HIGH PRESSURE LIQUID CHROMATOGRAPHIC FRACTIONATION AND DETERMINATION OF CAROTENOIDS AND CHLOROPHYLLS IN MANDARIN ESSENTIAL OILS

FRANCESCO GIONFRIDDO (*) - ENRICO POSTORINO (**)
GIUSEPPE CALABRÒ (**)

Abstract

A rapid and simple high pressure liquid chromatographic method for fractionation and determination of carotenoids and chlorophylls in three typologies of mandarin essential oil: yellow, red and green, industrially produced in Sicily and in Calabria (Italy), in the season 2007-2008, is reported.

The analysis doesn't require pre-extraction of the pigments from the essential oil and it is effected by injecting directly into the chromatograph the samples, previously filtered on a nylon filter 0.45 μm.

Under the used analytical conditions the components of a standard mixture of lycopene, α-carotene, β-carotene, lutein, zeaxanthin, β-cryptoxanthin, chlorophyll *a* and chlorophyll *b* are completely separated.

Riassunto

Viene riportato un metodo rapido e semplice per il frazionamento e la determinazione mediante HPLC di carotenoidi e clorofille in tre tipi di olio essenziale di mandarino, giallo, rosso e verde, prodotti industrialmente in Sicilia e in Calabria nella campagna agrumaria 2007-2008.

L'analisi non richiede la pre-estrazione dei pigmenti dall'olio essenziale e viene effettuata iniettando direttamente al cromatografo il campione, previamente filtrato su filtro di nylon 0.45μm.

Nelle condizioni analitiche utilizzate vengono nettamente separati i componenti di una miscela standard costituita da: licopene, α-carotene, β-carotene, luteina, zeoxantina, β-criptoxantina, clorofilla *a* e clorofilla *b*.

(*) Stazione Sperimentale per le Industrie delle Essenze e dei Derivati dagli agrumi – Reggio Calabria
(**) Università degli Studi di Messina, e-mail: calabro@unime.it

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Introduction

Fruits color, typical of every vegetable species, is due to the presence of pigments mainly belonging to the families of carotenoids, antocianis and chlorophylls; in citrus fruits these compounds are distributed, in various proportions, in the flavedo and in the endocarp.

In immature fruits chlorophylls are prevailing and with their green color they mask all other pigments. With the progress of maturation, the increase in the quantity of carotenoids confers to the fruits the characteristic yellow color. It has been, nevertheless, demonstrated (1), that development of carotenoids and antocianis doesn't necessarily involve diminution in chlorophylls content.

Carotenoids, together with α-tocoferol (vitamin E), flavonoids (vitamin P) and ascorbic acid (vitamin C), are generically defined "antioxidants"; they have broadly spread in vegetable world. They are formed during the process of secondary metabolism of plants as primary metabolism products: sugars, amino acids, lipids.

Carotenoids are also present in the animal organisms and in products of animal origin (yolk of eggs, feathers of some birds, shellfishes or fishes); their presence is exclusively correlated to food intakes.

Carotenoids are highly unsaturated linear molecules with minimum formula $C_{40}H_{56}$; they derive from the repetition of isoprene unit tied up between them through bonds type "head-tail" and to the center through bonds tail-tail, that give place to a perfectly symmetrical structure with respect to its center.

Color and strong reactivity of carotenoids are due to the presence of long chains with double conjugated bonds. For this reason the tonality of color of carotenoids varies, in relation to the extension of the conjugated system, from the pale yellow to the deep red more.

Carotenoids are grouped in six types in relationship to the central group, named carotene. Some natural carotenoids as zeaxanthin, lutein and violet xanthin, richer in oxygen, are named xanthophylls.

Carotenoids, furthermore to be responsible of color, perform also important biological activities; their intake interrupts chain reactions, provoked by oxygen free radicals, produced during the oxidative cellular

mechanism, that can result harmful to cellular membranes; they take part in different physiological activities as melanogenesis; they act on low density lipoproteines (LDL) and have protective effects on different types of carcinoma (2-5).

