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Table Olives from Portugal: Phenolic Compounds, Antioxidant Potential, and Antimicrobial Activity

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The phenolic compounds composition, antioxidant potential, and antimicrobial activity of different table olives from Portugal, namely, natural black olives "Galega", black ripe olive "Negrinha de Freixo", Protected Designation of Origin (PDO) "Azeitona de Conserva Negrinha de Freixo" olives, and "Azeitona de Conserva de Elvas e Campo Maior" Designation of Origin (DO) olives, were investigated. The analysis of phenolic compounds was performed by reversed-phase HPLC/DAD, and seven compounds were identified and quantified: hydroxytyrosol, tyrosol, 5-*O*-caffeoilquinic acid, verbascoside, luteolin 7-*O*-glucoside, rutin, and luteolin. The antioxidant activity was assessed by the reducing power assay, the scavenging effect on DPPH (2,2-diphenyl-1-picrylhydrazyl) radicals, and the β-carotene linoleate model system. The antioxidant activity was correlated with the amount of phenolics found in each sample. The antimicrobial activity was screened using Gram-positive (*Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus*) and Gram-negative bacteria (*Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*) and fungi (*Candida albicans*, *Cryptococcus neoformans*). PDO and DO table olives revealed a wide range of antimicrobial activity. *C. albicans* was resistant to all the analyzed extracts.

KEYWORDS: Table olives; phenolics; antioxidant potential, antimicrobial activity

INTRODUCTION

The olive tree (*Olea europaea* L.) is one of the most important fruit trees in the Mediterranean countries. Their products, olive oil and also table olives, are important components of the Mediterranean diet and are largely consumed in the world. Lower risks of coronary heart disease as well as certain cancers (of breast and colon) are associated with this diet (*1, 2*).

The preparation of table olives follows three main trades, namely green or Spanish-style olives, black ripe or Californian-style olives, and turning color and naturally black olives, but other industrial plans can be carried out (*3*). The production of green table olives includes a previous treatment with lye. After washing to eliminate the excess of alkali, lactic acid fermentation occurs spontaneously in brine (*4*). The processing method of black ripe olives includes fruit storage in brine (5−10% NaCl), during 2−6 months, with acidification to pH 4 with lactic and acetic acids and storage in anaerobic/aerobic conditions to prevent fermentation. The olives are then treated with dilute NaOH solution to debitter and are oxidized. After washing, the olives are placed in brine and ferrous gluconate or ferrous lactate to maintain their black color (*5*). The production of natural olives in brine, usually black olives, consists of a direct brining of olives without any debittering treatment. Olives are harvested when fully ripe or slightly before full ripeness. They are placed in brine and fermentation may be carried out in either anaerobic or aerobic conditions. In the anaerobic or traditional system, the natural fermentation is driven mainly by yeasts, due to the high salt concentration used (*3*). All these procedures influence the organoleptic properties of the product.

Table olives are well-known sources of compounds with important biological properties. These properties are related to fatty acid composition, mainly monounsaturated fatty acids (*6*), and to minor constituents, such as tocopherol and phenolic compounds (*7*). The natural antioxidants polyphenols are one of the main olive secondary metabolites, representing about 2−2.5% of the pulp (*8*).

The phenolics content of olive depends on several factors, such as cultivar (*9, 10*), climate (*11*), irrigation regimes (*12*), degree of ripeness of the fruit (*13*), and elaboration process (*14*). Recently there is an increasing interest in olive products and byproducts, due to their antioxidant properties. Many studies describe phenolic compounds as having a protective role in the oxidation of low-density lipoproteins (*15*) and in oxidative

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Table 1. Table Olive Samples

cultivar	processing	code	pulp (g)	stone (g)	pulp/stone
Galega	naturally black olives	G-NBO	2.24 ± 0.162	0.47 ± 0.071	4.8 ± 0.51
Negrinha de Freixo	black ripe olives	NF-BRO	3.39 ± 0.535	0.56 ± 0.043	6.0 ± 0.71
Negrinha de Freixo	turning color in brine	NF-PDO	4.25 ± 0.409	0.68 ± 0.093	6.3 ± 0.61
Azeiteira, Carrasquenha, Redondil and Conserva	green olives in brine	CE-DO	3.49 ± 0.415	0.60 ± 0.075	5.8 ± 0.52

alterations due to free radical and other reactive species (*16*). However, few studies reporting table olives antioxidant potential are available. Owen et al. (*17*) described the phenolic fraction antioxidant capacity of two Italian brined olive drupe kinds (one black and one green) using the hypoxanthine/xanthine oxidase assay, and Boskou et al. (*18*) estimated the total antioxidant capacity of five Greek table olives varieties using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method.

