0.67	0.35
3	camphene	940	1054	A	0.01	0.01	0.01	0.01	tr	0.01	tr	
4	sabinene	967	1118	A	0.17	0.19	0.17	0.17	0.13	0.19	0.12	0.12
5	$\beta$ -pinene	970	1102	A	1.12	1.09	1.02	1.04	0.80	1.24	0.76	0.55
6	$\beta$ -myrcene	989	1167	A	1.47	1.70	1.61	1.62	1.47	1.72	1.53	1.42
7	octanal	1003	nd <sup>d</sup>	A	0.29	0.20	0.21	0.14	0.12	0.12	0.10	0.07
8	$\alpha$ -terpinene	1015	1195	A	0.42	0.32	0.34	0.33	0.23	0.22	0.23	0.31
9	limonene	1034	1255	A	67.14	75.26	75.24	75.80	70.35	73.32	73.64	71.44
10	( <i>E</i> )- $\beta$ -ocimene	1049	1244	A	0.02	0.01	0.01	0.01	0.02	0.01	0.01	0.02
11	$\gamma$ -terpinene	1061	1472	A	16.80	14.18	13.67	14.34	19.76	17.20	17.53	17.28
12	<i>cis</i> -sabinene hydrate	1066	1568	A	0.05	0.07	0.04	0.04	0.08	0.06	0.06	0.11
13	1-octanol	1072	1278	A	0.16	0.08	0.05	0.02	0.02	0.01	tr	tr
14	$\alpha$ -terpinolene	1087	1554	A	0.86	0.68	0.67	0.69	0.96	0.71	0.81	0.98
15	<i>trans</i> -sabinene hydrate	1095	1560	B	0.11	0.17	0.09	0.07	0.22	0.14	0.16	0.19
16	linalool	1100	1394	A	0.73	0.39	0.53	0.38	0.21	0.18	0.19	0.25
17	nonanal	1105	nd	A	0.04	0.03	0.03	0.03	0.04	0.03	0.03	0.06
18	<i>cis</i> -pinene hydrate	1119	nd	B	0.06	0.03	0.04	0.03	tr	tr	tr	nd
19	<i>p</i> -menth-2-en-1-ol	1133	1506	B	0.04	0.01	0.02	0.01	nd	nd	nd	nd
20	camphor	1140	1483	A	0.01	0.02	0.02	0.01	tr	tr	tr	nd
21	citronellal	1154	nd	A	0.04	0.03	0.04	0.04	0.03	0.02	0.04	0.11
22	1-borneol	1162	1604	A	0.02	0.01	0.01	nd	0.01	tr	tr	nd
23	4-terpineol	1174	1705	B	0.88	0.32	0.52	0.35	0.10	0.07	0.09	0.14
24	$\alpha$ -terpineol	1188	1501	A	1.69	0.76	0.98	0.68	0.55	0.33	0.45	0.59
25	decanal	1205	1846	A	0.10	0.09	0.097	0.08	0.12	0.09	0.13	0.24
26	<i>trans</i> -carveol	1216	nd	A	0.02	0.02	0.02	0.02	0.02	0.01	0.02	0.03
27	octyl acetate	1219	1782	A	0.05	0.03	0.02	0.02	tr	tr	tr	nd
28	$\beta$ -citronellol	1229	1597	A	0.21	0.13	0.17	0.12	0.15	0.12	0.07	0.11
29	thymol methyl ether	1234	1685	B	0.03	0.02	0.01	0.01	nd	nd	nd	nd
30	neral	1240	1722	A	0.06	0.04	0.04	0.03	0.02	0.01	0.01	0.03
31	piperitone	1251	1863	A	0.02	0.01	0.01	0.01	tr	tr	tr	nd
32	geraniol	1255	1782	A	0.03	0.02	0.01	0.01	tr	tr	tr	nd
33	perilla aldehyde	1270	1737	A	0.32	0.21	0.28	0.19	0.15	0.11	0.16	0.23
34	geranial	1273	nd	A	0.03	0.02	0.01	0.02	tr	tr	tr	nd
35	<i>p</i> -cymen-7-ol	1287	nd	B	0.07	0.04	0.07	0.05	0.04	0.03	0.04	0.04
36	thymol	1294	2242	A	0.47	0.18	0.20	0.13	0.18	0.09	0.12	0.24
37	carvacrol	1300	nd	A	0.01	tr	tr	tr	0.02	0.02	0.02	0.03
38	undecanal	1307	1765	A	0.01	0.01	0.01	0.01	0.02	0.02	0.02	0.03
39	( <i>E,E</i> )-2,4-decadienal	1308	1667	B	tr	tr	0.01	nd	tr	tr	tr	nd
40	citronellyl acetate	1358	1731	A	0.01	nd	nd	0.01	0.01	tr	tr	nd
41	neryl acetate	1370	1480	A	tr	nd	0.01	nd	0.01	tr	tr	nd
42	$\alpha$ -copaene	1371	1764	B	0.01	0.01	0.01	0.01	0.01	tr	0.04	0.08
43	geranyl acetate	1386	1530	A	0.01	0.01	0.01	0.01	0.03	0.01	0.04	0.09
44	$\beta$ -cubebene	1388	nd	B	tr	nd	nd	nd	0.02	nd	tr	nd
45	methyl <i>N</i> -methylanthranilate	1404	nd	B	3.36	1.10	1.28	0.93	1.27	0.67	0.93	1.06
46	dodecanal	1408	1583	B	0.02	0.02	0.02	0.03	0.03	0.02	0.04	0.15
47	$\beta$ -caryophyllene	1413	1658	A	0.09	0.06	0.04	0.05	0.22	0.10	0.16	0.25
48	$\alpha$ -humulene	1445	1863	A	0.01	0.01	0.01	0.01	0.03	0.02	0.03	0.05
49	3-dodecen-1-al	1465	nd	B	0.02	0.01	0.02	0.02	0.02	0.02	0.03	0.12
50	germacrene D	1470	1713	B	0.01	0.01	nd	0.01	0.02	tr	0.03	0.07
51	$\alpha$ -selinene	1490	1753	B	0.06	0.05	0.07	0.04	0.13	0.07	0.13	0.30
52	( <i>E,E</i> )- $\alpha$ -farnesene	1508	1743	B	0.25	0.17	0.15	0.22	0.44	0.30	0.36	0.71
53	$\delta$ -cadinene	1520	1927	B	0.01	0.01	0.01	0.01	0.03	0.01	0.04	0.10
54	tetradecanal	1600	nd	B	tr	nd	nd	nd	0.01	tr	tr	nd
55	$\alpha$ -sinensal	1752	nd	A	0.45	0.23	0.19	0.23	0.42	0.26	0.39	0.82
	identified components				99.53	99.80	99.75	99.82	99.33	99.59	99.46	98.91
	grouped components											
	monoterpene hydrocarbons				89.67	95.25	94.42	95.83	94.53	96.65	95.53	92.61
	O-containing monoterpenes				4.97	2.49	3.15	2.19	1.83	1.20	1.47	2.19
	sesquiterpene hydrocarbons				0.89	0.54	0.47	0.56	1.32	0.76	1.18	2.38
	O-containing sesquiterpenes				0.45	0.22	0.19	0.23	0.42	0.26	0.39	0.82
	others				0.64	0.43	0.44	0.32	0.38	0.31	0.35	0.67
	aldehydes				0.94	0.64	0.76	0.58	0.58	0.46	0.58	1.07
	alcohols				4.55	2.20	2.74	1.88	1.60	1.06	1.22	1.73
	esters				3.38	1.11	1.29	0.95	1.32	0.68	0.97	1.15
	methyl <i>N</i> -methyl anthranilate/ $\alpha$ -sinensal				7.47	4.79	6.64	3.97	3.02	2.58	2.38	1.30