Some carotenoids furthermore are considered molecules of high pharmacological and nutritional interest, as they are precursory for the formation of retinol (vitamin A). In the intestinal mucous of animals for example β-carotene is turned into vitamin A (6).

Carotenoids in food industry are used as natural colorings of drinks and other foods, to confer them the characteristic orange color and to attract the consumer's attention. An high carotenoid content can constitute an advantage, for example, in the case of orange essential oils because it allows producers of drinks to resolve both the problem of aromatization and coloring.

To obtain orange essential oils richer in carotenoids, during the past appeal has been made to peeling machines that work on dry skin of whole fruits or to alternative technologies based on a prolonged contact between the essential oil and the residual flavedo of the peeling (7).

Many data concerning the presence of carotenoids in citrus are reported; for convenience of consultation and for better operating comparisons, data related to the content of such substances are often grouped according to the principal families: carotenic hydrocarbons and oxygenated derived (xanthophylls) (6).

Important researches on properties and structures of many carotenoids have been published by Karrer and Jucker (8) and Goodwin (9).

Development of chromatography allowed to deepen the knowledge on qualitative and quantitative composition of carotenoids in many products (10-14). At present, official analytical methods for determination of total carotenoids both in citrus juices (15) and essential oils (16) use spectrophotometric measurements of the absorbance at 452 nm of a petrol ether extract of citrus juices or of a 1% solution of the essential oil in petrol ether.

For calculation, the extinction coefficient of a standard solution of β-carotene 1% is used.

Spectrophotometric determination of total carotenoids represents a rapid and simple method but it doesn't give information on the identity and concentration of every component.

Some works carried out in Italian mandarin oils have shown that criptoxantin is the main component of the fruit pulp, while the violet xantin prevails in the peel (17-20).

Recently the HPLC-APSI-MS was used (21) for characterize the carotenoid esters fraction of three samples of mandarin oil obtained by fruits belonging to "Mediteranea" cultivar.

In this paper we report a simple method, that not requires expensive instrumentation that most industries can not afford or specialized technician, for the characterization of carotenoids and chlorophylls present in mandarin essential oils obtained by different extraction technologies and evaluate their concentration during maturation of the fruits.

This method provides the use of high pressure liquid chromatographic (HPLC), already used by Calabrò *et al.* (22), Verzera *et al.* (23) in citrus juices, by De Sio *et al.* (24), in vegetable matrixes and Bonaccorsi *et al.* (25) in citrus essential oils.

Materials and Methods

Analysis where conducted on samples of three different typologies of genuine mandarin essential oils, produced during the 2007-2008 citrus fruit season:

- Twelve samples: ten produced in Sicily and two in Calabria, of green mandarin essential oils, obtained by peeling the flavedo of green fruits picked from September to November 2007, with a "Speciale" machine.
- Nineteen samples: thirteen produced in Sicily and six in Calabria, of yellow mandarin essential oils, obtained by peeling the flavedo of yellow fruits picked from November 2007 to February 2008, with "press" machine.
- Eight samples: five produced in Sicily and three in Calabria, of red mandarin essential oils, obtained by peeling the flavedo of red fruits picked from December 2007 to February 2008, with a "Speciale" machine.

Each sample is representative of a batch of 250 kg of essential oil; on each sample of mandarin essential oil the following analyses have been carried out.

Chemical and physical analysis

According to AFNOR procedures (26) relative density (20 °C), optical activity (20 °C), refractive index (20 °C), evaporation residue, aldehydes content (expressed as decanal %) have been determined.

Gas-chromatographic analysis

Centesimal distribution of volatile fraction of the oils has been determined by using the gas-chromatography with capillary columns at the

following experimental conditions:

Gas-chromatograph: ThermoQuest GC 2000 series; Column: Restek DB5: length 30 m, internal diameter 0.25 mm; film tickness 0.25 μm; injection in split mode 1:100 at 230 °C; injected volume 0.8 μL of oil; detector: F.I.D. to 250 °C; gas carrier: He with a constant flow 1.5 mL/min; programmed column temperature from 60 ° to 140 °C with step of 3 °C/min, from 140 °C to 150 °C with step of 3.5 °C/min, from 150 °C to 180 °C with step of 10 °C/min, then isotherm at 180 °C for 18 minutes; acquirement software: Computer Compaq, with program data processing Chromquest v. 2.53.

