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Evolution of Minor Polar Compounds and Antioxidant Capacity during Storage of Bottled Extra Virgin Olive Oil

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We characterized 'Olivastra Seggianese' extra virgin olive oil (EVOO) and evaluated its chemical and sensory characteristics and antioxidant and antiradical activities during storage under novel conditions. Two oils (A and B) were analyzed for the commodity characteristics at blending (t_0) and after 9, 12, and 18 months; panel tests were performed and minor polar compounds (MPC) content was assessed at blending (t_0) and after 6, 9, 12, and 18 months. Antioxidant and antiradical activities in vitro were evaluated at t_0 and after 12 months, by human low density lipoprotein (LDL) and 1,1-diphenyl-2-picrylhydrazil radical (DPPH•) tests. Oil A, which had an initially higher MPC content, possessed "harder" organoleptic characteristics than oil B, which had a lower MPC content and was endowed with a "smoother" taste profile. Statistical analyses showed that secoiridoids, particularly deacetoxy-oleuropein aglycone, should be quantified to evaluate EVOO stability during storage. The antioxidant activity toward human LDL was linked to MPC content and to storage time. The tests on the stable free radical DPPH• confirmed the results on human LDL. We propose this as an additional parameter to evaluate olive oil quality and stability over time.

KEYWORDS: Extra virgin olive oil; HPLC/DAD; storage; shelf life; Mediterranean diet; antioxidants; polyphenols; sensory characteristics; hydroxytyrosol

INTRODUCTION

Extra virgin olive oil (EVOO) quality depends on many factors, such as condition of the fruits (which must be undamaged and rapidly processed), harvesting time, crushing and storage methods (*1*). EVOO quality is also strictly correlated with the properties of the cultivar used for its production. In the past few years, interest has focused on monocultivar extra virgin olive oils, especially from autochthonous cultivars, as their unique features are yet to be fully investigated and exploited.

In recent years, the definition of "EVOO quality" is shifting from commodity chemical values to sensory and health properties. The latter mainly concerns the activities of antioxidant molecules such as minor polar compounds (MPC). EVOO contains several different compounds with antioxidant activities, including polyphenolic compounds, to which the very strong natural antioxidant properties of olive oils are generally ascribed (*2*, *3*). A unique characteristic of EVOO is the equilibrium between stability and auto-oxidation phenomena (*4*, *5*), often evaluated by determining the peroxide content (*6*) and mainly dependent on the concentration of antioxidant molecules. In fact, as MPC are predominantly responsible for the olive oil resistance to oxidation—mainly owing to molecules containing an ortho-diphenolic group (e.g., 5-hydroxytyrosol) (*7*)—a method of assessing oil stability consists of the evaluation of MPC over time (*8*, *9*). Yet, their time-wise pattern of decrease and the specific antioxidant role of single MPC are still to be fully understood (*10*).

As mentioned, interest in the pharmacological activities of MPC, in particular 5-hydroxytyrosol and oleuropein, is constantly growing, as data that demonstrate the cardioprotective (and possibly chemopreventive) potential of such compounds are accumulating (*1*, *11*). Indeed, antioxidant molecules such as MPC might provide health benefits, but primarily discriminate the quality of the oil and maintain it over time (*9*). By investigating the time-wise MPC concentration profile in bottled oil, nutritional guidelines might be fine-tuned.

Both simple and complex phenolic antioxidants are detectable in the polar fraction of extra virgin olive oil: examples are 5-hydroxytyrosol and tyrosol derivatives (*12*−*14*), secoiridoids, and lignans (*15*, *16*). MPC concentration in extra virgin olive oil depends on several factors such as olive cultivar (*17*−*19*), and agronomic and technological aspects of production (*20*−*22*). Moreover, different storage conditions might decrease phenols concentration in the oil, adding to other factors such

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as temperature and exposure to light. Because of their antioxidant properties, phenolic compounds are of paramount importance to the shelf life of extra virgin olive oils.

