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Fatty Acid Profiles, Tocopherol Contents, and Antioxidant Activities of Heartnut (*Juglans ailanthifolia* Var. *cordiformis*) and Persian Walnut (*Juglans regia* L.)

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The fatty acid and tocopherol compositions of three heartnut (*Juglans ailanthifolia* var. *cordiformis*) varieties (Imshu, Campbell CW1, and Campbell CW3) were examined and compared with those of two Persian walnut (*Juglans regia* L.) varieties (Combe and Lake). The major fatty acids found in heartnuts and walnuts were identified by gas chromatography as linoleic (18:2n-6), α-linolenic (18:3n-3), oleic (18:1n-9), palmitic (16:0), and stearic acid (18:0). Polyunsaturated fatty acids were the main group of fatty acids found in both heartnut and walnut, ranging from 73.07 to 80.98%, and were significantly higher in heartnut than in Persian walnuts ($P < 0.001$). In addition, heartnuts had significantly higher levels of 18:2n-6 and lower levels of 18:3n-3 compared to the Persian walnuts. γ-Tocopherol was the main tocopherol homologue present in both types of nuts, followed by δ- and α-tocopherol. The highest concentration of γ-tocopherol was found in Combe Persian walnut at 267.87 μg/g, followed by Lake Persian walnut and Imshu, Campbell CW1, and CW3 heartnut at 205.45, 187.33, 161.84, and 126.46 μg/g, respectively. Tocopherols, particularly the γ-tocopherol, were found to contribute the most to the strong total antioxidant activities of both walnut and heartnut oils using either the free radical 2,2-diphenyl-1-picrylhydrazyl assay or the photochemiluminescence method.

KEYWORDS: Walnut; heartnut; fatty acid; tocopherol; antioxidant activities; *Juglans ailanthifolia* var. *cordiformis; Juglans regia* L.

INTRODUCTION

Walnuts (*Juglans regia* L.) are a good source of essential fatty acids and tocopherols that contribute to reduced risk of coronary heart disease (CHD) (*1*). The major fatty acids found in walnut are linoleic (18:2n-6), α-linolenic (18:3n-3), oleic (18:1n-9), palmitic (16:0), and stearic (18:0) acid (*1*−*6*). γ-Tocopherol has been identified as the major vitamin E homologue in walnut (*1*, *6*−*8*). The oil content and the fatty acid and tocopherol compositions have been found to vary significantly among different walnut cultivars (*2*−*8*).

The oxidation of lipids in food is responsible for the formation of off-flavors and undesirable chemical compounds that may be detrimental to health (*9*, *10*). Tocopherols as antioxidants can stabilize fatty acids in oil (*6*, *11*−*14*) and thus prevent the rancidity of oils during storage (*15*−*17*). α-Tocopherol has been found to be more active as an antioxidant than other vitamin E homologue in vivo (*18*, *19*). However, in some model systems γ-tocopherol has been found to have a higher antioxidant capacity than α-tocopherol (*11*, *20*, *21*). The antiradical capacity of oils from different food sources including walnut has been evaluated using the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•) induced free radical method (*22*). For walnut oils, the antiradical capacity has been attributed to the presence of tocopherols (*6*, *22*), and different homologues of vitamin E were shown to have different degrees of antioxidative efficiency (*11*, *18*−*21*).

The oils extracted from the Persian walnut (*Juglans regia* L.) have been widely studied for their fatty acid profiles, their tocopherol content, and their oxidative stability (*1*−*4*). Recently, heartnut (*Juglans ailanthifolia* var. *cordiformis*), a naturally occurring genetic oddity of the more common Japanese walnut, has become very popular among consumers and nut tree growers in southern Ontario, Canada. Heartnuts have a natural heart shape and are generally hardier, a characteristic that gives the heartnuts a great commercial potential in the Great Lakes region in Ontario. Information on the phytochemical composition of the heartnut is scarce. We have previously reported the phenolic content and antioxidant activity of heartnuts (*23*). In this study, we report for the first time on the fatty acid profiles and tocopherol contents of three heartnut varieties using gas chromatography (GC) and high-performance liquid chromatography (HPLC), respectively. The antioxidant activities of the