For the peaks identification of volatile fraction, a coupled system gas-chromatography (Trace 2000-Thermoquest mass spectrometry (GCQ Termoquest) has been used; peaks identification has been made through software Excalibur with library of spectra NIST 2.0.

HPLC analysis

The samples of the essential oil, filtered on a 0.45 μm nylon filter, have been injected directly into the liquid chromatograph.

The experimental conditions were as follows:

Liquid Chromatograph Thermofinnigan model Surveyor equipped with autosampler; injected volume: 10 μL ; column: YMC-Pack C30, 250 × 4.6 mm (5μm), made by YMC Inc., termostated at 30 °C. Employed solvents: Eluent A: methanol/water 95/5 (v/v); Eluent B: methylene chloride both containing 0.1% BHT (hydroxyl butyl toluene) and 0.05% triethylamine. Elution program: linear gradient in 35 minutes, from 95% of A to 30% of A, then 5 minutes of isocratic elution with 30% of A and 70% of B. After each chromatographic run, the column has been reconditioned by eluent A before the next injection. Detector: Diode array (PDA); Data acquirement by ethernet card and Chromquest 4.1 software. From the multidimensional signal coming out from the diode array the chromatograms at 450 nm were recorded.

Carotenoids standard where dissolved in chloroform containing BHT 0.1%, accounting the purity certificated by the producer. The solutions have been preserved under inert gas at –20° C; in these conditions the solutions result stable for about 15 days.

Chlorophyll *a* and chlorophyll *b* standards were dissolved in chloroform containing BHT 0.1% and further stabilized with a known quantity of β-carotene to protect them from oxygen and light.

Figure 1 shows the HPLC chromatogram of a standard mixtures: lutein concentration is 30 mg/L, chlorophyll *a* is 50 mg/L; for all the other components the concentration is 15 mg/L. Even if chlorophyll *a* and β-carotene are not completely separated they are perfectly distinguishable in order to obtain their quantification.

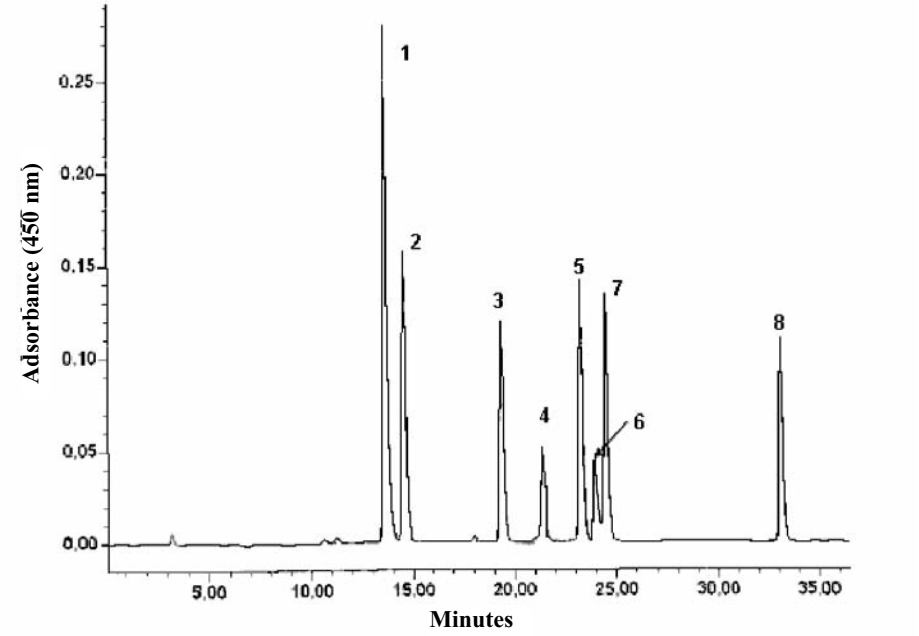

Fig. 1 – HPLC chromatogram of a mixture of standard used for the analyses: lutein (1), zeaxanthin (2), β-criptoxanthin (3), chlorophyll *b* (4), α-carotene (5), chlorophyll *a* (6), β-carotene (7), lycopene (8).

