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## Functional Lipid Characteristics of Turkish Tombul Hazelnut (Corylus avellana L.)

Cesarettin Alasalvar,<sup>\*,†</sup> Joana S. Amaral,<sup>‡,§</sup> and Fereidoon Shahidi<sup>||</sup>

TÜBİTAK Marmara Research Center, Food Institute, P.O. Box 21, 41470 Gebze, Kocaeli, Turkey, REQUIMTE, Serviços de Farmacognosia and Bromatologia, Faculdade de Farmácia, Universidade do Porto, R. Aníbal Cunha 164, 4050-047 Porto, Portugal, ESTIG, Instituto Politécnico de Bragança, Qta de Sta. Apolónia, Ap. 134, 5301-857 Bragança, Portugal, and Department of Biochemistry, Memorial University of Newfoundland, St. John's, Newfoundland A1B 3X9, Canada

The quality of crude oil extracted from Tombul (Round) hazelnut, grown in the Giresun province of Turkey, was evaluated for its fatty acid, triacylglycerol (TAG), tocol, and phytosterol compositions. Oleic acid contributed 82.78% to the total fatty acids, followed by linoleic, palmitic, and stearic acids. Among 12 TAGs separated, 11 were identified (including one unknown): LLL, OLL, PLL, OOL, POL, PPL, OOO, POO, PPO, SOO, and PSO (where P, palmitoyl; S, stearoyl; O, oleoyl; and L, linoleoyl). The main components were OOO (71.31%), OOL (12.26%), and POO (9.45%), reflecting the high content of oleic acid present in hazelnut oil. Seven tocol isoforms (four tocopherols and three tocotrienols) and eight phytosterols as well as cholesterol were positively identified and quantified; among these,  $\alpha$ -tocopherol (40.40 mg/100 g) and  $\beta$ -sitosterol (134.05 mg/100 g) were predominant in hazelnut oil and contributed 78.74 and 81.28% to the total tocols and phytosterols present, respectively. Tocotrienols were detected in small amounts (1.02% to the total tocols). The crude hazelnut oil extracted from Turkish Tombul hazelnut, thus, serves as a good source of nutrients, bioactives, and health-promoting components.

KEYWORDS: Tombul hazelnut; crude hazelnut oil; fatty acids; triacylglycerols; tocopherols; tocotrienols; phytosterols

## INTRODUCTION

The most recent recognition of nuts as heart-healthy foods by the FDA has provided a major boost to the image of nuts (1). Turkey is the world's largest producer of hazelnuts, contributing  $\sim$ 70% to the total global production, followed by Italy (12%), the United States (6%), and Spain (2%). Other countries contribute only 10% to the total production (2).

Hazelnut oil is becoming increasingly popular in Turkey and elsewhere. The main reason behind this demand is, of course, its nutritional composition and health-promoting components. Hazelnut oil is widely used for cooking, deep frying, salad dressings, and flavoring ingredients, among others. Annual production of hazelnut oil in Turkey reached 5800 tons in 2004 (3). Hazelnut oil can be found in the market either as crude (cold-pressed), refined, or a mixture of both. Currently, the price for refined hazelnut oil in Turkey is the same as virgin olive oil. It is, therefore, of great interest to assess the characteristics of hazelnut oil extracted from the Giresun (or premium) quality

Turkish Tombul variety, which has been famous for centuries among 17 commercial varieties in Turkey.

There is an increasing interest in the lipid characteristics of nut oils as they seem to be an interesting source of bioactive constituents and functional nutrients (4-6). Among nuts, the hazelnut has many beneficial health attributes. The benefits of inclusion of the hazelnut into the human diet is mainly related to its fat components (around 60%), most of which are highly rich in MUFA (primarily oleic acid), tocopherols ( $\alpha$ -tocopherol), phytosterols ( $\beta$ -sitosterol), polyphenols, and squalene (6–11).