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Table 1. Tocopherol Contents of Heartnut and Persian Walnut Seeds

sample	oil/nut (g/g)	α-tocopherol (μg/g)	β-tocopherol (μg/g)	γ-tocopherol (μg/g)	δ-tocopherol (μg/g)	total tocopherol (μg/g)
Walnuts, Mean Values of Duplicate Samples ± Standard Deviations						
Combe Persian walnut	0.59 ± 0.02	7.92 ± 0.04	ND[a]	267.87 ± 1.95[b]	31.79 ± 0.51	307.98 ± 2.5[c]
Lake Persian walnut	0.61 ± 0.04	5.06 ± 0.93	ND	205.45 ± 2.72[b]	11.64 ± 0.30	222.15 ± 3.95[c]
Heartnuts, Mean Values of Duplicate Samples ± Standard Deviations						
Campbell CW1 heartnut	0.49 ± 0.03	0.16 ± 0.19	ND	161.84 ± 1.01	5.20 ± 0.83	167.2 ± 2.03
Campbell CW3 heartnut	0.51 ± 0.06	0.33 ± 0.17	ND	126.46 ± 1.67	1.63 ± 0.88	128.42 ± 2.72
Imshu heartnut	0.53 ± 0.01	1.29 ± 0.07	ND	187.33 ± 2.29	11.71 ± 1.64	200.33 ± 4

[a] Not detectable. [b] Significant difference between mean values of heartnuts and walnuts at $P < 0.001$. [c] Significant difference between mean values of heartnuts and walnuts at $P < 0.01$.

lipid extracts of the heartnuts were evaluated using the DPPH• and photochemiluminescence (PCL) induced free radical methods. The oils extracted from two Persian walnut varieties grown in the same region were studied for comparison purposes.

MATERIALS AND METHODS

Chemicals, Solvents, and Nut Samples. Three heartnut varieties (Imshu, Campbell CW1, and Campbell CW3) and two Persian walnut varieties (Combe and Lake) were harvested in September 2005 and kindly provided by Ernie Grimo (Niagara-on-the-Lake, ON, Canada). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and α-, β-, γ-, and δ-tocopherols were from Sigma-Aldrich (Oakville, ON, Canada). Reagents for the PCL assay were purchased from Analytik Jena AG (Berlin, Germany). Standards of fatty acid methyl esters (FAMEs) (mixture 463, Nu-Chek-Prep, Inc., Elysian, MN) were obtained commercially. All solvents were of HPLC grade and purchased from Caledon Laboratories Ltd. (Georgetown, ON, Canada). Thin-layer chromatography (TLC) plates (250 μm, 20 × 20 cm, silica gel G) were from Fisher Scientific (Ottawa, ON, Canada). Trolox was obtained from Sigma-Aldrich.

Sample Preparation. All nuts were manually cracked and shelled and the seeds immediately placed into liquid nitrogen and ground in a coffee blender. For each sample, 5 g of finely ground nut from 10−15 fruits was accurately weighed in a 100 mL screw-capped glass flask and extracted twice with 50 mL of hexane for 2 h at room temperature in the dark with occasional manual shaking. The extract was filtered through a Whatman no. 1 filter paper, and the hexane was removed using a rotary evaporator (<30 °C) and finally flushed with nitrogen. The oil extract was kept in amber tubes and stored at −20 °C until being analyzed.