Results and Discussion

Table 1 and Table 2 show, respectively, chemical-physical analyses and centesimal composition of volatile fraction of each sample. All results are within the range fixed for cold pressed Italian mandarin oils (26-28).

TABLE 1

CHEMICAL-PHYSICAL PROPORTIES OF MANDARIN OILS EXAMINED

	Yellow mandarin oils (n = 19) (Nov. 2007 – Feb. 2008)				**Green mandarin oils (n = 12) (Sep. – Nov. 2007)**				**Red mandarin oils (n = 8) (Dec. 2007 – Feb. 2008)**			
	Min.	Max.	Aver.	Std. Dev.	Min.	Max.	Aver.	Std.Dev.	Min.	Max.	Aver.	Std. Dev.
Relative density at 20 °C	0.8516	0.8530	0.8522	0.0004	0.8516	0.8542	0.8528	0.0009	0.8525	0.8544	0.8537	0.0008
Optical rotation at 20 °C	71.21	72.90	72.09	0.41	71.28	73.36	72.25	0.72	73.25	74.77	74.19	0.60
Refractive index at 20 °C	1.4750	1.4769	1.4758	0.0005	1.4756	1.4782	1.4770	0.0010	1.4739	1.4762	1.4754	0.0008
Aldehydes (as decanal)	0.69	0.83	0.73	0.04	0.64	0.74	0.69	0.05	0.54	0.64	0.58	0.03
Evaporation residue	3.07	3.70	3.30	0.17	2.87	4.04	3.42	0.41	3.28	4.13	3.81	0.34

TABLE 2

CENTESIMAL COMPOSITION OF MANDARIN ESSENTIAL OILS VOLATILE FRACTION

	Yellow mandarin oils (n = 19) (Nov. 2007 – Feb. 2008)				**Green mandarin oils (n = 12) (Sep. – Nov. 2007)**				**Red mandarin oils (n = 8) (Dec. 2007 – Feb. 2008)**			
	Min.	Max	Mean	Dev.Std	Min.	Max	Mean	Dev. Std	Min.	Max	Mean	Dev.Std
α-thujene	0.770	0.827	0.803	0.017	0.727	0.810	0.773	0.022	0.751	0.827	0.786	0.027
α-pinene	2.200	2.310	2.261	0.034	2.015	2.283	2.183	0.079	1.982	2.321	2.121	0.123
camphene	0.019	0.027	0.021	0.002	0.013	0.039	0.024	0.008	0.022	0.03	0.027	0.003
sabinene	0.210	0.233	0.220	0.005	0.198	0.267	0.231	0.022	0.206	0.251	0.231	0.015
β-pinene	1.540	1.650	1.583	0.031	1.464	1.862	1.670	0.134	1.415	1.68	1.569	0.089
myrcene	1.690	1.730	1.710	0.013	1.694	1.989	1.900	0.108	1.683	1.858	1.738	0.059
α-phellandrene	0.230	0.250	0.239	0.008	0.202	0.277	0.231	0.021	0.202	0.246	0.223	0.017
α-terpinene	0.033	0.270	0.179	0.073	0.197	0.288	0.241	0.031	0.174	0.288	0.237	0.043
p-cymene	0.250	0.480	0.366	0.070	0.223	0.414	0.322	0.068	0.234	0.43	0.348	0.064
D-limonene	70.940	72.460	71.598	0.378	70.764	72.489	71.509	0.588	71.702	72.589	72.161	0.320
(Z) β-ocymene	0.000	0.030	0.014	0.012	0.013	0.028	0.020	0.006	0.002	0.028	0.014	0.010
γ-terpinene	17.410	18.680	18.166	0.304	17.728	18.861	18.398	0.385	17.371	18.213	17.875	0.362
trans sabinene hydrate	0.020	0.050	0.035	0.008	0.015	0.050	0.035	0.011	0.023	0.049	0.038	0.009
LInalool	0.656	0.820	0.751	0.056	0.599	0.773	0.662	0.053	0.665	0.781	0.723	0.047