A high MUFA diet tends to raise HDL cholesterol and lower TAG concentrations (12-14). Thus, hazelnut as an excellent source of MUFA may prove beneficial. In addition to MUFA, other components found in hazelnut oil have been reported to reduce plasma total and LDL cholesterol concentrations; these include PUFA (15-18) and tocotrienol (19). Besides favorable changes in plasma lipid profiles, a MUFA-rich diet instead of carbohydrates for SFA calories may favorably affect CVD risk (20) and has positive effects on atherosclerosis (21). Furthermore, phytosterols have been shown to decrease the risk of certain types of cancer (22, 23) and CVD (24) and enhance immune functions (25). Hazelnut oil, which is an excellent source of tocopherols, has also been shown to reduce the risk of CHD (26). In recent years, health effects of vitamin E

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<sup>\*</sup> To whom correspondence should be addressed. Tel.: +90 (0) 262 677 3272. Fax: +90(0)262 641 2309. E-mail: Cesarettin. Alasalvar@mam.gov.tr.

TÜBİTAK Marmara Research Center.

<sup>&</sup>lt;sup>‡</sup> Universidade do Porto.

<sup>§</sup> Instituto Politécnico de Bragança.

<sup>&</sup>lt;sup>II</sup> Memorial University of Newfoundland.

isoforms ( $\alpha$ -,  $\beta$ -,  $\gamma$ -, and  $\delta$ -tocopherols and tocotrienols) have been well-documented (27–29).

TAGs are a group of nonpolar lipids representing nearly 100% of the total nonpolar lipids in hazelnut oil (8). TAGs are increasingly used in the food industry as a tool to assess the quality and authenticity of vegetable oils (30), particularly the adulteration of olive oil with hazelnut oil (31).

Although lipid characteristics of the Tombul hazelnut cultivar have been reported (8), there is no information on TAG, tocotrienol, and complex phytosterol compositions of hazelnut oil extracted from the Turkish Tombul hazelnut cultivar. Thus, the objectives of this research were to investigate fatty acid, TAG, tocopherol, tocotrienol, and phytosterol compositions of crude hazelnut oil extracted from the Turkish Tombul hazelnut. The health aspects of these components, where possible, are also discussed.

#### MATERIALS AND METHODS

**Samples.** The sun-dried (commercial way of drying at 20-30 °C for  $\sim 3-4$  days) premium class natural Tombul (Round) hazelnut variety (*Corylus avellana* L.) was procured from the Giresun province of Turkey at the end of the 2005 harvest season and kept unshelled in a dark room at 5 °C until analyses were carried out. The hazelnuts were shelled before analysis.

**Reagents and Standards.** All chemical reagents and standards (sterols and TAGs) were obtained from Sigma-Aldrich-Fluka Co. Ltd. (Dorset, UK), unless otherwise specified. Tocopherols ( $\alpha$ -,  $\beta$ -,  $\gamma$ -, and  $\delta$ - isoforms) and tocotrienols ( $\alpha$ -,  $\beta$ -,  $\gamma$ -, and  $\delta$ - isoforms) were purchased from Calbiochem (La Jolla, CA), and tocol [2-methyl-2-(4,8,12-trimethyltridecyl)chroman-6-ol) as an IS was obtained from Matreya Inc. (Pleasant Gap, PA). The FAME standards were purchased from Supelco (Dorset).

**Sample Preparation.** Hazelnut samples were manually cracked and shelled and then chopped in a 643 MX coffee mill (Moulinex, Barcelona, Spain). Crude oil was obtained from finely ground nuts extracted with light petroleum ether (bp 40-60 °C) in a Universal extraction system B-811 (Büchi Labortechnik AG, Flawil, Switzerland); the residual solvent was removed by flushing with nitrogen. The extracted oil was used for the analyses of fatty acids, TAGs, and phytosterols. If delayed, it was stored in an amber vial (flushed with nitrogen) and stored at -40 °C for up to 2 weeks. Tocopherols and tocotrienols were analyzed directly on the ground sample as described next.

**Analysis of Total Fat Content.** The total fat content was determined in accordance with the official method of the Association of Official Analytical Chemists (*32*).

