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Evaluation of bioactive compounds in black table olives fermented with selected microbial starters

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ABSTRACT
BACKGROUND: Table olives have been a component of the Mediterranean diet for centuries, with the trend for their consumption currently increasing worldwide. They are rich in bioactive molecules with nutritional, antioxidant, anti-inflammatory or hormone-like properties. In the present study, the concentrations of phenolics, triterpenic acids, carotenoids and vitamins, as well as fatty acid profiles and antioxidant activity, were analyzed in the edible portion of black table olives (Olea europea L.) from Italian (Cellina di Nardò and Leccino) and Greek (Kalamàta and Conservolea) cultivars fermented with selected autochthonous starters and in the corresponding monovarietal olive oils.

RESULTS: On a fresh weight basis, Cellina di Nardò and Leccino table olives showed the highest total phenolic content. No significant differences were found with respect to the levels of total triterpenic (maslinic and oleanolic) acids and vitamin E among cultivars. All table olives were characterized by high amounts of oleic, linoleic and palmitic acids. Oils were richer in lipophilic antioxidants (carotenoids and tocochromanols) than table olives, which, instead, showed a higher content of polyphenols and triterpenic acids than oils.

CONCLUSION: The present study demonstrates that fermented table olives are an excellent natural source of unsaturated fatty acids, as well as being nutritionally important health-promoting bioactive compounds.

Keywords: carotenoids; fatty acids; olive oils; polyphenols; tocochromanols; triterpenic acids

INTRODUCTION
In addition to extra virgin olive oil, table olives have been important components of the Mediterranean diet for centuries and their consumption is now increasing worldwide. Among the Mediterranean countries, Italy, Greece and Spain contribute to 97% and 28% of the European and World table olive annual production, respectively.[1] Two procedures are commonly used for industrial production of table olives: (i) the chemical method, known as the Spanish or Californian style, consisting of alkaline treatment with sodium hydroxide, and (ii) the natural fermentation process, known as the Greek style.[2] Table olives provide a large amount of natural compounds with nutraceutical value (mainly unsaturated fatty acids, vitamin E, triterpenic acids, polyphenols and carotenoids) with antioxidant, anti-inflammatory or hormone-like properties.[3,4] The concentration of these compounds is very low in NaOH debittered olives compared to the naturally fermented.[5-7]

Previous studies conducted by our group have described the microbiological, biochemical and chemical evolution that occurs during the spontaneous fermentation process of Cellina di Nardò, Leccino, Kalamàta and Conservolea black table olives.[8, 9] These cultivars were selected because of their extensive use in the Mediterranean area: the Leccino, largely cultivated in Tuscany and Umbria regions, is one of the primary cultivars widespread in the Italian olive-growing areas, as well as all over the world; Cellina di Nardò is an autochthonous traditional cultivar of the Salento area in the southernmost part of Apulia characterized by high hardiness, resistance to adverse climatic conditions and pests, and high productivity; Kalàmata and Conservolea are the most important Greek cultivars for table olives.[2]

More recently, autochthonous yeast and lactic acid bacteria selected starters have been used to drive the fermentation process of the same cultivars, leading to an improvement in organoleptic characteristics of the final products and a substantial decrease of fermentation time.[10] In the present study, the above mentioned table olive products were characterized for the most important bioactive compounds (phenolics, triterpenic acids, carotenoids, tocochromanols and vitamins), as well as for the profiles of fatty acids and hydrophilic and lipophilic antioxidant activities, with the aim of highlighting differences in their nutritional value. The results were also compared with those obtained by analyzing the corresponding monovarietal oils.

MATERIALS AND METHODS

Fermentation process

Olives (150 kg; caliber 10–12 mm) were collected at the black stage of ripening of two Italian [Cellina di Nardò and Leccino, from Salento (South of Apulia)] and two Greek [Kalamàta from Amfissa (Phocis regional unit) and Conservolea from Messinia (Southwestern part of the Peloponnese)] cultivars. Olives were fermented as described previously by Tufariello et al.10 Briefly, olives were washed and placed in 50 L of a NaCl aqueous solution (12% or 8% w/v for the two Italian and Greek cultivars, respectively) at an industrial plant (Agricola Nuova Generazione) located in Salento (Martano, Lecce, South of Italy). Starter cultures, consisting of previously selected yeast and lactic acid bacteria strains,8, 9 were used to drive pilot-scale fermentations employing a sequential inoculation strategy. In particular, Leccino olives were inoculated firstly with Saccharomyces cerevisiae LI 180–7 and then with Lactobacillus plantarum L 180–11; Cellina di Nardò with Pichia anomala CL 30–29 and L. plantarum isolate C 180–34; Kalamàta with S. cerevisiae KI 30–16 and Leuconostoc mesenteroides K T5-1; and Conservolea with Debaryomyces hansenii A15-44 and L. plantarum A135-5. Fermentation was carried out at room temperature for 90 days.

Table olives preparation and sampling

At the end of the fermentation process, approximately 20 olive fruits for each cultivar were pitted. The flesh (exocarp and mesocarp) was cut into small pieces and homogenized in a blender (Waring Laboratory, Torrington, CT, USA) under liquid nitrogen. The fine powder was stored at −20 °C until analyses.

Monovarietal olive oils preparation

Monovarietal oils were extracted from olives originating from the same fresh product lots of those used for table olive production using a mini olive press (Spremioliva C30 milling machine; Toscana, Enologica Mori, Tavernelle Val di Pesa, Italy) as described previously by Binetti et al.[11] The olive oils were stored in dark glass bottles at −20 °C under a nitrogen atmosphere until the analyses were performed.

Extraction and high-performance liquid chromatography (HPLC) analysis of polyphenols

Phenolic compounds were extracted from triplicate aliquots (1 g) of each table olive samples, as described by Hajimahmoodi et al.,[12] and from oil samples (0.1 g) as reported by Garcia et al.[13]

Polyphenols extracts were analyzed quali-quantitatively as described by Bleve et al.[8] using an 1100 Series HPLC system (Agilent Technologies Inc., Santa Clara, CA, USA) equipped with a Luna 5 µm C18(2) 100 Å column (250 × 4.6 mm) (Phenomenex, Torrance, CA, USA). The wavelengths used for quantification of phenol compounds were 280, 295, 320 and 350 nm.

Extraction and HPLC analysis of triterpenic acids

Triterpenic acids were extracted as described by Romero et al.5 from triplicate aliquots (1 g) of each table olive sample and from oil samples (0.1 g) as reported by García et al.[14] Triterpenic acids extracts were analyzed quali-quantitatively using the method of Lozano-Mena et al.,[15] slightly modified, using an 1100 Series HPLC system (Agilent) equipped with a Luna 5 µm C18(2) 100 Å column (250 × 4.6 mm) (Phenomenex). The mobile phases were: acetonitrile (A) and 1% (v/v) H_3PO_4 in water (B). The gradient elution was: 0 min, 60% A and 40% B; 0–5 min, 50% A and 50% B; 5–10 min, 40% A and 60% B; 10–20 min, 30% A and 70% B; 20–25 min, 25% A and 75% B; 25–30 min, 20% A and 80% B; 30–35 min, 80% A and 20% B; and 40 min, 0% A and 100% B. The flow rate was 1.0 mL min^{-1} and the column temperature was maintained at 30 °C. The absorbance was registered at a wavelength of 210 nm.