TABLE 2 - continued

	Yellow mandarin oils (n = 19) (Nov. 2007 – Feb. 2008)				**Green mandarin oils (n = 12) (Sep. – Nov. 2007)**				**Red mandarin oils (n = 8) (Dec. 2007 – Feb. 2008)**			
Nonanal	0.000	0.022	0.008	0.006	0.000	0.017	0.007	0.006	0.001	0.009	0.005	0.004
Citronellal	0.000	0.021	0.013	0.006	0.009	0.024	0.015	0.005	0.004	0.019	0.013	0.006
terpinen-4-ol	0.030	0.075	0.050	0.012	0.022	0.053	0.037	0.009	0.03	0.054	0.043	0.009
α-terpineol	0.100	0.190	0.156	0.028	0.117	0.167	0.147	0.016	0.083	0.205	0.137	0.046
Decanal	0.100	0.145	0.113	0.015	0.106	0.159	0.137	0.019	0.091	0.126	0.106	0.015
Methyl N-methyl antrhanylate	0.350	0.574	0.434	0.065	0.243	0.416	0.319	0.059	0.293	0.462	0.361	0.070
β-cariophyllene	0.070	0.092	0.081	0.006	0.063	0.126	0.099	0.022	0.061	0.104	0.091	0.014
α-humulene	0.010	0.019	0.013	0.004	0.010	0.025	0.016	0.006	0.01	0.01	0.01	0.000
2-dodecen-l-al	0.006	0.023	0.015	0.005	0.001	0.019	0.007	0.006	0.001	0.016	0.011	0.005
α-selinene	0.030	0.046	0.037	0.005	0.025	0.056	0.038	0.010	0.021	0.038	0.03	0.008
α-farnesene	0.120	0.152	0.140	0.009	0.090	0.151	0.115	0.016	0.083	0.161	0.124	0.030
α-farnesol	0.010	0.018	0.013	0.004	0.002	0.029	0.014	0.010	0.001	0.009	0.005	0.002
α-sinensal	0.280	0.710	0.511	0.139	0.242	0.328	0.270	0.026	0.232	0.446	0.359	0.065

The results obtained by yellow mandarin essential oils (Table 3), evidence that the sum of each carotenoid varies between 2.9 and 47.3 mg/L with a mean content of 11.2 mg/L; the increase is enough regular during the maturation.

The sample G19 clearly differentiates from the others: this difference can be attributed both to the advanced maturation of the fruits (February), and to the possible influence of the production technology (pressing) that foresees a prolonged contact between the juice and the essential oil, with additional solubility of carotenoids in the oil. The chlorophylls content varies between 5.1 and 29.3 mg/L. It doesn't follow a regular course.

Regarding to the green mandarin essential oils, of which in Figure 2 we give, as an example, the HPLC chromatogram of a green mandarin oil sample (V1), the carotenoids content (Table 4) ranges between 5.8 and 48.4 mg/L, with a regular decrement in time. The chlorophylls are abundant in comparison to the red and yellow essential oils; the concentration range varies between 21.0 and 146.5 mg/L; also in this case the essential oils produced in the first period of the season are richer in chlorophyll. The ratio between carotenoids and chlorophylls concentrations is in the range 0.30-0.35, unless for two essential oils samples produced in Calabria, pointing out that the variation in time of the two pigments proceeds in parallel.

The concentration of every carotenoid is more homogeneous in comparison to the other examined essential oils. During the maturation the content in a-carotene and (β-carotene decreases in evident way and regularly with time; equally evident and regular is the content increase of cryptoxantin.