Analysis of Fatty Acids. FAMEs were prepared from hazelnut oil and determined by GC according to the method described by Slover and Lanza (33) and Alasalvar et al. (8). FAMEs were prepared using BF<sub>3</sub> in methanol (20% of BF<sub>3</sub> in methanol) and extracted with *n*-haxene and then analyzed by GC. For this purpose, samples (1  $\mu$ L) were injected into a Supelcowax 10 column (60 m  $\times$  0.25 mm i.d., 0.25  $\mu$ m film thickness; Supelco, Bellefonte, PA) coated with poly(ethylene glycol). The column was connected to a Hewlett-Packard 5890 Series II (Little Falls, Willmington, DE) GC equipped with a FID. The oven temperature was programmed as follows: 180 °C for 2 min, raised to 200 °C at 2 °C/min, held at 200 °C for a further 10 min, and then raised to 215 °C at 2 °C/min and kept there for 10 min. The injector and detector temperatures were 200 and 250 °C, respectively. Helium was used as the carrier gas at a flow rate of 1.5 mL/min. FAME identification was based on retention times as compared with those of standard FAMEs.

**Analysis of Triacylglycerols.** TAGs were determined by HPLC/ ELSD based on the methodology reported by Amaral et al. (*34*). A 0.2 g oil sample was dissolved in 4.0 mL of acetone and homogenized by stirring. The extract was filtered through a GELMAN Acrodisc LC13 PVDV 0.45  $\mu$ m pore size syringe filter (PALL Life Sciences, Ann Arbor, MI) before analysis. The chromatographic separation of the compounds was achieved with a Kromasil 100 C<sub>18</sub> column, 250 mm

 $\times$  4.6 mm i.d., 5  $\mu$ m particles (Teknokroma, Barcelona, Spain) operated at ambient temperature (around 20 °C). The injection loop was set up to  $10 \,\mu$ L. The chromatographic analyses were performed with the HPLC equipment which consisted of a PU-1580 quaternary pump and an AS-950 auto-sampler (Jasco, Tokyo, Japan). Detection was performed with a model 75 ELSD (Sedere, Alfortville, France). The mobile phase (filtered through a 0.45  $\mu$ m Millipore filter and degassed prior to use) used was a mixture of acetone/acetonitrile (70:30, v/v) (A) and acetone/ acetonitrile (80:20, v/v) (B). Elution was performed at a solvent flow rate of 1 mL/min with a two step gradient, starting with 0% B, changing to 100% B at 35 min, maintaining these conditions for 15 min, and then returning to the initial conditions. The ELSD was programmed with the following settings: evaporator temperature, 40 °C; air pressure, 3.5 bar; and photomultiplier sensitivity, 5. Data were recorded and processed using the Borwin-PDA controller software (JMBS Developments, Fontaine, France). Peaks were identified taking into account relative retention times of OOO. Peak identification was also supported by literature data on hazelnut oil, based on MALDI-TOF mass spectrometry (35) and APCI mass spectrometry (31, 35). Quantification of the peaks was made by internal normalization, assuming that the detector response was the same for all compounds (36).

Analysis of Tocopherols and Tocotrienols. The sample extraction and standards preparation were carried out according to the HPLC method of Amaral et al. (37, 38). BHT as an antioxidant and tocol as an IS were added to the ground hazelnut sample prior to the extraction procedure. The final extract was filtered through a GELMAN Acrodisc LC13 PVDV 0.45 µm pore size syringe filter (PALL Life Sciences) before auto-injection. The chromatographic separation of the compounds was achieved with a INERTSIL 5 SI normal phase column, 250 mm  $\times$  3 mm i.d., 5  $\mu$ m particles (Varian, Middelburg, Netherlands) operated at ambient temperature (around 20 °C). The injection loop was set up to  $10 \,\mu$ L. The HPLC equipment consisted of an integrated system with a PU-980 pump, AS-950 auto-sampler, MD-910 multi-wavelength DAD, and FP-920 programmable fluorescence detector (Jasco). Data were recorded and processed using the Borwin-PDA controller software (JMBS Developments). The effluent was monitored with a DAD connected in series with a fluorescence detector programmed at the excitation and emission wavelengths of 290 and 330 nm, respectively. The gains set in the fluorescence detector were as follows: from 0 to 11.5 min, gain 10; from 11.5 to 14.3 min, gain 100; and afterward, gain 10. The mobile phase (filtered through a 0.45  $\mu$ m Millipore filter and degassed prior to use) was a mixture of n-hexane and 1,4-dioxane at a ratio of 95.5:4.5 (v/v) at 0.7 mL/min for 20 min. The compounds were identified by comparing their retention times and UV spectra with authentic standards. The identified compounds were quantified by fluorescence detection based on the IS method. Tocopherol and tocotrienol levels in hazelnut oil were calculated using the percentage of oil content measured in Tombul hazelnut.