Some researches have also demonstrated that biocompounds present in olive products, such as oleuropein (*19*, *20*) and hydroxytyrosol (*19*) and aliphatic aldehydes (*21*), inhibit or delay the rate of growth of a range of bacteria and microfungi, so that they might be used as alternative food additives or in integrating pest management programs. The increasing resistance to antibiotic represents the main factor justifying the need to find and/or develop new antimicrobial agents. Thus, many studies have been focused on antimicrobial agents and on the antimicrobial properties of plant-derived active principles, such as spices and essential oils, which have been used for a long time in traditional medicine to overcome infections (*22*).

The phenolic fraction of table olives has been studied in different works (*23*, *24*). Nevertheless, no information is available about Portuguese table olives, which are recognized by the European Union as having Protected Designation of Origin (PDO), that possess a characteristic composition. Herein, we intended to evaluate the phenolic compounds of different table olives produced in Portugal. One of the studied samples were from Galega cultivar, which is the most important Portuguese table olive cultivar, representing more than 50% of the national market. The others samples belong to the PDO "Azeitona de Conserva Negrinha de Freixo" and "Azeitona de Conserva de Elvas e Campo Maior" Designation of Origin (DO) olives. The analysis of phenolic compounds was performed by reversed-phase HPLC/DAD. We also intended to correlate the phenolics levels with the antioxidant and antimicrobial activity of the table olive extracts. The antioxidant activity was evaluated by several chemical assays: reducing power, scavenging effects on DPPH radicals, and β-carotene linoleate model system. The antimicrobial activity was screened using Gram-positive (*Bacillus cereus*, *B. subtilis*, *Staphylococcus aureus*) and Gram-negative bacteria (*Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella penumoniae*) and fungi (*Candida albicans*, *Cryptococcus neoformans*).

MATERIALS AND METHODS

Samples. Four table olive samples were studied (**Table 1**). Two samples were obtained in a supermarket (G-NBO and CE-DO), and two were obtained directly from the producers, NF-BRO from an industrial producer, and NF-PDO from a traditional producer both in Mirandela region (Northeast Portugal). One sample (G-NBO) was classified as naturally black olives from Galega cultivar. Two samples were from Negrinha de Freixo cultivar: NF-BRO, Negrinha de Freixo ripe black olives, produced by the Californian method, and NF-PDO, produced by the traditional method and classified as turning color in brine according to the parameters of the PDO "Azeitona de Conserva Negrinha de Freixo", recognized by the European Union (*25*). The last sample (CE-DO) was from green olives in brine, belonging to "Azeitona de Conserva de Elvas e Campo Maior" Designation of Origin (DO). According to the DO, these olives were made from fruits of Azeiteira, Carrasquenha, Redondil, and Conserva cultivars. The registration of CE−DO for PDO is in course and was already published (*26*). For table olives' characterization, ten fruits per sample were weighed. Then the stones were removed and weighed separately. The pulp fraction was obtained by the difference between the two weights and the pulp/stone ratio was calculated.

Samples Preparation. For all table olive samples, the pulp (~100 g) was frozen, lyophilized, and stored at 4 °C, protected from light until further use. The samples were then reduced to a fine powder (20 meshes) and stored in an exsiccator protected from light.

Identification and Quantification of Phenolic Compounds. *Standards.* The standards used were from Sigma (St. Louis, MO) or Extrasynthèse (Genay, France). Methanol and formic acid were obtained from Merck (Darmstadt, Germany). The water was treated in a Milli-Q water purification system (Millipore, Bedford, MA) before use.

Extraction of Phenolic Compounds. The extraction was achieved as previously reported (*27*) with some modification: each powdered sample (ca. 1.5 g) was thoroughly mixed with several volumes of methanol, until complete extraction of phenolic compounds (negative reaction to NaOH 20%). The methanolic extracts were gathered, filtered, evaporated to dryness under reduced pressure (40 °C), and redissolved in methanol (2 mL) of which 20 μL was injected for HPLC analysis.