For fatty acid analysis 10 mg of oil was methylated in a 15 mL culture tube equipped with a Teflon-lined screw cap under N_2 and anhydrous conditions. $NaOCH_3$/methanol (0.5 mL, 0.5 N) was added, and the tube was heated at 50 °C for 15 min. The tubes were then cooled to room temperature, and 1 mL of distilled water and 2 mL of hexane were added. The hexane layer was removed and reduced in volume before the FAME mixture was applied onto TLC plates for purification using the developing solvent system of hexane/diethyl ether/acetic acid (85:15:1, v/v/v). The FAME band was visualized after the TLC plates had been sprayed with 2′,7′-dichlorofluoroscein/methanol (0.1% w/v) and viewed under UV light (254 nm). The corresponding FAME band was removed and eluted with chloroform. After removal of the chloroform using a stream of nitrogen, the FAMEs were dissolved in an appropriate volume of hexane (1−2 μg/μL) and analyzed by GC (*24*).

Analyses of Tocopherols by HPLC. An Agilent Technology 1100 series HPLC system equipped with a quaternary pump, a degasser, a thermostatic autosampler, and a photodiode array detector (PAD) was used for the analysis of tocopherols in the hexane extracts of nuts. Each sample (0.2 g of oil) was accurately weighed in an amber vial and dissolved in 1 mL of hexane for HPLC analysis of tocopherols. The analysis was carried out using a Phenomenex silica column (250 × 4.6 mm, 5 μm) and a mobile phase containing 10% *tert*-butyl methyl ether in hexane (v/v). The flow rate was kept constant at 1.0 mL/min for a total run time of 25 min. The sample injection volume was 10 μL. Peaks of interest were monitored at 294 nm.

Analyses of FAMEs by GC. FAMEs were analyzed using a GC (model 5890; Hewlett-Packard, Palo Alto, CA) equipped with a splitless injection port (flushed after 0.3 min), a flame ionization detector (FID), an autosampler (Hewlett-Packard, model 7673), a 100 m CP-Sil 88 fused capillary column (Varian Inc., Mississauga, ON, Canada), and a Hewlett-Packard ChemStation software system (version A.09). The injector and detector temperatures were both set at 250 °C; H_2 served as carrier gas at a flow of 1 mL/m. The column was initially operated at 45 °C for 4 min and then temperature-programmed at 13 °C/min to 175 °C, held for 27 min, increased at 4 °C/min to 215 °C, and finally held for 35 min; total run time was 86 min. The GC reference standard FAME mixture 463 from Nu-Chek Prep was used to identify the FAMEs (*24*). Quantification of the FAMEs was based on the FID response expressed as percent of total FAMEs.

Radical Scavenging Method. The DPPH• assay was carried out according to the procedures used by Brand-Williams et al. (*9*) with minor modifications. In this study, DPPH• (2.5 mg/100 mL), all standards, and samples were dissolved in a mixture of solvents (hexane/EtOAc/MeOH 1:1:2, v/v/v) instead of methanol. This solvent mixture was used as a blank check as well. Samples were prepared in duplicate for each of the three concentrations used. A standard or sample solution (0.5 mL) was added to 3.5 mL of DPPH• solution. The absorbance of the mixture was then determined at 515 nm in a Varian Cary 3C spectrophotometer (Varian, Palo Alto, CA) at 0 min, 5 min, and every 10 min until the reaction reached a plateau at room temperature. The antioxidant (antiradical) activity was expressed as EC_{50}, which is defined as the concentration of an antioxidant at which 50% of the initial absorbance is reduced. A lower EC_{50} value indicates a higher antiradical activity (*9*, *22*, *25*, *26*).

PCL Assay. The PCL assay followed the procedures reported by Tsao et al. (*27*) with minor modifications. A commercially available ACL-Kit (antioxidant capacity of lipid-soluble compounds, Analytik Jene AG) from the manufacturer was used. Briefly, the test mixture consisted of 1.0 mL of reaction buffer containing 0.1 M sodium carbonate (pH 10.5), 0.1 mM Na_2-EDTA, 2.3 mL of methanol, 25 μL of 1 mM luminol, and 10 μL of a standard or a sample solution. When necessary, samples were diluted so that the PCL curves fell within the linear range of the standard Trolox (0.5−3.0 nmol). All samples were tested in duplicate. The antioxidant capacity of the sample was calculated as equivalent units of Trolox.