Oil consumption usually occurs within 1 year from its production, but, according to the current legislation (RIS-2/78-IV/98 COI/T.15/NC n 2/Rev. 8, del 25/11/1998), extra virgin olive oil can be consumed within 18 months from bottling. Consequently, we took an 18-month period of storage as approximation of an extended shelf life, and investigated chemical and organoleptic changes of bottled oil during this period.

Aims of this study were to fully characterize 'Olivastra Seggianese' extra virgin olive oil and to evaluate its chemical and sensory characteristics and antioxidant and antiradical activity during storage under novel conditions. Furthermore, we studied the correlation of MPC concentration with their antioxidant capacity and calculated how this could be predictive of olive oil stability.

MATERIALS AND METHODS

Olive Oil Production and Storage Conditions. The EVOOs used in this study were obtained from the autochthonous cultivar 'Olivastra Seggianese'. The 'Olivastra Seggianese' is well-known for its frost tolerance; volatiles obtained from leaves, fruits, and virgin oil have recently been investigated (*17*). The samples used in this study have been collected from within the area that was awarded the Tuscan IGP labels EC no. 644/98.

The two oils utilized in this study were produced from the cultivar 'Olivastra Seggianese', with 5% of 'Moraiolo' cultivar (Manni, Grosseto, Italy). The plants were cultivated under standard organic practices, according to the directive EEC/91/2092. The fruits were hand-harvested directly from the plants, between the last week of October and the first half of November 2001. After harvesting, the fruits were processed within 24 h in a continuous two-phases line (Jumbo 3 Pieralisi, Jesi, Italy). The oils were temporarily stored in five different containers and only at the end of the harvesting a blend was performed to obtain two oils (hereafter referred to as oil A and oil B) with different organoleptic features: higher and lower fruity/bitter taste, respectively. After blending, an aliquot of the two oils was bottled into 100 mL glass bottles capped with nitrogen and sealed with synthetic stoppers. The bottles were made of special glass, which screens from 99.99% UV light radiation. Samples were stored in the dark at constant temperature (18 °C).

During the 18 month period, two bottles of each oil were analyzed for both their chemical and organoleptic properties, according to EEC/91/2568. New bottles were opened at any time point. In particular, samples of the two oils were analyzed for the commodity characteristics at blending (t_0) and after 9, 12, and 18 months; panel tests were performed, and MPC content was assessed at blending (t_0) and after 6, 9, 12, and 18 months. Antioxidant and antiradical activities in vitro were evaluated at t_0 and after 12 months, by LDL and DPPH• tests (see below).

Extraction, Identification, and Quantification of Individual MPC. Fifty milliliters of each oil sample were extracted with 150 mL of EtOH/H_2O (70:30 v/v). The water was acidified (pH = 2.5) with formic acid. A defatting with *n*-hexane was performed to completely remove the lipid fraction. The raw alcoholic extract of each sample was brought to dryness under reduced pressure, redissolved in 2 mL of extraction solvent, and analyzed by HPLC equipped with a DAD detector and an MSD API-electrospray. This extract was used for the determination of single MPC and for the LDL and DPPH• tests, respectively.

Identification of individual MPC was carried out by using both HPLC/DAD and HPLC/MS, using retention time, UV−visible detection, and mass spectra. The quantitative evaluation of individual MPC was performed by HPLC/DAD through the use of authentic standards, such as tyrosol, oleuropein, luteolin, and apigenin, which were used to prepare four-point regression curves ($r^2 \geq 0.99$). Tyrosol and 5-hydroxytyrosol amounts were calculated at 280 nm using tyrosol as the reference standard; oleuropein aglycone, deacetoxy-oleuropein aglycone, and secoiridoid compounds were calculated at 280 nm using oleuropein as the reference standard; elenolic acid and elenolic acid derivatives were evaluated at 240 nm using oleuropein as the reference standard. For oleuropein aglycone, deacetoxy-oleuropein aglycone, elenolic acid, and its derivatives, the correction of the molecular weight was applied. The flavonoid aglycones luteolin and apigenin were evaluated measuring the absorbance at 350 nm of the pure standards. Lignans were identified and analyzed as previously described (*23*).