In the red mandarin essential oils (Table 5) the concentration of the carotenoids ranges between 25.7 and 77 mg/L with an average content of 45.4 mg/ L. Also in this case the content in carotenoids increases during the season, but evolution is not so regular as in the other cases. The concentration of the chlorophylls, that range between 2.0 and 87.8 mg/L, doesn't follow a regular pattern.

TABLE 3

CAROTENOID AND CHLOROPHYLLS FPOUND IN YELLOW MANDARIN ESSENTIAL OIL SAMPLES (mg/L)

Sample	Origin	Period	Lutein	Zea-xan-tin	β-cripto-xantin	α-caro-tene	β-caro-tene	Licopene	Chloro-phyll b	Chloro-phyll a	Sum of carote-noids	Sum of chloro-phylls
G1	Sicily	Nov. 2007	0.1	0.1	2.5	0.2	0.4	< QL	0.7	7.2	3.3	7.9
G2	Sicily	Nov. 2007	0.1	0.1	3.1	0.3	0.4	< QL	0.6	7.1	4.0	7.7
G3	Sicily	Nov. 2007	0.1	< QL	2.0	0.2	0.4	0.2	0.3	10.9	2.9	11.2
G4	Calabria	Nov. 2007	0.4	0.1	3.9	0.7	0.6	< QL	1.9	6.4	5.7	8.3
G5	Calabria	Nov. 2007	0.5	0.1	4.0	0.6	0.6	0.2	1.8	6.3	6.0	8.1
G6	Sicily	Nov. 2007	0.4	0.1	4.1	0.5	0.6	0.1	1.6	5.9	5.8	7.5
G7	Sicily	Nov. 2007	0.4	0.2	5.2	0.6	0.6	< QL	1.7	12.2	7.0	13.9
G8	Calabria	Nov. 2007	0.2	0.2	6.6	0.8	2.7	0.7	1.2	7.0	11.2	8.2
G9	Calabria	Nov. 2007	0.2	0.1	6.8	0.8	4.2	0.8	1.3	7.0	12.9	8.3
G10	Sicily	Dec. 2007	0.1	0.1	6.1	0.8	1.7	0.4	1.4	20.0	9.2	21.4
G11	Sicily	Dec. 2007	0.2	0.2	5.2	0.5	2.1	0.3	2.0	4.2	8.5	6.2

TABLE 2 - continued

Sample	Origin	Period	Lutein	Zea-xantin	β-criptoxantin	α-carotene	β-carotene	Licopene	Chlorophyll b	Chlorophyll a	Sum of carotenoids	Sum of chlorophylls
G12	Sicily	Dec. 2007	0.2	0.1	7.6	0.8	0.8	0.2	1.5	22.5	9.7	24.0
G13	Calabria	Dec. 2007	0.5	0.3	9.6	1.2	0.9	0.1	2.7	7.7	12.6	10.4
G14	Sicily	Dec. 2007	0.1	0.1	7.8	1.5	1.4	0.2	1.8	7.5	11.1	9.3
G15	Sicily	Dec. 2007	0.1	0.1	8.3	1.4	1.7	0.3	1.0	4.5	11.9	5.5
G16	Sicily	Dec. 2007	0.5	0.2	8.2	0.9	1.2	< QL	2.2	8.8	11.0	11.0
G17	Sicily	Dec. 2007	0.7	0.4	12.9	1.2	1.2	< QL	2.3	27.0	16.4	29.3
G18	Sicily	Jan. 2008	0.4	0.3	10.7	1.2	2.8	0.4	1.0	24.1	15.8	26.1
G19	Calabria	Feb. 2008	0.1	0.5	12.2	1.7	31.4	1.4	0.6	5.3	47.3	5.9
		Min. value	0.1	0.1	2.0	0.2	0.4	0.1	0.3	4.2	2.9	5.5
		Max value	0.7	0.5	12.9	1.7	31.4	1.4	2.7	27.0	47.3	29.3
		Mean	0.3	0.2	6.7	0.8	2.9	0.4	1.5	10.6	11.2	12.1
		Std. Dev.	0.2	0.1	3.2	0.4	7.0	0.4	0.6	7.1	9.6	7.2