HPLC/DAD System for Analysis of Phenolic Compounds. Chromatographic separation was carried out as reported previously (*27*), with an analytical HPLC unit (Gilson), using a Spherisorb ODS2 column (250 × 4.6 mm, RP-18, 5 μm particle size, Merck, Darmstadt, Germany) using a solvent system of water−formic acid (19:1) (A) and methanol (B), starting with 5% methanol and installing a gradient to obtain 15% B at 3 min, 25% B at 13 min, 30% B at 25 min, 35% B at 35 min, 40% B at 39 min, 45% B at 42 min, 45% B at 45 min, 47% B at 50 min, 48% B at 60 min, 50% B at 64 min, 100% B at 66 min, and 100% B at 68 min. The flow rate was 0.9 mL/min. Detection was achieved with a diode-array detector, and chromatograms were recorded at 280, 320, 350, and 500 nm. Spectral data from all peaks were accumulated in the range 200−600 nm. The data were processed on Unipoint system software (Gilson Medical Electronics, Villiers le Bel, France). Peak purity was checked by software contrast facilities.

Phenolic compounds quantification was achieved by the absorbance recorded in the chromatograms relative to external standards at the maximum wavelength absorption of each phenolic compound class, so this was done at 280 nm for hydroxytyrosol and tyrosol, at 320 nm for verbascoside and 5-*O*-caffeoilquinic acid, and at 350 nm for luteolin 7-*O*-glucoside, rutin, and luteolin.

Antioxidant Activity. *Materials and Reagents.* Standards BHA (2-*tert*-butyl-4-methoxyphenol), TBHQ (tert-butylhydroquinone), and α-tocopherol were purchased from Sigma (St. Louis, MO). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was obtained from Alfa Aesar. All other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO). Methanol was obtained from Pronalab (Lisboa, Portugal). Water was treated in a Milli-Q water purification system (TGI Pure Water Systems).

Extract Preparation. Each powdered sample (~5 g) was extracted with 250 mL of boiling water for 45 min and filtered through Whatman no. 4 paper. The aqueous extract was frozen, lyophilized, redissolved in water at a concentration of 10 mg/mL, and analyzed for antioxidant activity.

Reducing Power Assay. The reducing power was determined according to a described procedure (*28*). Various concentrations of

sample extracts (2.5 mL) were mixed with 2.5 mL of 200 mmol/L sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated at 50 °C for 20 min. After incubation, 2.5 mL of 10% tricloroacetic acid (w/v) was added and then the mixture was centrifuged at 1000 rpm in a refrigerated centrifuge (Centorion K24OR-2003), for 8 min. The upper layer (5 mL) was mixed with 5 mL of deionized water and 1 mL of 0.1% of ferric chloride, and the absorbance was measured spectrophotometrically at 700 nm. The extract concentration providing 0.5 absorbance (EC_{50}) was calculated from the graph of absorbance registered at 700 nm against the correspondent extract concentration. BHA and α-tocopherol were used as reference compounds.

Scavenging Effect Assay. The capacity to scavenge the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical was monitored according to a method reported before (*29*). Various concentrations of sample extracts (0.3 mL) were mixed with 2.7 mL of methanolic solution containing DPPH radicals (6×10^{-5} mol/L). The mixture was shaken vigorously and left to stand in the dark until stable absorption values were obtained. The reduction of the DPPH radical was measured by monitoring continuously the decrease of absorption at 517 nm. The DPPH scavenging effect was calculated as a percentage of DPPH discolouration using the equation % scavenging effect = $[(A_{DPPH} - A_S)/A_{DPPH}] \times 100$, where A_S is the absorbance of the solution when the sample extract has been added at a particular level and A_{DPPH} is the absorbance of the DPPH solution. The extract concentration providing 50% inhibition (EC_{50}) was calculated from the graph of scavenging effect percentage against extract concentration. BHA and α-tocopherol were used as reference compounds.

Antioxidant Assay Using β-Carotene Linoleate Model System. The antioxidant activity of olive table extracts was evaluated by the β-carotene linoleate model system (*30*). A solution of β-carotene was prepared by dissolving 2 mg of β-carotene in 10 mL of chloroform. Two milliliters of this solution was placed in a 100-mL round-bottom flask. After chloroform removal at 40 °C under vacuum, 40 mg of linoleic acid, 400 mg of Tween 80 emulsifier, and 100 mL of distilled water were added to the flask under vigorous shaking. Aliquots (4.8 mL) of this emulsion were transferred into different test tubes containing 0.2 mL of different concentrations of olive table extracts. The tubes were shaken and incubated at 50 °C in a water bath. As soon as the emulsion was added to each tube, the zero time absorbance was measured at 470 nm using a spectrophotometer. Absorbance readings were then recorded until the control sample had changed color. A blank assay, devoid of β-carotene, was prepared for background subtraction. Antioxidant activity was calculated using the following equation: antioxidant activity = (β-carotene content after 2 h of assay/initial β-carotene content) × 100. The assays were carried out in triplicate and the results were expressed as mean values ± standard deviations. The extract concentration providing 40% antioxidant activity (EC_{40}) was calculated from the graph of antioxidant percentage against extract concentration. TBHQ was used as reference compound.