HPLC-DAD Analysis. HPLC/DAD analyses were performed with a HP 1100L liquid chromatograph equipped with HP DAD (Agilent Technologies, Palo Alto, CA). The experimental conditions were similar to those previously reported (*24*). In details, the analytical column was a 4.6 × 250 mm LiChrosorb RP18, 5 mm (Merck), maintained at 26 °C, equipped with a 10 × 4 mm LiChrosorb RP18 precolumn. The HPLC/DAD analyses were performed with solvents of analytical grade purchased from Carlo Erba (Milano, Italy). The eluent was H_2O (pH 3.2 by H_3PO_4)/CH_3CN. A four-step linear solvent gradient was used starting from 100% H_2O up to 100% CH_3CN, during a 106 min period, at a flow rate of 1 mL/min.

HPLC-MS Analysis. The HPLC-MS analyses were performed using an HP 1100L liquid chromatograph equipped with a DAD detector and 1100 MS detectors. The interface was an HP 1100 MSD API-electrospray (Hewlett-Packard). The HPLC-MS analyses were performed according to a previous report (*25*).

Reference Compounds. The following commercial products were used: tyrosol, oleuropein, luteolin, and apigenin, all from Extrasynthèse (Genay, France).

Antioxidant Activity. LDL oxidation was investigated in vitro by incubating isolated LDL with an oxidative agent, i.e., copper sulfate; the effect of the olive oil extracts on the oxidation process was evaluated by the formation a marker, namely short−chain aldehydes, evaluated as thiobarbituric acid-reacting substances (TBARS). Human low-density lipoproteins (LDL, $d = 1.021-1.063$) were isolated by sequential ultracentrifugation from plasma obtained from healthy, normolipidemic volunteers ($n = 4$). Before initiation of the experiments, LDL samples were desalted by size exclusion chromatography and their protein contents determined according to the method of Lowry et al. (*26*). LDL samples were diluted with PBS to 200 μg/mL and oxidation was started by the addition of 5 μM copper sulfate. Control samples were added with 5 μL of ethanol, which was the vehicle employed to dissolve the extracts (final ethanol concentration: 0.5%). Incubation was carried out at 37 °C in a shaking bath, and aliquots were withdrawn at different times for the assessment of TBARS formation (*27*). The results are expressed as nanomoles of TBARS per milligram of LDL.

Antiradical Activity. Free radical scavenging activity was evaluated by the DPPH• (1,1-diphenyl-2-picrylhydrazil radical) assay. The antiradical capacity of the sample extracts was estimated according to the procedure reported by Brand-Williams (*28*), with slight modifications. Two milliliters of the sample solution, suitably diluted with ethanol, was added to 2 mL of an ethanolic solution of DPPH• (25 mg/100 mL), and the mixture was allowed to stand. After 20 min, the absorption was measured at 517 nm (LAMBDA 25, Perkin-Elmer spectrophotometer) versus ethanol as the blank. Each day, the absorption of the DPPH• solution was checked. The antiradical activity is expressed as IC_{50}, i.e. the concentration required to cause a 50% discoloration of the DPPH• solution. IC_{50} was calculated plotting the ratio. $(A_{blank} - A_{sample}/A_{sample}) \times 100$, where A_{blank} is the absorption of the DPPH• solution and A_{sample} is the absorption of the DPPH• solution after the addition of the sample, against the concentration of the sample. IC_{50} is expressed as mg sample/mg DPPH• (*29*).

Statistical Analyses. Data of the chemical analysis were investigated using the MANOVA procedure of Statgraphics Plus statistical package (Manugistic, Rockville, MD). Total variance was calculated assuming the two oils and the time as sources of variation, *F* ratio, and probability calculated to check significance of variation in relation to the source and confidence level.