Statistical Analysis. Statistical analysis was performed using a linear correlation and *T* test (two-sample assuming equal variances) functions of Microsoft Excel (v. 10.5815.4219 SP-2).

RESULTS AND DISCUSSION

Separation, Identification, and Quantification of Tocopherols by HPLC. All standard and sample preparation procedures were carried out in subdued light in a nitrogen atmosphere at room temperature to avoid oxidation. The total oil content of the two Persian walnut and three heartnut varieties ranged from 49 to 61%, with the Lake Persian walnut yielding the highest percentage of oil, whereas the Campbell CW1 heartnut yielded the lowest (**Table 1**). The average yield of total oil of the three heartnut varieties was slightly lower (51.0%) than that of the walnuts (60.0%). In general, the values for the

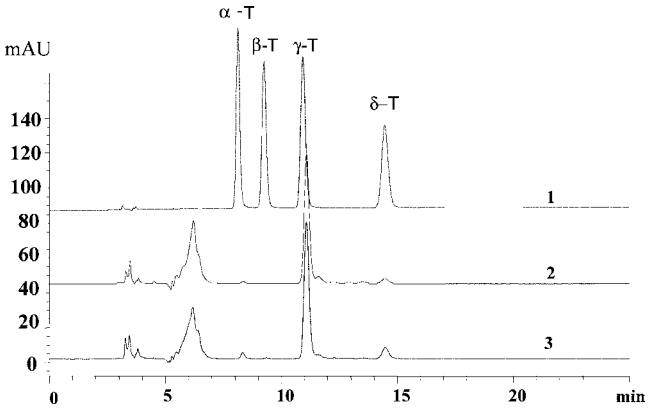

Figure 1. Separation of the four different tocopherol homologues using a normal-phase HPLC column (1) and the tocopherol profiles of Campbell CW1 heartnut (2) and Combe Persian walnuts (3).

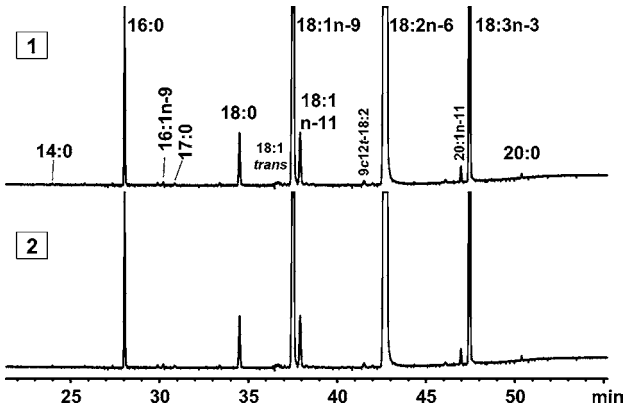

Figure 2. Partial GC chromatogram obtained from the oils of Campbell CW3 heartnut (1) and Combe Persian walnut (2). For GC column and conditions see Materials and Methods.

two Persian walnuts were in agreement with those published previously (*2*, *3*, *6*, *28*).

Although sample preparation for liposoluble vitamins commonly involved alkaline hydrolysis, several studies indicated that the method without hydrolysis was simple and efficient for the analysis of tocopherols (*7*, *28*, *29*). In this study, the preparation for tocopherols was achieved by direct hexane extraction. HPLC has been widely used for the determination of tocopherols in walnuts, and both normal- (*8*) and reversed-phase columns (*7*, *28*−*30*) have been used to separate the vitamin E homologues. However, the latter does not usually allow for complete separation of the β- and γ-tocopherols. The present method using a normal-phase column gave a good separation of all vitamin E homologues within 25 min (**Figure 1**). Identification and confirmation of the tocopherols were based on comparison of the retention times and UV spectra with authentic standards. Even though there were marked differences in the content of the individual tocopherols in heartnut and Persian walnut, the relative proportions of the major tocopherols were rather similar (**Figure 1**). The major tocopherols detected in both heartnut and walnut oils were identified as α-, γ-, and δ-tocopherol (**Figure 1**).