<sup>a</sup> LRI based on a series of *n*-hydrocarbons reported according to their elution order on SE-52. <sup>b</sup> A, identities confirmed by comparing mass spectra and retention time with those of authentic standards supplied by Aldrich (Milwaukee, WI) and Extrasynthese (Genay Cedex, France);  $\alpha$ -sinensal (Sinensals, Nat.) was supplied by International Flavors & Fragrances (New York). B, identities tentatively assigned by comparing mass spectra with those obtained from the following literature: Adams, R. P. *Identification of Essential Oil Components by Gas Chromatography/Quadrupole Mass Spectroscopy*; Allured: Carol Stream, IL, 2001. McLafferty, F. W.; Stauffer, D. B. *The Wiley/NBS Registry of Mass Spectral Data*, 5th ed.; Wiley: New York, 1991. Verzera, A.; Trozzi, A.; Cotroneo, A.; Lorenzo, D.; Dellacassa, E. *Uruguayan Essential Oil*. 12. Composition of Nova and Satsuma Mandarin Oils. *J. Agric. Food Chem.* **2000**, *48*, 2903–2909. Dellacassa, E.; Rossini, C.; Verzera, A.; Trozzi, A.; Dugo, G. *Uruguayan essential oils. Part IV. Composition of lemon oil. Flavour Fragrance J.* **1997**, *12*, 247–255 (28). <sup>c</sup> For quantitative evaluation, all GC peak areas were compared relative to the internal standard (*n*-tetradecane). Repeatability of the measuring system showed variation coefficients under 5% for all components. <sup>d</sup> Not determined.