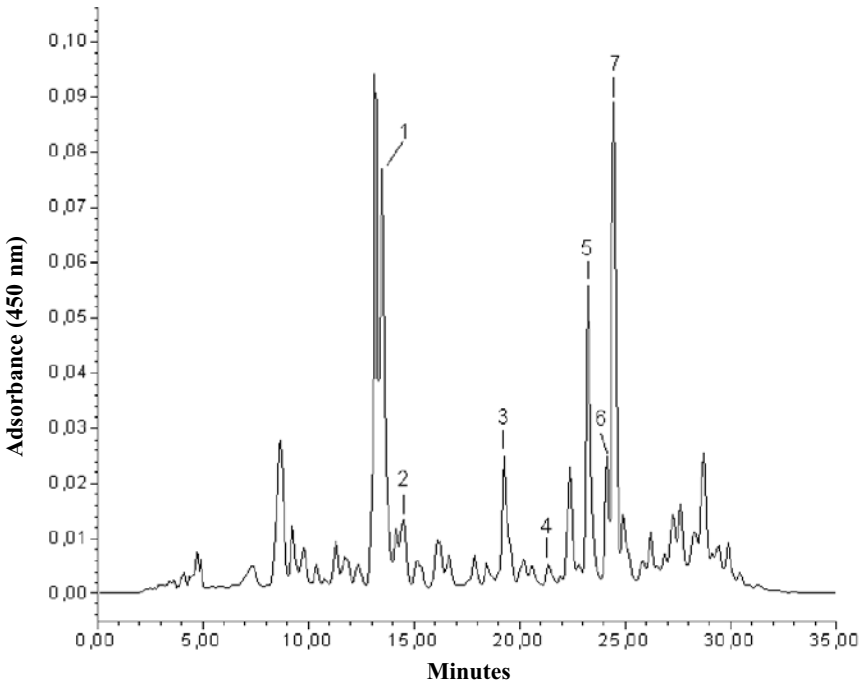

Fig.2 – Chromatogram of V1 sample (green mandarin oil): (1) lutein; (2) zeaxanthin; (3) β-criptoxanthin; (4) chlorophyll *b*; (5) α-carotene; (6) chlorophyll *a*; (7) β-carotene.

TABLE 4

CAROTENOIDS AND CHLOROPHYLL CONTENT IN GREEN MANDARIN ESSENTIAL OIL SAMPLES (mg/L)

Sample	Origin	Period	Lutein	Zeaxantin	β-cripto-xantin	α-carotene	β-carotene	Licopene	Chlorophyll b	Chlorophyll a	Sum of carotenoids	Sum of chloro-phylls
V1	Sicily	Sept. 2007	5.2	0.3	2.3	7.7	15.0	0.0	1.6	114.8	30.5	116.4
V2	Sicily	Sept. 2007	18.6	3.2	4.1	7.9	14.6	0.0	1.4	145.1	48.4	146.5
V3	Sicily	Oct. 2007	3.8	1.0	1.8	6.1	10.6	Trace	0.5	69.8	22.3	70.3
V4	Sicily	Oct. 2007	4.5	0.4	2.4	7.4	14.5	0.0	1.1	102.2	29.2	103.3
V5	Sicily	Oct. 2007	103.4	1.6	4.4	6.6	10.4	0.0	1.4	92.2	33.4	93.6
V6	Sicily	Oct. 2007	13.4	2.3	3.9	6.7	11.4	0.0	1.4	97.3	37.7	98.7
V7	Sicily	Oct. 2007	9.2	1.3	1.8	5.3	7.3	0.0	1.2	71.6	24.9	72.8
V8	Sicily	Oct. 2007	8.0	1.1	2.9	5.2	8.4	0.0	1.1	89.1	25.6	90.2
V9	Calabria	Oct. 2007	2.6	0.2	8.9	2.2	5.9	Trace	4.1	38.6	19.8	42.7
V10	Calabria	Oct. 2007	2.2	0.6	8.9	2.2	5.2	0.1	1.5	72.2	19.2	28.7
V11	Sicily	Nov. 2007	1.7	0.4	2.8	2.6	6.2	0.0	0.6	43.4	13.7	44.0
V12	Sicily	Nov. 2007	0.7	0.2	1.0	1.2	2.7	0.0	1.9	19.1	5.8	21.0
		Min. value	0.7	0.2	1.0	1.2	2.7	0.0	0.5	19.1	5.8	21.0
		Max value	18.6	3.2	8.9	7.9	15.0	0.1	4.1	145.1	48.4	146.5
		Mean	6.7	1.1	3.8	5.1	9.4	0.0	1.5	75.9	26.0	77.4
		Std. Dev.	5.4	0.9	2.6	2.4	4.1	0.0	0.9	38.1	11.2	37.9