Antimicrobial Activity. *Materials and Reagents.* Ampicillin and cycloheximide were of the highest available quality and purchased from Merck (Darmstadt, Germany). Water was treated in a Milli-Q water purification system (TGI Pure Water Systems).

Extract Preparation. Powdered sample (~5 g) was extracted with 250 mL of boiling water for 45 min and filtered through Whatman no. 4 paper. The aqueous extract was frozen, lyophilized, redissolved in water at a concentration of 100 mg/mL, and analyzed for antimicrobial activity.

Microorganisms and Culture Conditions. Microorganisms labeled CECT were obtained from the Spanish type culture collection (CECT) of Valencia University, while microorganisms labeled ESA were clinically isolated strains identified by Microbiology Laboratory of Escola Superior Agrária de Bragança.

Three Gram-positive (*Bacillus cereus* CECT 148, *Bacillus subtilis* CECT 498, and *Staphylococus aureus* ESA 7) and three Gram-negative (*Escherichia coli* CECT 101, *Pseudomonas aeruginosa* CECT 108, and *Klebsiella pneumoniae* ESA 8) bacterial strains were used. The fungi strains used were *Candida albicans* CECT 1394 and *Cryptococcus neoformans* ESA 3. Microorganisms were cultured aerobically at 37 °C (Scientific 222 oven model, 2003) in nutrient agar medium for bacteria, and at 30 °C (Scientific 222 oven model, 2003) in sabouraud dextrose agar medium for fungi.

Test Assays for Antimicrobial Activity. A screening of antibacterial activities against the Gram-negative and Gram-positive bacteria and fungi was performed, and the minimal inhibitory concentration (MIC) was determined by an adaptation of the agar streak dilution method based on radial diffusion (*31*, *32*). Suspensions of the microorganism were prepared to contain approximately 10^8 cfu/mL, and the plates containing agar medium were inoculated (100 μL). A 50 μL volume of each sample was placed in a hole (depth 3 mm, diameter 4 mm) made in the centre of the agar. Under the same conditions, different solutions of ampicillin (antibacterial) and cycloheximide (antifungal) were used as standards. The assays with the standards were carried out using water, methanol, and DMSO solutions, the latter being chosen as the best solvent. After comparative toxicity assays, this solvent was shown to be nontoxic. The MIC was considered to be the lowest concentration of the tested sample able to inhibit the growth of bacteria or fungi, after 24 h. The diameters of the inhibition zones corresponding to the MICs were measured using a ruler, with an accuracy of 0.5 mm. Each inhibition zone diameter was measured three times (three different plates) and the average was considered. Controls using only inoculation and only DMSO were also carried out.

Statistical Analysis. All the determinations were carried out in triplicate and the results are expressed as mean values and standard deviations. A regression analysis, using Exel for Windows Software, was established between phenolic contents of different olive samples and EC_{50} values obtained by different antioxidant assays.

RESULTS AND DISCUSSION

Identification and Quantification of Phenolic Compounds. The HPLC/DAD analysis of the studied samples revealed different chemical profiles, in which seven phenolic compounds were identified and quantified: hydroxytyrosol, tyrosol, 5-*O*-caffeoilquinic acid, verbascoside, luteolin 7-*O*-glucoside, rutin, and luteolin (**Figure 1**, **Table 1**). The existence of several other compounds, namely, cyanidin 3-*O*-glucoside, cyanidin 3-*O*-rutinoside, oleuropein, apigenin 7-*O*-glucoside, and quercetin 3-*O*-rhamnoside, previously described in unprocessed fruits of the same olive cultivars (*9*), was also checked, but they were not detected in the analyzed samples. Extraction solvents other than methanol were used, namely, hydromethanol and water, presenting all the extracts the same qualitative composition and similarities in the quantitative analysis. Solvent extraction was done exhaustively until there was a negative reaction with 20% NaOH, which confirms the absence of phenols in the olive sample that remained following the solvent extraction.

