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Antioxidant and Antiradical Activities in Extracts of Hazelnut Kernel (*Corylus avellana* L.) and Hazelnut Green Leafy Cover

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Phenolic compounds in the aqueous systems were extracted, from hazelnut kernel (HK) and hazelnut green leafy cover (HGLC), with 80% (v/v) ethanol (HKe and HGLCe) or 80% (v/v) acetone (HKa and HGLCa). The extracts were examined for their phenolic and condensed tannin contents and phenolic acid profiles (free and esterified fractions) as well as antioxidant and antiradical activities by total antioxidant activity (TAA), antioxidant activity in a β-carotene-linoleate model system, scavenging of DPPH (2,2-diphenyl-1-picrylhydrazyl) radical, and reducing power. Significant differences ($p < 0.05$) in the contents of total phenolics, condensed tannins, and TAA existed among the extracts that were examined. HGLCa extract had the highest content of total phenolics (201 mg of catechin equivalents/g of extract), condensed tannins (542 mg of catechin equivalents/g of extract), and TAA (1.29 mmol of Trolox equivalents/g of extract) followed by HGLCe, HKa, and HKe extracts, respectively. Five phenolic acids (gallic acid, caffeic acid, *p*-coumaric acid, ferulic acid, and sinapic acid) were tentatively identified and quantified, among which gallic acid was the most abundant in both free and esterified forms. The order of antioxidant activity in a β-carotene−linoleate model system, the scavenging effect on DPPH radical, and the reducing power in all extracts were in the following order: HGLCa > HGLCe > HKa > HKe. These results suggest that both 80% ethanol and acetone are capable of extracting phenolics, but 80% acetone was a more effective solvent for the extraction process. HGLC exhibited stronger antioxidant and antiradical activities than HK itself in both extracts and could potentially be considered as an inexpensive source of natural antioxidants.

KEYWORDS: Hazelnut kernel; hazelnut green leafy cover; total phenolics; condensed tannins; phenolic acids; antioxidant activity; antiradical activity; DPPH; free-radical scavenging

INTRODUCTION

Recent recognition of nuts as "heart-healthy" foods by the Food and Drug Administration (FDA) has provided a major boost to the image of nuts, including hazelnut. Turkey is the world's largest hazelnut producer, contributing approximately 70% to the total global production, followed by Italy (12%), the United States (6%), and Spain (2%) (*1*). Hazelnut green leafy cover (HGLC) is a byproduct of hazelnut shell processing (**Figure 1**), which is removed from hazelnut soon after harvesting with no current commercial value. However, it is occasionally used as fertilizer for the hazelnut trees upon composting.

Plant-derived products (edible and nonedible) contain a wide range of phytochemicals and phenolic compounds (such as phenolic acids, flavonoids, tannins, and lignans, among others) that possess substantial antioxidant and antiradical activities (*2*), anticarcinogenic and antimutagenic effects (*3*), and antiproliferative potential (*4*). These phenolics provide protection against harmful effects of free radicals and are known to reduce the risk of certain types of cancer, coronary heart disease (CHD), cardiovascular disease (CVD), stroke, atherosclerosis, osteoporosis, inflammation, and other neurodegenerative diseases associated with oxidative stress (*2*, *3*, *5*−*9*). Moreover, plant phenolic compounds are known to have multifunctional properties, such as acting as reducing agents (free-radical terminator/scavenger), metal chelators, singlet oxygen, and free-radical quenchers (*10*).

Evaluation of antioxidant and antiradical activities of fruits, vegetables, and other plant products cannot be carried out accurately by any single universal method or extraction solvent system due to the complex nature of phytochemicals present (*2*, *11*, *12*). Numerous methods (*2*, *12*−*17*) and different solvent systems (*18*−*20*) have been used to evaluate and estimate the

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Figure 1. Hazelnut kernel and hazelnut green leafy cover.

antioxidant and antiradical potential of foods and dietary supplements. To accurately evaluate the potential of antioxidants and antiradicals in foods, at least two different methods and solvent systems should be employed.

The antioxidant activities of nut hulls from almond (*21*−*23*), pistachio (*24*), and peanut (*25*, *26*) have been reported. These studies have acknowledged that nut hulls are rich sources of natural antioxidant and phenolic compounds, which may render beneficial biological activities. Although several publications dealing with antioxidant activity and phenolic constituents of hazelnut kernel (HK) (*19*, *27*, *28*) and its brown skin or testa (*29*) have appeared, no such information is available about HGLC. Extracts of natural antioxidants from inexpensive or processing byproducts could potentially be used as nutraceuticals, dietary supplements, and pharmaceuticals or cosmetic ingredients, among others. Thus, investigation of phenolic constituents and measurement of antioxidant and antiradical activities of HK and HGLC are of particular interest. The objectives of this study were to investigate the phenolic constituents and their antioxidant and antiradical activities in the aqueous ethanol and acetone extracts obtained from both HK and HGLC.