Extraction and HPLC analysis of carotenoids and tocochromanols

Carotenoids and tococromanols (tocopherols and tocotrienols) were extracted as described by Minguez-Mosquera and Garrido-Fernandez16 from triplicate aliquots (1 g) of each table olive sample. Triplicate aliquots (0.1 g) from oils were dissolved in 1 mL of ethyl acetate, filtered through a 0.45 µm syringe filter (Millipore Corporation, Billerica, MA, USA) and immediately analyzed by HPLC, without extraction and saponification.17 Quali-quantitative analyses of carotenoids and tococromanols were carried out using a 1100 Series HPLC system (Agilent) as described by Durante et al.[18]

Vitamin A determination

The vitamin A values, expressed as retinol equivalents (RE), were calculated according to NRC,[19] where 6 µg of β-carotene corresponds to 1 µg RE.

Extraction and HPLC analysis of vitamin B1 and B2

Vitamins B1 and B2 were extracted from triplicate aliquots (1 g) of fermented table olives as described by Hasan et al.[20] Quali-quantitative analyses of vitamins B1 and B2 were carried out using a 1100 Series HPLC system (Agilent) equipped with a ProntoSIL 120–5 C18 AQ (150 × 3 mm, 5µm; Bischoff Chromatography, Leonberg, Germany) column. The mobile phases were: 50 mmol L^{-1} H3PO4 in water (adjusted to pH 2.5) (A) and acetonitrile (B). The gradient elution was: 0 min, 99% A and 1% B; 0–2 min, 99% A and 1% B; 2–8.5 min, 30% A and 70% B; 8.5–11 min, 30% A and 70% B; and 11–15 min, 99% A and 1% B. The flow rate was 0.6 mL min^{-1} and the column temperature was maintained at 40 °C. Absorbance was registered at a wavelength of 268 nm.

Vitamin C determination

Vitamin C content was evaluated using the method described by Ferreira et al.[21] with some modifications. Triplicate aliquots (100 mg) of fermented table olives were extracted with 10 mL of metaphosphoric acid (1%, w/v) for 45 min at room temperature on a magnetic stirrer and then centrifuged at 4000 × g for 10 min. The supernatant (1 mL) was mixed with 9 mL of 2,6-dichlorophenolindophenol solution (0.005%, w/v) and the absorbance was measured within 30 min at 515 nm against a blank. The content of vitamin C was calculated on the basis of the calibration curve of authentic L-ascorbic acid (25–250 µg mL^{-1}; $y = -0.0048x + 1.2708$; $r2 = 0.9994$).

Measurement of antioxidant activity

Antioxidant extracts were sequentially extracted from triplicate aliquots (1 g) of fermented table olives with 5 mL of absolute methanol (hydrophilic extract) and hexane (lipophilic extract) at 4 °C, under constant shaking (300 rpm) on a magnetic stirrer overnight. Samples were centrifuged at 10 000 g and supernatants were used for subsequent analyses. Antioxidant activity was measured in both the hydrophilic and lipophilic fractions using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay method and calculated as the percentage of discoloration = [1 – (A of sample t = 30/A of control t = 0)] × 100.[22]

Lipid extraction and fatty acid profiles determination by gas chromatography-mass spectroscopy

Total lipids were extracted from triplicate aliquots (1 g) of fermented table olives using the modified method of Bligh and Dyer.[23] Lipids from table olives and olive oil samples (0.1 g) were subjected to fatty acid derivatization in accordance wiuth the German Society for Fat Research (DGF – C-VI 11a) method.24 The analyses were performed on an 5977E gas chromatograph/mass spectrometer (Agilent) as described by Durante et al.[25]

Statistical analysis

All data represent the mean ± SD of three independent replicates (n = 3).

Statistical analysis was based on one-way analysis of variance. Tukey's post-hoc method was applied to establish significant differences among means ($P < 0.05$).

Correlations were calculated using Pearson's correlation coefficient (r). All statistical comparisons were performed using SigmaStat, version 3.11 (Systat Software Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Phenolic profile

Plant-derived phenolics are one of the main natural compounds considered to be responsible for several beneficial effects on human health and well-being, including the prevention and prophylaxis of cancers and cardiovascular diseases.[26,27] Table olives, especially black ones, are important dietary sources of phenolics; indeed, they ranked 19th and 28th in the top 100 richest foods in polyphenols by concentration and content per serving, respectively, although significant variations were reported among cultivars.[28,29] It is also well known that simple and complex phenolics contribute to olive oil stability.[30]

Significant differences in the content of total polyphenols of the fermented table olives were demonstrated among cultivars ($P < 0.05$), within a range of values between 316.48 (Conservolea) and 1300 μg g^{-1} fresh weight (FW) (Cellina di Nardò), whereas, in the corresponding oils, phenol levels were much lower, ranging from 49.71 μg g^{-1} oil (Conservolea) to 558.13 μg g^{-1} oil (Cellina di Nardò) (Fig. 1A). These values are in accordance with those reported by Owen et al.,[31] Blekas et al.[32] and Piscopo et al.[33]