RESULTS AND DISCUSSION

The oils obtained from the 'Olivastra Seggianese' cultivar, as analyzed at the beginning of the experimentation (t_0), had

Table 1. Commodity Characteristics of Oils A and B, Evaluated at Different Times Starting from the Blending (t_0)[a]

	t_0	9 months	12 months	18 months
		oil A		
acidity %	0.36 (0.06)	0.37 (0.06)	0.37 (0.05)	0.33 (0.03)
no peroxides	6.2 (1)	13 (1)	13 (1)	11.5 (0.5)
K 232	1.964 (0.037)	2.015 (0.047)	2.262 (0.033)	1.775 (0.026)
K 262	0.127 (0.012)	0.192 (0.013)	0.177 (0.046)	0.3 (0.119)
K 268	0.117 (0.013)	0.183 (0.012)	0.17 (0.053)	0.283 (0.097)
K 270	0.116 (0.013)	0.183 (0.012)	0.171 (0.054)	0.29 (0.101)
K 274	0.116 (0.012)	0.183 (0.012)	0.168 (0.055)	0.279 (0.091)
ΔK	−0.005 (0.002)	−0.004 (0.001)	−0.002 (0.002)	−0.009 (0.011)
		oil B		
acidity %	0.39 (0.05)	0.41 (0.05)	0.44 (0.06)	0.40 (0.010)
no peroxides	8.6 (1.6)	10.1 (1.9)	9.2 (0.550)	9.9 (2.150)
K 232	1.973 (0.114)	2.234 (0.105)	2.088 (0.115)	1.988 (0.012)
K 262	0.132 (0.023)	0.186 (0.024)	0.175 (0.043)	0.175 (0.014)
K 268	0.122 (0.023)	0.176 (0.023)	0.17 (0.049)	0.177 (0.013)
K 270	0.12 (0.024)	0.176 (0.023)	0.17 (0.050)	0.177 (0.012)
K 274	0.121 (0.032)	0.174 (0.023)	0.167 (0.046)	0.176 (0.014)
ΔK	−0.0045 (0.001)	−0.004 (0)	−0.004 (0.001)	0.001 (0.001)

[a] Data are the means (SD) of three determinations, each performed in triplicate.

commodity characteristics that pointed to their high quality, in accordance to EC 2568/91 and EC 1513/01 regulations. In particular, as shown in **Table 1**, although both samples were of high quality, there were differences between the single parameters of the two oils. The organoleptic characteristics (panel test) showed that oil A was more fruity and bitter than oil B and that bitterness was intensively high for the former, whereas it was judged to be low−medium for the latter (data not shown). The total MPC values (**Figure 1**) of oil A were higher than those of oil B, as reflected by their different organoleptic characteristics. Accordingly, some recent works on MPC studied the correlation between their concentration in the oil and specific organoleptic properties: the molecules responsible for bitter and pungent attributes appear to be the secoiridoids containing tyrosol and 5-hydroxytyrosol (*30*). In agreement with this hypothesis, oil A, which has a higher MPC content, demonstrates "harder" organoleptic characteristics than oil B, which has a lower MPC content and is endowed with "smoother" organoleptic characteristics.

The oil samples, accurately stored (see Materials and Methods), were tested for their stability over the 18 month period. Analyses were performed at regular intervals, testing commodity characteristics (**Table 1**), performing a panel test, and measuring the MPC content (**Figure 1**). The commodity characteristics (**Table 1**) were analyzed at the beginning and after 9, 12, and 18 months. Many commodity standards classify an olive oil as extra virgin; the chemical parameters we report in this work, namely peroxides number, acidity, and Δ*K* (**Table 1**), are indicators of oil stability and oxidation level. The maximum limits, in accordance with the current regulations (IGP Toscano), are as follows: 0.60% (expressed as oleic acid), 16 mEq 0.2/Kg, and 0.01 (2.50 for K232 and 0.22 for K270), respectively. All the tested parameters complied with the IGP and the European Community regulations (2081/92) over the 18 months. The panel test of the two oils revealed that the organoleptic characteristics were maintained during the whole period of our research, as no defects were detected at any time. The higher polyphenol content oil (oil A) always exhibited a higher fruity intensity than the other, but both of them were suitable to be classified as extra virgin under the current legislation (EC 2568/92).

The following compounds were identified by HPLC: 5-hydroxytyrosol, tyrosol, elenolic acid (EA), and two elenolic acid derivatives calculated as a sum (EA der.), deacetoxy-oleuropein aglycone (DacOLagl), oleuropein aglycone, four secoiridoids derivatives calculated as a sum (secoir. der.), and the flavones luteolin and apigenin. During the whole 18 months, the most represented components were DacOLagl, oleuropein aglycone, and the secoir. der., as previously reported for Italian virgin olive oils (*18*, *31*).

Figure 1 reports the total MPC content of the EVOOs samples at different times: at t_0 and after 6, 9, 12, and 18 months. In every stage of the trial, oil A exhibited a higher total MPC content as compared with oil B. The total MPC amount decreased over time (**Figure 2**), following well-fitting first-order kinetics, as already reported by Gutierrez and Fernandez (*32*). The rate of MPC decrease (**Figure 2**) of oil A was lower than that of oil B (lower initial amount of MPC); in particular, after 18 months, oil A lost 22% of its MPC whereas oil B lost 48% of their initial concentration.

Recent works were published on the relation between oil stability and antioxidants (namely, MPC and α-tocopherol) content. Hrncirik and Fritsche studied three extra virgin olive oils subjected to accelerated (60 °C) storage conditions, which in 88 days exhibited an 88% decrease of both MPC and α-tocopherol content (*9*). Gutierrez and Fernandez studied two different storage conditions (2 °C with darkness and 30 °C with illumination): α-tocopherol and MPC concentrations were stable and decreased by 10%, respectively, at 2 °C (*32*). In our work we employed a novel method of storage, by using glass bottles, which screen EVOO from 99.99% UV light radiation. Considering the individual MPC of the two oils (**Figure 1**), the class of molecules that plays the major role in the decrease of total MPC of both oils is secoir. der., DacOLagl, and oleuropein aglycone. In fact, if we consider the total amounts of those compounds, evaluated for each time of the trial, a decrease, as compared to the total MPC, of 50% for oil A and of 60% for oil B was recorded: this reduction was mainly due to DacOLagl. The decrease of those three molecules (secoir. der., DacOLagl, and oleuropein aglycone) was presumably due to their hydrolysis, as a correlation with a concomitant increase in tyrosol and 5-hydroxytyrosol was noted.

The evaluation of the percentage of hydrolysis is a parameter recently taken into consideration to monitor the rate of oil aging (*18*), and it is estimated as the percentage of tyrosol + 5-hydroxytyrosol over total MPC. **Figure 1** reports the percent-

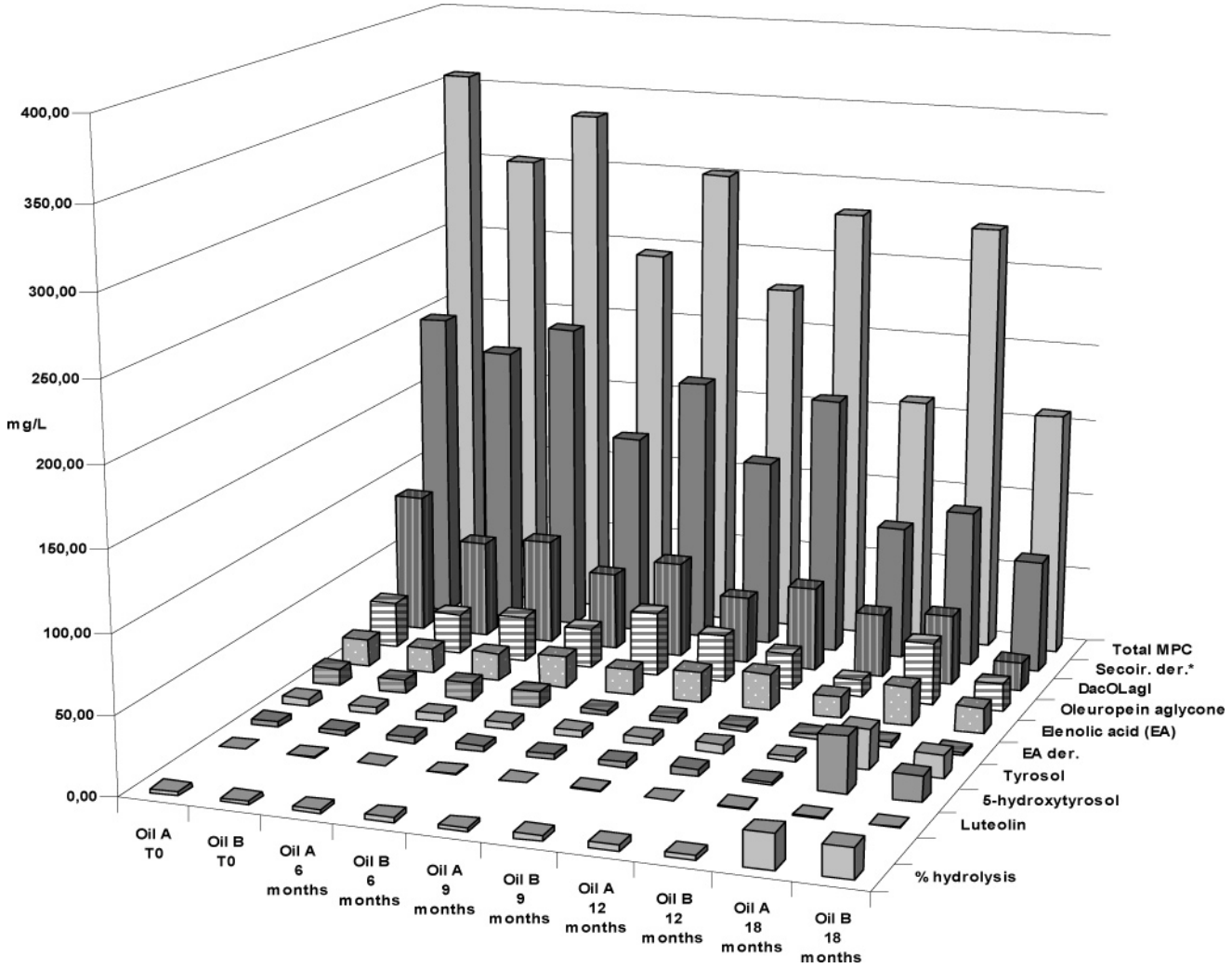

Figure 1. Minor polar compounds (MPC) evaluated at different times, starting from the blending (t_0), in mg L^{-1}. Data are the means of three determinations, each performed in triplicate; SE was in the range 1–3%; *molecules composed by a secoiridoidic group bound to tyrosol or 5-hydroxytyrosol; EA der.: elenolic acid derivatives; DacOLagl: deacetoxy-oleuropein aglycone; total MPC: sum of single MPC.

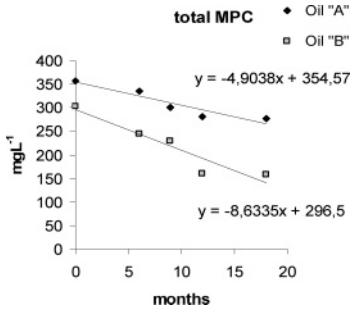

Figure 2. Total MPC concentration of oils A and B. Data are the means of three determinations, each performed in triplicate; SE was in the range 1–3%.

age of hydrolysis of the A and B oils, evaluated at the different times of the trial: the recorded values were quite homogeneous during the first 12 months, whereas a steep rise between 12 and 18 months was recorded. However, this parameter alone is not sufficient to assess oil stability in time because 5-hydroxytyrosol concentrations do not exhibit a regular time-wise trend (*7*).

Statistical Analyses. Statistical parameters are reported in **Table 2**. The two oils (A and B), obtained after mixing bulks containing fruits at different ripening stages (t_0) in order to obtain different products (accordingly, the *panel test* reported different characteristics of the two oils), had significantly different total MPC contents ($p = 0.002$). The difference was mainly due to the total secoiridoid derivatives (sum secoir) calculated as sum of DacOLagl, secoir. der., and oleuropein; variations were present in the levels of DacOLagl, secoir. der., and oleuropein (**Figure 1**). The two commercial products we tested were then truly different, the difference being mainly due to the secoiridoids.

In order to determine which class of molecules should be best selected to evaluate stability over time, the individual MPC concentrations over the 18 months were statistically compared. The molecules that showed highly significant differences in values within the 18 month period of the research (**Table 2**) were the secoiridoids (sum secoir), particularly DacOLagl ($p = 0.003$). Concentrations of tyrosol and 5-hydroxytyrosol were quite stable within the first 12 months and then rapidly decreased between 12 and 18 months. The tyrosol content, although less sensitive than DacOLagl, can be used as another indicator of stability, since its diminishing over time was 97% significant ($p = 0.029$).

Evaluation of Antioxidant and Antiradical Activity. The increasing interest for the antioxidant properties of natural compounds and food components is due to their hypothetical

Table 2. Statistical Analyses of Single and Total MPC (mg/L^{-1})

source of variation	total MPC	5-hydroxy-tyrosol	tyrosol	EA der.	EA	DacO-Lagl	secoir. der.	oleuropein aglyco ne	luteolin	EA der. + EA	sum secoir.
oil											
A	310.4	10.16	8.94	6.79	19.96	63.68	168.2	32.48	0.198	26.76	264.4
B	218.8	5.60	6.08	5.47	17.20	43.09	118.6	22.30	0.476	22.68	184.0
F ratio	50.6	1.49	1.72	3.56	1.04	59.04	17.61	8.53	1.86	1.78	72.33
p	0.002	0.289	0.259	0.132	0.366	0.002	0.014	0.043	0.244	0.253	0.001
time											
0	329.9	3.11	4.28	9.60	16.97	76.36	191.3	28.03	0.35	26.57	295.7
6	290.1	3.69	4.35	10.99	18.85	58.85	165.7	27.48	0.22	29.85	252.0
9	264.8	3.55	4.16	3.51	18.23	52.37	146.9	35.56	0.51	21.75	234.8
12	220.5	3.24	4.42	2.84	18.43	47.44	126.7	17.09	0.38	21.26	191.2
18	217.8	25.86	20.33	3.74	20.44	31.91	86.5	28.78	0.23	24.18	147.2
F ratio	10.9	5.82	8.66	23.96	0.17	29.40	9.02	2.89	0.27	1.09	29.11
p	0.019	0.058	0.029	0.005	0.942	0.003	0.028	0.165	0.880	0.469	0.003

Table 3. LDL and DPPH• Tests

sample	nmol TBARS/mg LDL	sample	EC_{50} DPPH test
	at t_0		
oxidized	18.72 ± 2.11		
oil A 10^{-5} M	0.64 ± 0.03	oil A	7.89×10^{-7}
oil A 5×10^{-6} M	4.27 ± 0.91		
oil A 10^{-6} M	23.34 ± 1.65		
oil B 10^{-5} M	1.55 ± 0.23	oil B	2.78×10^{-6}
oil B 5×10^{-6} M	17.44 ± 1.32		
oil B 10^{-6} M	22.44 ± 1.88		
	after 12 months		
oxidized	19.37 ± 2.86		
oil A 10^{-5} M	0.28 ± 0.12	oil A	3.45×10^{-7}
oil A 5×10^{-6} M	3.65 ± 1.32		
oil A 10^{-6} M	18.25 ± 2.01		
oil B 10^{-5} M	0.87 ± 0.63	oil B	5.67×10^{-7}
oil B 5×10^{-6} M	3.99 ± 0.66		
oil B 10^{-6} M	16.12 ± 1.05		

prevention of the deleterious effects of free radicals on the human body and of the oxidation of fats and other constituents of food.

Currently, the market prefers antioxidants from natural rather than from synthetic sources (*33*). In this respect, olive oil phenolic constituents are being actively investigated, as accumulating in vitro and in vivo data demonstrate their biological activities and suggest that they might play a role in the lower incidence of cardiovascular diseases observed in the Mediterranean area (*11*).

To our knowledge, this is the first report that comprises MPC content, biological, and organoleptic properties during olive oil stability evaluation. When tested on LDL oxidation, i.e., one of the early steps in atherogenesis (*34*), both phenolic extracts (**Table 3**) retained their antioxidant properties after 12 months (at concentrations of 5×10^{-6} to 10^{-5} M). However, all the extracts exhibited, at baseline, pro-oxidant activities at low concentrations, probably due to the strong reducing proprieties of the extracts that, at low concentrations, can accelerate, but not inhibit, the starting phases of the oxidation process. Conversely, after 12 months an antioxidant activity was recorded, possibly due to the release of free 5-hydroxytyrosol from secoiridoids. Last, higher concentrations exhibit marked antioxidant activities. Indeed, oil A extract was more potent compared with oil B extract, confirming how the antioxidant potency was dose-dependently related with total MPC, as previously reported (*35*). In fact, Franconi et al. (*35*). studied the antioxidant activity of oil extracts on human LDL, as evaluated by measuring malondialdehyde and conjugate diene formation induced by copper ions. In both tests, the oil extracts dose-dependently reduced malondialdehyde and conjugate diene generation.

The free radical scavenging activities of the phenolic extracts were also tested at t_0 and after 12 months of storage, by means of the DPPH• test. As shown in **Table 3**, this potent scavenging activity (EC_{50}s in the 10^{-7} M range) was maintained at 12 months, indicating proper storage conditions. In turn, the antioxidant and free radical scavenging properties of the two oils remained stable during the 12 months preservation period, indicating that the decrease observed in the MPC content does not appreciably diminish the beneficial properties of the oils.

In conclusion, this paper reports on the study of two EVOOs from the Seggianese cultivar, with different organoleptic features and MPC content, bottled into 100 mL bottles made of special UV-filtering, capped with nitrogen, and sealed with synthetic stoppers. Under these packaging conditions, the MPC content, the biological properties, and the organoleptic characteristics of the two EVOOs were rather stable for 12 months after bottling. Statistical analyses indicated that secoiridoids, particularly DacOLagl, should be selected to evaluate EVOO stability during storage; the antioxidant activity toward human LDL was linked to MPC content and to storage time. The tests on the stable free radical DPPH• confirmed the results on human LDL, and we propose it as a method to evaluate olive oil quality and stability.

In synthesis, the long-term preservation of the potentially beneficial properties of olive oil and of its organoleptic characteristics, so much appreciated by consumers, might be improved by optimizing all of the production steps, with special attention paid to bottling.

ABBREVIATIONS USED

EVOO, extra virgin olive oil; MPC, minor polar phenolic compounds; HPLC/DAD/MS, high performance liquid chromatography/diode array detection/mass spectrometry; LDL, human low density lipoproteins; DPPH•, 1,1-diphenyl-2-picrylhydrazil radical; DacOLagl, deacetoxy-oleuropein aglycone; EA der., elenolic acid derivatives; secoir. der., secoiridoid derivatives.

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