The quantification of the three major tocopherols is presented in **Table 1**; β-tocopherol was identified only in trace amounts. Although the levels of tocopherols differed among nut varieties, γ-tocopherol was the predominant vitamin E homologue present in both nuts, followed by δ- and α-tocopherol. The total tocopherol contents ranged from 128.42 to 307.98 μg/g in all nut oils, but walnuts had significantly higher concentrations of total tocopherols than the heartnuts ($P < 0.01$). The γ-tocopherol content was significantly higher ($P < 0.001$) in the Persian walnuts than in heartnuts (**Table 1**), but there was greater variability when it was expressed in relative abundance, with the Combe Persian walnut variety showing the lowest level (87%) and both Campbell varieties showing the highest levels (97−98%). Although no data have been reported on the tocopherol contents of heartnuts, the concentrations obtained in this study for walnuts were consistent with previous reports (*1*, *6*−*8*).

Identification and Quantification of the FAMEs by GC. The use of walnut oil for cosmetic purposes may also be due to its high content in essential fatty acids and in particular linoleic and linolenic acids (*31*). GC is the technique of choice for analyzing the composition of fatty acids in walnuts (*1*−*6*), and typical separations of a heartnut and walnut are shown in **Figure 2**. The major fatty acids identified in heartnut and walnut were 18:2n-6, 18:3n-3, 18:1n-9, 16:0, 18:0, and 18:1n-11 (**Table 2**). The fatty acid compositions of the different nuts showed greater variability between the heartnut and walnut varieties than among the different varieties within each group. The heartnuts

Table 2. Fatty Acid Composition (Relative Percent) of Oil Extracted from Heartnuts and Persian Walnuts

	walnut		heartnut		
fatty acid	Combe Persian	Lake Persian	Campbell CW1	Campbell CW3	Imshu
14:0	0.03	0.03	0.02	0.01	0.02
15:0	0.02	0.02	0.02	0.02	0.02
16:0	5.87	5.59	2.63	2.42	2.71
7c-16:1	0.06	0.05	0.04	0.04	0.03
9c-16:1	0.06	0.06	0.05	0.04	0.05
17:0	0.05	0.01	0.03	0.03	0.03
7c-17:1	0.02	0.05	0.02	0.01	0.01
9c-17:1	0.02	0.01	0.04	0.04	0.04
18:0	3.24	2.83	0.96	0.91	1.03
6-8t-18:1	0.02	0.02	0.03	0.03	0.02
9t-18:1	0.05	0.05	0.06	0.05	0.05
10t-18:1	0.07	0.07	0.07	0.06	0.06
11t-18:1	0.04	0.05	0.05	0.05	0.04
12t-18:1	0.03	0.04	0.03	0.03	0.03
13t/14t-18:1	0.02	0.02	0.02	0.02	0.02
9c-18:1	15.73	16.39	13.02	13.50	13.91
11c-18:1	0.76	0.82	1.22	1.09	1.16
12c-18:1	0.04	0.04	0.06	0.05	0.04
13c-18:1	0.03	0.03	0.04	0.02	0.03
19:0	0.02	0.02	0.00	0.00	0.02
9t12t-18:2	0.02	0.02	0.02	0.02	0.02
9c13t-/8t12c-18:2	0.03	0.02	0.02	0.03	0.02
9c12t-18:2	0.10	0.09	0.11	0.11	0.09
9t12c-18:2	0.04	0.04	0.04	0.04	0.04
18:2n-6	57.29[a]	60.96[a]	68.96	70.57	72.08
20:0	0.11	0.05	0.02	0.02	0.03
9c12c15t-18:3	0.07	0.06	0.07	0.06	0.04
9c12t15c-18:3	0.03	0.02	0.02	0.02	0.01
11c-20:1/9t12c15c-18:3	0.19	0.19	0.26	0.23	0.24
18:3n-3	15.75[b]	12.11[b]	11.96	10.34	7.97
21:0	0.02	0.02	0.00	0.01	0.02
20:2n-6	0.03	0.03	0.05	0.06	0.05
22:0	0.03	0.03	0.00	0.00	0.01
23:0	0.01	0.01	0.00	0.00	0.00
24:0	0.01	0.00	0.00	0.00	0.00
ΣSFA[c]	9.40[b]	8.61[b]	3.68	3.42	3.87
Σ*cis*-MUFA[c]	16.92	17.64	14.75	15.03	15.51
Σ*trans*-MUFA	0.25	0.25	0.26	0.23	0.23
ΣPUFA[c]	73.07[a]	73.10[a]	80.98	80.96	80.10
Σ*trans*-PUFA	0.48	0.58	0.58	0.54	0.48
n-3/n-6 ratio	0.28	0.20	0.17	0.15	0.11