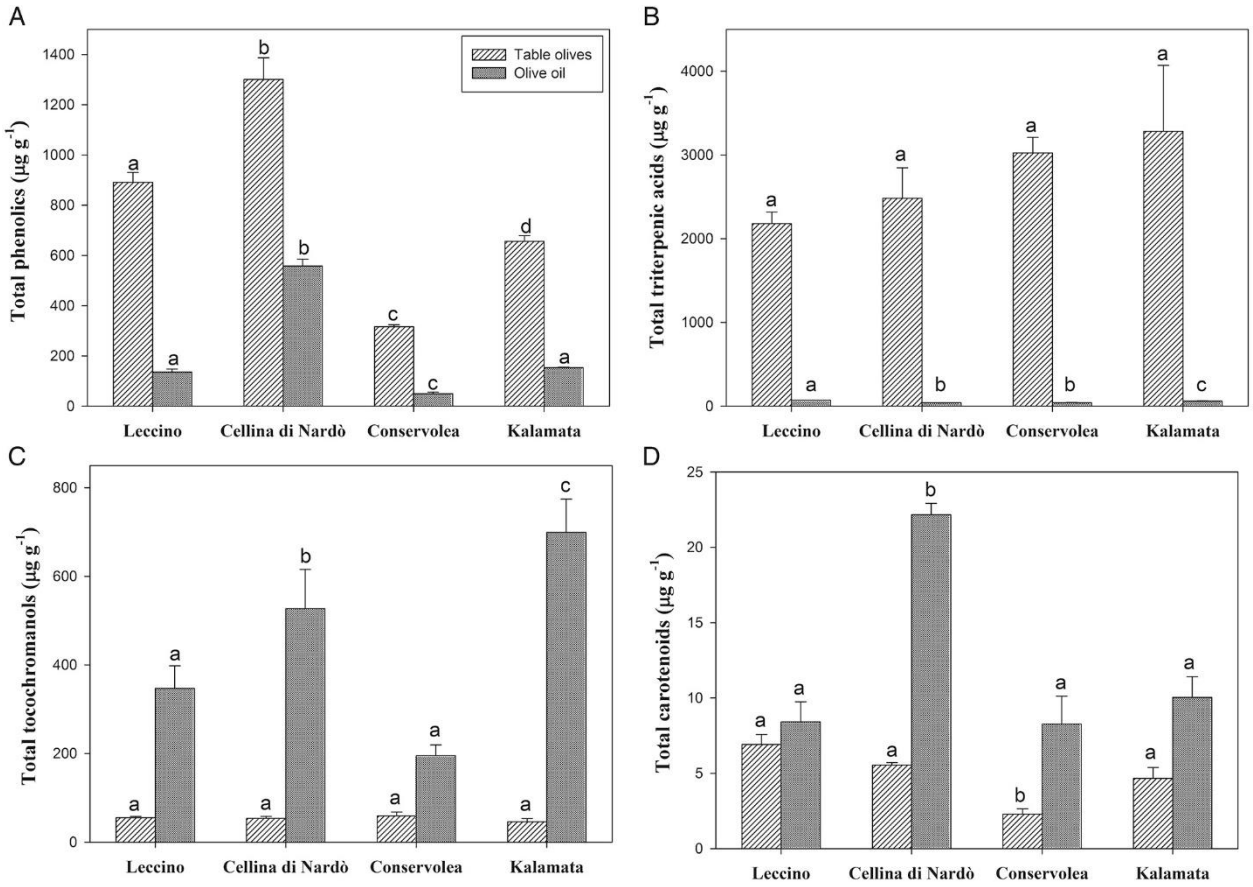

Figure 1. Total contents of (A) phenolics, (B) triterpenic acids, (C) tocochromanols and (D) carotenoids in fermented table olives from Cellina di Nardò and Leccino, Conservolea and Kalamàta cultivars and in the resulting monovarietal olive oils. Values are expressed as μg g^{-1} FW and μg g^{-1} oil for table olive and olive oil, respectively, and represent the mean ± SD of three independent replicates (n = 3). Data were submitted to one-way analysis of variance. Significant differences between cultivars were detected using the Tukey's post-hoc test ($P < 0.05$) as indicated using different letters above the bars.

Several studies reported the roles of β-glucosidase, esterase and polyphenol oxidase activities with respect to lowering oleuropein content and increasing phenolic alcohols (hydroxytyrosol and/or tyrosol) of olives during drupe development and ripening and fermentation.[28,34-36] Regarding the processing of table olives, Tataridou and Kotzekidou[36] reported a higher concentration of hydroxytyrosol and tyrosol in black olives (Kalamàta) and green olives (Chalkidikis) fermented with oleuropeinolytic strains of L. plantarum than in those industrially processed with NaOH. Hydroxytyrosol, which is well known for its antioxidant, hypoglycemic and hypocholesterolemic properties,[37,38] is one of the most powerful polyphenol antioxidants identified in olives and is well absorbed, distributed and metabolized by the human body.[39] In the specific table olive batches that were investigated in the present study, the highest amount of hydroxytyrosol was detected in Cellina di Nardò (832.97 μg g^{-1} FW), followed by Leccino (553.69 μg g^{-1} FW), Kalamàta (477.06 μg g^{-1} FW) and Conservolea (205.49 μg g^{-1} FW) cultivars (Table 1), with the latter two showing values in very good agreement with those reported by Zoidou et al.[40] for Kalamon (555.1 μg g^{-1} FW) and Chondrolies (200.9 μg g^{-1} FW) Greek olives.

Table 1. Content of bioactive compounds and vitamins in table olives from Cellina di Nardò, Leccino, Conservolea and Kalamàta cultivars, fermented using selected autochthonous microbial starters

	Leccino	Cellina di Nardò	Conservolea	Kalamàta
Phenolic compounds (μg g^{-1} FW)				
Hydroxytyrosol	553.69±4.58^{a}	832.97±26.92^{b}	205.49±5.53^{c}	477.06±10.11^{d}
Pyrocathecol	21.17±2.26^{a}	151.90±23.30^{b}	55.68±1.15^{c}	nd
Verbascoside	258.55±23.69^{a}	207.61±20.31^{a}	53.31±0.78^{b}	18.60±5.52^{b}
Isoverbascoside	13.58±1.42^{a}	nd	nd	50.11±1.15^{b}
Rutin	41.88±6.91^{a}	46.31±6.81^{a}	nd	nd
Luteolin-7-O-glucoside	2.63±0.35^{a}	8.71±0.21^{b}	nd	nd
Tyrosol	nd	nd	nd	110.67±5.52
Cyanidin 3-rutinoside	nd	35.41±4.32	nd	nd
Cyanidin 3-glucoside	nd	18.73±1.92	nd	nd
Triterpenic acids (μg g^{-1} FW)				
Maslinic acid	1637.82±102.81^{a}	2117.02±353.42^{a}	2132.68±85.60^{a}	1950.46±429.82^{a}
Oleanolic acid	541.74±35.46^{a}	366.42±11.54^{b}	891.63±101.17^{a}	1333.34±355.84^{c}
Tococromanols (μg g^{-1} FW)				
ß-Tocotrienol	8.99±0.27^{a}	1.63±0.21^{b}	nd	6.73±1.95^{a}
γ-Tocopherol	3.44±0.16^{a}	6.59±0.11^{b}	nd	4.45±0.87^{a}
α-Tocopherol	42.90±2.44^{a}	45.42±4.14^{a,b}	59.14±8.79^{b}	34.97±4.26^{a}
Carotenoids (μg g^{-1} FW)				
Lutein	5.79±0.59^{a}	4.82±0.11^{a,b}	1.57±0.17^{c}	4.26±0.62^{b}
Zeaxanthin	0.28±0.06^{a}	0.11±0.01^{b}	nd	0.09±0.001^{b}
β-carotene	0.84±0.01^{a}	0.62±0.04^{a}	0.72±0.16^{a}	0.32±0.09^{b}
Vitamin A (μg RE g^{-1} FW)	0.07±0.001^{a}	0.05±0.001^{b}	0.06±0.01^{a,b}	0.03±0.007^{c}
Vitamin B1 (μg g^{-1} FW)	3.80±0.33^{a}	4.27±0.83^{a}	3.95±1.41^{a}	5.26±0.29^{a}
Vitamin B2 (μg g^{-1} FW)	2.59±0.85^{a}	2.20±0.68^{a}	1.48±0.59^{a}	3.05±0.46^{a}
Vitamin C (μg g^{-1} FW)	<1	<1	<1	<1

FW, fresh weight; ND, not detected; RE, retinol equivalents. Data are the mean ± SD of three independent replicates (n = 3). Different lowercase letters indicate significant differences between cultivars ($P < 0.05$).