MATERIALS AND METHODS

Samples. The premium class natural Turkish Tombul hazelnuts (*Corylus avellana* L.) were harvested from the Giresun province of Turkey at the beginning of the harvest season of 2004. Briefly, HGLCs were removed by hand from hazelnuts soon after harvesting. Both unshelled hazelnuts and HGLCs were separately sun-dried simultaneously for 3 days at ∼20−25 °C. The sun-dried HGLCs were green/brownish in color. Both sun-dried samples were dispatched by DHL World Wide Express to the Food Research Center, University of Lincoln, and then kept in a dark room at 5 °C until they were analyzed. The unshelled hazelnuts were cracked prior to analysis to remove hazelnut hard shells. The remaining hazelnut kernels with skin (**Figure 1**) were used for the analysis.

Chemicals. All chemicals were obtained from Sigma-Aldrich Co. Ltd. (Dorset, U.K.), unless otherwise specified.

Preparation of Defatted Samples. All samples that were tested (HK and HGLC) were ground separately in a coffee mill (Moulinex Corp., Toronto, ON) for 3 min and then defatted by blending with hexane (1:10, w/v, 3 × 5 min) in a Waring blender (model 33BL73, Waring Products Division, Dynamic Corp. of America, New Hartford, CT) at ambient temperature. Defatted samples were subsequently air-dried for 12 h and stored in vacuum-packaged polyethylene pouches at −20 °C until they were used for further analysis.

Preparation of Extracts. Defatted HK and HGLC were transferred to dark-colored flasks, extracted using two different solvent systems [100 mL of an 80:20 (v/v) ethanol/water mixture (HKe and HGLCe) and an 80:20 (v/v) acetone/water mixture (HKa and HGLCa)] at a solid:solvent ratio of 1:10 (w/v), and subsequently placed in a shaking Magni Whirl constant-temperature water bath (model MSG-1122A-1, Blue M Electric Co., Blue Island, IL) at 50 °C for 30 min (*18*). The resulting slurries were centrifuged at 4000*g* for 15 min, and the supernatant was collected. The residue was re-extracted twice under the same conditions, and supernatants were combined. Then, the solvent was removed from the combined supernatants under vacuum at 40 °C (model Büchi Rotavapor R-114, Büchi Labortechnik AG, Flawil, Switzerland), and the remaining water in the concentrated extract was removed by lyophilization for 72 h at −48 °C and 0.046 mbar (Freezone 6, model 77530, Labconco Co., Kansas City, MO). Finally, the prepared crude extracts were stored at −20 °C in vacuum-sealed pouches (in the dark) until they were used for further analysis.

UV Spectra. Ultraviolet (UV) spectra of the extracts were recorded using a Beckman DU 7500 diode array spectrophotometer (Beckman Instruments Inc., Fullerton, CA), as reported by Amarowicz et al. (*30*).

Determination of the Total Phenolic Content. The content of total phenolics in extracts was determined according to a modified version of the procedure described by Singleton and Rossi (*31*) using the Folin and Ciocalteu's phenol reagent. The content of total phenolics was determined from a standard curve using catechin as a standard and expressed as milligrams of catechin equivalents (CE) per gram of extract.

Determination of the Condensed Tannin Content. The content of condensed tannins in extracts was determined according to a modified version of the vanillin assay described by Price et al. (*32*) and expressed as milligrams of CE per gram of extract.

Determination of Total Antioxidant Activity (TAA). The TAA in extracts was determined according to the Trolox equivalent antioxidant capacity (TEAC) assay described by van den Berg et al. (*33*) and Liyana-Pathirana and Shahidi (*34*). The TAA was expressed as millimoles of Trolox equivalents (TE) per gram of extract.

Extraction, Hydrolysis, Identification, and Quantification of Phenolic Acids. Phenolic acids in extracts of HK and HGLC were assessed according to the high-performance liquid chromatographic (HPLC) method of Amarowicz and Weidner (*35*), with slight modifications. An aqueous suspension of the extract (100 mg in 10 mL) was adjusted to pH 2 (using 6 M HCl), and free phenolic acids were extracted five times, each into 10 mL of diethyl ether, using a separatory funnel. The combined extract was then evaporated to dryness under vacuum at room temperature. The aqueous solution was neutralized

and then lyophilized. The residue was dissolved in 10 mL of 2 M NaOH and hydrolyzed for 4 h at room temperature under a stream of nitrogen. After acidification to pH 2 (using 6 M HCl), phenolic acids liberated from esters were extracted five times, each into 15 mL of diethyl ether, using a separatory funnel. Ether from the combined extracts was then evaporated to dryness. The dry residues of free and esterified phenolic acids were dissolved separately in 2 mL of methanol and finally filtered through a GELMAN Acrodisc LC13 PVDV 0.45 μm pore size syringe filter (PALL Life Sciences, Ann Arbor, MI) for the HPLC analysis.