and 0.60% (w/w), respectively, for all samples collected during the 2002 season.

**Identification and Quantification of Mandarin Essential Oils.** Whereas the essential oils extracted by either steam distillation or cold-pressing from mandarin fruit peel differed considerably both qualitatively and quantitatively, the oils extracted from the fruits at different stages of maturity changed only quantitatively (Tables 1 and 2). The percent composition of single components for Cai and Montenegrina mandarins is reported in Tables 1 and 2, and a classification based on functional groups is also summarized. The data are mean values of eight analyses performed during the whole season. As a whole, 55 compounds, representing ~99.8% of the GC profile for both varieties and extraction methods, were identified. The composition for both mandarin varieties showed similar patterns at different stages. In agreement with earlier reports for other mandarin varieties (7–11, 22–26), monoterpene hydrocarbons accounted for 90–97% of the oils; limonene,  $\gamma$ -terpinene,  $\beta$ -myrcene,  $\alpha$ -pinene, and  $\beta$ -pinene were the major components. Other monoterpene hydrocarbons occurred only in small amounts (<0.5%). The sesquiterpene hydrocarbons, including  $\beta$ -caryophyllene and (*E,E*)- $\alpha$ -farnesene as the main components, accounted for 0.36–2.51%. The main aldehydes were  $\alpha$ -sinensal (0.19–1.05%), octanal (0.06–0.38%), perilla aldehyde (0.08–0.32%), and decanal (0.08–0.30%). Monoterpene alcohols were the most abundant oxygenated compounds in both mandarins, and 4-terpineol, linalool, and  $\alpha$ -terpineol were the predominant components. The total ester content of Montenegrina oils was higher than that found in Cai oils, methyl *N*-methylantranilate being the major ester.

Concerning the qualitative and quantitative composition, on the whole, the composition of the essential oil of Montenegrina mandarin is close to that of Cai, and the contribution of methyl *N*-methylantranilate, linalool,  $\alpha$ -sinensal, and the ester composition explains their pleasant aroma as previously reported (27). Particular mention has to be ascribed to the value of the ratio between methyl *N*-methylantranilate and  $\alpha$ -sinensal, the values of which were dependent on the growing season but with higher values for Montenegrina cultivar, indicating also a possible index to distinguish the oils from both cultivars of mandarin.

**Effect of Extraction Method and Seasonal Differentiation of Mandarin Essential Oil Constituents.** Another issue facing the industry is determining what constitutes seasonal variation (23, 24). In our sampling, significant differences among mandarin cultivars and time sampling were assessed for hydrodistilled oils using a two-way ANOVA (two mandarin cultivars  $\times$  four sampling periods). The results indicated that a large number of compounds showed significant differences for the different sampling periods. Moreover, seven compounds also showed significant differences between the cultivars considered [octanal, nonanal, decanal, geraniol, (*E,E*)-2,4-decadienal, (*E,E*)-farnesene, and  $\alpha$ -sinensal].

