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Determination and Quantification of Carotenoids in EVOOs the Province of Messina Italy

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Abstract

This paper presents the investigation of the pigments composition in monovarietal virgin olive oils produced from the three main olive varieties (Minuta, Ottobratica and Calabrese) cultivated in Sicily (southern Italy). In all, 19 compounds were identified and quantified in 18 olive oil samples. The application of reversed-phase liquid chromatography with photodiode array detection using a C-30 column in the simultaneous qualitative–quantitative analysis of virgin olive oils pigments, has been shown. The qualitative pigment pattern was similar among the varieties investigated, whereas quantitative differences were found among the different cultivars, which can all be considered as having an high pigment content. β-Carotene was the major component (1.13 – 9.10 ppm), followed by Pheophytin a (0.17– 8.13 ppm). Lutein (0.80 – 2.80 ppm), Neoxanthin (0.30 – 1.03 ppm) and Pheophytin a1 (0.35–1.90 ppm), were also well represented. The presence of carotenoid esters was also detected. This may be due to genetic factors and/or geographical differences. The ratio between the two isochromic pigment fractions, namely the chlorophyll and the carotenoid fractions, was around one in all varieties, showing that they were in balance. These parameters, along with other analytical parameters, could be used as indicators of typicality in olive oils. The presence of a specific pigment profile in olive oils could in fact be used to guarantee the genuineness of the product, since the quality control of food requires a precise knowledge of the pigments composition of the original products.

Keywords

Chlorophyll; β-Carotene; Carotenoids; Sicilian oil; pigments; UPLC/PDA.

Introduction

Extra virgin olive oil (EVOO) is one of the most representative food of Mediterranean diet (1-2) for its high nutritional value. The beneficial effects of EVOO on human health are related to its characteristic composition in fatty acids and minor components (1-2%), such as squalene and phytosterols, aldehydes, polyphenols, pigments, vitamins, minerals, etc. (3-7). The first high performance liquid chromatographic studies on the pigments fraction of monovarietal virgin olive oils from some Spanish cultivar were done by Minguez-Mosquera, Gandul-Rojas and Gallardo-Guerrero (8-9), cultivar Greek (10), cultivar Italian (4-5-11-12) and cultivar Tunisian (13), have been reported. With the aim to increase the database on monovarietal virgin olive oils composition, which are oils obtained only by mechanical pressure of olives of a single variety, it is important to increase the number of studies. Chlorophylls and derivatives have a pro-oxidant effect in the presence of light, since transfer the energy of light and oxygen to the molecules, triggering the processes of oxidation, while the dark act as antioxidants. Carotenoids are characterized by the presence of more isoprene groups, their color varies from yellow to orange to red-purple. The most common are the α-, the β- and γ-carotene and are also called pro-vitamin A as precursors of the same, and so have an important nutritional value. Beside the carotenoids may consider other pigments related to them, such as lutein, violaxanthin and neoxanthin. In the maturation of the drupe succeed numerous

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color variations: initially dominates the intense green, tending to clear up to yellow, and then red patches appear, until the total black-purple color of the fruit. During the extraction process of oil from olives occurs a decrease in the total amount of pigments, chlorophylls decrease becoming phaeophytins by the removal of the Mg2+ (4). Even in the process of conservation of the oil it occurs a reduction of the concentrations in pigments (10). The content of carotenoids and chlorophylls in olive oil virgin depends on many factors, such as the environment, the technology of extraction and conservation system (14). Several epidemiologic studies have shown that an increase in the consumption of foods rich in carotenoids is correlated with a reduction in risk of development of various degenerative chronic diseases (15-16). Were attributed to carotenoids numerous biological effects such as antioxidant activity, improved immune response, control of cell growth and differentiation (15), the promotion of anti-inflammatory and anti-tumor (17), the decrease in the risk of cardiovascular disease (15), the protective action of epithelium (18) and positive action on eyesight (19). Finally, carotenoids together with polyphenols and tocopherols provide oxidative stability to olive oils and have a synergistic antioxidant and anticarcinogenic action in physiological concentrations. The carotenoid profile of extra virgin olive oil can be used as a quality parameter and genuineness for this product, since the presence of other different carotenoids or the detection of a high level of carotenoids transformation may be indicative for the use of practical incorrect or fraudulent techniques (20). The quality and the nutritional and beneficial properties of the product can be altered by conservation inadequate ways, such as exposure to high temperatures or light, causing variations of these compounds through isomerization reactions and discoloration. These reactions are mainly present in deodorization treatments, to which an olive oil with poor organoleptic quality may be subjected ("deodorized oils"). Recent work on the kinetic study and characterization of the thermodynamic parameters, responsible for the thermal degradation reactions of lutein, β-carotene and β-cryptoxanthin in the EVOOs (21), demonstrate the thermal stability of these compounds in oily matrices and allow, through mathematical models, to predict the degradation of carotenoids during storage and / or heat treatment of EVOOs. The aim of this study is to evaluate the chlorophyll and carotenoid pigments composition in monovarietal extra virgin olive oils from the three main olives varieties (Minuta, Ottobratica and Calabrese), cultivated in province of Messina (Sicily-Italy).