Phenolic acids in each fraction were analyzed using a Shimadzu HPLC system (Shimadzu Corp., Kyoto, Japan) consisting of a LC-10AD pump, a SCTL 10A system controller, and a SPD-M 10A photodiode array detector (DAD). Twenty microliters of the sample extracts was automatically injected into a prepacked LiChrospher 100 RP-18 column (250 mm × 4 mm × 4 mm inner diameter, 5 μm particles, Merck, Darmstadt, Germany) at room temperature. Isocratic elution (filtered through a 0.45 μm Millipore filter prior to use) was employed with a mobile phase consisting of HPLC-grade water, acetonitrile, and acetic acid (88:10:2, v/v/v) at a flow rate of 1 mL/min. The wavelengths of the DAD were set at 280 and 320 nm for monitoring phenolic acids. Tentatively identified phenolic acids were quantified on the basis of their peak areas and comparison with a calibration curve obtained with the corresponding standards (gallic acid, caffeic acid, *p*-coumaric acid, *o*-coumaric acid, *m*-coumaric acid, ferulic acid, sinapic acid, vanillic acid, protocatechuic acid, syringic acid, gentisic acid, and salicylic acid). The results from free and esterified hydrolysates were calculated to represent the total phenolic acid content. Phenolic acids are expressed as micrograms of phenolic per gram of extract.

β-Carotene−Linoleate Model System. The antioxidant activity of the extracts was evaluated in a β-carotene−linoleate model system (*36*). Briefly, methanolic solutions (0.2 mL) containing 2 mg of the extract were added to a series of tubes containing 5 mL of previously prepared emulsion of linoleate and β-carotene stabilized with Tween 40, prepared as described by Wanasundara et al. (*37*). A reference compound was carried out using 0.5 mg of BHA (butylated hydroxyanisole). Immediately after the addition of the emulsion to the tubes, the zero-time absorbance at 470 nm was recorded using a Beckman spectrophotometer (Beckman Instruments Inc.). Absorbance values were recorded over a 120 min period at 15 min intervals the samples were kept in a water bath at 50 °C.

Determination of DPPH Radical Scavenging Activity. The method described by Amarowicz et al. (*38*) was used to assess DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity of the extracts. Briefly, an aliquot (0.1 mL) of methanolic solution containing 0.02−0.1 mg of extracts was mixed with 2 mL of methanol, and then a methanolic solution of DPPH radical (1 mM, 0.250 mL) was added. The mixture was vortexed for 15 s and then left to stand at room temperature for 30 min. Finally, the absorbance of the resulting solution was read spectrophotometrically (Beckman Instruments Inc.) at 517 nm. Results were expressed as the content of extract (milligram per assay) versus absorbance at 517 nm. From the graph, IC_{50} was read as milligrams of extract required to scavenge the initial DPPH radical by 50%.

Determination of the Reducing Power. The reducing power of the extracts was determined as described by Amarowicz et al. (*16*) and Oyaizu (*39*). Briefly, a suspension of each extract (0.02−0.1 mg) in 1 mL of distilled water to which were added 2.5 mL of a 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of a 1% (w/v) solution of potassium ferricyanide was used for the determination of reducing power. After incubation in a water bath at 50 °C for 20 min, 2.5 mL of a 10% (w/v) trichloroacetic acid solution was added, and the mixture was then centrifuged at 1750*g* for 10 min. Following this, 2.5 mL of the supernatant was combined with 2.5 mL of distilled water and 0.5 mL of a 0.1% (w/v) solution of ferric chloride. Finally, the absorbance of the reaction mixture was recorded spectrophotometrically (Beckman Instruments Inc.) at 700 nm; the increased absorbance of the reaction mixture indicates a greater reducing power. Results were expressed as content of extract (milligram per assay) versus absorbance at 700 nm.

Table 1. Contents of Phenolics, Condensed Tannins, TAA, and IC_{50} in Extracts of HK and HGLC[a]

extracts[b]	total phenolics[c]	condensed tannins[d]	TAA[e]	IC_{50}[f]
HKe	23.2 ± 0.5g	40.5 ± 0.8g	0.20 ± 0.01g	0.504 ± 0.015g
HKa	103 ± 1.0h	320 ± 6.3h	0.62 ± 0.03h	0.098 ± 0.003h
HGLCe	156 ± 1.0i	385 ± 2.1i	1.14 ± 0.05i	0.074 ± 0.002i
HGLCa	201 ± 2.0j	542 ± 2.1j	1.29 ± 0.03j	0.065 ± 0.002j

[a] Data are expressed as means ± the standard deviation ($n = 3$) on an extract. Means ± standard deviations followed by the same letter, within a column, are not significantly different ($p > 0.05$). [b] HKe, hazelnut kernel extracted with ethanol; HKa, hazelnut kernel extracted with acetone; HGLCe, hazelnut green leafy cover extracted with ethanol; and HGLCa, hazelnut green leafy cover extracted with acetone. [c] Total phenolics, expressed as milligrams of CE per gram of extract. [d] Condensed tannins, expressed as milligrams of CE per gram of extract. [e] TAA, expressed as millimoles of TE per gram of extract. [f] IC_{50}, expressed as milligrams of extract required to scavenge the initial DPPH radical by 50%.