Analysis of Phytosterols. Phytosterol composition was analyzed by a GC/FID methodology as reported by Amaral et al. (11, 39), with slight modifications. The chromatographic separation of the sterol compounds was achieved with a DB-5MS column, 30 m  $\times$  0.25 mm i.d., 0.25 µm film thickness (J&W Scientific, Folsom, CA) at a maximum operating temperature of 325 °C. The column was connected to a Chrompack CP 9001 GC (Chrompack, Middelburg, Netherlands) equipped with a Chrompack CP-9050 autosampler, split-splitless injector, and FID. The injector and the detector temperatures were both set at 320 °C. The column temperature was 250 °C and programmed to increase at a rate of 2 °C/min to 300 °C and then held for 12 min. The carrier gas used was helium at an internal pressure of 100 kPa. The split ratio was 1:50, and the injected volume was 1.5  $\mu$ L. The total phytosterol content was determined considering all peaks of sterol eluted between cholesterol and  $\Delta^7$ -avenasterol. Identification was achieved by comparing the relative retention times from samples with those obtained with standards. Clerosterol,  $\Delta^5$ -avenasterol,  $\Delta^7$ -stigmastenol, and  $\Delta^7$ -avenasterol were tentatively identified by comparison with references (11, 39).  $\beta$ -Sitostanol and  $\Delta^5$ -avenasterol eluted very closely, and therefore, they were quantified as  $\Delta^5$ -avenasterol.

**Statistical Analysis.** The mean and SD (either n = 3 or n = 6) were determined using the Microsoft Excel statistical software (Microsoft Office Excel 2003, Microsoft Corp., Redmond, WA).

 Table 1. Fatty Acid Composition of Oil Extracted from Tombul Hazelnut<sup>a</sup>

$0.04 \pm 0.00$ $0.03 \pm 0.00$ $4.81 \pm 0.04$ $0.18 \pm 0.01$
$4.81 \pm 0.04$
$0.19 \pm 0.01$
0.10 ± 0.01
$0.06 \pm 0.00$
$0.07 \pm 0.00$
$2.69 \pm 0.02$
$82.78 \pm 0.09$
$8.85 \pm 0.04$
$0.12 \pm 0.01$
$0.13 \pm 0.01$
$0.17 \pm 0.01$
$0.02 \pm 0.00$
$0.02 \pm 0.01$
$0.01 \pm 0.00$
$0.02 \pm 0.00$
7.79
83.24
8.97

<sup>a</sup> Data are expressed as mean  $\pm$  SD (n = 3).

#### **RESULTS AND DISCUSSION**

Fatty Acid Composition. The fatty acid profile of hazelnut oil is shown in Table 1. Sixteen fatty acids were identified among which oleic acid (18:1 $\omega$ 9) contributed 82.78% to the total, followed by linoleic acid (18:2 $\omega$ 6) at 8.85%, palmitic acid (16:0) at 4.81%, and stearic acid (18:0) at 2.69%. The remaining 12 fatty acids contributed only 0.87% to the total amount of fatty acids present. The total SFA made up a small proportion (7.79%) of the fatty acids of hazelnut oil, whereas total MUFA was the highest (83.24%). Unsaturated fatty acids (MUFA + PUFA) accounted for 92.21% of the total fatty acids present. Hazelnut oil, in terms of its high proportion of unsaturated fatty acids, is much more desirable than other vegetable oils. The major fatty acids found in the present study are, in general, comparable to those reported in the literature on different hazelnut varieties (6, 7, 10, 11, 40-43). However, the composition and amount of fatty acids both between and within the same hazelnut varieties may be influenced by a number of factors such as variety, geographic origin, growing condition, maturity, fertilization, time of harvest, season, soil type, climate, latitude, and storage conditions, among others (7, 11, 44, 45).