[a] Significant difference between mean values of heartnuts and walnuts at $P < 0.001$. [b] Significant difference between mean values of heartnuts and walnuts at $P < 0.01$. [c] Abbreviations: SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

Table 3. DPPH Radical Scavenging Power of Standards and Seeds of Different Varieties of Heartnuts and Walnuts

sample or standard	DPPH• EC_{50} (mg of antioxidant/mg of DPPH)[a]	% contribution by γ-tocopherol[b]	% contribution by total tocopherols[c]
α-tocopherol	0.23		
β-tocopherol	0.27		
γ-tocopherol	0.20		
δ-tocopherol	0.27		
Combe Persian walnut	508.57	65	75
Lake Persian walnut	954.86	96	99
Campbell CW1 heartnut	1106.29	89	91
Campbell CW3 heartnut	1342.86	85	86
Imshu heartnut	920.00	84	90

[a] Milligrams of standard or seed sample required to reduce absorbance of 1 mg of the initial DPPH• concentration by 50%. Values are means of duplicates. [b] % contribution = [(mg EC_{50} of γ-tocopherol in nut/mg EC_{50} of standard γ-tocopherol) × 100]. [c] % contribution = [(mg EC_{50} of total tocopherol in nut/mg EC_{50} of average of all standards of tocopherol homologues) × 100].

had significantly higher levels ($P < 0.001$) of 18:2n-6 (69.77 ± 1.56%) and a significantly lower content ($P < 0.01$) of SFAs (3.55 ± 0.23%; 16:0 and 18:0) compared to the two walnut varieties. In general, the walnut varieties had higher levels of 18:3n-3 than the heartnuts, and the latter also showed wide differences among the three varieties ranging from 8 to 12%. The n-3/n-6 ratio ranged from 0.28 in the Combe Persian walnut variety to 0.11 in the Imshu heartnut. Increased consumption of n-3 fatty acids has been recommended in the North American diet (*32*). The amounts of fatty acids in walnuts found in our study were similar to values reported in the literature (*2*−*6*). To the best of our knowledge, this is the first report on the fatty acid composition of heartnut.

Antioxidant Activities. As mentioned above, walnuts are an excellent source of tocopherols and essential fatty acids. However, the high content of unsaturated fatty acids makes the walnut lipids prone to oxidation, which may lead to unpleasant odors and flavors (*6*). The antioxidative properties of tocopherols have been shown to prevent the oxidation of PUFAs in walnut and stabilize the free fatty acids in different oils including walnuts (*6*, *11*−*14*). Although lipids extracted from the Persian walnut have been examined for their antioxidant activities using the DPPH• method (*22*), no report has been published on the antioxidant activity of lipids from the heartnut. The DPPH• method measured the disappearance of DPPH•, which is a useful index to estimate total free radical scavenging (RSC) capacity in a given medium (*9*, *10*, *22*). Methanol and ethanol have been most commonly used in the DPPH• model for different foods and extracts, and occasionally ethyl acetate has been used (*9*, *10*, *22*, *33*). However, extracts rich in lipid content, such as the oil extracted from walnuts, often leave a precipitate in single-solvent solutions, such as methanol, that interferes with the spectrometric measurements and consequently affects the accuracy of the assay. Different solvent mixtures were examined to improve the solubility of oils. A mixture of hexane/ethyl acetate/methanol in the ratio of 1:1:2 (by volume) was found to be the best solvent for walnut and heartnut extracts in the DPPH• assay. The stability and molar absorptivity of DPPH• were not affected by using the mixed solvent system compared to methanol by itself. To the best of our knowledge, a mixture of solvent has not been previously reported for the DPPH• model.

The RSC of α-, β-, γ-, and δ-tocopherol standards and the nut oils were expressed as EC_{50} in milligrams of antioxidant per milligrams of DPPH• and are shown in **Table 3**. The antioxidant capacity of the hexane extracts of heartnut and walnut ranged widely from 508.57 to 1342.86 mg of antioxidant/mg of DPPH•. Significant differences were found among α-, β-, γ-, and δ-tocopherol ($P < 0.01$), and the RSC of the homologues followed the order $\gamma > \alpha > \beta \approx \delta$ (**Table 3**). The EC_{50} of α-tocopherol in our study agreed well with the value reported by Sánchez-Moreno et al. (*24*). Although α-tocopherol has been found to be more active as an antioxidant in vivo (*18*, *19*), several papers have indicated that γ-tocopherol is a stronger antioxidant in some model systems than α-tocopherol (*11*, *20*, *21*). This may be due to differences in the mechanisms involved in the in vivo and in vitro antioxidative processes.

In general, Persian walnuts had a higher average antioxidant power than the heartnuts, although not statistically significant. However, among the different varieties of heartnut the Imshu heartnut showed stronger antioxidant power than the Lake Persian walnut variety (**Table 3**). The highest RSC was observed for the Combe Persian walnut, followed by the Imshu heartnut variety and the least in the Campbell heartnut varieties. The EC_{50} values of all nuts (both walnut and heartnut) obtained from the DPPH• assay correlated significantly ($P < 0.001$) and inversely with the total tocopherol contents ($r^2 = 0.97$), indicating that tocopherols are the principal components responsible for the antioxidant effect. Espín et al. (*22*) also suggested that the RSC of the lipid fraction of the different plant oils including walnut oil was mainly due to the content of all the tocopherols. The EC_{50} values were also highly correlated with the γ-tocopherol content ($r^2 = 0.97$), the predominant vitamin E homologue in walnut and heartnut oils. Therefore, γ-tocopherol is considered to be the main contributor to the total antioxidant activity of walnut and heartnut oils. The tocopherols contributed 75−99% to the total antioxidant activity of all nut oils in the DPPH• assay, and γ-tocopherol alone contributed 65−96% (**Table 3**). Contributions of tocopherols were obtained by calculating the γ-tocopherol or total tocopherol content in the amount of EC_{50} of the nut seed and then dividing by the EC_{50} value of γ-tocopherol or average tocopherols and, finally, presented in percentage (**Table 3**).

