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Characterization of Carrot Root Oil Arising from Supercritical Fluid Carbon Dioxide Extraction

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Carrot root oil (SCO), obtained by supercritical fluid carbon dioxide (SC-CO_2) extraction, was characterized and compared to a commercial carrot oil (MCO) and a virgin olive oil (VOO) (cv. Coratina). SCO showed much higher contents of carotenes, phenolics, waxes, phytosterols, and sesquiterpene and monoterpene volatiles. In SCO, the most prominent components present in the fully investigated analytical fractions (fatty acids, triglycerides, waxes, phytosterols, long-chain aliphatic alcohols, superior triterpene alcohols, and volatiles) were, respectively, linolenic acid, trilinolein, waxes C_{38}, β-sitosterol, campesterol and stigmasterol, 1-hexacosanol, 24-methylencycloartanol and cycloartenol, β-caryophyllene, α-humulene, α-pinene, and sabinene. In VOO, the major constituents of the above analytical classes were, respectively, oleic acid, trilinolein, waxes C_{36}, unsaturated volatile C_6 aldehydes (*trans*-2-hexenal most markedly), and the same prominent sterols and superior alcohols found in SCO. In MCO, which also contained a proportion of unknown plant oil, several components showed magnitudes that were lower compared to SCO but higher with respect to VOO. The last had the aliphatic and triterpene alcohol concentration higher compared to that of both SCO and MCO. Several chemometric methods, applied to different analytical data sets, proved to be effective in grouping the three oil kinds.

KEYWORDS: Carrot root oil; supercritical fluid carbon dioxide extraction; analytical composition; market carrot oil; virgin olive oil; chemometrics

INTRODUCTION

Carrot root (*Daucus carota* L.) contributes significantly to the overall agricultural income of several European countries and the United States. It is valuable for its good digestibility and high contents of provitamin A and other nutraceutical molecules. Both epidemiological and nutritional studies have pointed out its positive impact on human health (*1*). Among 38 edible vegetables and fruits tested for their dietetic value or contribution to nutrition, it has been ranked 10th and 7th, respectively (*2*). Such a functional food is considered to be the major dietary source of carotenes for humans, providing >17% of the total vitamin A requirements (*2*). According to some authors, vitamin A deficiency is the leading cause of blindness in children in developing countries (*3*). A carrot intake of only 100 g can satisfy the daily carotene demand for our organism, ensuring the bioavailability of this important provitamin (*1*−*3*). More than 600 carotenoids have been detected in this commodity, even though α- and β-carotenes (trans forms) are the most important compounds (*3*). These are endowed with 50 and 100% vitamin A activity [expressed as retinol activity equivalent (RAE)], respectively. Other quantitatively significant carotenoids are γ- and ξ-carotenes, lycopene, and zeacarotene (*3*, *4*). Two bioactive carotenoids, such as retinol and retinal, are biogenerated through rearrangement of the β-carotene, which is mediated by the cytostolic enzyme β-carotenoid-15,15-dioxygenase (*2*, *4*).

High concentrations of antioxidizing phenol compounds also occur in carrot. These are essentially hydroxycinnamic derivatives, among which prevail chlorogenic, *p*-hydroxybenzoic, and caffeic acids. Two bioactive phenol-like polyacetylenic compounds, falcarinol (caratoxina) and falcarindiol, are claimed to exert cytotoxic actions against human tumoral cells (*2*, *5*). The phenol fraction, noticeably 6-methoxymellein (isocoumarin), is largely responsible for the characteristic bitterness and harshness

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attributes, whereas the characteristic sweetness flavor is ascribable to the significant concentrations of sugars (α- and β-glucoses, fructose, sucrose, and possibly maltose) (*6*). Behind the complex aroma of carrot root are many monoterpene and sesquiterpene volatiles, including either hydrocarbon or oxygenate compounds. Among the latter, a compound called carotol (which also occurs largely in carrot seed) is present in significant amounts (*7*). In addition to the above compounds, 20 amino acids (the major ones being aspartic acid, α-alanine, serine, glutamic acid, glucosamine, arginine, valine, and threonine) as well as organic acids (malic, citric, pyroglutamic, and succinic acids) were detected (*8*).

This produce is mostly a fresh-consumed food crop, and only a minor proportion of the whole production is processed for exploitation in agrofood, pharmaceutical, nutraceutical, and cosmetic industries (especially for making preparations to protect the skin against diseases) (*9*). Carrot oil, extracted with hexane, is often used as a natural food colorant. An essential oil is produced from carrot seeds. Generally, carrot oils are mixed with jojoba, corn, sunflower, or other plant oils before marketing (*10*−*12*). Such carrot oils are high-added-value products; therefore, they deserve to be studied in depth in relation mainly to extraction yield and chemical composition (*13*−*15*). In this work, the supercritical fluid carbon dioxide (SC-CO_2) extraction method was investigated. The analytical composition of the oil (SCO) obtained by this procedure was well elucidated (by evaluating a large range of classes of components, both glyceridic and nonglyceridic) in comparison with that of a market carrot oil (MCO) as well as of a virgin olive oil (VOO). The last is an excellent reference product because of its recognized high nutritional and health values (*16*). To our knowledge, no similar original investigations on this topic have been carried out so far. The results of this study should provide stakeholders with an industrial nonconventional extraction procedure to recover the valuable oil fraction from carrot root.