Beneficial effects of a MUFA-rich hazelnut-enriched diet for human health have been reported (14, 46–48). As compared to other nut and vegetable oils, hazelnut oil has been reported to contain the highest proportion of oleic acid (4, 6, 49, 66).

Amaral et al. (10) reported that hazelnut oil contained trace amount of trans fatty acids (0.02%). Similar to SFA, evidence suggests that the consumption of a high amount of trans fatty acids raises LDL cholesterol and lowers HDL cholesterol concentrations, therefore increasing the risk of developing heart disease, stroke, and certain types of cancer, among others (50– 52). The FDA recently published a final rule that trans fatty acids be declared in the nutrition label of conventional foods and dietary supplements. If a serving contains less than 0.5 g, the content, when declared, must be expressed as 0 g (53).

**Triacylglycerol Composition.** In a previous study, Alasalvar et al. (8) reported that the crude lipid extract from Turkish Tombul hazelnut composed of nonpolar (98.8%) and polar (1.2%) constituents and TAGs was the major nonpolar lipid class and contributed nearly 100% to the total amount.

Figure 1 shows a chromatogram of TAGs obtained under the experimental conditions described. Twelve TAGs (including

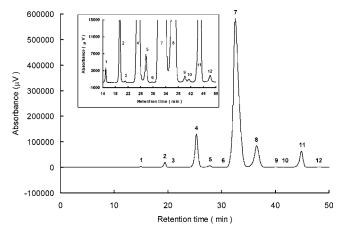


Figure 1. Triacylglycerol profile of hazelnut oil obtained by HPLC/ELSD. Peaks: 1, LLL; 2, OLL; 3, PLL; 4, OOL; 5, POL; 6, PPL; 7, OOO; 8, POO; 9, PPO; 10, unknown; 11, SOO; and 12, PSO.

Parcerisa et al., using an RI detector (44, 55) and MS detector (31), have reported higher values for LLL ((0.5-3.6%)), OLL ((1.8-10.8%)), POL ((2.7-6.5%)), and PPO ((0.7-2.4%)) and much lower values for OOO ((32.3-57.0%)) than those reported herein. Nevertheless, our data are in fairly good agreement with results reported by the same authors when studying the TAG composition during hazelnut development using an ELSD detector (45).

Parcerisa et al. (44, 45, 55) detected trace amounts of PPP. In contrast, Ayorinde et al. (35) have analyzed hazelnut oil by MALDI-TOF mass spectrometry and pointed out that minor quantities of SLL and SOL could be coeluted with OLL and OOO, respectively. The data herein analyzed indicate that minor amounts of SLL and SOL can exist but are undetectable by the methodology employed. Amaral et al. (34) have reported that the TAG composition in hazelnuts can be influenced by genetic and environmental factors.

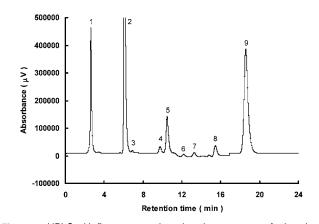
**Tocols (Tocopherol and Tocotrienol) Composition.** The content of total tocols (51.31 mg/100 g) in fresh Tombul hazelnut oil is shown in **Table 2**. Seven tocol isoforms were detected and quantified ( $\alpha$ -,  $\beta$ -,  $\gamma$ -, and  $\delta$ -tocopherols and  $\alpha$ -,  $\beta$ -, and  $\gamma$ -tocotrienols) (**Figure 2**). Among the tocols identified,  $\alpha$ -tocopherol was most abundant (40.40 mg/100 g), accounting for 78.74% of the total, followed by  $\gamma$ -tocopherol (8.33 mg/100 g),  $\beta$ -tocopherol (1.53 mg/100 g), and a small amount of  $\delta$ -tocopherol (0.53 mg/100 g). Tocotrienols contributed only 1.02% to the total tocols present. In a previous study, Alasalvar et al. (8) identified four tocopherols in freshly extracted oil from Tombul hazelnut. The extraction procedure, HPLC conditions, detector, column, and mobile phase used were different from those published earlier.

Several researchers have only detected and quantified tocopherols in crude, refined, virgin, mixed, and pressed hazel nuts, among which  $\alpha$ -tocopherol was the predominant tocopherol with concentrations ranging from 11.9 to 61.88 mg/100 g (7, 31, 41, 42, 49, 56, 57). Small amounts of  $\alpha$ -,  $\beta$ -, and  $\gamma$ -tocotrienols

Table 2. Triacylglycerol, Tocol, and Phytosterol Compositions of Oil Extracted from Tombul Hazelnut<sup>a</sup>

triacylglycerols <sup>b</sup>	relative %	tocols <sup>c</sup>	mg/100 g of oil	phytosterols	mg/100 g of oil
LLL	$0.24 \pm 0.02$	α-tocopherol	$40.40\pm0.56$	cholesterol	$0.30\pm0.03$
OLL	$1.85 \pm 0.10$	$\beta$ -tocopherol	$1.53 \pm 0.02$	campesterol	$9.92 \pm 0.55$
PLL	$0.05 \pm 0.00$	$\gamma$ -tocopherol	$8.33 \pm 0.16$	stigmasterol	$1.61 \pm 0.14$
OOL	$12.26 \pm 0.38$	$\delta$ -tocopherol	$0.53 \pm 0.01$	clerosterol	$1.80 \pm 0.11$
POL	$0.79\pm0.07$	$\alpha$ -tocotrienol	$0.20 \pm 0.02$	$\beta$ -sitosterol	$134.05 \pm 5.23$
PPL	$0.01 \pm 0.00$	$\beta$ -tocotrienol	$0.09 \pm 0.00$	$\Delta^5$ -avenasterol <sup>d</sup>	$13.93 \pm 0.18$
000	71.31 ± 1.11	$\gamma$ -tocotrienol	$0.23 \pm 0.01$	$\Delta^7$ -stigmastenol	$2.09 \pm 0.16$
POO	$9.45 \pm 0.76$			$\Delta^7$ -avenasterol	$1.22 \pm 0.11$
PPO	$0.08 \pm 0.01$				
unknown	$0.07 \pm 0.01$				
SOO	$3.79 \pm 0.24$				
PSO	$0.10 \pm 0.01$				
total	$100.00 \pm 0.00$	total	$51.31 \pm 0.51$	total	$164.92 \pm 5.92$

<sup>a</sup> Data are expressed as means  $\pm$  SD (n = 6). <sup>b</sup> Trilinoleoylglycerol (LLL), oleoyl-dilinoleoylglycerol (OLL), palmitoyl-dilinoleoylglycerol (PLL), dioleoyl-linoleoylglycerol (POL), apalmitoyl-oleoylglycerol (POL), dipalmitoyl-linoleoylglycerol (PPL), trioleoylglycerol (OOO), palmitoyl-dioleoylglycerol (POO), dipalmitoyl-oleoylglycerol (PPO), stearoyl-dioleoylglycerol (SOO), and palmitoyl-stearoyl-oleoylglycerol (PSO). <sup>c</sup> Vitamin E activity (expressed as  $\alpha$ -tocopherol equivalents) = 41.92  $\pm$  0.55 mg/100 g of oil. (The conversion factors for vitamin E activity were as follows:  $\alpha$ -tocopherol × 1.00,  $\beta$ -tocopherol × 0.40,  $\gamma$ -tocopherol × 0.10,  $\delta$ -tocopherol × 0.01,  $\alpha$ -tocotrienol × 0.30,  $\beta$ -tocotrienol × 0.05, and  $\gamma$ -tocotrienol × 0.01 (*65*). <sup>d</sup>  $\Delta$ <sup>5</sup>-Avenasterol contains  $\beta$ -sitostanol.