Statistical Analysis. Results were expressed as means ± the standard deviation (SD) ($n = 3$) on an extract. The statistical significance (*t*-test, two-sample equal variance, using a two-tailed distribution) and coefficient of determinations (r^2) were determined using Microsoft Excel statistical software and chart (add trendline), respectively (Microsoft Office Excel 2003, Microsoft Corp., Redmond, WA). Differences ($p < 0.05$) were considered to be significant.

RESULTS AND DISCUSSION

Total Phenolics, Condensed Tannins, and TAA. Phenolic compounds in the aqueous systems were extracted from HK and HGLC, using 80% (v/v) ethanol (HKe and HGLCe) or 80% (v/v) acetone (HKa and HGLCa). Significant differences ($p < 0.05$) in total phenolics, condensed tannins, and TAA existed among the extracts. HGLCa extract had the highest content of total phenolics (201 mg of CE/g of extract), condensed tannins (542 mg of CE/g of extract), and TAA (1.29 mmol of TE/g of extract), followed by HGLCe, HKa, and HKe extracts, respectively (**Table 1**). Correlation existed between the total phenolic contents of the extracts and their TAA ($r^2 = 0.97$).

Total Phenolics. The total phenolic content of HK and HGLC extracts ranged from 23.2 to 103 mg of CE/g of extract and from 156 to 201 mg of CE/g of extract, respectively (**Table 1**). Extracts obtained from 80% ethanol were characterized as having a lower content of total phenolics compared to those of extracts obtained from 80% acetone. Siriwardhana and Shahidi (*15*) found that the 80% (v/v) ethanol extracts of whole almond seed (kernel) and its green shell (leafy) cover had a total phenolic content of 8.1 and 71.1 mg of CE/g of extract, respectively. A higher concentration of total phenolics in almond seed using 80% (v/v) acetone extract (16.1 mg of CE/g of extract) was obtained by Amarowicz et al. (*40*). Compared to cited studies, a content of total phenolics in HK extracts, much higher than that of almond seed extracts, was detected. Recently, Madhujith and Shahidi (*41*) studied the antioxidant potential of four bean varieties and their hull fractions, which were extracted with 80% (v/v) acetone. The total phenolic content of whole seed and bean hull extracts ranged from 4.9 to 93.6 mg of CE/g of extract and from 6.7 to 270 mg of CE/g of extract, respectively. Subsequently, Shahidi et al. (*42*) studied the antioxidant activity of white and black sesame seeds and their hull fractions, which were extracted with 80% (v/v) ethanol. The total phenolic contents of whole black sesame seeds and hull extracts were 29.9 and 146.6 mg of CE/g of extract, respectively. The corresponding values for white sesame were 10.6 and 29.7 mg of CE/g of extract, respectively. Both HK and HGLC were

Table 2. Contents of Free and Esterified Phenolic Acids (micrograms per gram) in Extracts of HK and HGLC[a]

	free phenolic acids					esterified phenolic acids				
extracts[b]	gallic	caffeic	*p*-coumaric	ferulic	sinapic	gallic	caffeic	*p*-coumaric	ferulic	sinapic
HKe	nd[c]	nd	nd	nd	nd	158 ± 5d	nd	13 ± 1d	nd	39 ± 2d
HKa	nd	nd	5 ± 1d	nd	nd	204 ± 6e	nd	21 ± 1e	nd	52 ± 2e
HGLCe	253 ± 25d	nd	38 ± 5e	nd	nd	1244 ± 40f	376 ± 8d	180 ± 12f	55 ± 2d	22 ± 1f
HGLCa	269 ± 27e	nd	41 ± 5e	nd	nd	1450 ± 44g	352 ± 8e	202 ± 14f	58 ± 3d	18 ± 1g

[a] Data are expressed as means ± the standard deviation ($n = 3$) on an extract. Means ± standard deviations followed by the same letter, within a column, are not significantly different ($p > 0.05$). [b] HKe, hazelnut kernel extracted with ethanol; HKa, hazelnut kernel extracted with acetone; HGLCe, hazelnut green leafy cover extracted with ethanol; and HGLCa, hazelnut green leafy cover extracted with acetone. [c] Not detected.

shown to be good sources of phenolics compared with those of almond, bean, and sesame seeds (*40*−*42*).