Table 4. Antioxidant Activities of Walnuts and Heartnuts Measured by the PCL–ACL Assay (Average of Duplicate Samples)

equivalent units of standard (Trolox) (nmol/nmol of tocopherol)				equivalent units of standard (Trolox) (nmol/mg of nuts)				
α-tocopherol	β-tocopherol	γ-tocopherol	δ-tocopherol	Combe Persian walnut	Lake Persian walnut	Campbell CW1 heartnut	Campbell CW3 heartnut	Imshu heartnut
1.27	1.10	1.09	1.47	2.10	1.06	0.94	0.95	1.36
% contribution by γ-tocopherol[a]				35	52	47	36	37
% contribution by total tocopherols[b]				41	58	49	37	41

[a] % contribution = [(nmol Trolox equivalent units of γ-tocopherol in nut/nmol Trolox equivalent units of standard γ-tocopherol) × 100]. [b] % contribution = [(nmol Trolox equivalent units of average total tocopherols in nut/nmol average Trolox equivalent units of all standards of tocopherol homologues) × 100].

The antioxidant activity of walnut and heartnut oils was further evaluated using the PCL−ACL method. In this assay, a sample was prepared so that the lag phase of the solution fell within the linear range of the standard curve, which was generated by measuring the PCL of Trolox at 0, 0.5, 1, 2, and 3 nmol. The PCL−ACL antioxidant activities of the nut oils and α-, β-, γ-, δ-tocopherols are shown in **Table 4**. The antioxidant activities of the tocopherols were in the order $\delta > \alpha > \beta \approx \gamma$; however, significant differences were found among the different homologues ($P = 0.01$). Even though the PCL−ACL activities between the Persian walnut and heartnut were not significant ($P = 0.48$), the order of the antioxidant activities of the individual nut oils was the same as that observed using the DPPH• assay, that is, Combe Persian walnut > Imshu heartnut > Lake Persian walnut > Campbell CW1 heartnut > Campbell CW3 heartnut (**Table 4**). The antioxidant activity in the PCL−ACL experiment also showed good correlation with the total and γ-tocopherol contents with correlation coefficients of $r^2 = 0.81$ and 0.77, respectively. Tocopherols contributed 41−58% to the total antioxidant activity of all nut oils in the PCL−ACL assay, and γ-tocopherol alone contributed 35−52% (**Table 4**). This suggests that although tocopherols, particularly γ-tocopherol, are the most important contributors to the total antioxidant activity of walnut and heartnut oils, other compounds may have also played a role in this assay, which is based on the scavenging activity against the superoxide radical $^{\bullet}O_2^-$.

Additional evidence was provided by measuring the antioxidant activities of the major fatty acids in the oils, that is, 18:3n-3, 18:2n-6, and 18:1n-9, using the same two model systems. None of these unsaturated fatty acids in their free form possessed antioxidant activities. The antioxidant activity from the tocopherols, particularly γ-tocopherol, together with the strong antioxidant activity of the ellagic acid derivatives found in the methanolic extracts of heartnuts and walnuts (*23*) contributes to the total antioxidative capacity in these nuts. These antioxidative phytochemicals in heartnuts and walnuts can provide good health benefits to the consumer.

Conclusion. Results obtained in the present study clearly indicate differences in the total oil content, fatty acid and tocopherol compositions, and the antioxidant activities among the different Persian walnut and heartnut varieties and between the two types of nuts. Although the three heartnut varieties on average contained lower tocopherol contents and showed lower antioxidant activities using the two assay systems than the two Persian walnut varieties, some heartnut varieties, such as the Imshu variety, can be better than certain walnuts. In addition, although the n-6/n-3 ratio of the two essential fatty acids 18:3n-3 and 18:2n-6 favored the Persian walnut from a nutritional point of view (higher 18:3n-3/18:2n-6 ratio), heartnuts had the benefits of a significantly lower content of SFA compared to walnuts. Considering recent developments in heartnut breeding and growing, better quality heartnuts with improved nutritional value are just a matter of time.

ABBREVIATIONS USED

FAME, fatty acid methyl ester; GC, gas chromatography; HPLC, high-performance liquid chromatography; PCL, photochemiluminescence; PUFA, polyunsaturated fatty acid; RSC, free radical scavenger; SFA, saturated fatty acids.

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