MATERIALS AND METHODS

Chemicals. These were mostly of chromatographic grade and were almost all supplied by Farmitalia C. Erba (Milan, Italy). Bis(trimethylsilyltrifluoroacetamide) and resorcinol for capillary gas chromatography analysis (cGC) of individual phenols, as well as Folin−Ciocalteu reagent for colorimetric analysis of total phenols, and silica gel 60 for cGC analysis of waxes were provided by Merck (Darmstadt, Germany). Hexane and chloroform for cGC analysis of both sterols and waxes were obtained from Baker (Deventer, Holland). Pyridine for cGC analysis of sterols was from Fluka (Buchs, Switzerland). Nonan-1-ol for cGC analysis of volatiles and deuterated chloroform and tetramethylsilane for ^{13}C NMR analyses of total triglycerides and diglycerides were purchased from Sigma-Aldrich Chemical (St. Louis, MO). Finally, carbon dioxide (99% purity) was supplied by Son (Rome, Italy).

Preparation of Oil Samples. Carrot roots, having a good state of preservation, were purchased from a supermarket (Avezzano, Italy). After pelletization and dehydration, for which a combined apparatus model Z333 (Sandvik Process Systems, Milan, Italy) was used, they were processed in a 5-L SC-CO_2 extraction pilot apparatus (Separex, Chanpigneulles, France). The extracting conditions used were as follows: pressure, 330 bar; temperature, 40 °C; CO_2 flow, 12 kg/h; ethanol entrainer, 50 mL. Preliminary experimental runs established that these were the optimal conditions for achieving larger extraction yields and better physicochemical characteristics of the extracts. A test lasted 4 h, during which 2 kg of pellets was processed. The extraction cell containing the raw material was adapted into the SC unit. After reaching thermal equilibrium, the system was slowly pressurized by opening the valve at the extractor's inlet and allowing CO_2 plus cosolvent to flow across the extraction cell. Once the system reached and stabilized at the operating pressure, the valves from the extractor's outlet were opened and the extraction process began. The thermostatic bath controlling the CO_2 inlet flow to the pump was set to −10 °C. The depressurization included three steps, during which the column pressure was gradually reduced to 100, 80, and 60 bar, respectively. The cosolvent was removed from the oil extract by a Büchi 461 rotary evaporator (Flawil, Switzerland) at a bath temperature of 40 °C. Such an extract appeared to be quite dense and had a yellow-brown color with reddish-bright orange reflexes; it also gave off a strong odor of carrot and contained 15−20% of solid substances, which seemed to be mostly of a wax nature. This solid fraction was quantified by filtration on Whatman 91 paper (Carlo Erba, Milan, Italy) followed by drying in an oven at 105 °C for 3 h.

The opercula (Natur Farma, Verona, Italy), containing carrot oil mixed to another unknown crop oil, were purchased from an herbalist's shop (Pescara, Italy). They were cut open with a bistoury in the laboratory to recover the contained oil. VOO was obtained by processing olive fruits of cv. Coratina (Olea europaea L.) with a Novoil EDJ/1 two-way centrifuging system (Rapanelli, Foligno, Italy) in the experimental oil mill. The same processing conditions described in an earlier work were used (*17*). The above cultivar is very popular in Italy, being employed for producing "Terra di Bari" virgin olive oil, which received a European POD (protect origin denomination) trademark. A homogeneous sample of 1.2 ton of olives, mechanically harvested during the crop year 2002, was processed. This was divided into four equal 300 kg parts, which were processed as replicate batches. For each test, six samples of oil were drawn for analyses. These were stored frozen (at −20 °C) in darkness until analyzed, using green screw-capped bottles (250 mL) without headspace, which after filling were hermetically sealed. For SCO and MCO oils, four replicates were likewise planned.

Analyses of Oil Samples. The analytical procedures used were mostly those outlined in earlier works (*17*, *18*). Free acid content and peroxide value were assessed by titrimetric methods. Resistance to autoxidation was measured by a Rancimat apparatus model 679 (Basel, Switzerland) operated at 120 °C with an air flow rate of 20 L/h. The flex point of the peroxidation curve (recorded at the paper speed of 1 cm/h) was considered as an oxidation mark (induction time). The carbonyl index was assessed by a colorimetric method, using phloroglucin reagent and taking the absorbance values at 540 nm. The ultraviolet (UV) spectrophotometric indices (specific extinctions), k_{232} and k_{270}, were evaluated on an oil sample dissolved in isooctane. Carotenes and chlorophylls were determined colorimetrically, after preparation of a calibration curve with β-carotene and chlorophyll *a*, respectively. The chromatic parameters, such as chroma, brightness, and hue, from which the integral color index was evaluated, were determined by transmittance measurements.

Phytosterols, diterpene dialcohols, long-chain aliphatic alcohols, and superior triterpene alcohols were determined by cGC, using as internal standard either α-cholestanol or arachidilic alcohol and for the separation a 25 m × 0.30 mm i.d., 0.20 μm, glass column coated with phenyldimethylpolysiloxane (Supelco Inc., Bellefonte, PA). To evaluate the alcholic index, an algorithm involving some of the above alcohol compounds (both aliphatic and triterpene) was used (*17*). Total triglycerides, as well as 1,2- and 1,3-diglycerides (expressed as percent of total glyceridic classes) from which the qualitative 1,2-diglycerides-to-1,3-diglycerides ratio was assessed, were determined by ^{13}C nuclear magnetic resonance (NMR), using a UNITY INOVA narrow-bore 500 MHz NMR spectrometer equipped with a Unix-based Sun Microsystems workstation (Varian NMR Instruments, Palo Alto, CA). The spectra were recorded at 25 °C by using the DEPT pulse sequence under the constraints (the proton pulse was set at 45°) that all of the protonated carbons, CH, CH_2, and CH_3, of the acyl chains were detected as positive resonances. Their intensities (i.e., areas) were determined by integration using the software provided with the NMR spectrometer (*19*). The triglyceride composition (expressed as percent of total triglycerides) was determined by high-performance liquid chromatography (HPLC), using an LDC_4 100 Ms model system equipped with a Shodex RF Se-61 differential refractometer and a chromoject integrator (Thermo Separation Products, Schaumburg, IL). Separation was carried out by a 250 mm × 4.5 mm i.d., 5 μm, reversed-phase column, coated with Supelcosil LC-18 (Supelco Inc.). The mobile phase consisted of an acetone/acetonitrile (60:40, v/v) mixture, which was pumped at 1

mL/min at room temperature (*18*). Fatty acid composition (expressed as percent of total fatty acids) was determined by cGC, after conversion of these compounds to methyl esters (FAMEs), using for separation a 25 m × 0.35 mm i.d., 0.25 μm, fused-silica capillary column coated with biscyanopropyl-cyanopropylphenylsiloxane (Nordion Ltd., Helsinki, Finland). Hydrogen was the carrier gas (column pressure = 50 kPa) (*17*).

Waxes were analyzed by cGC using a SPBTH-5 30 m, 0.32 mm i.d., 0.25 μm capillary column (Supelco Inc.), after extraction with a 70−230 mesh hydrated silica gel column and *n*-hexane/diethyl ether (99:1, v/v) as eluent. The internal standard was lauryl arachidate. Volatiles were quantified by a dynamic headspace (DHS)-cGC procedure, after 2 h of stripping at 37 °C in a nitrogen stream, entrapping by activated charcoal, and elution with diethyl ether. A 25 m × 0.32 mm i.d., 0.20 μm, Carbowax 20 M capillary column, coated with ethylene glycol (Nordion Ltd.), was used for their separation. The internal standard was nonan-1-ol (>99% purity). Tocopherols were quantified by HPLC with a 300 mm × 3.9 mm i.d., 10 μm, direct-phase M-porasil column (Waters Corp., Milford, MA), using a hexane/propan-2-ol (98.5:1.5, v/v) eluent and a UV detector at 292 nm. Finally, phenolics were quantified colorimetrically, taking the absorbance readings at 725 nm wavelength and using the Folin−Ciocalteu reagent for developing the color. Some individual phenols, such as tyrosol and hydroxytyrosol, were quantified by cGC, after extraction with methanol, using a 25 m × 0.32 mm i.d., 0.20 μm, capillary column coated with dimethylpolysiloxane (Lab. Service Analitica Ltd., Anzola Emilia, Bologna, Italy). The internal standard was resorcinol (>99% purity) (*17*, *18*).

Statistics. A simple experimental design including three oil kinds (SCO, MCO, and VOO) was adopted. The significance of the means was tested by one-way analysis of variance (ANOVA) with replications. When a significant *F* value was found, means were separated using Duncan's post hoc pairwise test (*20*). On the basis of analytical data sets, parametric chemometric methods, such as principal component analysis (PCA), hierarchical cluster analysis (HCA), and canonical discriminant analysis CDA), as well as a nonparametric multivariate method (CART's classification), were used to discriminate the samples. The statistical software packages Minitab release 13.0 (Minitab Inc., State College, PA), SPSS release 12.0 (SPSS Inc., Chicago, IL), and Statistica release 6.0 (Statsoft Inc., Tulsa, OK) were used (*20*−*22*). A Pentium IV processor was used under Windows X_p Professional operating system.

RESULTS AND DISCUSSION

Eight kilograms of carrot roots gave ∼200 mL of SCO. The analytical characterization of the three kinds of oils led to many experimental data, most of which are presented in **Tables 1**−**7** as means ± standard deviation (SD) along with the statistical significances.

SCO shows higher magnitudes of peroxide index, UV and carbonyl indices, and free fatty acid content (data not shown) compared to VOO. This means that in VOO the hydrolytic and oxidation reactions take place much more slowly (*16*). **Table 1** shows that a valuable feature of SCO is its high contents of phenols and secoiridoid phenol derivatives, such as tyrosol and hydroxytyrosol. However, its induction time value (not shown) as well as that of MCO is lower, owing likely to the extraction procedure employed for these oils and possibly also to the greater rate of oxidation products (including oxidized carotenes) (*23*). In contrast, the contents of tocopherols in SCO (and MCO) are lower with respect to those in VOO. SCO is mainly characterized by highest levels of carotenes and values of the integral color index. The latter seems to be mostly linked to the lowest values of brightness. The concentrations of chlorophyll pigments are similar in SCO and VOO and lower in MCO. In SCO, the marked yellow-orange color, attributable to the highest contents in carotenes, seems to fully overcome the green color (as confirmed by higher values of the hue parameter).

Table 1. Magnitudes of Natural Antioxidants and Color-Related Variables in Supercritical Fluid Carbon Dioxide Extracted Carrot Root Oil (SCO), Commercial Carrot Oil (MCO), and Mechanically Extracted Virgin Olive Oil (VOO)[a]

analytical variable	SCO	MCO	VOO
total polyphenols (as caffeic acid, mg/kg)	623.2 ± 38.0a	45.5 ± 2.4b	137.0 ± 7.5c
tyrosol (as resorcinol, mg/kg)	23.3 ± 1.4a	7.3 ± 0.4b	5.1 ± 0.2c
hydroxytyrosol (as resorcinol, mg/kg)	1.8 ± 0.1a	0.7 ± 0.0b	0.9 ± 0.0c
chlorophylls (as chlorophyll *a*, mg/kg)	23 ± 1a	17 ± 1b	21 ± 1a
total carotenes (as β-carotene, mg/kg)	1850 ± 141a	170 ± 12b	27 ± 2c
brightness (%)	1.4 ± 0.1a	10.1 ± 0.7b	69.5 ± 5.5c
chroma (%)	82.8 ± 6.7a	97.7 ± 7.3b	64.4 ± 4.3c
hue (nm)	595 ± 45a	597 ± 30a	577 ± 28a
integral color index	153.4 ± 8.4a	97.2 ± 6.4b	10.2 ± 0.7c
tocopherols (mg/kg)	160.1 ± 12.2a	182.3 ± 12.0b	208.2 ± 16.9c

[a] Data are means of three independent samples ± standard deviation (SD). Means within the same row with different letters are significantly different (Duncan's multiple-range test, $p \leq 0.05$).

Table 2. Content of Glycerides (as Percent of Total Glyceridic Classes) (As Determined by ^{13}C NMR Method) in Supercritical Fluid Carbon Dioxide Extracted Carrot Root Oil (SCO), Commercial Carrot Oil (MCO), and Mechanically Extracted Virgin Olive Oil (VOO)[a]

glyceride compound	SCO	MCO	VOO
1,2-diglycerides	0.77 ± 0.04a	0.58 ± 0.04b	1.50 ± 0.12c
1,3-diglycerides	1.14 ± 0.08a	1.10 ± 0.09a	1.80 ± 0.13b
total diglycerides	1.91 ± 0.13a	1.68 ± 0.10b	3.30 ± 0.18c
ratio of 1,2-diglycerides to 1,3-diglycerides	0.68 ± 0.03a	0.53 ± 0.04a	0.83 ± 0.04b
ratio of 1,2-diglycerides to total diglycerides	0.40 ± 0.03ab	0.35 ± 0.02a	0.45 ± 0.03b
triglycerides	80.90 ± 5.82a	98.32 ± 7.28b	98.50 ± 6.70b

[a] Data are means of three independent samples ± SD. Means within the same row with different letters are significantly different (Duncan's multiple-range test, $p \leq 0.05$).

Table 2 shows how the contents percent in 1,2-diglycerides, 1,3-diglycerides, total diglycerides, and total triglycerides and the value of the qualitative ratio of 1,2-diglycerides to 1,3-diglycerides are higher in VOO. The values of these glyceridic variables are often similar in SCO and MCO.

Table 3 displays the contents of individual triglycerides in the three kinds of oils. In SCO, the major triglyceride is trilinolein (LLL), followed in order by 1,2-dilinoleoin-3-palmitoylglycerol (LLP), 1,2-dilinoleoyl-3-oleoylglycerol (LLO), 1-palmitoyl-2-oleoyl-3-linoleoylglycerol (POL), and 1,2-dioleoyl-3-palmitoylglycerol (OOP). In VOO, the major triglyceride compounds are in order triolein (OOO), OOP, 1,3-dilinoleoyl-2-linoleoylglycerol (OLO), POL, and 1-stearoyl-2,3-dioleoylglycerol (SOO). Finally, in MCO the more prominent triglyceride compounds are LLL, LLO, LLP, POL, and OLO. We believe that the triglyceride composition in SCO could be mainly related to the genetic variation. Up to now, such a composition, as well as that of fatty acids, waxes, phytosterols, and superior alcohols (discussed below), was unknown.

Table 4 gives the wax composition of the oils. SCO appears to be very rich in waxes compared to both MCO and VOO. In

Table 3. Concentration (as Percent of Total Triglycerides) of Individual Triglycerides (As Determined by HPLC Method) in Supercritical Fluid Carbon Dioxide Extracted Carrot Root Oil (SCO), Commercial Carrot Oil (MCO), and Mechanically Extracted Virgin Olive Oil (VOO)[a]

triglyceride component	SCO	MCO	VOO
trilinolein (LLL)	47.8 ± 3.2a	24.4 ± 1.3b	0.4 ± 0.0c
1,2-dilinoleoyl-3-oleoylglycerol (LLO)	13.9 ± 0.8a	20.1 ± 1.0b	2.3 ± 0.1c
1,2-dilinoleoyl-3-palmitoylglycerol (LLP)	16.6 ± 1.0a	17.7 ± 1.2a	0.7 ± 0.0b
1,3-dilinoleoyl-2-linoleoylglycerol (OLO)	3.1 ± 0.1a	10.6 ± 0.5b	14.9 ± 0.9c
1-palmitoyl-2-oleoyl-3-linoleoylglycerol (POL)	8.3 ± 0.5a	14.0 ± 1.0b	7.6 ± 0.3c
triolein (OOO)	2.1 ± 0.2a	3.7 ± 0.2b	39.4 ± 2.4c
1,2-dioleoyl-3-palmitoylglycerol (OOP)	5.6 ± 0.3a	6.7 ± 0.3b	25.3 ± 1.6c
1,2-dipalmitoyl-3-oleoylglycerol (PPO)	2.5 ± 0.1a	1.7 ± 0.1b	3.8 ± 0.2c
1-stearoyl-2,3-dioleoylglycerol (SOO)	0.1 ± 0.0a	1.1 ± 0.1b	5.6 ± 0.3c

[a] Data are means of three independent samples ± SD. Means within the same row with different letters are significantly different (Duncan's multiple-range test, $p \leq 0.05$).

Table 4. Concentration (Milligrams per Kilogram) of Waxes in Supercritical Fluid Carbon Dioxide Carrot Root Extracted Oil (SCO), Commercial Carrot Oil (MCO), and Mechanically Extracted Virgin Olive Oil (VOO)[a]

wax class	SCO	MCO	VOO
waxes C_{34}	78.7 ± 5.6a	98.3 ± 6.4b	84.6 ± 5.1c
waxes C_{36}	389.5 ± 22.6a	198.1 ± 15.5b	220.6 ± 15.2c
waxes C_{38}	2112.7 ± 177.5a	63.4 ± 4.4b	144.7 ± 8.4c
waxes C_{40}	535.7 ± 34.8a	83.2 ± 5.8b	63.3 ± 4.7c
waxes C_{42}	102.7 ± 5.0a	57.8 ± 2.2b	60.1 ± 3.1b
waxes C_{44}	42.7 ± 2.9a	14.1 ± 1.1b	13.6 ± 1.0b
waxes C_{46}	37.5 ± 2.9a	29.3 ± 2.4b	25.6 ± 2.0c
total waxes	3299.4 ± 171.6a	544.1 ± 34.3b	612.5 ± 47.8c

[a] Data are means of three independent samples ± SD. Means within the same row with different letters are significantly different (Duncan's multiple-range test, $p \leq 0.05$).

Table 5. Concentration (as Percent of Total Fatty Acids) of Individual Fatty Acids in Supercritical Fluid Carbon Dioxide Extracted Carrot Root Oil (SCO), Commercial Carrot Oil (MCO), and Mechanically Extracted Virgin Olive Oil (VOO)[a]

fatty acid component	SCO	MCO	VOO
myristic ($C_{14:0}$)	0.4 ± 0.0a	0.1 ± 0.0b	nd[b]
palmitic ($C_{16:0}$)	16.6 ± 1.0a	11.1 ± 0.7b	9.9 ± 0.5c
palmitoleic ($C_{16:1}$)	1.8 ± 0.1a	0.2 ± 0.0b	0.4 ± 0.0c
heptadecanoic ($C_{17:0}$)	0.4 ± 0.0a	0.1 ± 0.0b	nd
heptadecenoic ($C_{17:1}$)	0.8 ± 0.0	nd	nd
stearic ($C_{18:0}$)	1.8 ± 0.1a	3.7 ± 0.2b	2.8 ± 0.1c
oleic ($C_{18:1}$)	11.6 ± 0.6a	25.9 ± 1.6b	78.8 ± 4.7c
linoleic ($C_{18:2}$)	60.1 ± 2.1a	52.3 ± 1.7b	6.8 ± 0.4c
arachidonic ($C_{20:0}$)	0.6 ± 0.0a	0.5 ± 0.0a	0.3 ± 0.0b
linolenic ($C_{18:3}$)	4.9 ± 0.3a	5.9 ± 0.3b	0.6 ± 0.0c
eicosenoic ($C_{20:1}$)	0.4 ± 0.0a	0.2 ± 0.0b	0.3 ± 0.0ab
behenic ($C_{22:0}$)	0.3 ± 0.0a	nd	0.1 ± 0.0b
lignoceric ($C_{24:0}$)	0.2 ± 0.0	nd	nd
SFAs[c]	20.4 ± 1.3a	15.5 ± 1.0b	13.1 ± 0.8a
MUFAs[d]	14.6 ± 0.9a	26.3 ± 2.0b	79.5 ± 5.2c
PUFAs[e]	65.0 ± 4.2a	58.2 ± 4.0a	7.4 ± 0.4b
ratio of FAω_6 to FAω_3[f]	12.3 ± 0.8a	8.9 ± 0.5b	11.3 ± 0.5c

[a] Data are means of three independent samples ± SD. Means within the same row with different letters are significantly different (Duncan's multiple-range test, $p \leq 0.05$). [b] None detected at a limit of 0.01%. [c] Saturated fatty acids. [d] Monounsaturated fatty acids. [e] Polyunsaturated fatty acids. [f] Fatty acids.

SCO, the major wax compounds are C_{38}, C_{40}, C_{36}, and C_{42}; in VOO they are C_{36}, C_{38}, C_{34}, and C_{40}; finally, in MCO they are C_{36}, C_{34}, C_{40}, and C_{42}.

Table 5 summarizes the figures related to the fatty acid composition of the oils, which is obviously linked to that regarding triglycerides. In fact, from our data it emerges that in SCO the major component of this fraction is linoleic acid, whereas in VOO it is oleic acid. This latter, however, is abundant also in SCO. This oil contains higher percentages of palmitic and linolenic acids, but lower concentrations of stearic acid. As far as the minor fatty acids (myristic, palmitoleic, heptadecanoic, heptadecenoic, arachidonic, eicosenoic, behenic, and lignoceric acids) are concerned, these, in total, appear to be present in greater amounts in SCO. The monounsaturated fatty acids-to-saturated fatty acids ratio (one related to the nutritional and health properties of an oil) (*24*) is ~8-fold higher in VOO. On the contrary, the fatty acids ω_6-to-fatty acids ω_3 ratio, which according to the results of epidemiological investigations plays an important role in preventing some human diseases, appears to be slightly higher in SCO, the optimal value corresponding to ~10 (*24*, *25*). SCO displays higher percentages of polyunsaturated fatty acids (PUFAs) and saturated fatty acids (SFAs), but lower relative values of monounsaturated fatty acids (MUFAs). Concerning MCO, this shows higher proportions of stearic acid (compared to the other two types of oils), as well as nondected percentages of heptadecenoic, behenic, and lignoceric acids. These fatty acids (as well as myristic acid) are not even detected in VOO. SCO, due to its higher contents in PUFAs, is more susceptible to autoxidation than VOO; however, PUFAs include some essential fatty acids exerting important physiological effects (*25*, 26).

Table 6 reports the concentrations of phytosterols and triterpene dialcohols in the oils. A remarkable characteristic of SCO is its highest value of total sterols, which compared to that concerning VOO and MCO, appears to be almost 30- and 17-fold higher, respectively. In all three kinds of oils, especially in SCO, β-sitosterol is by far the most prominent compound present in this analytical fraction, followed by campesterol and stigmasterol. SCO is also rich in sitostanol, chlerosterol, Δ^5-24-stigmastadienol, Δ^5-avenasterol, Δ^7-stigmastenol, and Δ^7-avenasterol. This oil also contains significant amounts of cholesterol; its low-density lipoprotein (LDL) form is involved in the atherogenesis process (*24*−*27*). In VOO, Δ^5-23-stigmastadienol and brassicasterol are not detectable. MCO does not contain detectable amounts of triterpene dialcohols (erythrodiol and uvaol). The sum of these two compounds is slightly higher in SCO, even though the levels of uvaol are higher in VOO.

Table 7 shows the analytical composition of both long-chain aliphatic alcohols and superior triterpene alcohols. The individual alcohols display a concentration that increases according to the order VOO > MCO > SCO. The only exceptions are 1-tetracosanol and 24-methylencycloartanol, which in SCO have a value higher compared to those in MCO, as well as β-amyrin plus butyrospermol, the level of which in MCO is higher compared even to VOO. The levels of total aliphatic alcohols and of total triterpene alcohols are, respectively, ~5- and 4-fold

Table 6. Concentration (Milligrams per Kilogram) of Individual Sterols and Triterpene Dialcohols in Supercritical Fluid Carbon Dioxide Extracted Carrot Root Oil (SCO), Commercial Carrot Oil (MCO), and Mechanically Extracted Virgin Olive Oil (VOO)[a]

sterol and triterpene dialcohol component	SCO	MCO	VOO
cholesterol	65.9 ± 4.0a	6.5 ± 0.4b	5.8 ± 0.4b
brassicasterol	78.4 ± 4.6a	5.1 ± 0.3b	nd[b]
24-methylencholesterol	46.8 ± 3.7a	5.3 ± 0.3b	1.7 ± 0.1c
campesterol	4159.8 ± 341.1a	294.4 ± 22.7b	31.5 ± 2.1c
campestanol	30.2 ± 2.2a	8.9 ± 0.6b	1.7 ± 0.1c
stigmasterol	3203.8 ± 208.2a	278.8 ± 24.3b	13.5 ± 1.1c
δ^7-campesterol	209.6 ± 15.9a	13.3 ± 1.1b	1.9 ± 0.1c
δ^{5-23}-stigmastadienol	125.7 ± 8.7a	5.6 ± 0.4b	nd
chlerosterol	654.3 ± 54.3a	7.2 ± 0.6b	13.9 ± 0.8c
β-sitosterol	19785.9 ± 1642.2a	950.0 ± 52.3b	790.0 ± 54.5c
sitostanol	742.4 ± 56.4a	26.1 ± 2.1b	14.8 ± 1.3c
δ^5-avenasterol	185.0 ± 12.4a	19.8 ± 0.9b	118.1 ± 5.0c
δ^5-24-stigmastadienol	526.8 ± 34.8a	19.1 ± 1.4b	10.1 ± 0.8c
δ^7-stigmastenol	196.4 ± 17.1a	62.8 ± 4.6b	3.7 ± 0.2c
δ^7-avenasterol	237.4 ± 17.6a	23.3 ± 1.9b	7.6 ± 0.4c
total sterols	30248.4 ± 2571.1a	1726.2 ± 120.8b	1014.3 ± 77.1c
campesterol/stigmasterol	1.4 ± 0.1a	1.1 ± 0.1a	2.3 ± 0.1b
erythrodiol	25.4 ± 1.6a	nd	15.5 ± 1.1b
uvaol	14.2 ± 0.9a	nd	21.2 ± 1.5b
erythrodiol + uvaol	39.7 ± 3.2a	nd	36.7 ± 2.3b

[a] Data are means of three independent samples ± SD. Means within the same row with different letters are significantly different (Duncan's multiple-range test, $p \leq 0.05$). [b] None detected at a limit of 0.01 mg/kg.

Table 7. Concentration (Milligrams per Kilogram) of Individual Superior Aliphatic/Triterpene Alcohols and Other Alcohol Components in Supercritical Fluid Carbon Dioxide Extracted Carrot Root Oil (SCO), Commercial Carrot Oil (MCO), and Mechanically Extracted Virgin Olive Oil (VOO)[a]

alcohol component	SCO	MCO	VOO
1-docosanol (C_{22})	13.0 ± 1.0a	13.7 ± 1.0a	47.3 ± 3.3b
1-tetracosanol (C_{24})	10.1 ± 0.8a	6.0 ± 0.5b	75.8 ± 6.0c
1-hexacosanol (C_{26})	17.7 ± 1.3a	63.4 ± 4.3b	107.8 ± 6.1c
1-octacosanol (C_{28})	12.9 ± 1.0a	14.1 ± 0.8a	40.7 ± 2.4b
total aliphatic alcohols	53.7 ± 4.5a	97.2 ± 6.3b	271.6 ± 10.5c
β-amyrin + butyrospermol	101.0 ± 4.9a	306.7 ± 18.7b	285.7 ± 16.6c
cycloartenol	166.0 ± 12.1a	293 ± 17.3b	666.7 ± 32.0c
24-methylenecycloartanol	176.1 ± 10.4a	63.0 ± 4.3b	826.8 ± 47.1c
total triterpene alcohols	443.1 ± 31.9a	663.4 ± 53.7b	1779.3 ± 160.1c
phytol	849.3 ± 50.1a	517.6 ± 31.1b	190.2 ± 15.6c
geranylgeraniol	193.8 ± 12.8a	164.9 ± 9.6b	19.2 ± 1.1c
citrostadienol	33.1 ± 2.2a	294.0 ± 17.1b	137.7 ± 8.4c
alcoholic index	0.01 ± 0.00	0.00 ± 0.00	0.50 ± 0.00

[a] Data are means of three independent samples ± SD. Means within the same row with different letters are significantly different (Duncan's multiple-range test, $p \leq 0.05$).

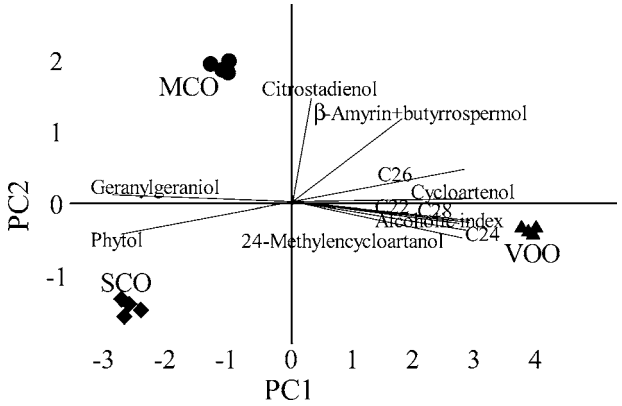

Figure 1. Score and loading plot (based on the superior aliphatic and triterpene alcohol composition) by dimensions 1 and 2 from PCA, showing groupings, such as supercritical fluid carbon dioxide extracted carrot root oils (SCO, ◆), market carrot oils (MCO, ●), and mechanically extracted virgin olive oils (VOO, ▲).

higher in VOO compared to SCO (the latter having the lower content of total alcohols among the three kinds of oil examined). In contrast, geranylgeraniol, a newly identified aliphatic alcohol, as well as phytol, seems to display higher values in SCO. Finally, citrostadienol, another alcohol present in a typical chromatogram of the alcohol fraction, but having characteristics different from those of the above-mentioned aliphatic and triterpene alcohol compounds, shows higher values in MCO. The qualitative alcoholic index is equal to 0 in MCO and close to 0 in SCO and VOO.

A complex volatile fraction, including a very large number of peaks (many of which have not yet been identified) and corresponding mainly to sesquiterpenes and monoterpenes, is present in the studied oils (especially in SCO). From the data obtained (not shown), it appears that the major sesquiterpene hydrocarbon volatiles present in SCO are β-caryophyllene, α-humulene, (*E*)-γ-bisabolene, and (*Z*)-γ-bisabolene. Among the monoterpene volatiles, the more prominent compounds in SCO are α-pinene, sabinene, β-myrcene, limonene, γ-terpinene, *p*-cymene, and terpinolene. Finally, within the carbonyl volatiles, the more prominent compounds in SCO are 2-nonenal, octanal, 2-decenal, and heptanal. The oxygenate volatiles contribute more to the aroma of SCO compared to hydrocarbons (*28*). The above findings are consistent with the literature data concerning the qualitative−quantitative volatile composition of carrot root (*1*, *7*, *13*, *23*). VOO contains significant levels of esters, alcohols, hydrocarbons, and C_6, C_5 and C_9 aldehydes. Among these aldehyde components, the unsaturated compound *trans*-2-hexenal, arising from the lipoxygenase pathway (and having as a precursor the linolenic acid) (*18*), accounts for >60% of the whole volatile fraction. MCO, even though in lower proportion, contains most of the volatiles occurring in SCO (including the unidentified aromatic molecules). According to some experimenters (*1*−*8*), in carrot root and related products, the composition of the volatiles and of the other analytical fractions (above-discussed) is strongly modified by a large number of different variables, such as genotype (genetic background), root color, harvest period, seasonal conditions, soil and climate, cultivation techniques, length and conditions of storage, industrial processing, cooking, various types of stress, and others. The genetic

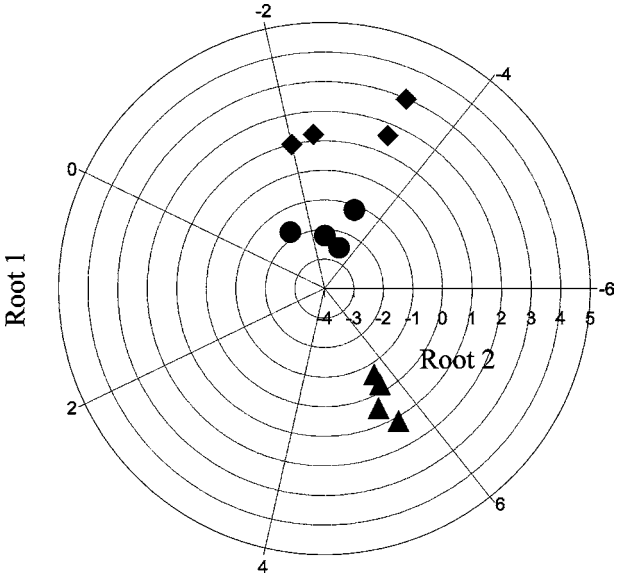

Figure 2. Plot by the roots (canonical functions) 1 and 2 from CDA, based on the fatty acid composition, showing groupings, such as supercritical fluid carbon dioxide extracted carrot root oils (SCO, ◆), market carrot oils (MCO, ●), and mechanically extracted virgin olive oils (VOO, ▲).

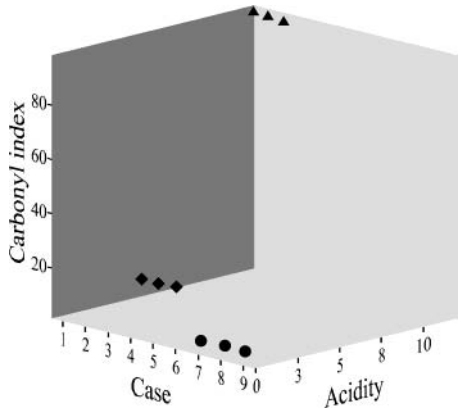

Figure 3. Three-dimensional plot from CDA, based on the variables acidity and carbonyl index, showing groupings, such as supercritical fluid carbon dioxide extracted carrot root oils (◆), market carrot oils (●), and mechanically extracted virgin olive oils (▲).

variation magnitude is somewhat higher compared to that of other factors (*6*, *23*).

Results of Chemometric Analyses. The biblot in **Figure 1** shows how the PCA method, applied to the data of the superior aliphatic and triterpene alcohol composition, is capable of characterizing the three kinds of oils. In fact, along the first dimension (accounting for 73.8% of the total variance) are discriminated both VOO (positive half) and SCO (negative half) samples, whereas along the second dimension (accounting for 16.6% of the total variance) are differentiated the MCO (positive half) samples. This biblot also highlights the alcohols mainly contributing to the discrimination of each oil kind. The dendrogram (not shown) produced by HCA analysis, applied to the triglyceride composition data, confirms the results achieved by PCA analysis. The plot in **Figure 2** displays that the CDA method, applied to the data of fatty acids composition, is likewise effective in discriminating the three oil kinds, when considering the first and second roots (canonical functions). The plot in **Figure 3** (produced from 9 rather than 12 cases), arising itself from CDA analyses, highlights the role of the analytical

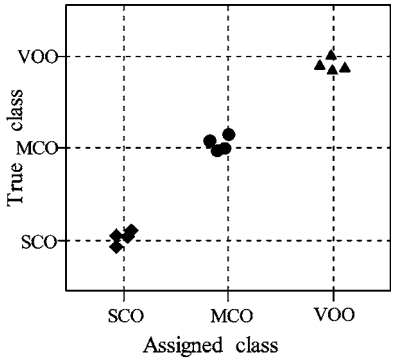

Figure 4. Classification of supercritical fluid carbon dioxide extracted carrot root oils (SCO, ◆), market carrot oils (MCO, ●), and mechanically extracted virgin olive oils (VOO, ▲), based on values of the wax composition, using CART's nonparametric method.

variables acidity and carbonyl index in discriminating the three types of oils. Finally, on the basis of the data of wax composition, the CART nonparametric method also appears to be very effective in characterizing the three oil kinds (**Figure 4**).

From the analytical findings summarized in this work, it can be concluded that the carrot root oil arising from SC-CO_2 extraction, because of its marked nutritional and health-promoting properties, as confirmed by comparison of its composition with that of virgin olive oil (a highly functional crop oil), may play a role in the prevention of cardiovascular and other human diseases. Due to its highest contents in carotenes, it also may be exploited, after purification, in the nutraceutical and cosmetic industries, two sectors capable likewise of ensuring a marked increase of its added value.

ABBREVIATIONS USED

SCO, carrot root oil; SC-CO_2, supercritical fluid carbon dioxide extraction; MCO, market carrot oil; VOO, virgin olive oil; RAE, retinol activity equivalent; FAMEs, fatty acid methyl esters; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; SFAs, saturated fatty acids; DHS, dynamic headspace; LDL, low-density lipoproteins; PCA, principal component analysis; HCA, hierarchical cluster analysis; CART, classification and regression tree; ANOVA, analysis of variance; SD, standard deviation.

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