Condensed Tannins. The content of condensed tannins, expressed as milligrams of CE per gram of extract, varied quite markedly, from a low of 40.5 for HKe to a high of 542 for HGLCa (**Table 1**). Extracts from HGLCa and HGLCe contained larger amounts of condensed tannins than extracts obtained from HKa and HKe. In addition, acetone extract (80%) was a more effective solvent for the extraction of condensed tannins that ethanol extract (80%). According to Naczk et al. (*43*), a higher level of crude tannins was detected in wild blueberry leaves extracted with acetone [70% (v/v)] compared with that extracted with ethanol [95% (v/v)]. The reason acetone extract is more effective than ethanol extract is that tannins are relatively high-molecular weight compounds and the polarity of ethanol is too low for total extraction of these polar compounds from plant sources. Acetone extract [80% (v/v)] provides the most complete extraction of phenolic compounds from lentil seeds, especially flavonols and condensed tannins (*18*), and this is expected to extend to other high-tannin plant material.

Tannins, partly responsible for the astringent taste as well as the brown color, are water-soluble naturally occurring complex polyphenols that are present in many plant foods, including nuts, their skins, and hulls (*2*, *44*). According to Venkatachalam et al. (*45*), total tannins in nine commercially important edible tree nuts (hazelnut, almond, Brazil nut, cashew, macadamia, pecan, pine nut, pistachio, and walnut) varied from 0.01 to 0.88% on an "as is" weight basis, expressed as CE by the vanillin assay. The health aspect of tannins has been reviewed well by Chung et al. (*44*). Hagerman et al. (*46*) reported that tannins were 15−30 times more effective in quenching peroxyl radicals than simple phenolics and Trolox. Therefore, tannins should be considered as potentially important biological antioxidants.

TAA. The total antioxidant activities of HKe and HKa extracts were lower than those of HGLCe and HGLCa extracts (**Table 1**). The data indicated that TAA values of HK and HGLC extracts at the same extract concentration were in the following order: HGLCa > HGLCe > HKa > HKe. Moreover, TAA values of HGLCe and HGLCa extracts were 5.7- and 2.1-fold greater than those of their corresponding HKe and HKa extracts, respectively. The contents of total phenolic and condensed tannin extracts were closer; hence, at a given extract concentration, HGLC extracts would be more antioxidative than HK extracts. The TAA of HKa was 2.6- and 2.1-fold higher than those of crude acetone extracts [80% (v/v)] of almond seed (*40*) and pea (*30*), respectively.

Wu et al. (*28*) measured the lipophilic and hydrophilic antioxidant capacities of common foods in the U.S. markets and found that hazelnut had the third highest value (96.45 μmol of TE/g on an as is basis) of total antioxidant capacity (by combining lipophilic and hydrophilic) among 10 nut samples that were examined, with pecan and walnut having the highest. Due to different expressions used in this study (on an extract basis) and those in the cited study (on an as is weight basis), no direct comparison could be made on a quantitative basis.

Phenolic Acids. The contents of free and esterified phenolic acids in extracts of HK and HGLC are listed in **Table 2**. A total of five phenolic acids were tentatively identified, one of which was a hydroxylated derivative of benzoic acid (gallic acid) and four of which were cinnamic acid derivatives (caffeic acid, *p*-coumaric acid, ferulic acid, and sinapic acid). In addition, there were several unknown compounds in both free and esterified phenolic acids. Among the identified phenolic acids, gallic acid was most abundant in both free and esterified forms, and all five phenolic acids were present only in the HGLCe and HGLCa extracts. In contrast, no phenolic acid was detected in HKe extract. Caffeic acid, ferulic acid, and sinapic acid were not detected in free phenolic acids. The order of total phenolic acid concentration (both free and esterified) was as follows: HGLCa > HGLCe > HKa > HKe. The esterified phenolics were the predominant form of phenolic acids and comprised 100, 98.2, 86.6, and 87% of the total soluble phenolic acids present in HKe, HKa, HGLCe, and HGLCa extracts, respectively.

Senter et al. (*29*) compared phenolic acids of nine edible tree nuts produced in the United States. The extracts of nut samples showed great diversity in the phenolics that were present. Qualitative and quantitative differences existed among nut samples in the phenolic acids present, with gallic acid being predominant except in pine nut, almond, and hazelnut (filbert). A total of eight phenolic acids were isolated and identified among nine nuts (*p*-hydroxybenzoic acid, *p*-hydroxyphenylacetic acid, vanillic acid, protocatechuic acid, syringic acid, gallic acid, caffeic acid, and ferulic acid). Protocatechuic acid has been reported to be the predominant phenolic acid in testa (brown skin) of hazelnut with a concentration of 0.36 μg/g. This phenolic acid was not detected in our study. This could be due to either different extraction solvents used between the two studies (we used 80% ethanol or acetone, whereas they used 0.1 M HCl in methanol) or the possibility that amounts of brown skin present in HK might have not been sufficient for detection of protocatechuic acid, since brown skin represents a very minor proportion of the whole HK. It has been reported that caffeic acid, sinapic acid, ferulic acid, and *p*-coumaric acid are more antioxidative than protocatechuic acid, syringic acid, vanillic acid, and protocatechuic acid (*47*).