2. Materials and methods

2.1. Chemicals

HPLC-grade solvents, organic solvents and reagents were purchased from Sigma–Aldrich (Milan, Italy). Chlorophylls a and b standards were supplied by Sigma. Pheophytins a and b were obtained by acidification with hydrochloric acid from the respective solutions of chlorophylls (22). Chlorophyll a and b C-10 epimers, were obtained according to the method of Watanabe et al. (23). Pyropheophytin a was obtained by heating in pyridine as described by Schwartz, Woo and von Elbe (24).β-Carotene, lutein and β-cryptoxanthin were purchased from Extrasynthese (Genay, France). Violaxanthin and neoxanthin were obtained after extraction with cold acetone and purification by OCC from lattuce according to the method reported by Kimura and Rodriguez-Amaya (25); the purity of standards evaluated by HPLC was of 96% for violaxanthin and 95% for neoxanthin. In order to avoid contaminations among the bands during OCC, only the main portion of each band of carotenoids was collected. Auroxanthin was obtained as described by Davies (26). Standards were stored under nitrogen in the dark at -20 °C.

2.2. Materials and pigments extraction

18 samples of monovarietal virgin olive oils from the five main olive varieties (Minuta, Ottobratica and Calabrese) cultivated in Sicily (Italy), were obtained from selected mills during the 2015–2016 season. Olive oils were extracted from green fruits harvested at the same time using the centrifugal or continuos system technology. Fresh oil samples were stored at 20 °C in the dark before analysis in triplicate. Analyses were carried out within 6 months from olive oils production. Six different samples of each different cultivar were analysed. Each sample, was extracted by liquid-phase distribution (LPD) between N,N-dimethyl-formamide (DMF) and hexane, according to a method described by Giuffrida (4). The samples of virgin olive oils (25 g) were dissolved directly in DMF (150 ml) and treated with five successive 50 ml portions of hexane in a decanting funnel. Chlorophylls, chlorophyllic derivatives and xanthophylls were retained in the DMF phase. The hexane phase contained lipids and carotenes. The DMF phase was treated with a 2% Na2SO4 solution at 0 °C and extracted two times with a 100 ml of a mixture of hexane/ethyl ether (1:1; v/v). The aqueous phase was discarded, eliminating polyphenols and other water-soluble compounds. The organic phase was evaporated to dryness in a rotovapor at 30 °C. The dry residue was dissolved in an appropriate volume of methanol, and analysed by UPLC/PDA. The five hexane phases were combined, concentrated, filtrated, and made up to a known volume of hexane, to directly spectrophotometrically measure the β-carotene concentration, using a previously prepared calibration curve, as this phase only contains this pigment (4).

2.3. Pigments analyses

Pigments in the samples were identified by comparison with standards and from their spectral characteristics, both absorption maxima and peak ratios. UPLC/PDA quantification was carried out using the external standard method; standard curves were calculated by linear regression analyses, based on the available standards calibration curves. The carotenoids analysis was carried out using an Acquity UPLC® Waters liquid chromatography system equipped with a column heater,

a photodiode array detector ACQ-PDA, a quaternary solvent manager Xevo-TQD and a sample manager ACQ-FTN, controlled by Waters® EmpowerTM chromatographic software. In all analyses, an Acquity UPLC® Waters Totally porous C-30 column of 5 mm (4.6 x 250 mm), protected by 0.2 µm stainless steel In-Line Filter with a Holder Waters, was used. The mobile phase consisted of a binary gradient of methanol/methyl-tert-butyl ether/water, MeOH/MTBE/H2O (90:8:2; v/v/v) (A) and MeOH/MTBE/H2O (8:90:2; v/v/v) (B), starting with 0% B, followed by a linear gradient to 30% B in 20 min, to 80% B at 35 min, to 100% B at 65 min and to 100% B at 75 min, then re-equilibrating the column to initial B conc., at a flow rate of 1mL/min. The carotenoids was detected and quantified using the PDA set at 450 nm wavelength.

3. Results and discussion

This paper presents the investigation of the chlorophyll and carotenoid pigments composition in monovarietal virgin olive oils from the three main olive varieties (Minuta, Ottobratica and Calabrese) cultivated in Sicily in the province of Messina (southern Italy). In all, 19 compounds were identified and quantified in 18 olive oil samples as reported in Table 1. Among the varieties investigated in this paper, Minuta oils showed the highest chlorophyll a content (2.00 ppm on average) and chlorophyll b (0.60 ppm on average), followed by Ottobratica oils with values 0.40 ppm on average for chlorophyll a and 1.00 ppm on average for chlorophyll b). In Calabrese oils was seen only in the presence of chlorophyll a (0.4 ppm on average). Interestingly, all the Sicilian olive oils investigated in this work were produced from cultivars growing in the east side of the island, in the province of Messina. Their pigment contented is lower to other cultivars of Sicilian oils studied by Giuffrida et al. (4) for monovarietal virgin olive oils obtained from cultivars growing in the west area of the island. In general, β-carotene was the major component (range 1.13 to 9.05 ppm), followed by lutein (range 1.03 to 2.80 ppm) and Pheophytin a (range 0.17 to 6.20 ppm). In cultivars studied, the variation of the content in the pheophytin a shows a substantial variability. This is mainly due to the different type of cultivar, the type of maturation of the olives, as too mature olives are subject to an oxidation of chlorophylls and therefore an increase phaeophytins. Table 1 shows the quantitative composition of chlorophylls and carotenoids of the olive oils investigated. Minuta oils studied in this work can be considered as having an high pigments content, with a green hue as the prevailing colour. Although, the cultivars Minuta studied in this work showed the lowest pigment content among the cultivars investigated, the cultivars Ottobratica and Calabrese showed the highest β-carotene amounts (8.05 e 7.30 ppm, respectively). Quantitative differences may be attributed to geographical differences and/or to characteristic biosynthetic or catabolic pathways differences (27). The new data on the pigments composition from cultivars reported in this paper represent a contribution to guarantee the genuineness and and typicality of the products, and then the food quality control.

Table 1: Concentrations (ppm) of individual carotenoids and chlorophylls from monovarietal extra virgin olive oils from various sicilian olive varieties.

Pigments		Minuta (ppm)						Ottobratica (ppm)						Calabrese (ppm)					
	sample	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
β-Carotene		1,42	2,07	1,13	4,02	1,40	1,30	8,90	9,05	8,50	6,80	7,40	6,80	4,50	6,80	7,26	8,90	9,10	8,90
Neoxanthin-isomer		0,28	0,29	0,17	0,31	0,35	0,30	0,12	0,27	0,20	0,32	0,20	0,32	0,12	0,26	0,29	0,25	0,27	0,27
Neoxanthin		0,65	0,79	0,70	1,03	0,55	0,60	0,58	0,48	0,45	0,90	0,76	0,90	0,30	0,58	0,60	0,55	0,53	0,58
Violaxanthin		0,39	0,42	0,42	0,30	0,40	0,35	0,37	0,37	0,40	0,42	0,34	0,18	0,15	0,29	0,33	0,40	0,37	0,37
Luteoxanthin		0,31	0,36	0,33	0,45	0,27	0,29	0,32	0,32	0,37	0,36	0,29	0,30	0,10	0,30	0,32	0,37	0,32	0,32
Antheraxanthin		0,38	0,40	0,35	0,34	0,30	0,32	0,35	0,31	0,32	0,33	0,25	0,40	0,12	0,28	0,34	0,32	0,33	0,35
cis-Violaxanthin-isomer		0,40	0,42	0,38	0,26	0,46	0,44	0,34	0,32	0,36	0,34	0,30	0,40	0,17	0,34	0,36	0,36	0,34	0,34
Chlorophyll b		0,60	0,65	0,62	0,81	0,50	0,56	nd	nd	nd	1,40	1,11	1,14	nd	nd	nd	nd	nd	nd
Lutein		2,56	2,63	2,70	2,80	2,53	2,43	1,44	1,36	1,45	2,50	2,53	2,00	0,80	1,45	1,50	1,45	1,40	1,44
Chlorophyll a		2,30	2,20	2,50	1,90	2,40	2,35	0,45	nd	0,40	0,50	0,56	0,40	0,13	0,30	0,42	0,40	0,35	0,45
β-cryptoxanthin		0,40	0,40	0,25	0,30	0,30	0,35	0,35	0,33	0,36	0,37	0,30	0,36	0,14	0,33	0,30	0,36	0,35	0,35
cis-Lutein		0,34	0,35	0,37	0,31	0,29	0,35	0,29	0,30	0,29	0,30	0,28	0,31	0,12	0,40	0,32	0,29	0,30	0,29
Neoxanthin-ester		0,31	0,32	0,25	0,30	0,27	0,31	0,28	nd	0,23	0,26	0,20	0,22	0,09	0,29	0,33	0,23	0,25	0,28
Pyropheophytin a		nd	0,80	nd	1,12	nd	nd	0,35	0,25	nd	0,60	nd	nd	nd	0,28	0,28	nd	nd	0,35
Pheophytin a[1]		nd	1,08	nd	1,60	nd	nd	1,08	1,10	1,15	1,90	2,01	2,20	0,35	1,10	1,20	1,15	1,10	1,08
Pheophytin a		1,12	3,51	1,02	6,20	1,10	1,20	5,82	0,17	5,32	8,13	7,64	7,32	3,20	5,80	6,02	5,30	5,12	5,82
Pheophytin b		nd	0,05	nd	0,05	nd	nd	nd	5,22	0,15	nd	nd	nd	0,06	nd	nd	0,15	0,12	nd
Lutein ester		nd	0,20	nd	0,09	nd	nd	nd	nd	nd	0,12	0,10	0,14	nd	nd	nd	nd	nd	nd
β-cryptoxanthin ester		nd	0,09	nd	0,10	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

- The values refer to the average obtained from three repetitions for each sample.
- Expressed as ppm values (mg/kg).
- nd: not determinable.

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