The phenolic amounts found in table olives ranged from ~0.9 to 5 g/kg, following the order G-NBO > CE-DO > NF-PDO >> NF-BRO (**Table 2**). Hydroxytyrosol, tyrosol, and luteolin were the prevailing phenols in all samples, which is in accordance with previous works (*23*). The quantification of the identified phenolics revealed that hydroxytyrosol was the compound present in the highest amount, varying from ~60.7 in NF-PDO sample to 85.9% of total phenolics in CE-DO table olives. In fact, this is in good agreement with the literature, in which hydroxytyrosol was found to be the most abundant identified phenolic compound in table olives (*14*, *23*). This compound results from the hydrolysis of oleuropein (*33*), which is the major phenolic in the fresh fruits (*9*, *24*). Oleuropein is responsible for the bitter taste of unprocessed olives and, to become edible, the fruits need to lose, at least partially, their natural bitterness. Consequently, oleuropein cannot be found in processed fruits.

Some differences were observed in the phenolic composition of the studied table olives. Rutin only was detected in G-NBO, and CE-DO does not exhibit either 5-*O*-caffeoilquinic acid or

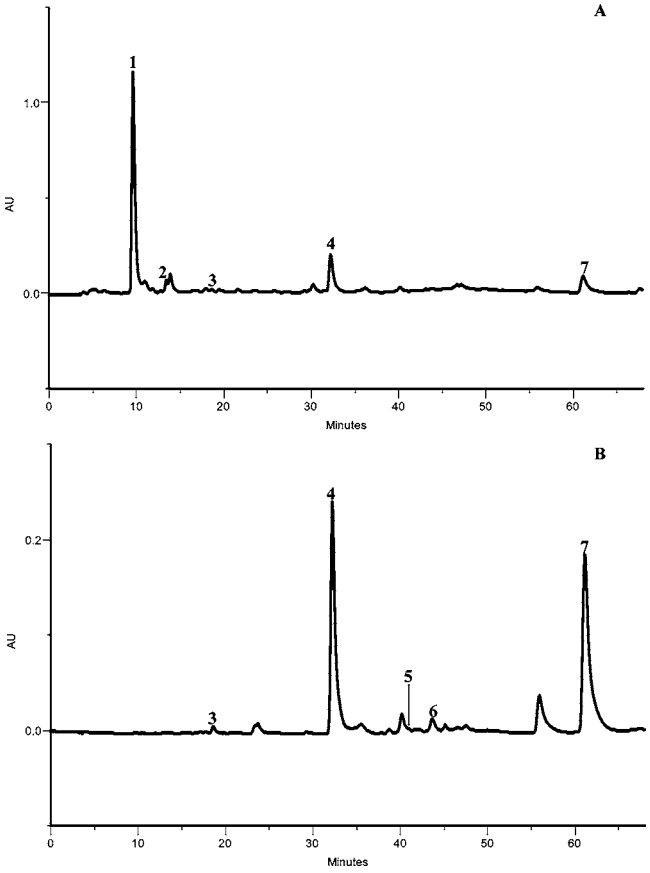

Figure 1. HPLC phenolic profile of G-NBO sample. Detection at (**A**) 280 and (**B**) 350 nm (*1*) hydroxytyrosol; (*2*) tyrosol; (*3*) 5-*O*-caffeoilquinic acid; (*4*) verbascoside; (*5*) luteolin 7-*O*-glucoside; (*6*) rutin; (*7*) luteolin.

verbascoside, which are present in all the other samples. According to previous studies, this fact could be related with the cultivar (*9*, *24*) and/or the processing method (*23*). Natural black olives in brine obtained from Galega cultivar (sample G-NBO) presented the highest amount of phenolics (**Table 2**). When a comparison is made with this table olive sample and fresh unprocessed "Galega" olive samples previously studied (*9*), its noticed that the phenolics content is similar, indicating that there is no substantial loss of phenolics during the manufacturing process.

Comparing the results obtained with treated (NF-BRO and NF-PDO) and fresh unprocessed Negrinha do Freixo olive samples of the same geographic origin (*9*), the absence of anthocyanic compounds can be observed. This can be attributed to the lower maturation index of the fruits used in this study. For black ripe olives and those turning color in brine, the fruits are collected in green and cherry maturation index (around 2). In fact, it is well-known that the anthocyanins content rises with the maturation of the fruit (*24*). In the present work the analyzed samples had a low maturation index, which is considerably lower than that of the unprocessed olives used for oil production

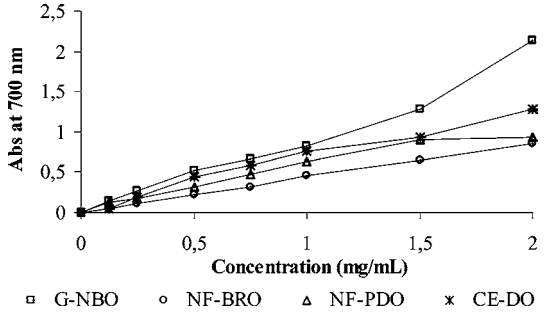

Figure 2. Reducing power values of different table olive extracts. Each value is expressed as mean ± standard deviation.

(*9*). So, anthocyanic compounds may not be detected. On the other hand, the processing method may also lead to a decrease of anthocyanins. The processing method of black ripe or Californian-style (sample NF-BRO) involves an oxidation step, which may result in the loss of this kind of compounds due to their instability. Also the anaerobic fermentation process used to obtain natural olives in brine (NF-PDO) can be responsible for the loss of anthocyanins, as reported before (*34*).

On a quantitative level, in opposition to the results observed in a previous work (*14*), the manufacturing by Californian-style of Negrinha de Freixo cultivar olives (NF-BRO) had a significant influence on the amount of phenolics. This kind of olive exhibited a reduced content, only ~0.9 mg/kg (**Table 2**), which corresponds to less than 30% of the total phenolics observed in the other analyzed samples. If we compare the same cultivar subjected to two different trade preparations (samples NF-PDO and NF-BRO), we can see that Californian-style manufacturing only maintains 28.8% of the total phenolics presented by natural fermentation in brine. The Californian process consists of three consecutive treatments, penetrating the skin with dilute sodium hydroxide solutions, washing, and aeration. Finally, irons salts are added to improve the development of the color (*5*). During this process oxidation reactions occur, involving the oxidation of natural *o*-difenol in olives to quinones, followed by the transformation of quinones into different dark compounds (*35*). The alkali aerobic treatments result in a darkening of the fruits and a decrease in phenolic compounds, with loss of nutritional value (*5*). Thus, it seems that the Californian treatment is more drastic for phenolic compounds.

Green olives in brine (CE-DO) showed a high amount of total phenolics, but only hydroxytyrosol, tyrosol, luteolin 7-*O*-glucoside, and luteolin were determined (**Table 2**). These findings are in accordance with those from other authors (*17*) who have identified a small number of compounds in green table olives.

Antioxidant Potential. The reducing power of the table olive extracts increased in a concentration-dependent way, as shown in **Figure 2**. In this assay, the yellow color of the test solution changes to various shades of green and blue, depending on the reducing power of each extract. The presence of reducers (i.e., antioxidants) causes the reduction of the Fe^{3+}/ferricyanide

Table 2. Phenolic Compounds Content in Table Olive Samples (mg/kg, Dry Basis)[a]

sample	hydroxytyrosol	tyrosol	5-*O*-caffeoil-quinic acid	verbascoside	luteolin 7-*O*-glucoside	rutin	luteolin	Σ
G-NBO	3833.0 (180.9)	139.1 (24.0)	10.9 (1.6)	475.8 (18.0)	nq	20.5 (0.1)	163.0 (6.2)	4642.2
NF-BRO	672.4 (75.4)	161.3 (15.5)	4.5 (0.5)	11.3 (2.8)	7.5 (0.3)	–	9.6 (2.4)	866.7
NF-PDO	1822.2 (23.0)	217.1 (0.5)	40.3 (0.1)	756.4 (8.4)	nq	–	164.7 (6.6)	3000.7
CE-DO	2783.6 (398.1)	179.7 (48.7)	–	–	25.9 (4.3)	–	249.9 (19.6	3239.0

[a] Results are expressed as mean (standard deviation) of three determinations. Σ: sum of the determined phenolic compounds. nq: not quantified.

Table 3. EC Values (mg/mL) of Table Olive Samples

samples	reducing power (EC_{50})	DPPH (EC_{50})	β-carotene bleaching (EC_{40})
G-NBO	0.48	0.47	3.25
NF-BRO	1.11	0.94	10.00
NF-PDO	0.80	0.64	8.47
CE-DO	0.59	0.60	3.32

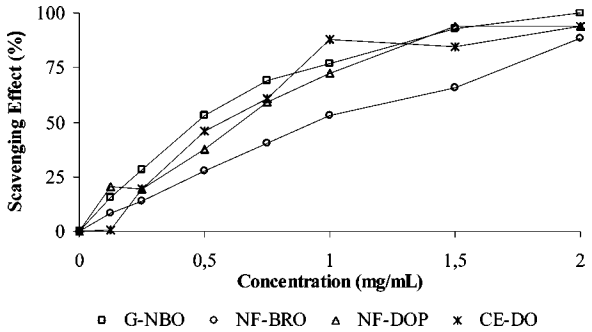

Figure 3. Scavenging effect of different table olive extracts. Each value is expressed as mean ± standard deviation.

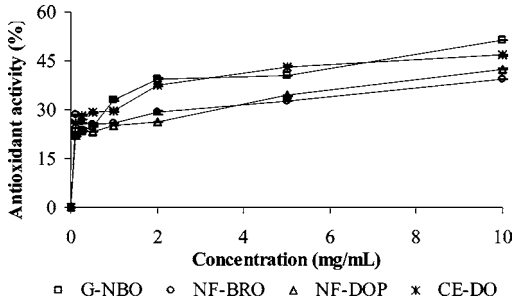

Figure 4. Antioxidant activity (%) of different table olive extracts determined by the β-carotene bleaching method. Each value is expressed as mean ± standard deviation.

complex to the ferrous form. Therefore, measuring the formation of Perl's Prussian blue at 700 nm can be used to monitor the Fe^{2+} concentration. Table olives showed high reducing powers at very low concentrations (<2 mg/mL), being even more potent than BHA (0.12 at 3.6 mg/mL) and α-tocopherol (0.13 at 8.6 mg/mL) standards. The reducing power for the different table olives followed the order G-NBO > CE-DO > NF-PDO > NF-BRO (**Table 3**).

The radical scavenging activity (RSA) assay constitutes a screening method currently used to provide basic information on the antiradical activity of extracts. RSA values of the extracts were expressed as the ratio percentage of sample absorbance decrease and the absorbance of DPPH solution in the absence of extract at 517 nm. The table olive extracts displayed a concentration-dependent RSA (**Figure 3**), with all the samples exhibiting a considerable capacity at low concentrations. G-NBO and NF-BRO showed the highest and the lowest activities, respectively (**Table 3**). These results are better than those obtained for BHA (96% at 3.6 mg/mL) and α-tocopherol (95% at 8.6 mg/mL).

The antioxidant activity of table olive extracts measured by the bleaching of β-carotene is presented in **Figure 4**. The linoleic acid free radical attacks the highly unsaturated β-carotene models. The presence of different antioxidants can hinder the extent of β-carotene-bleaching by neutralizing the linoleate-free radical and other free radicals formed in the system (*36*). Accordingly, the absorbance decreased rapidly in samples without antioxidant, whereas in the presence of an antioxidant they retained their color and, thus, absorbance for a longer time. The results indicate a concentration-dependent antioxidant capacity (**Figure 4**), with G-NBO and CE-DO samples as the most active (**Table 3**). However, the protection of β-carotene bleaching by the samples is lower than that of TBHQ standard (82.2% at 2 mg/mL).

The results obtained in the antioxidant activity assays are, at least partially, related with the amount of phenolics found in olive extracts, which were higher for G-NBO and lower for NF-BRO samples (**Table 2**) and followed the orders obtained for each assay (**Table 3**). This fact was proved when we correlated phenolic contents of olive samples and EC_{50} values. For the three tested methods, significant correlation curves with high linear coefficient regressions for reducing power was obtained for the samples ($R^2 = 0.931$; $p = 0.035$) and for DPPH ($R^2 = 0.987$; $p = 0.007$). However for β-carotene the regression established was not statistically significant ($R^2 = 0.708$; $p = 0.159$) due to the reduced number of samples and a worse performance for antioxidant evaluation of this method.

Hydroxytyrosol, most probably, contributes to an important extent to the observed effects, as one of the phenolic compounds with higher antioxidant activity (*17*). Nevertheless, other compounds, such as α-tocopherol, which is abundant in olive products and presents a high antioxidant activity, may be involved (*37*).

Antimicrobial Activity. The different table olive samples were screened for their antimicrobial activity against *B. cereus*,

Table 4. Antimicrobial Activity of the Table Olive Samples[a]

	MIC (mg/mL)							
samples	*B. cereus*	*B. subtilis*	*S. aureus*	*P. aeruginosa*	*E. coli*	*K. peumoniae*	*C. albicans*	*C. neoformans*
G-NBO	10 (++)	100 (–)	50 (++)	100 (–)	75 (++++)	50 (++)	100 (–)	100 (–)
NF-BRO	25 (+++)	50 (++)	100 (–)	100 (–)	100 (+++)	100 (–)	100 (–)	100 (–)
NF-PDO	25 (++++)	50 (++++)	100 (–)	100 (++)	75 (++++)	100 (++++)	100 (–)	100 (++++)
CE-DO	25 (+++)	100 (–)	100 (++++)	100 (++)	100 (++++)	75 (+)	100 (+++)	100 (++)
ampicillin	0.00313 (++++)	0.0125 (++++)	0.00625 (++++)	0.00625 (++++)	0.00625 (++++)	0.00625 (++++)	NT	NT
cycloheximide	NT	NT	NT	NT	NT	NT	0.0125 (++)	0.00625 (++++)

[a] No antimicrobial activity (–), inhibition zone < 1 mm. Slight antimicrobial activity (+), inhibition zone 2–3 mm. Moderate antimicrobial activity (++), inhibition zone 4–5 mm. High antimicrobial activity (+++), inhibition zone 6–9 mm. Strong antimicrobial activity (++++), inhibition zone > 9 mm. Standard deviation ± 0.5 mm. NT, not tested.

B. subtilis, *S. aureus* (Gram-positive), *E. coli*, *P. aeruginosa*, and *K. pneumoniae* (Gram-negative) bacteria and *C. albicans* and *C. neoformans* (fungi) (**Table 4**). The MICs for bacteria and fungi were determined as an evaluation of the antimicrobial activity of the tested table olives.

Despite all the table olive extracts revealing antimicrobial activity, the response for each microorganism tested was different. In general, olives produced according to the traditional process (NF-PDO and CE-DO) showed the best results (**Table 4**). CE-DO extracts, inhibited all the tested microorganisms, with the exception of *B. subtilis* (Gram-positive). Olive extracts from NF-PDO exhibited considerable growth inhibitions for *B. cereus*, *B. subtilis*, *P. aeruginosa*, *E. coli*, *K. pneumoniae*, and *C. neoformans* (**Table 4**). However, the same olive cultivar (Negrinha de Freixo) submitted to other treatment (Californian-style) only inhibited *B. cereus*, *B. subtilis*, and *E. coli* growth and with lower capacity (**Table 4**). This fact could be related to modifications that occurred along the process that led to the reduction of levels of phenolics such as oxidation process with air exposition and ferrous gluconate or ferrous lactate additions (**Table 2**).

B. cereus and *E. coli* were the most sensitive microorganisms, being inhibited by all the extracts tested. Nevertheless, the G-NBO sample was the most effective, with lower MIC (10 mg/mL). On the other hand, *C. albicans* was the most resistant, only being susceptible to CE-DO sample. The antimicrobial activity of different extracts was modest, with high MICs concentrations (ranging between 10 and 100 mg/mL) when compared with aliphactic aldehydes isolated from olives (*21*) that exhibits antifungal activity against *Tricophyton mentagrophytes* and *Microsporum canis*, dermatophytes responsible for infection of keratinized tissue, with lower MICs values (ranged from 1.9 to 125 μg/mL). Also, oleuropein and hydroxytyrosol have shown antimicrobial activity against *Salmonella* spp., *Vibrio* spp., and *Staphylococcus aureus* with MICs between 62.5 and 125 μg/mL for ATCC strains and between 31.25 and 250 μg/mL for clinical isolates (*19*). As expected, the standards ampiciline (antibacterial) and cycloheximide (antifungal) presented lower MICs than the table olive extracts. Usually, pure active compounds reveal more activity than crude extracts.

Certainly the chemical composition of the table olive extracts impacted the antimicrobial effects observed. In fact, the mode of action of phenolics has been shown to be concentration dependent (*20*, *22*). Additionally, the antimicrobial action of these compounds is well-known and is related to their ability to denature proteins, which in general renders them to be classified as surface-active agents (*38*). These results are important against several pathogenic microorganisms resistant to a number of phytochemicals. Thus, it seems that dietary intakes of table olives, specially with Protected Designations of Origin, may lower the risk of bacterial infections, particularly in the intestinal tract, mainly due to the protective action provided by its phenolic compounds.

In conclusion, the results obtained in the present work denote that table olives may constitute a good source of healthy compounds, especially phenolics, in the diet, suggesting that their consumption could be useful in the prevention of diseases in which free radicals are implicated. Also, these important products produced in a traditional way, with DO or PDO appellation, inhibited some medically important microorganisms, suggesting that these kinds of table olives may be good candidates for application as antimicrobial agents against bacteria responsible for human gastrointestinal and respiratory tract infections. As far as we know, this is the first report considering the antioxidant and antimicrobial potential of table olives from Portugal.

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