The amount of tyrosol in ripe unprocessed olive drupes from five different cultivars grown in Tuscany (Frantoio, Rossellino, Ciliegino, Cuoricino and Grossolana) has previously been reported by Romani et al.[41] Although with concentrations 0.4–10 times lower than hydroxytyrosol, all cultivars showed a substantial amount (from ~100 to ~1200 mg g^{-1} FW) of tyrosol. In the present study, tyrosol was detected only in Kalamàta table olive (110.67 μg g^{-1} FW) (Table 1). Very low concentrations of tyrosol have been reported in Greek-style naturally black olives in brine (13–41 μg g^{-1} FW) of the cultivar Conservolea by Blekas et al.,[32] whereas amounts of tyrosol similar to those in the present study were reported for Greek-style Kalamàta olives (53–101 μg g^{-1} FW). Among the main phenolic compounds identified by Malheiro et al.[42] in twenty-four samples of naturally fermented table olives, tyrosol was absent in only one black olive sample (cultivar Cobrançosa) and varied between 0.37 and 10.48 μg mg^{-1} of extract. Pyrocathecol was observed at a high content in Cellina di Nardò (151.90 μg g^{-1} FW), followed by Conservolea (55.68 μg g^{-1} FW) and Leccino (21.17 μg g^{-1} FW) table olives. Verbascoside, the major hydroxycinnamic acid derivative in olive pulp,[43] is reported to possess pharmacologically beneficial activities for human health, including antioxidant, anti-inflammatory, antineoplastic and neuroprotective properties.[44] Generally, after olive fermentation, the verbascoside concentration increases in the brine and reduces in the olive fruit.[9,45] In the present study, verbascoside content ranged between 18.60 μg g^{-1} FW (Kalàmata) and 258.55 μg g^{-1} FW (Leccino), whereas its positional isomer isoverbascoside was found only in Kalàmata (50.11 μg g^{-1} FW) and Leccino (13.58 μg g^{-1} FW) table olives (Table 1). Flavonoids, such as rutin and luteolin-7-O-glucoside, were observed only in Leccino (41.88 and 2.63 μg g^{-1} FW, respectively) and Cellina di Nardò (46.31 and 8.71 μg g^{-1} FW, respectively) table olives. The chromatographic profile of polyphenols revealed a quantitatively relevant peak with a retention time and ultraviolet–visible spectrum similar to quercetin. Although, to the best of our knowledge, there are no reports regarding the presence of quercetin as major flavonol in table olives, we cannot exclude the possibility that the fermentation process could lead to the metabolic conversion of other flavonoids into quercetin. Nevertheless further investigations are required to confirm the identity of the compound.

The purple and black color of fresh olives is a result of the presence of monomeric anthocyanins, mainly cyanidin 3-rutinoside and cyanidin 3-glucoside, which account for more than 90% of the total anthocyanins in fresh naturally black ripe drupes. During table olive fermentation, part of the monomeric anthocyanins diffuses in the brine, although most polymerizes within the drupe into stable pigments contributing to the color of the processed product.[46,47] Variations in the content of these compounds depend on the cultivar and on fermentation process. Cellina di Nardò table olives were the only cultivar characterized by the presence of the monomeric anthocyanins cyanidin-3-glucoside (35.41 μg g^{-1} FW) and cyanidin-3-rutinoside (18.73 μg g^{-1} FW) (Table 1).

Triterpenic acids

Pentacyclic triterpenes are secondary plant metabolites widespread in fruit peel, leaves and stem bark.[48] Because of the proven health benefits of triterpenic acids, acting as antitumor, anti-diabetic, antioxidant, cardioprotective, neuroprotective, anti-hypertensive, anti-hyperlipidemic, antiviral and anti-inflammatory agents, there is a growing interest in identifying natural dietary sources of these compounds.[15,49] Maslinic and oleanolic acids have been quantified in very high concentration in table olives (1319 and 841.4 μg g^{-1} FW, respectively), which represent the main dietary source of these compounds.[5] Accordingly, in all analyzed table olives, maslinic and oleanolic acids were present at a high concentration (Table 1). Maslinic acid ranged from 1637.82 μg g^{-1} FW (Leccino) to 2132.68 μg g^{-1} FW (Conservoea), although these values did not differ significantly ($P > 0.05$). By contrast, the content of oleanolic acid varied significantly ($P < 0.05$) among samples, ranging from 366.42 μg g^{-1} FW (Cellina di Nardò) to 1333.34 μg g^{-1} FW (Kalamata). In all samples, maslinic acid was higher than oleanolic acid, in agreement with the data reported by Romero et al.,[5] Medina et al.[50] and Alexandraki et al.[6] Interestingly, the latter study reported that the natural processing of debittering of Greek table olives (Kalàmata and Conservolea) did not influence the triterpenic acid content in the final product. Moreover, Romero et al.[5] found that the concentration of maslinic and oleanolic acids in the olive flesh was reduced after alkaline olive debittering. Furthermore, alkaline-treatment led to the release of triterpenic acids in the washing solutions. In the present study, the concentration of maslinic and oleanolic acids in brine was not detectable (data not shown), indicating that the fermentation process carried out by the selected autochthonous microorganisms did not affect the levels of triterpenic acids in the final product.

It is worth noting that the levels of total triterpenic acids in the table olives are quite higher than those reported in the corresponding olive oils, which ranged from 40.18 μg g⁻1 oil (Conservolea) to 71.59 μg g^{-1} oil (Leccino) (Fig. 1B). These values are in agreement to those reported in virgin olive oil by Allouche et al.[51] and Pérez-Camino and Cert.[52]

Tococromanols, carotenoids and vitamins

Fermented table olives are also a source of tococromanols (tocopherol and tocotrienol forms),[53] known to have vitamin E activity and to play a role in preventing oxidant-related chronic pathologies such as cardiovascular diseases, atherosclerosis and cancers.[54] The total tococromanol concentration in table olives and in the corresponding monovarietal oils is reported in Fig. 1(C). Table olives showed considerably lower amounts of total tocochromanols (on average approximately 57 μg g^{-1} FW, with no significant differences among cultivars) than the corresponding monovarietal olive oils, where total tocochromanols significantly varied between the 195 μg g^{-1} oil of Conservolea and 699.01 μg g^{-1} oil of Kalamata cultivars. These data were in agreement with those reported by Condelli et al.[55] and Piscopo et al.[33] who studied 75 commercial extra virgin olive oils and four different monovarietal oils produced in the Mediterranean basin.