TABLE 5

CAROTENOIDS AND CHLOROPHYLL CONTENT IN RED ESSENTIAL OILS (mg/L)

Sample	Origin	Period	Lutein	Zeaxantin	β-cripto-xantin	α-carotene	β-carotene	Licopene	Chlorophyll b	Chlorophyll a	Sum of carotenoids	Sum of chlorophylls
R1	Calabria	Dec. 2007	0.3	0.4	8.5	1.1	14.4	1.0	0.4	3.7	25.7	4.1
R2	Calabria	Dec. 2007	0.3	0.3	9.7	1.5	19.0	1.1	2.7	10.7	31.9	13.4
R3	Calabria	Dec. 2007	0.3	0.3	9.3	1.4	17.6	0.7	0.3	9.6	29.6	9.9
R4	Calabria	Dec. 2007	0.1	0.2	14.6	2.0	27.2	1.7	1.3	0.7	45.8	2.0
R5	Sicily	Dec. 2007	0.1	0.4	12.9	1.6	12.6	1.3	1.1	5.1	28.9	6.2
R6	Sicily	Jan. 2008	1.6	1.0	32.7	4.3	34.1	3.3	1.4	86.4	77.0	87.8
R7	Sicily	Feb. 2008	0.6	0.7	33.2	5.5	32.0	2.4	0.7	36.0	74.4	36.7
R8	Sicily	Feb. 2008	0.4	0.4	15.6	2.1	29.8	1.8	3.0	14.3	50.1	17.3
		Min. value	0.1	0.2	8.5	1.1	12.6	0.7	0.3	0.7	25.7	2.0
		Max value	1.6	1.0	33.2	5.5	34.1	3.3	3.0	86.4	77.0	87.8
		Mean	0.5	0.5	17.1	2.4	23.3	1.7	1.4	20.8	45.4	22.2
		Std. Dev.	0.5	0.3	10.1	1.6	8.4	0.9	1.0	28.7	20.5	28.7

Conclusions

The proposed HPLC method which requires an equipment normally evaluable in most citrus industries, is very simple and rapid. It furnishes a detailed information both on qualitative and quantitative composition of carotenoids and chlorophylls.

From the comparison of the obtained results for the three types of mandarin essential oils, we can conclude that:

- the average content in carotenoids increases from 11.2 mg/L of the yellow mandarin essential oils, to 26.0 mg/L for the green mandarin essential oils, and to 45.4 mg/L for the red mandarin essential oils;
- the ratio carotenoids/chlorophylls in the green mandarin essential oils produced in Sicily is nearly constant 0.30-0.35. This ratio is very high in the red mandarin essential oils (average 5.63) and it assumes intermediate values in the yellow mandarin essential oils (average 1.25);
- the mandarin essential oils produced in Sicily result, generally, richer in carotenoids in comparison to those produced in Calabria;
- the green color is not a function of the chlorophylls content; it depends from the preponderance of chlorophylls in respect to carotenoids. (The sample R6 is red although it has a concentration more than double in chlorophylls in comparison to the samples V10, V11, V12);
- it is confirmed that there isn't any correlation between the variation of the content in carotenoids and in chlorophylls.

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