**Figure 2.** HPLC with fluorescence detection chromatogram of a hazelnut sample. Peaks: 1, BHT; 2,  $\alpha$ -tocopherol; 3,  $\alpha$ -tocotrienol; 4,  $\beta$ -tocopherol; 5,  $\gamma$ -tocopherol; 6,  $\beta$ -tocotrienol; 7,  $\gamma$ -tocotrienol; 8,  $\delta$ -tocopherol; and 9, IS (tocol).

together with four tocopherols in hazelnut oils have recently been reported (10, 38, 40, 43, 58). Varietal differences in tocopherol and tocotrienol content of hazelnut cultivars have been reported in the previous studies. In addition, geographic origin, climate, harvesting year, storage conditions, culture conditions, and soil type, among others, might also affect the tocopherol and tocotrienol compositions and their contents in hazelnut oils.

Table 2 (footnotes) indicates that hazelnut oil is an excellent source of vitamin E (41.92 mg/100 g). Consuming approximately 24 g of hazelnut oil per day supplies 100% of the RDA of vitamin E (59) for adults. The health benefits of vitamin E are strongly supported by numerous reports (26, 27, 29). Vitamin E is a powerful lipid-soluble antioxidant, capable of protecting the body's cells from free radical damage (29). This means they may help to protect humans from certain chronic diseases, while delaying the body's aging process. The latest research showed that although the antioxidant activity of tocotrienols is higher than that of tocopherols, tocotrienols have a lower bioavailability after digestion (60). In addition, Qureshi et al. (19) reported that tocotrienols lower serum cholesterol in hypercholesterolemic humans. Kornsteiner et al. (57) studied the tocopherol content of oil extracted from different nuts and found that vitamin E was highest in hazelnut oil (33.1 mg/100 g), followed by, in descending order, almond > peanut > pistachio > pine nut >

walnut > Brazil nut > pecan > cashew > macademia. Hazelnut oil has been reported to contain the highest  $\alpha$ -tocopherol level among nut oils (49, 57). Depending on varieties, hazelnut oil also contained 2–3 times more  $\alpha$ -tocopherol than olive oil (31, 40, 56).

**Phytosterol Composition.** Among the sterols identified and quantified (cholesterol, campesterol, stigmasterol, clerosterol,  $\beta$ -sitosterol,  $\Delta^5$ -avenasterol +  $\beta$ -sitostanol,  $\Delta^7$ -stigmastenol, and  $\Delta^7$ -avenasterol) in hazelnut oil,  $\beta$ -sitosterol comprised 81.28% of the total, while  $\Delta^5$ -avenasterol +  $\beta$ -sitostanol and campesterol were the second and third components of the group with values of 8.45 and 6.02%, respectively (**Table 2**). The remaining phytosterols contributed only 4.25% to the total phytosterol present. The same number of sterols in 19 cultivars of hazelnut oils was identified by Amaral et al. (11). In a previous study, Alasalvar et al. (8) identified and quantified only the three most common phytosterols ( $\beta$ -sitosterol, campesterol, and stigmasterol) in freshly extracted Tombul hazelnut oil. The extraction procedure and GC conditions used were different from the previous publication.

Bada et al. (41) identified 10 sterols in 15 hazelnut oils collected from different sources. In addition to the present study, they detected a small amount of campestanol (0.2–0.4% to the total) and  $\Delta^5$ -stigmastadienol (0.6–1.0% to the total). No cholesterol was found in their study. Moreover, 11 sterols in hazelnut oil were extracted using hexane and supercritical fluid extractions (54) in five hazelnut oils obtained from different countries (43). They detected a small amount of campestanol and  $\Delta^5$ -24-stigmastadienol in addition to those reported in the present study. Among all hazelnut oils studied,  $\beta$ -sitosterol was the predominant compound.