In all assayed table olives, α-tocopherol was the prevalent form of tococromanols, accounting for more than 60% of the total, followed by γ-tocopherol in Cellina di Nardò and β-tocotrienol in Leccino and Kalàmata (Table 1). These differences are related to genotype characteristic and/or to different environmental growth conditions. Hassapidou et al.[56] have reported that the fermentation process did not affect the α-tocopherol content in Conservolea and Kalàmata black olives which was

approximately 34 and 40 μg g^{-1} FW, respectively. An appreciable content of α-tocopherol (64.4 μg g^{-1} FW) was also reported by Lanza et al.[57] in the pericarp of spontaneously fermented table olives from the Italian cultivar Intosso d'Abruzzo. These values are very similar to those we found in our samples, ranging from 59.14 μg g^{-1} FW (Conservolea) to 34.97 μg g^{-1} FW (Kalàmata). The 'Scientific opinion on dietary reference values for vitamin E' document, recently released by the EFSA Panel on Dietetic Products, Nutrition and Allergies, sets the adequate intake (AI) of vitamin E (only as α-tocopherol) at 11 and 13 mg day^{-1} for women and men, respectively.[58] On the basis of the data obtained in this study, a serving (15 g) of table olives can provide from 4.0% (Kalàmata) to 8.0% (Conservolea) of the vitamin E AI for adults. Obviously, the contribution of olive oil to vitamin E AI is much higher. Bayram et al.[59] reported that the daily consumption of about 50 mL of extra virgin olive oils may be sufficient to fulfill the dietary recommendation for vitamin E. Therefore, especially considering the Mediterranean diet, table olives in combination with olive oil represent an important dietary source of vitamin E.

Carotenoids are valuable minor components present in table olives, they play an important antioxidant role in protecting the cell against oxidative damage and counteracting lipid peroxidation.[60] In the present study, the total content of carotenoids in fermented table olives was significantly lower than in the monovarietal olive oils produced from the same cultivars ($P < 0.05$) (Fig. 1D). Leccino, Cellina di Nardò and Kalàmata table olives showed significantly higher amounts of total carotenoids (6.91, 5.55 and 4.67 μg g^{-1} FW respectively) than Conservolea (2.29 μg g^{-1} FW). Lutein resulted the most abundant carotenoid followed by β-carotene in all assayed cultivars (Table 1). Many carotenoids, including β-cryptoxanthin, α-, β- and γ-carotenes have pro-vitamin A activity, whereas others, such as the xanthophylls lutein and zeaxanthin, counteract age-related macular degeneration by quenching the singlet oxygen and blue light at the retina layer level.[61] In our samples, the only detectable carotenoid with pro-vitamin A activity was β-carotene whose amount varied between 0.32 μg g^{-1} FW (Kalàmata) and 0.84 μg g^{-1} FW (Leccino), corresponding to 0.03–0.07 μg RE g^{-1} FW depending on cultivars (Table 1), so that a 15-g serving of fermented table olives contributes very marginally (<0.2%) to the population reference intake for vitamin A fixed to 650 and 750 μg RE day^{-1} for adult women and men, respectively by EFSA.[62]

Table olives provide also small amounts of vitamins of the B group and C, considered to have great antioxidant effects, besides their known essential roles for the normal growth and development of the humans. In our samples, vitamins B1 (3.80–5.26 μg g^{-1} FW) and B2 (1.48–3.05 μg g^{-1} FW), as well as traces of vitamin C (<1 μg g^{-1} FW), were also detected, with no significant differences among cultivars (Table 1)

Fatty acid composition

The nutritional value and the positive effects of olive oil and table olives on human health are also attributed to the presence of large amounts of monounsaturated fatty acids (MUFAs), particularly oleic acid. Several studies have reported the positive correlation between the dietary intake of MUFAs and a reduced risk of cardiovascular diseases and breast cancer, as well as normal cholesterol levels.[63,64] Moreover, table olives are a valid source of essential fatty acids, mainly linoleic (ω-3) and linolenic (ω-6) acids, which the human body requires but is unable to synthesize.

In the present study, the fatty acid profile of fermented table olives revealed significant qualitative and quantitative differences among the assayed cultivars (Table 2). In general, myristic, palmitic, margaric, stearic, arachidic and behenic acids (saturated fatty acids: SFAs), palmitoleic, margaloreic, oleic and gadoleic acids (MUFAs), as well as linoleic and linolenic acids (polyunsaturated fatty acids: PUFAs), were detected in all samples, with some minor exceptions. Differences in the fatty acids composition probably depend on the metabolic behavior of each cultivar in relation to the characteristics of the genotype. Independently from the cultivar, the most abundant fatty acid was oleic acid, ranging from 53.30% (Conservolea) to 67.87% (Kalamata) of total fatty acids, followed by palmitic and linoleic acids, ranging from 12.79% (Conservolea) to 18.07% (Cellina di Nardò) and from 8.57% (Kalàmata) to 26.01% (Conservolea), respectively.

Table 2. Fatty acids composition in table olives from Cellina di Nardò, Leccino, Conservolea and Kalamàta cultivars, fermented using selected autochthonous microbial starters

	Leccino	Cellina di Nardò	Conservolea	Kalamàta
Fatty acids (%)				
Myristic acid (C12:0)	0.12±0.06^{a}	0.06±0.01^{a}	0.29±0.01^{b}	0.14±0.02^{a}
Palmitic acid (C16:0)	15.34±0.62^{a}	18.07±0.01^{b}	12.79±1.61^{c}	15.40±0.19^{a}
Palmitoleic (C16:1)	1.87±0.14^{a}	2.32±0.01^{b}	0.78±0.01^{c}	0.93±0.05^{c}
Margaric acid (C17:0)	0.13±0.05^{a}	0.16±0.01^{a,b}	0.18±0.01^{a,b}	0.24±0.06^{b}
Margaroleic acid (C17:1)	0.21±0.07^{a,b}	0.30±0.01^{a,b,c}	0.18±0.01^{b}	0.49±0.13^{c}
Stearic (C18:0)	2.60±0.07^{a}	2.85±0.02^{a}	4.20±0.61^{b}	4.36±0.55^{b}
Cis Oleic (C18:1)	59.89±3.56^{a,b}	62.26±1.03^{b}	53.30±4.08^{a}	67.85±1.29^{c}
Linoleic (C18:2 n-6)	17.66±2.67^{a}	11.81±0.01^{a}	26.01±5.31^{b}	8.57±0.05^{c}
Linolenic (C18:3 n-3)	1.03±0.01^{a}	1.25±0.01^{b}	1.25±0.07^{b}	0.99±0.06^{c}
Arachidic acid (C20:0)	0.48±0.05^{a}	0.11±0.01^{b}	0.46±0.02^{a}	0.31±0.08^{c}
Gadoleic acid (C20:1)	0.51±0.02^{a,b}	0.60±0.02^{a}	0.44±0.01^{b,c}	0.33±0.09^{c}
Behenic acid (C22:0)	0.16±0.01^{a}	0.21±0.01^{b}	0.12±0.01^{c}	0.39±0.01^{d}
∑SFA	18.83±0.86^{a}	21.46±1.06^{a}	18.04±2.27^{a}	20.84±0.91^{a}
∑MUFA	62.48±3.79^{a,b}	65.48±1.09^{a,b}	54.70±4.11^{c}	69.60±1.56^{b}
∑PUFA	18.69±2.68^{a}	13.06±0.02^{a,b}	27.27±5.38^{c}	9.56±0.11^{b}
∑PUFA/SFA	0.99	0.61	1.51	0.46
ω-6/ω-3	17.44	9.44	20.81	8.65

ND, not detected.
Data are the mean ± SD of three independent replicates (n = 3).
Different lowercase letters indicate significant differences between cultivars ($P < 0.05$).