Yurttas et al. (*27*) isolated and tentatively identified six phenolic aglycones in Turkish and American hazelnut extracts; these were gallic acid, *p*-hydroxybenzoic acid, epicatechin and/or caffeic acid, sinapic acid, and quercetin. However, the variety of hazelnut and extraction solvents used in this study was different from that used by Yurttas et al. (*27*), who used 300 mL of a methanol/water mixture (2:1, v/v) for extraction solvents. Variety and extraction exerted a great influence on the concentration and variability of phenolic acids present.

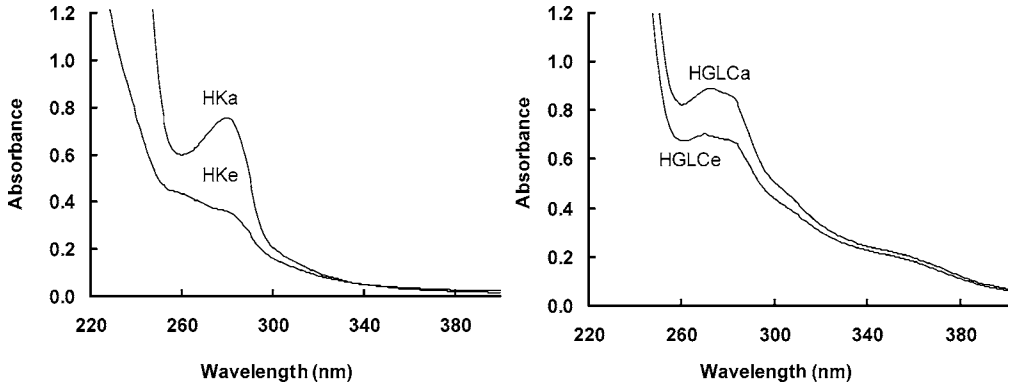

Figure 2. UV spectra in extracts of HK and HGLC.

Ten phenolics (including triterpenoids) in almond hulls (*21*, *22*, *48*, *49*) and four phenolic acids in almond seed (*40*) have been reported following base-assisted hydrolysis. Like hazelnut and almond kernels (seeds), some of other tree nut kernels and their processing byproducts have been reported to contain different patterns and levels of phenolic acids (*2*).

Ultraviolet Spectra. Figure 2 depicts UV spectra of phenolic compounds extracted from HK and HGLC. The UV spectra of extracts depended upon the extraction solvent that was employed. HKa extract exhibited maximum absorbance at 280 nm, whereas HKe extract showed no maximum absorption. With regard to HGLC, the UV spectra of both HGLCa and HGLCe extracts exhibited maximum absorbance at 274 and 272 nm, respectively. The UV spectra of the extracts showed that both acetone and ethanol were capable of extracting phenolics, but acetone was a more effective solvent for extracting hazelnut samples. This could be due to the presence of large amounts of condensed tannins that are more soluble in acetone than ethanol and might have brought about their better extraction and hence a higher absorbance value (**Table 1**). The excellent ability of acetone to extract phenolic compounds in this study is in agreement with published data for lentil seeds and may be due to better solubilization of condensed tannins in this solvent (*18*).

Antioxidant Activity in the β-Carotene−Linoleate Model System. To study the antioxidant efficacy of samples, the active components must be extracted effectively from the defatted samples. A number of solvent systems have been used successfully for extraction of antioxidants and bioactives from oilseeds (*19*, *20*, *40*, *50*) and plant species (*16*, *43*). Therefore, two solvent systems (80%, v/v, ethanol and 80%, v/v, acetone) were evaluated for their effectiveness in the preparation of HK and HGLC extracts. These solvent systems were chosen because they were less polar than pure water and might extract more hydrophobic compounds.

The antioxidant activity of both HK and HGLC, as measured by the β-carotene−linoleate model system, is presented in **Figure 3**. As oxidation progressed, the absorbance of β-carotene at 470 nm decreased and its yellow color faded. The reference compound, BHA, exhibited a more powerful antioxidant activity than all extracts that were examined. The antioxidant activity in this model system was in the following order: BHA > HGLCa > HGLCe > HKa > HKe > control. However, acetone extracts were more effective compared than the corresponding ethanol extracts; a higher level of condensed tannins, which are more soluble in acetone, may have brought about the stronger antioxidant activity observed for acetone extracts. A similar trend of UV spectra was also reported when wild blueberry leaves were extracted with 70% (v/v) acetone (*43*). More research is needed to assess the contribution of tannin and non-

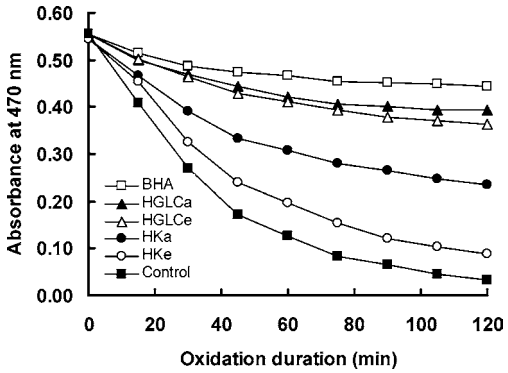

Figure 3. Antioxidant activity in extracts of HK and HGLC in a β-carotene–linoleate model system.

tannin fractions of phenolics to the observed antioxidant activity of the prepared extracts from hazelnut samples.

Yurttas et al. (*27*) studied antioxidant activity of nontocopherol phenolics of hazelnut and found that nonhydrolyzed extracts of hazelnut phenolics exhibited greater antioxidant activities than the corresponding hydrolyzed extracts. They suggested that some phenolics may have been destroyed during hydrolysis. Antioxidant activity in 80% (v/v) acetone extract of almond seed in the same model system showed a weaker antioxidant activity for almond seed extract (*40*) compared to that of 80% acetone extract of HK. The antioxidant activities of several plant-derived foods and their byproducts using the same model system with different extraction solvents have been published (*13*, *16*, *20*, *43*, *50*). The antioxidant activities of extracts determined herein exhibited similar patterns, with some exceptions.

Scavenging of DPPH Radicals. The DPPH radical scavenging assay is commonly employed in evaluating the ability of antioxidants to scavenge free radicals. The change in absorbance at 517 nm is used as a measure of the scavenging effect of a particular extract for DPPH radicals (*43*). The absorbance at 517 nm decreases as the reaction between antioxidant molecules and DPPH radical progresses. Hence, the more rapidly the absorbance decreases, the more potent the antioxidant activity of the extract in terms of its hydrogen atom-donating capacity (*16*). As shown in **Figure 4**, HGLCa and HGLCe extracts exhibited a greater scavenging activity than their HKa and HKe counterparts. No significant difference ($p > 0.05$) was noted between HGLCa and HGLCe extracts at all concentrations that were tested, except for 0.08 and 0.1 mg/assay at which HGLCa was a stronger ($p < 0.05$) scavenger than HGLCe. Similar trends were also observed for IC_{50} (**Table 1**). The scavenging potentials of the extracts that were tested (HGLCa, HGLCe, HKa, and HKe), reported as milligrams of extract required to reduce the

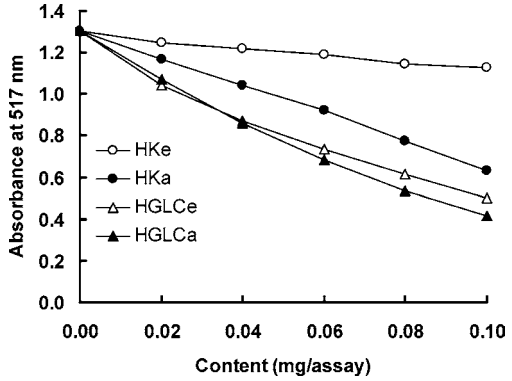

Figure 4. Scavenging activity in extracts of HK and HGLC on DPPH radical.

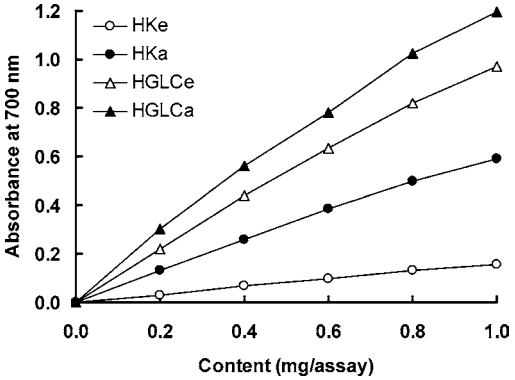

Figure 5. Reducing power in extracts of HK and HGLC.

concentration of DPPH radical in the reaction mixture by 50%, were 0.065, 0.074, 0.098, and 0.504, respectively.

Amarowicz et al. (*40*) studied the antioxidant activity of almond seed extract and its fractions (nontannin and tannin) and found that acetone extract [80% (v/v)] of crude almond seed exhibited a stronger DPPH radical scavenging activity than its non-tannin fraction but rendered a weaker activity than its tannin fraction. The antiradical activity against DPPH radical in the same acetone extract of hazelnut (HKa) was higher than that of crude acetone extract of almond seed and its non-tannin fraction but lower than that of its tannin fraction separated from crude almond extract. In addition, Siriwardhana and Shahidi (*15*) reported that DPPH radical scavenging activities at 100 and 200 ppm were 21 and 100% for whole almond seed and 35 and 100% for green shell (leafy) cover ethanol extracts [80% (v/v)], respectively. The results obtained for both almond seed (*15*, *40*) and HK in this study clearly show that HGLC extracts scavenged organic free radicals more effectively than seed/kernel extracts.

Reducing Power. Figure 5 depicts the reducing power of the extracts of HK and HGLC examined as a function of their concentrations. The coefficients of determination (r^2) obtained for HGLCa, HGLCe, HKa, and HKe were 0.994, 0.996, 0.997, and 0.998, respectively. In this assay, the yellow color of the test solution changes to various shades of green and blue depending upon the reducing power of each extract. At the same dose, the reducing power of HGLCa extract was superior to that of any other extract that was investigated. The lower reducing power of HKe extract was due to a content of phenolic acids lower than those present in the extract of HGLCa (**Table 2**). Thus, phenolics present in extracts of HGLCa and HGLCe displayed a considerable reducing power, primarily due to their effect as electron donors and thereby suppressing radical chain reactions by converting free radicals to more stable products. Thus, reducing activity leads to termination of the radical chain reactions that may otherwise be very damaging (*51*).

The results obtained from the extracts of HGLC were similar to those reported for crude tannins of canola (*52*) and blueberry leaf extracts (*43*). The reducing powers of HK extracts (HKa and HKe) determined in this study were found to be higher than that of 80% (v/v) acetone extract of almond seed (*40*).

In summary, different assays used for examining antioxidant and antiradical activities of HK and HGLC extracts revealed that HGLC exhibited stronger antioxidant and antiradical activities than HK and could potentially be considered as an inexpensive source of natural antioxidants. Acetone was found to be an effective solvent for extraction of antioxidants from both HK and HGLC. Further research is required for qualitative and quantitative determination of free, esterified, glycoside, and ester-bound phenolic acids in HK and HGLC. Furthermore, it is also needed to evaluate antioxidant and antiradical activities of various phenolic fractions of these extracts as well as to isolate and identify the active components of HK and HGLC.

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LITERATURE CITED

(1) Food and Agriculture Organization. FAOSTAT data. http://faostat.fao.org/faostat (December 2005).
(2) Shahidi, F.; Naczk, M. *Phenolics in Food and Nutraceuticals*; CRC Press: Boca Raton, FL, 2004.
(3) Surh, Y.-J. Cancer chemoprevention with dietary phytochemicals. *Nat. Rev. Cancer* **2003**, *3*, 768−780.
(4) Yang, J.; Halim, L.; Liu, R. H. Antioxidant and antiproliferative activities of common nuts. Presented at the Institute of Food Technologists Annual Meeting and Food Expo, New Orleans, LA, July 16−20, 2005; Paper 35-5.
(5) Hertog, M. G. L.; Feskens, E. J. M.; Hollman, P. C. H.; Katan, M. B.; Kromhout, D. Dietary antioxidant flavonoids and risk of coronary heart disease: The Zutphen Elderly Study. *Lancet* **1993**, *342*, 1007−1011.
(6) Ness, A. R.; Powles, J. W. Fruit and vegetables, and cardiovascular disease: A review. *Int. J. Epidemiol.* **1997**, *26*, 1−13.
(7) Joseph, J. A.; Shukitt-Hale, B.; Denisova, N. A.; Bielinski, D.; Martin, A.; McEwen, J. J.; Bickford, P. C. Reversals of age-related declines in neuronal signal transduction, cognitive, and motor behavioral deficits with blueberry, spinach, or strawberry dietary supplementation. *J. Neurosci.* **1999**, *19*, 8114−8121.
(8) Mazza, G.; Fukumoto, L.; Delaquis, P.; Girard, B.; Ewert, B. Anthocyanins, phenolics, and color of Cabernet Franc, Merlot, and Pinot Noir wines from British Colombia. *J. Agric. Food Chem.* **1999**, *47*, 4009−4017.
(9) Watson, R. R. *Functional Foods & Nutraceuticals in Cancer Prevention*; Blackwell Publishing: Oxford, U.K., 2003.
(10) Pratt, D. E. Natural antioxidants from plant material. In *Phenolic Compounds in Food and their Effects on Health. (Volume II). Antioxidants and Cancer Prevention*; Huang, M.-T., Ho, C.-T., Lee, C., Eds.; ACS Symposium Series 507; American Chemical Society: Washington, DC, 1992; pp 54−71.
(11) Chu, Y.-H.; Chang, C.-L.; