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Antioxidant Activity of Sicilian Pistachio (*Pistacia vera* L. Var. Bronte) Nut Extract and Its Bioactive Components

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Pistacia vera L. is the only species of *Pistacia* genus producing edible nuts. This paper investigates the antioxidant potential of a Sicilian variety of pistachio nut by chemical as well as biological assays and measured antioxidant vitamins and a number of antioxidant polyphenols in either the hydrophilic and/or the lipophilic nut extract. In accordance with the majority of foods, the total antioxidant activity, measured as a TAA test, was much higher (50-fold) in the hydrophilic than in the lipophilic extract. Substantial amounts of total phenols were measured. The hydrophilic extract inhibited dose-dependently both the metal-dependent and -independent lipid oxidation of bovine liver microsomes, and the Cu^{+2}-induced oxidation of human low-density lipoprotein (LDL). Peroxyl radical-scavenging as well as chelating activity of nut components may be suggested to explain the observed inhibition patterns. Among tocopherols, γ-tocopherol was the only vitamin E isomer found in the lipophilic extract that did not contain any carotenoid. Vitamin C was found only in a modest amount. The hydrophilic extract was a source of polyphenol compounds among which *trans*-resveratrol, proanthocyanidins, and a remarkable amount of the isoflavones daidzein and genistein, 3.68 and 3.40 mg per 100 g of edible nut, respectively, were evaluated. With the exception of isoflavones that appeared unmodified, the amounts of other bioactive molecules were remarkably reduced in the pistachio nut after roasting, and the total antioxidant activity decreased by about 60%. Collectively, our findings provide evidence that the Sicilian pistachio nut may be considered for its bioactive components and can effectively contribute to a healthy status.

KEYWORDS: Sicilian pistachio; antioxidant activity; bioactive components; vitamin E; vitamin C; *trans*-resveratrol; daidzein; genistein

INTRODUCTION

Studies of dietary patterns have shown that the traditional Mediterranean diet has to be considered the preferred dietary program for its health-promoting properties (*1*−*4*). Among other beneficial effects, the Mediterranean-style diet including large amounts of plant foods and derivatives, such as fruits, vegetables, nuts, seeds, olive oil, and red wine is considered to modulate body oxidative stress, on the basis of the high levels of antioxidant components (*5*−*7*). Pistachio (*Pistacia vera* L.), a member of the *Anacardiaceae* family, is native of arid zones of Central and West Asia and distributed throughout the Mediterranean basin. The genus *Pistacia* contains only 11 species among which *P. vera*, cultivated for its edible nuts, is by far the most important economically. It is a long-lived tree up to 10 m high, and has tough leaves, tiny brownish green flowers, and clusters of oblong fruits containing the seeds or pistachio nuts, with a wooden shell and a yellow or green kernel. The pistachio nut is mainly used as a snack food, both raw and toasted, and it is a confectionery ingredient in fermented meats, ice cream, bread, sauces, and pudding manufacturing. In Italy, the pistachio is grown mainly in Sicily, and a pistachio cultivar of high quality is typical of Bronte, an area around the Etna volcano, where the lava land and climate allow the production of a nut with an intense green color and aromatic taste, very appreciated in international markets. Bronte's annual pistachio production is 30 tons, which represents 80% of the total Sicily production.

The *Pistacia* species have been used in folk medicine as a remedy for various diseases (*8*). Recent studies showed that essential oils and lipophilic extracts of leaves, fruits, gum, and galls may exert various characteristics such as anti-microbial, anti-inflammatory, insecticidal, and anti-nociceptive activities, with various terpenes playing a major role in the observed activities (*8*−*14*). Only a few data have been reported about the antioxidant content of preparations from the *Pistacia* species. Galloyl quininic derivatives were identified in aqueous extracts from leaves and gum of *P. lentiscus* (*15*, *16*), and α-tocopherol was evaluated in lipophilic extracts from the leaves of *P. lentiscus* and *P. therebintus* (*17*). Interestingly, the edible

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pistachio nut has recently been ranked among the first 50 food products highest in antioxidant potential (*18*). The polyphenol phytoalexin *trans*-resveratrol was detected in the aqueous extracts from the edible nut of five Turkish cultivars of *P. vera* (*19*). In this context, evaluating antioxidants and other bioactive components in pistachio nuts could provide this fruit with a remarkable value and have a significant economic impact on the pistachio food industry.

The present study investigated antioxidant properties of extracts of the Sicilian Bronte's cultivar of *P. vera* nuts by established screening assays, including the [2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)] diamminium salt radical cation ($ABTS^{+\bullet}$) decolorization assay, total phenols (TP), the metal-dependent and -independent lipid peroxidation of bovine liver microsomes, and the Cu^{2+}-stimulated oxidation of human low-density lipoprotein (LDL). In addition, antioxidant vitamins, phytoestrogens, and proanthocyanidins were evaluated. Finally, the total antioxidant activity and the bioactive components were also measured in nuts after roasting to evaluate the effect of the heat treatment used in the preparations of the toasted snack.

MATERIALS AND METHODS

Chemicals. Ascorbic acid, cyanidin chloride, daidzein, and daidzin ethylenediaminetetraacetic acid (EDTA), gallic acid, genistein, genistin, potassium persulfate ($K_2S_2O_8$), *trans*-resveratrol, Sephadex G-25 (Fine), 2,6-di-*tert*-butyl-4-methylphenol (BHT), thiobarbituric acid (TBA), α-, β-, γ-tocopherol, and tricholoroacetic acid (TCA) were from Sigma Chemical Co. (St. Louis, MO). [2,2′-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid)] diammonium salt (ABTS) and 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (Trolox) were from Aldrich Chemical Co. (Gillingham, UK). 2,2′-Azobis(2-amidinopropane)-dihydrochloride (AAPH) was from Polyscience, Inc. (Warrington, PA). All other materials and solvents were of the highest purity or high-performance HPLC grade.

Preparation of Nut Extracts. *P. vera* L. nuts, Bronte's cultivar, collected in September 2005 in Sicily (Italy) were obtained from a local grower and were stored in the dark at 4 °C. The nuts were shelled, and the kernels with their skin (seed coat) were powdered with a mortar and a pestle. Samples of 25 g were then extracted with 200 mL of a mixture of methanol/water (2:1; v/v) or with 100 mL of dichloromethane, over 24 h at 4 °C. After a cleanup step via centrifugation and filtration through a Millex HV 0.45 μm filter (Millipore, Billerica, MA), the extracts were subjected to rotary evaporation to remove the organic solvents.

Industrial processing of pistachio includes roasting, which is usually performed at 160 °C, for 40 min. This condition was then reproduced at the laboratory scale. Accordingly, pistachio nuts with their ligneous shell were toasted in a dried incubator (Binder GmbH, Tuttlinger, Duch). Then, the nuts were shelled, weighed, and submitted to methanolic extraction as stated previously. Under our conditions, the heat exposure caused a weight loss of about 25%. All extracts were portioned and stored at −80 °C until the analyses that were carried out within 2 months.

Total Antioxidant Potential. The total antioxidant activity (TAA) of either the hydrophilic or the lipophilic extract was evaluated using the ABTS radical cation decolorization assay (*20*). $ABTS^{+\bullet}$ was prepared by reacting ABTS with $K_2S_2O_4$ (*21*). Samples were analyzed in duplicate, at five different dilutions, within the linearity range of the assay. The assay was standardized with the synthetic antioxidant Trolox, and results were expressed as μmol of Trolox equivalents/g of edible nut.

Total Phenols. Hydrophilic extracts were submitted to a solid-phase extraction. The extract was loaded on top of a 500 mg Supelco Discovery DSC-18 column, previously washed with 2 mL of methanol followed by 2 mL of 5 mM H_2SO_4, to clean up the polar substances. The phenolic compounds were then eluted with 2 mL of methanol and evaluated by the reduction of phosphotungstic−phosphomolybdic acid (Folin−Ciocalteu's reagent) to blue pigments, in alkaline solution according to Folin and Denis (*22*). Quantitation was by reference to curves constructed with gallic acid, and the results were expressed as mg of gallic acid equiv (GAE)/100 g of edible nut.

Biological Models of Lipid Peroxidation. Metal-Dependent and -Independent Lipid Peroxidation of Bovine Liver Microsomes. Bovine liver microsomes were prepared from a tissue homogenate in phosphate buffered saline, pH 7.4 (PBS), by differential centrifugation. Microsomes were incubated at 37 °C in PBS either in the absence (control) or in the presence of hydrophilic extracts of pistachio nuts, and lipid peroxidation was stimulated by either 20 mM AAPH or a mixture of 10 μM $FeCl_3$ and 100 μM ascorbic acid, added to the assay mixture after a 2 min preincubation at 37 °C. Lipid peroxidation was monitored by the formation of a TBA reactive substance (TBA-RS) (*23*). Aliquots (1 mL) of the reaction mixture were added to 2 mL of a solution containing 15% TCA (v/v), 0.375% TBA (w/v), 0.25 N HCl, and 0.02% BHT (w/v) to prevent formation of nonspecific TBA-RS and decomposition of AAPH during the subsequent boiling (*24*). The mixture was incubated in a boiling water bath for 30 min. After cooling, the precipitate was removed by centrifugation, and TBA-RS in the supernatant was determined at 532 nm. The results were expressed as nmol of MDA equiv/mg of protein, using the molar extinction coefficient of 156 000 (*23*).

Copper-Mediated LDL Oxidation. Blood samples were obtained from apparently healthy individuals ($n = 3$) by venipuncture, with informed consent. EDTA (1 mg/mL of blood) was used as an anticoagulant. Blood cells were sedimented at 1.000 g for 10 min, plasma was collected, and LDL (d 1.019−1.063 g/mL) was isolated by stepwise ultracentrifugation at 4 °C in a Beckman L8-70 M ultracentrifuge, fitted with a 50 Ti rotor using potassium bromide for density adjustments, according to Kleinveld et al. (*25*). The LDL fraction was shown to be free of other lipoproteins by electrophoresis on an agarose gel. EDTA and salts were removed from LDL by gel filtration on Sephadex G-25. LDL (100 μg of protein) was incubated either in the absence (control) or in the presence of various amounts of hydrophilic extract of pistachio nut, in PBS, supplemented with 10 μM $CuCl_2$ as a prooxidant, in a 1 mL quartz cuvette. The pistachio extracts were added immediately before the addition of the oxidant. LDL oxidation was followed by continuously monitoring the formation at 37 °C of conjugated diene (CD) lipid hydroperoxides at 234 nm (*26*) in a Beckman DU 640 spectrophotometer equipped with a temperature control system. The time-course of LDL oxidation exhibited a period during which oxidation did not occur (lag phase) followed by a net rise of absorbance due to the CD hydroperoxide production (propagation phase). The resistance of LDL to oxidation is usually estimated in terms of length of lag phase and rate of lipoperoxide production during the propagation phase. The lag phase was determined graphically as the intercept with the extrapolation of the part of the curve representing the lag and the propagation phase.

Proteins were determined by the Bio Rad colorimetric method (*27*).

HPLC Procedures. A Gilson modular liquid chromatographic system (Gilson Inc., Middleton, WI) equipped with M 302 and 305 pumps, an injector Model 77-25 (Rheodyne, Berkely, CA) with a 100 μL injector loop, and a M 802c manometric module was used. The chromatographic column was a 5 μm Chromsep C_{18} column (250 mm × 4.6 mm i.d.) with a 5 μm guard column (10 mm × 4 mm) (Varian, Palo Alto, CA). Detection was by a M 118 UV−vis detector used along with the Gilson 712 HPLC System Controller Software. Sensitivity was 0.05% AUFS, unless specified.

Tocopherols. The lipophilic extract was mixed with one volume of water, and two volumes of absolute ethanol, followed by extraction with eight volumes of petroleum ether. The extracts were gathered, dried under nitrogen, resuspended in several microliters of methanol, and injected on top of the HPLC column eluted with methanol at 1.5 mL min^{-1}. Spectrophotometric revelation was at 290 nm. Under the conditions described, δ-tocopherol eluted at 13.40 min, γ-tocopherol at 16.00 min, and α-tocopherol at 18.15 min.

Vitamin C. Evaluation of ascorbic acid in the hydrophilic extract was performed by reversed-phase HPLC, with spectrophotometric detection at 266 nm, as reported by Lazzarino et al. (*28*) with minor changes. These included length of the column (250 mm × 4.6 mm i.d.), particle size (5 μm), and isocratic elution with 10 mM KH_2PO_4

Table 1. Antioxidant *Potential of P. vera* L. Nut[a]

	TAA[b]	TP[c]	*n*
water-soluble extract	27.55 ± 2.30	175.23 ± 5.62	4
lipid-soluble extract	0.51 ± 0 .08	not determined	3

[a] Each value is the mean ± SD of *n* determinations performed in duplicate. [b] μmol of Trolox equivalents/g of edible nut. [c] mg of GAE/100 g of edible nut.

buffer, pH 7.0, containing 1% methanol and 10 mL/L of tetrabutylammonium bromide, at 1.2 mL min^{-1}. The retention time of ascorbate was 11.90 min.

***trans*-Resveratrol**. Evaluation of *trans*-resveratrol in the hydrophilic extract was performed by reversed-phase HPLC, as reported by Tokusoglu et al. (*19*). Isocratic elution with acetonitrile/water (40:60; v/v) containing 0.1% trifluoroacetic acid (v/v) was carried out at a flow rate of 1.0 mL min^{-1}. Spectrophotometric revelation was at 308 nm, and sensitivity was 0.01% AUFS. Under the conditions described, *trans*-resveratrol eluted at 10.60 min.

Isoflavones. Evaluation of phytoestrogenic isoflavones daidzein and genistein, and of their glycoside derivatives daidzin and genistin, respectively, in the hydrophilic extract from pistachio nuts was performed by reversed-phase HPLC as reported by Griffith et al. (*29*), with minor changes, including the length of the column (250 mm × 4.6 mm i.d.), the particle (size 5 μm), and the elution gradient. Solvent A was 0.1% (v/v) acetic acid in water, solvent B was 0.1% (v/v) acetic acid in acetonitrile, and the flow rate was 1 mL min^{-1}. The gradient was started immediately upon injection, and gradient elution was from 10 to 70% B in a linear gradient over 60 min. The column was washed at 90% B for 3 min and equilibrated 10 min between runs at 10% B. Detection was by UV absorbance at 260 nm. Under the conditions described, daidzin eluted at 24.50 min, genistin at 30.50 min, daidzein at 41.00 min, and genistein at 53.90 min.

All compounds were quantified by reference to standard curves constructed with 1−100 ng of each pure commercial compound. All procedures including extraction and analysis of nut components were performed under dim red light to preserve light sensitive vitamins.

Proanthocyanidins. The proanthocyanidins were evaluated in the hydrophilic extract by the modified method of Porter et al. (*30*), after conversion to anthocyanidins by acid hydrolysis in presence of iron ions. Briefly, 0.5 mL of extract was added to 1.5 mL of EtOH/HCl (95:5; v/v) and 50 μL of a 2 mM $FeCl_3$ dissolved in 2 M HCl. The reaction mixture was capped and heated in a water bath at 95 °C, for 40 min, then it was rapidly cooled by immersion in cold water, and the spectrum from 400 to 700 nm was recorded. The formed anthocyanidins were evaluated by measuring the peak height at 543 nm over a baseline between 400 and 700 nm. To subtract the contribution of natural anthocyanins in the sample, the extract was processed as stated previously and put in ice instead of warming, then the spectrum was subtracted to obtain the net value of absorbance. The proanthocyanidin concentration in the extract was expressed as the amount of cyanidin formed according to a calibration curve with cyanidin chloride.

Statistical Analysis. Conventional methods were used for calculation of means and standard deviations. Comparison between individual group means was performed by the unpaired Student's *t*-test.

RESULTS AND DISCUSSION

This study evaluated the antioxidative efficacy of extracts from *P. vera* L. cultured in Bronte's area in Sicily and assessed antioxidant vitamins and other bioactive compounds of this nut. A global picture of the antioxidant potential of the extracts was achieved by the ABTS and TP assays (**Table 1**). The antioxidant activity of the lipophilic extract was much lower than that of the hydrophilic one, a result consistent with literature data showing that water-soluble components contribute >90% to the total antioxidant potential of most foods (*31*). According to our measurements, one serving of 28.4 g of shelled nuts contains about 50 mg of GAE of TP. The antioxidant capacity of the Sicilian pistachio is of the same order than that reported by other authors (*18*) but lower than that measured by Wu et al. (*31*) in pistachio nut marketed in the United States. The higher values of the TP reported by Wu et al. (*31*) may account for this finding.

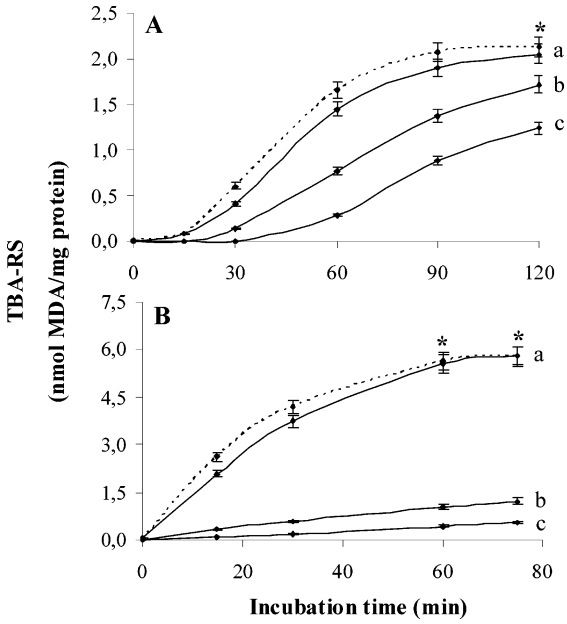

Figure 1. Time-course of TBA-RS formation during the AAPH-induced (**A**) and Fe^{3+}/ascorbate-induced (**B**) microsomal oxidation, either in the absence (dotted line) or in the presence (solid lines) of hydrophilic extracts from 0.25 (a), 0.5 (b), or 1.0 (c) mg of pistachio nut per mL of reaction mixture. Microsomes at 0.5 mg of protein, and 1.5 mg of protein per mL of reaction mixture, were submitted to peroxidation by AAPH and Fe^{3+}/ascorbate, respectively, as reported in the Materials and Methods. Each value is the mean ± SD of four determinations performed in duplicate. With respect to control, asterisk signifies nonsignificant values (Student's *t*-test).

The activity of the hydrophilic extract from the pistachio nut was investigated in biological models of lipid oxidation, including bovine liver microsomal membranes and human LDL. When lipid oxidation was induced in microsomes by the hydrophilic azo-initiator AAPH in the absence of nut extract, the production of TBA-RS was delayed for a short period due to the endogenous antioxidants. Then, lipid oxidation started, and TBA-RS reached a plateau after a 90 min incubation (**Figure 1A**). Hydrophilic extracts from 0.25 to 1.0 mg of pistachio nut extended the inhibition period and slowed down TBA-RS formation in a dose-dependent manner (**Figure 1A**). The metal-dependent microsome oxidation pattern is shown in **Figure 1B**. In the presence of Fe^{+3}/ascorbate, the production of TBA-RS started immediately, with a maximum at 60 min. The co-incubation with the extract from 0.25 mg of nut resulted in a weak inhibition of lipid oxidation; however, the extracts from 0.5 and 1.0 mg of nut almost totally inhibited TBA-RS formation within a 75 min observation time (**Figure 1B**). The higher protective activity observed in the metal-dependent oxidation model could be the expression of combined peroxyl radical-scavenging abilities and metal-chelating activity of one or more extract components.

The hydrophilic extract from pistachio nut increased the resistance of human LDL to copper-induced oxidation. A typical experiment is shown in **Figure 2**. With respect to LDL incubated in the absence of nut extract, a dose-dependent elongation of the lag phase was observed in the presence of extracts from 30 to 100 μg. Remarkably, when an extract from 500 μg of nuts

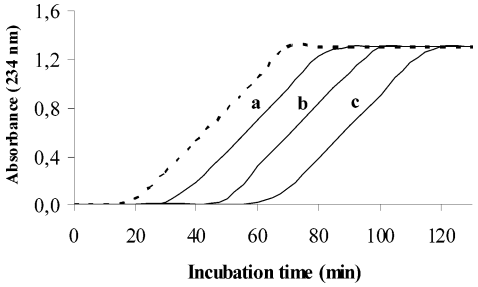

Figure 2. Time-course of CD lipid hydroperoxide formation during Cu^{2+}-mediated LDL oxidation either in the absence (dotted line), or in the presence (solid lines), of hydrophilic extracts from 30 (a), 60 (b), or 100 (c) μg of pistachio nut. Oxidation conditions were as reported in the Materials and Methods.

Table 2. Antioxidant *Vitamins and Polyphenol Compounds in 100 g of Edible Nut of P. vera* L.

	amount[a] (mg)
vitamin E	
α-tocopherol	0.51 ± 0.04
γ-tocopherol	10.54 ± 1.03
vitamin C	3.48 ± 0.23
proanthocyanidins[b]	268.12 ± 8.31
trans-resveratrol	$12.0 \times 10^{-3} \pm 1.2 \times 10^{-3}$
isoflavones	
daidzein	3.68 ± 0.41
genistein	3.40 ± 0.37

[a] Each value is the mean ± SD of four determinations performed in duplicate. [b] mg of cyanidine chloride equivalents.

was included in the incubation mixture, LDL oxidation was totally prevented for at least 6 h (not shown). The kinetic patterns observed during the oxidation of LDL in the presence of the lowest amounts of nut extracts suggest the activity of lipoperoxyl radical-scavenging components. However, the complete prevention of LDL oxidation by 500 μg of nut extract may be rather rationalized by a metal-chelating activity. Although the antioxidant contribution of other yet undetermined components in the pistachio nut extracts cannot be ruled out, the polyphenol content suggests that these compounds, via their dual ability to act as chain-breaking antioxidants and chelate transition metal ions (*32*−*34*), may play a significant role in preventing lipid peroxidation in our experimental systems.

The pistachio nut provides a ready source of nutritionally valuable oil. On a fresh weight base, it contains about 60% of lipids, among which monounsatured plus polyunsatured fatty acids account for 85% of the total fatty acids (*35*). Our measurements of the major lipophilic antioxidant vitamins extend the knowledge on the lipid pattern of the pistachio nut. Substantial amounts of tocopherols were found (**Table 2**), whereas the main dietary carotenoids such as α- and β-carotene, and all-*trans*-licopene, were absent or below the detection level of our assay. The total amount of vitamin E is comparable to that reported in hazelnuts and about one-half of that found in almonds and walnuts (*36*). Similar to walnuts, the major isomer in pistachio nuts is γ-tocopherol (*37*), a compound to be considered as an important biofunctional compound of our diet. Indeed, nutritional studies demonstrated that γ-tocopherol is absorbed as efficiently as α-tocopherol (*38*) and that it may act as an efficient endogenous antioxidant (*39*), also promoting the cellular uptake of α-tocopherol (*40*). On the other hand, the vitamin C found in the Sicilian pistachio nut was modest (**Table 2**), although comparable to that measured in other nuts, such as pecans (*41*).

Table 3. Effects of *Toasting on Antioxidant Potential and on Bioactive Components of P. vera* L. Nut[a]

	% of raw nut
TAA	39.37 ± 2.30
TP	37.40 ± 2.07
vitamin C	absent
isoflavones	125.14 ± 9.15
proanthocyanidins	12.80 ± 0.65

[a] Values are the mean ± SD of three separate determinations performed on the same sample.

The flavonoid proanthocyanidins, also known as condensed tannins, are flavan-3-ol polymers, occurring in a wide variety of foods including berries, chocolate, apples, red wines, and nuts (*42*). We found milligram amounts of proanthocyanidins per 100 g of Sicilian pistachio nuts (**Table 2**). Other authors (*35*) measured appreciable amounts of tannins in several nuts, including pistachios from California. Unfortunately, the data reported are not easily comparable with our measurements because of the different assay methods. Proanthocyanidins are known to bind metals through complexation involving their *o*-diphenol groups (*43*), a property that may account for the observed inhibition of the metal-induced lipid oxidation in our models.

Isoflavones and *trans*-resveratrol are important bioactive polyphenols known as phytoestrogens in that they are capable of interacting with estrogenic receptors (*44*, *45*). Isoflavones are mostly abundant in soybeans but also present in appreciable amounts in a variety of sprouts, legumes, and nuts (*46*), whereas resveratrol is concentrated in grape (*Vitis vinifera* L.) and wine (*47*, *48*) and recently found in the edible peanut (*Arachys hypogaea* L.) and in pistachio nut (*P. vera* L.) grown in Turkey (*19*). Our study shows that pistachio nuts from Sicily are a very rich source of daidzein and genistein (**Table 2**). When compared to a wide variety of fruits and nuts (*48*), the amount in our nuts is only lower than that reported in soybeans (*49*). Differently from soy, where isoflavones are present mainly as glycoside conjugates, the isoflavone-glycosides daidzin and genistin were not detected in our pistachios. Substantial amounts of *trans*-resveratrol were found in the Sicilian pistachio nuts (**Table 2**). However, it should be mentioned that the amount in our variety is much lower than that found in the Turkish pistachio (*19*). Differences in the genotype, environmental conditions, including exposure to fungi and bacteria and UV light, processing, and storage may affect nutrient and mineral composition as well as the flavonoid content of plants (*50*).

Toasting is one the most frequently utilized pistachio nut technologies; however, such reactive molecules as radical scavengers and antioxidants may be easily damaged during thermal treatment. The antioxidant capacity and the levels of the main bioactive compounds were compared in the same lot of Bronte's pistachio nuts, before and after exposure at 160 °C, for 40 min. The antioxidant potential was substantially affected by toasting. The heat treatment reduced by about 60% both TAA and TP (**Table 3**). As a result of the known thermal instability (*51*), a total loss of vitamin C was evident. The proanthocyanidin content was reduced by about 90%, whereas isoflavones, when considering the weight loss caused by roasting, appeared unmodified (**Table 3**). This appears in accordance with other reports showing that no decay of various isoflavones was observed after thermal exposition of soy and red clover (*52*).

The relationship between the amount of total phenols and antioxidant properties of many fruits and vegetables has been investigated in several studies, and phenolic components are

believed to account significantly for the antioxidant capacity (*18*, *53*). In accordance, the percent decrease of the total antioxidant activity observed in preparations from toasted nuts is consistent with the percent decrease of total phenols in the same preparation.

In the past decade, a correlation between consumption of nuts and a reduced incidence of ischemic heart disease has been observed, only partially ascribable to a favorable influence on the plasma fatty acid profile (*54*−*59*). In addition, positive effects of pistachio nuts on the oxidative status of healthy individuals have recently been reported (*60*). In this context, our findings that the Sicilian pistachio nuts are a rich source of bioactive antioxidant compounds, especially phytoestrogens, the dietary consumption of which is associated with a decreased incidence of cardiovascular diseases and some tumors (*61*−*64*), may further confirm the beneficial health effects of nuts.

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