Conservolea table olives showed significantly lower MUFA (54.70%) and higher PUFA (27.27%) levels than all other cultivars. The ratios of PUFA to SFA (PUFA/SFA) and of ω-6 to ω-3 fatty acids (ω-6/ω-3) were also evaluated, being important parameters currently used to assess the nutritional quality of the lipid fraction of foods. World Health Organization/Food and Agriculture Organization guidelines considered a PUFA/SFA ratio > 0.4–0.5 as being optimal in a balanced diet. All tested fermented table olives exceeded these suggested values: 0.99 (Leccino), 0.61 (Cellina di Nardò), 1.51 (Conservolea) and 0.46 (Kalamata) (Table 2). Furthermore, The World Health Organization has recommended an ω-6 and ω-3 ratio of from 4 to 10 especially during child growth and development because the long-chain ω-3 series are fundamental for brain and retina development.65 Cellina di Nardò and Kalàmata fermented table olives fell in this range, highlighting their nutritional value.

Hydrophilic and lipophilic antioxidant activity

In the present study, the hydrophilic and lipophilic antioxidant activities were estimated by the DPPH assay. As shown in Fig. 2, the hydrophilic antioxidant activity of all tested table olives was higher than the lipophilic one. Furthermore, Leccino and Cellina di Nardò showed higher hydrophilic (75.33% and 84.68% of DPPH discoloration, respectively) and lipophilic (47.78% and 48.83% of DPPH discoloration, respectively) antioxidant activities than Kalamàta and Conservolea cultivars. The differences observed in hydrophilic antioxidant activity may depend on the composition and profile of phenolic compounds rather than their total amount. A good correlation between hydrophilic antioxidant activity and hydroxytyrosol ($r = 0.988$; $P = 0.0118$) was found in the studies by Owen et al.66 and Pereira et al.[67] No correlation was found between lipophilic antioxidant activity and lipophilic compounds, suggesting that other hexane soluble molecules not considered in the present

study, such as sterols and chlorophylls, may mostly contribute to the observed lipophilic antioxidant activity of table olive extracts.

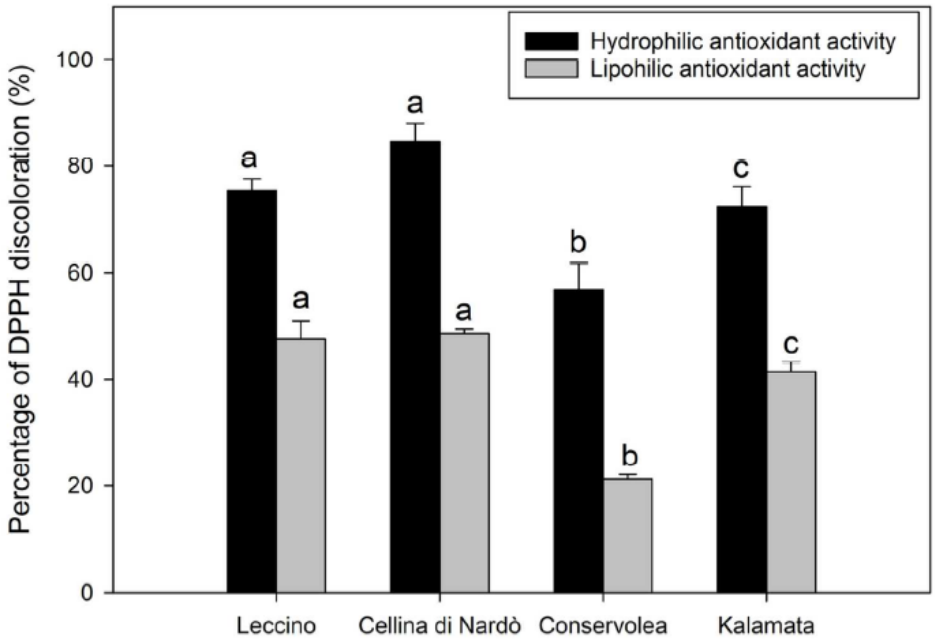

Figure 2. Hydrophilic and lipophilic antioxidant activities evaluated by the DPPH assay in fermented table olives from two Cellina di Nardò, Leccino, Conservolea and Kalamàta cultivars. Values are expressed as a percentage of DPPH discoloration (%) and represent the mean ± SD of three indepen- dent replicates (n = 3). Data were submitted to one-way analysis of vari- ance. Differences among groups were detected using Tukey's post-hoc test ($P < 0.05$). Different lowercase letters indicate significant differences among extracts ($P < 0.05$).

CONCLUSIONS

The present study provides information regarding some bioactive compounds and fatty acids of black table olive, belonging to different cultivars (Leccino, Cellina di Nardò, Conservolea and Kalamàta), fermented with selected microbial starters. Although the results obtained refer to specific batches of table olive, they nevertheless indicate differences among the olive samples with respect to the content of most of the assayed compounds. Cellina di Nardò table olives had the highest content of total phenolics. Cellina di Nardò and Leccino showed greater antioxidant activity than Kalamàta and Conservolea table olives. The hydrophilic antioxidant activity of table olives was significantly correlated with hydroxytyrosol in the present study. Additionally, Cellina di Nardò and Kalamàta olive oils had the highest carotenoid and vitamin E contents, respectively. As a general conclusion, the results obtained in the present study confirm that table olives are a rich source of high quality polyphenols, triterpenic acids, vitamin E and MUFA and also have the potential, in combination with olive oil, to provide numerous health benefits.

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REFERENCES

1. International Olive Council (IOC), Markets Newsletter September (2015). Available: http://www.internationaloliveoil.org [26 May 2016].