When the same statistical model was applied to data from cold-pressed oils, the results showed significant differences among mandarin cultivars and time sampling for the same compounds. The data show that the aldehyde compounds were the most significant. These results demonstrated that the extraction methods proposed make no difference in establishing significant differences among mandarin cultivars when using the data of each essential oil composition.

**Enantiomeric Analysis.** Enzymatic reactions are commonly characterized by a high degree of stereospecificity; thus, the enantiomeric distribution of the components of citrus essential oils can provide useful information on the genuineness of the oils, their quality, the extraction technique employed, the

**Table 3.** Enantiomeric Ratios for  $\alpha$ -Pinene, Sabinene,  $\beta$ -Pinene, Limonene, Linalool, 4-Terpeneol, and  $\alpha$ -Terpineol in Cold-Pressed Cai and Montenegrina Mandarin Oils<sup>a</sup>

	Cai				Montenegrina			
	March	April	May	June	March	April	May	June
1 <i>R</i> (+)- $\alpha$ -pinene	46.0	48.1	48.2	47.0	44.7	46.4	48.1	48.6
1 <i>S</i> (-)- $\alpha$ -pinene	54.0	51.9	51.8	53.0	55.3	53.6	51.9	51.4
1 <i>R</i> ,5 <i>R</i> (+)-sabinene	81.2	82.9	82.5	82.1	80.7	77.6	82.3	83.3
1 <i>S</i> ,5 <i>S</i> (-)-sabinene	18.8	17.1	17.5	17.9	19.3	22.4	17.7	16.7
1 <i>R</i> (+)- $\beta$ -pinene	98.8	98.9	98.8	98.8	98.5	98.8	98.8	98.7
1 <i>S</i> (-)- $\beta$ -pinene	1.2	1.1	1.2	1.2	1.5	1.2	1.2	1.3
4 <i>R</i> (+)-limonene	98.2	98.3	98.6	98.4	98.0	98.1	98.4	98.4
4 <i>S</i> (-)-limonene	1.8	1.7	1.4	1.6	2.0	1.9	1.6	1.6
3 <i>S</i> (+)-linalool	81.8	77.3	80.8	82.7	81.7	83.8	80.7	81.2
3 <i>R</i> (-)-linalool	18.2	22.7	19.2	17.3	18.3	16.2	19.3	18.8
4 <i>S</i> (+)-4-terpineol	11.1	10.8	12.7	12.4	11.1	10.7	11.0	11.8
4 <i>R</i> (-)-4-terpineol	88.9	89.2	87.3	87.6	88.9	89.3	89.0	88.2
8 <i>R</i> (+)- $\alpha$ -terpineol	27.3	30.9	29.8	29.2	25.9	27.6	29.9	30.7
8 <i>S</i> (-)- $\alpha$ -terpineol	72.7	69.1	70.2	70.8	74.1	72.4	70.1	69.3

<sup>a</sup> The order of elution of the different compounds and their enantiomers from the chiral column was as indicated in the table. Identities were confirmed by comparing retention time with those of authentic standards supplied by Aldrich (Milwaukee, WI). The order of elution of the enantiomers was checked under the same chromatographic conditions previously described by Lorenzo et al. (27).

**Table 4.** Enantiomeric Ratios for  $\alpha$ -Pinene, Sabinene,  $\beta$ -Pinene, Limonene, Linalool, 4-Terpeneol, and  $\alpha$ -Terpineol in Hydrodistilled Cai and Montenegrina Mandarin Oils<sup>a</sup>

	Cai				Montenegrina			
	March	April	May	June	March	April	May	June
1 <i>R</i> (+)- $\alpha$ -pinene	52.9	50.7	50.9	51.4	53.7	51.0	50.8	51.1
1 <i>S</i> (-)- $\alpha$ -pinene	47.1	49.3	49.1	48.6	46.3	49.1	49.2	49.0
1 <i>R</i> ,5 <i>R</i> (+)-sabinene	82.1	98.8	98.6	98.6	98.8	98.7	98.6	98.8
1 <i>S</i> ,5 <i>S</i> (-)-sabinene	17.9	1.3	1.4	1.4	1.2	1.3	1.4	1.3
1 <i>R</i> (+)- $\beta$ -pinene	82.1	83.3	83.5	83.0	80.6	73.8	83.2	82.9
1 <i>S</i> (-)- $\beta$ -pinene	17.9	16.8	16.5	17.1	19.4	26.3	16.8	17.1
4 <i>R</i> (+)-limonene	98.2	98.4	98.4	98.4	98.1	98.4	98.4	98.4
4 <i>S</i> (-)-limonene	1.8	1.7	1.6	1.6	1.9	1.6	1.6	1.6
3 <i>S</i> (+)-linalool	81.0	81.0	82.3	81.4	80.7	79.9	81.7	83.6
3 <i>R</i> (-)-linalool	19.0	19.0	17.7	18.7	19.3	20.2	18.3	16.4
4 <i>S</i> (+)-4-terpineol	26.9	26.1	26.4	27.1	26.6	24.8	26.6	26.2
4 <i>R</i> (-)-4-terpineol	73.1	73.9	73.6	72.9	73.4	75.3	73.4	73.8
8 <i>R</i> (+)- $\alpha$ -terpineol	28.7	31.7	31.7	31.7	28.1	31.5	31.7	31.2
8 <i>S</i> (-)- $\alpha$ -terpineol	71.3	68.3	68.3	68.4	71.9	68.6	68.3	69.1

<sup>a</sup> The order of elution of the different compounds and their enantiomers from the chiral column was as indicated in the table. Identities were confirmed by comparing retention time with those of authentic standards supplied by Aldrich (Milwaukee, WI). The order of elution of the enantiomers was checked under the same chromatographic conditions previously described by Lorenzo et al. (27).

geographical origin, and their biogenesis. Determination of the enantiomeric distribution of the components of citrus oils has been the object of numerous studies carried out by gas chromatography with chiral capillary columns coated with cyclodextrins. The best results have been obtained by multidimensional gas chromatography (MDGC), using two GC ovens. With this system a heart-cut of the relevant fractions can be made and these fractions transferred from the nonchiral column to the chiral one under the experimental conditions described above. Using this design, and in order to better characterize these oils, the enantiomeric ratio of seven components ( $\alpha$ -pinene, sabinene,  $\beta$ -pinene, limonene, linalool, terpinen-4-ol, and  $\alpha$ -terpineol) was determined by subsequent transfers during the same analysis using authentic samples of each enantiomer to determine their order of elution. Tables 3 and 4 report the enantiomeric ratios of the components analyzed.

It can therefore be concluded that the examination carried out on the essential oils of mandarins Cai and Montenegrina

allowed us to identify compounds that characterize these oils with respect to those reported from other species. Furthermore, the enantiomeric distribution of the selected compounds allows a chemotaxonomic characterization of both mandarin oils from Brazil. However, no significant differences were found among mandarin cultivars and time sampling for the enantiomeric distribution of the selected compounds using a two-way ANOVA (two mandarin cultivars  $\times$  four sampling periods). As indicated above, these results demonstrated that the extraction methods proposed make no difference in establishing significant differences among mandarin cultivars when using the enantiomeric distribution of selected compounds of each essential oil. The similarities found between the *C. deliciosa* cultivars considered are strong evidence for the hypothesis that Montenegrina originated from Cai as previously reported from agronomic studies (4, 14).

In conclusion, Cai and Montenegrina mandarin oils have chemical characteristics similar to those of the Italian mandarin oil, indicating the opportunity of processing these cultivars separately from others. Furthermore, by focusing the attention on high fruit quality, it is possible to satisfy consumer requirements and to obtain a high qualitative standard for industrial processing. In this context the mandarin cultivars here analyzed are expected to experience a good reception from both producers and the market.

#### ABBREVIATIONS USED

HRGC, high-resolution gas chromatography; GC, gas chromatography; FID, flame ionization detector; MDGC, multidimensional gas chromatography, MS, mass spectrometry.

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