The total content of phytosterol (including cholesterol) found in the present study was 164.92 mg/100 g, which is in good agreement with those reported for hazelnut oils (11, 40, 42, 43). In contrast, the level found in this work is slightly lower than those reported by Savage et al. (7) and Bada et al. (41) for some hazelnut oils. Significant differences in individual and total phytosterol contents in hazelnut oils among some cultivars have been reported (7, 11, 43). Amaral et al. (11) compiled four olive oils from different geographical origins and observed that total phytosterol contents ranged from  $\geq 100$  to 230 mg/100 g, which are within the same range or lower than those found in most hazelnut oils.  $\beta$ -Sitosterol has also been reported to be the main sterol in olive oil (75–90% to the total sterol) (61). Phillips et al. (62) studied the phytosterol composition of nuts consumed in the U.S. and quantified six phytosterols. Among 10 economically important nuts (hazelnut, almond, walnut, pistachio, pine nut, Brazil nut, cashew, pecan, macademia, and peanut), the total phytosterol content ranged from 95 to 279 mg/100 g of nut, being the lowest in the Brazil nut and the highest in the pistachio nut. Hazelnut had a phytosterol content of 121 mg/100 g of nut in their study.

Numerous well-designed studies have documented the beneficial role of phytosterols and phytostanols on serum cholesterol (17, 18, 63, 64). The consumption of margarine containing 10% phytosterols resulted in a 13% reduction in total and LDL cholesterols in normal and mildly hypercholesterolemic patients (18). Hence, the consumption of foods such as hazelnut oil that contain phytosterols provides potential health benefits.

In summary, hazelnut oil serves as a good source of natural antioxidants and bioactives, thus reflecting its nutraceutical potential in different food and specialty applications. In addition, hazelnut oil, a rich source of MUFA (oleic acid) and phytosterols ( $\beta$ -sitosterol), may be considered as a supplement for daily diet plannings to reduce the risk of CHD by lowering total, LDL, VLDL, and TAG levels and increasing HDL cholesterol levels (14). Diet seems to play an important role in health maintenance and disease prevention, and potential benefits are ascribed to vitamin E isomers, so qualitative and quantitative vitamin E compositions herein reported for this matrix should encourage the consumption of the hazelnut and its oil.

### ABBREVIATIONS USED

APCI, atmospheric pressure chemical ionization; BHT, butylated hydroxytoluene; CHD, coronary heart disease; CVD, cardiovascular disease; DAD, diode array detector; ELSD, evaporative light scattering detector; FAME, fatty acid methyl ester; FAMEs, fatty acid methyl esters; FDA, Food and Drug Administration; FID, flame-ionization detector; GC, gas chromatography; GC/FID, gas chromatography/flame-ionization detector; HDL, high-density lipoprotein; HPLC/ELSD, highperformance liquid chromatography/evaporative light scattering detector; IS, internal standard; LDL, low-density lipoprotein; MALDI-TOF, matrix-assisted laser desorption ionization-timeof-flight; MS, mass spectrometry; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; RDA, recommended dietary allowances; RI, refractive index; SD, standard deviation; SFA, saturated fatty acids; TAG, triacylglycerol; TAGs, triacylglycerols; TAG compounds (L, linoleoyl; O, oleoyl; P, palmitoyl; S, stearoyl; LLL, trilinoleoylglycerol; OLL, oleoyldilinoleoylglycerol; PLL, palmitoyl-dilinoleoylglycerol; OOL, dioleoyl-linoleoylglycerol; POL, palmitoyl-oleoyl-linoleoylglycerol; PPL, dipalmitoyl-linoleoylglycerol; OOO, trioleoylglycerol; POO, palmitoyl-dioleoylglycerol; PPO, dipalmitoyloleoylglycerol; SOO, stearoyl-dioleoylglycerol; PSO, palmitoylstearoyl-oleoylglycerol; PPP, tripalmitoylglycerol; SLL, stearoyldilinoleoylglycerol; and SOL, stearoyl-oleoyl-linoleoylglycerol); UV, ultraviolet; VLDL, very low-density lipoprotein.

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