2. Garrido Fernández A, Fernández Díaz MJ and Adams RM, Table Olives. Production and Processing. Chapman & Hall, London (1997).
3. Kemal K and Nergiz C, The effect of table olive preparing methods and storage on the composition and nutritive value of olives. Grasas y Aceites 54: 71– 76 (2003).
4. Tufariello M, Mita G and Bleve G, Biotechnology can improve a traditional product as table olives, in Products from Olive Tree, ed. by D Boskou. AOCS Press, Urbana, IL, pp. 235– 260 (2016).
5. Romero C, García A, Medina E, Ruíz-Méndez Ma V, de Castro A and Brenes M, Triterpenic acids in table olives. Food Chem 118: 670– 674 (2010).
6. Alexandraki V, Georgalaki M, Papadimitriou K, Anastasiou R, Zoumpopoulou G, Chatzipavlidis I et al., Determination of triterpenic acids in natural and alkaline-treated Greek table olives throughout the fermentation process. LWT – Food Sci Technol 58: 609– 613 (2014).
7. D'Antuono I, Garbetta A, Ciasca B, Linsalata V, Minervini F, Lattanzio VM et al., Biophenols from table olive cv Bella di Cerignola: chemical characterization, bioaccessibility, and intestinal absorption. J Agric Food Chem 64: 5671– 5678 (2016).
8. Bleve G, Tufariello M, Durante M, Perbellini E, Ramires FA, Grieco F et al., Physico-chemical and microbiological characterization of spontaneous fermentation of Cellina di Nardò and Leccino table olives. Front Microbiol 15: 19092– 19105 (2014).
9. Bleve G, Tufariello M, Durante M, Grieco F, Ramires FA, Mita G et al., Physico-chemical characterization of natural fermentation process of Conservolea and Kalamàta table olives and developement of a protocol for the pre-selection of fermentation starters. Food Microbiol 46: 368– 382 (2015).
10. Tufariello M, Durante M, Ramires FA, Grieco F, Tommasi L, Perbellini E et al., New process for production of fermented black table olives using selected autochthonous microbial resources. Front Microbiol 6: 1– 15 (2015).
11. Binetti G, Del Coco L, Ragone R, Zelasco S, Perri E, Montemurro C et al., Cultivar classification of Apulian olive oils: use of artificial neural networks for comparing NMR, NIR and merceological data. Food Chem 219: 131– 138 (2017).
12. Hajimahmoodi M, Sadeghi N, Jannat B, Oveisi MR, Madani S, Kiayi M et al., Antioxidant activity, reducing power and total phenolic content in Iranian olive cultivar. J Biol Sci 8: 779– 783 (2008).
13. García A, Brenes M, Martínez F, Alba J, García P and Garrido A, High-performance liquid chromatography evaluation of phenols in virgin olive oil during extraction at laboratory and industrial scale. J Am Oil Chem Soc 78: 625– 629 (2001).
14. García A, Brenes M, Dobarganes MC, Romero C and Ruiz-Méndez MV, Enrichment of pomace olive oil in triterpenic acids during storage of 'Alpeorujo' olive paste. Eur J Lipid Sci Technol 110: 1136– 1141 (2008).
15. Lozano-Mena G, Juan ME, García-Granados A and Planas JM, Determination of maslinic acid, a pentacyclic triterpene from olives, in rat plasma by high-performance liquid chromatography. J Agric Food Chem 60: 10220– 10225 (2012).
16. Minguez-Mosquera IM and Garrido-Fernandez J, Chlorophyll and carotenoid presence in olive fruit (Olea europaea). J Agric Food Chem 37: 1– 7 (1989).
17. Puspitasari-Nienaber NL, Ferruzzi MG and Schwartz SJ, Simultaneous detection of tocopherols, carotenoids, and chlorophylls in vegetable oils by direct injection C30 RP-HPLC with coulometric electrochemical array detection. J Am Oil Chem Soc 79: 633– 640 (2002).
18. Durante M, Lenucci MS, Laddomada B, Mita G and Caretto S, Effects of sodium alginate bead encapsulation on the storage stability of durum wheat (Triticum durum Desf.) bran oil extracted by supercritical CO2. J Agric Food Chem 60: 10689– 10695 (2012).
19. National Research Council (NRC) Committee on Dietary Allowances, Food and Nutrition Board NRC. Recommended Dietary Allowances, 10th edn. National Academy Press, Washington, DC, pp. 78– 92 (1988).

20. Hasan Nazmul, MD, Akhtaruzzaman M and Sultan Zakir MD, Estimation of vitamins B-complex (B2, B3, B5 and B6) of some leafy vegetables indigenous to Bangladesh by HPLC method. J Anal Sci Meth Instrum 3: 24– 29 (2013).
21. Ferreira ICFR, Aires E, Barreira, JCM and Estevinho LM, Antioxidant activity of Portuguese honey samples: different contributions of the entire honey and phenolic extract. Food Chem 114: 1438– 1443 (2009).
22. Beta T, Nam S, Dexter JE and Sapirstein HD, Phenolic content and antioxidant activity of pearled wheat and roller-milled fractions. Cereal Chem 82: 390– 393 (2005).
23. Bligh EG and Dyer WJ, A rapid method of total lipid extraction and purification. J Physiol Biochem 37: 911– 917 (1959).
24. Lange E, German Standard Methods for the Analysis of Fats and Other Lipids. German Society for Fat Science (DGF). Wissenschaftliche Verlagsgesellschaft, Stuttgart (2000).
25. Durante M, Lenucci MS, Marrese PP, Rizzi V, De Caroli M, Piro G et al., α-Cyclodextrin encapsulation of supercritical CO2 extracted oleoresins from different plant matrices: a stability study. Food Chem 199: 684– 693 (2016).
26. Visioli F and Bernardini E, Extra virgin olive oil's polyphenols: biological activities. Curr Pharm Design 17: 786– 804 (2011).
27. Shaidi F and Ambigaipalan P, Phenolics and polyphenolics in foods, beverages and spices: antioxidant activity and health effects – a review. J Funct Foods 18: 820– 897 (2015).
28. Amiot MJ, Fleuriet A and Macheix JJ, Importance and evolution of phenolic compounds in olive during growth and maturation. J Agric Food Chem 34: 823– 826 (1986).
29. Pèrez-Jimènez Lòpez-Miranda J and Mata P, Protective effect of dietary monounsaturated fat on arteriosclerosis: beyond cholesterol. Atherosclerosis 163: 385– 398 (2002).
30. Gallina-Toschi T, Cerretani L, Bendini A, Bonoli-Carbognin M and Lercker G, Oxidative stability and phenolic content of virgin olive oil: an analytical approach by traditional and high resolution techniques. J Sep Sci 28: 859– 870 (2005).
31. Owen RW, Mier W, Giacosa A, Hule WE, Spiegelhalder B and Bartsch H, Phenolic compounds and squalene in olive oils: the concentration and antioxidant potential of total phenols, simple phenols, secoroids, lignans and squalene. Food Chem Toxicol 38: 647– 659 (2000).
32. Blekas G, Vassilakis C, Harizanis C, Tsimidou M and Boskou D, Biophenols in table olives. J Agric Food Chem 50: 3688– 3692 (2002).
33. Piscopo A, De Bruno A, Zappia A, Ventre C and Poiana M, Characterization of monovarietal olive oils obtained from mills of Calabria region (Southern Italy). Food Chem 213: 313– 318 (2016).
34. Briante R, Patumi M, Limongelli S, Ferdinando F, Vaccaro C, Di Salla A et al., Changes in phenolic and enzymatic activities content during fruit ripening in two Italian Cultivars of Olea europaea L. Plant Science 162: 791– 798 (2002).
35. Cardoso SM, Guyot S, Marnet N, Lopes-da-silva JA, Silva MSA, Renard CMGC et al., Identification of oleuropein oligomers in olive pulp and pomace. J Sci Food Agric 86: 1495– 1502 (2006).
36. Tataridou M and Kotzekidou P, Fermentation of table olives by oleuropeinolytic starter culture in reduced salt brines and inactivation of Escherichia coli O157:H7 and Listeria monocytogenes. Int J Food Microbiol 208: 122– 130 (2015).
37. Aruoma OI, Deiana M, Jenner A, Halliwell B, Kaur H, Banni S et al., Effect of hydroxytyrosol found in extra virgin olive oil on oxidative DNA damage and on low-density lipoprotein oxidation. J Agric Food Chem 46: 5181– 5187 (1998).
38. Manna C, Della Ragione F, Cucciolla V, Borriello A, D'Angelo S, Galleti P et al., Biological effects of hydroxytyrosol, a polyphenol from olive oil endowed with antioxidant activity, in Advances in Nutrition and Cancer 2, Vol. 42: Advances in Experimental Medicine and Biology, ed. by V Zappia, F Della Ragione, A Barbarisi, A Russo and GL Dello Iacovo. Kluwer Academic/Plenum Publishers, New York, NY, pp. 115– 130 (1999).

39. Incani A, Deiana M, Corona G, Atzeri A, Loru D, Rosa A et al., Protective effect of hydroxytyrosol against oxidative stress in kidney cells. Progr Nutr 9: 198– 203 (2007).
40. Zoidou E, Melliou E, Gikas E, Tsarbopoulos A, Magiatis P and Skaltsounis A, Identification of Throuba Thassos, a traditional Greek table olive variety, as a nutritional rich source of oleuropein. J Agric Food Chem 58: 46– 50 (2010).
41. Romani A, Mulinacci N, Pinelli P, Vincieri FF and Cimato A, Polyphenolic content in five Tuscany cultivars of Olea europaea L. J Agric Food Chem 47: 964– 967 (1999).
42. Malheiro R, Mendes P, Fernandes F, Rodrigues N, Bento A and Pereira JA, Bioactivity and phenolic composition from natural fermented table olive. Food Funct 5: 3132– 3142 (2014).
43. Vinha AF, Ferreres F, Silva BM, Valentao P, Goncalves A, Pereira JA et al., Phenolic profiles of Portguese olive fruits (Olea europaea L.): influences of cultivar and geographical origin. Food Chem 89: 561– 568 (2005).
44. Alipieva K, Korkina L, Orhan IE and Georgiev MI, Verbascoside – a review of its occurrence, (bio)synthesis and pharmacological significance. Biotechnol Adv 32: 1065– 1076 (2014).
45. Servili M, Settanni L, Veneziani G, Esposto S, Massitti O, Taticchi A et al., The use of Lactobacillus pentosus 1MO to shorten the debittering process time of black table olives (Cv. Itrana and Leccino): a pilot-scale application. J Agric Food Chem 54: 3869– 3875 (2006).
46. Vlahov G and Solinas M, Anthocyanin polymerization in black table olives. Agricoltura Mediterranea 123: 7– 11 (1993).
47. Romero C, Brenes M, Yousfi K, Garcıa P, Garcıa A and Garrido A, Effect of cultivar and processing method on the contents of polyphenols in table olives. J Agric Food Chem 52: 479– 484 (2004).
48. Jäger S, Trojan H, Kopp T, Laszczyk MN and Scheffler A, Pentacyclic triterpene distribution in various plants – rich sources for a new group of multi-potent plant extracts. Molecules 14: 2016– 2031 (2009).
49. Lin C, Wen X and Sun H, Oleanolic acid derivatives for pharmaceutical use: a patent review. Expert Opin Ther Pat 26: 643– 655 (2016).
50. Medina E, Morales-Sillero A, Ramirez EM, Rallo P, Brenes M and Romero C, New genotypes of table olives: profile of bioactive compounds. IJFST 47: 2334– 2341 (2012).
51. Allouche Y, Jiménez A, Uceda M, Aguilera MP, Gaforio JJ and Beltrán G, Triterpenic content and chemometric analysis of virgin olive oils from forty olive cultivars. J Agric Food Chem 57: 3604– 3610 (2009).
52. Pérez-Camino MC and Cert A, Quantitative determination of hydroxyl pentacyclic triterpene acids in vegetable oils. J Agric Food Chem 47: 1558– 1562 (1999).
53. Sakouhi F, Harrabi S, Absalon C, Sbei K and Boukhchina Kallel H. α-Tocopherol and fatty acids contents of some Tunisian table olives (Olea europea L.): changes in their composition during ripening and processing. Food Chem 108: 833– 839 (2008).
54. Brigelius-Flohé R and Traber MG, Vitamin E: function and metabolism. FASEB J 13: 1145– 1155 (1999).
55. Condelli N, Caruso MC, Galgano F, Russo D, Milella L and Favati F, Prediction of the antioxidant activity of extra virgin olive oils produced in the Mediterranean area. Food Chem 177: 233– 239 (2015).
56. Hassapidou MN, Balatsouras GD and Manoukas AG, Effect of processing upon the tocopherol and tocotrienol composition of table olives. Food Chem 50: 112– 114 (1994).
57. Lanza B, Di Serio MG, Iannucci E, Russi F and Marfisi P, Nutritional, textural and sensorial characterisation of Italian table olives (Olea europaea L. cv. 'Intosso d'Abruzzo'). IJFST 45: 67– 74 (2010).
58. EFSA (European Food Safety Authority), Parma, Italy, Scientific Opinion on Dietary Reference Values for vitamin E as α-tocopherol. EFSA J 13: 4149 (2015).

59. Bayram B, Esatbeyoglu T, Schulze N, Ozcelik B, Frank J and Rimbach G, Comprehensive analysis of polyphenols in 55 extra virgin olive oils by HPLCECD and their correlation with antioxidant. Plant Foods Hum Nutr 67: 326– 336 (2012).
60. Stahl W and Sies H, Bioactivity and protective effects of natural carotenoids. Biochim Biophys Acta 1740: 101– 107 (2005).
61. 61Landrum JT and Bone RA, Lutein, zeaxanthin and the macular pigment. Arch Biochem Biophys 385: 28– 40 (2001).
62. EFSA (European Food Safety Authority) Parma, Italy, Scientific Opinion on Dietary Reference Values for vitamin A. EFSA J 13: 4028 (2015).
63. Pèrez-Jimènez Lòpez-Miranda J and Mata P, Protective effect of dietary monounsaturated fat on arteriosclerosis: beyond cholesterol. Atherosclerosis 163: 385– 398 (2002).
64. Kastorini CM, Milionis HJ, Goudevenos JA and Panagiotakos DB, Mediterranean diet and coronary heart disease: is obesity a link – a systematic review. Nutr Metab Cardiovas 20: 536– 551 (2010).
65. Food and Agriculture Organization (FAO), Fats and Oils in Human Nutrition. Report of a Joint FAO/WHO Expert Consultation. FAO Food and Nutrition Paper 57. Food and Agriculture Organization of the United Nations, Rome (1994).
66. Owen RW, Haubner R, Mier W, Giacosa A, Hull WE, Spiegelhalder B et al., Isolation, structure, elucidation and antioxidant potential of the major phenolic and flavonoid compounds in brined olive drupes. Food Chem Toxicol 41: 703– 717 (2003).
67. Pereira JA, Pereira AP, Ferreira IC, Valentão P, Andrade PB, Seabra R et al., Table olives from Portugal: phenolic compounds, antioxidant potential, and antimicrobial activity. J Agric Food Chem 54: 8425– 8431 (2006).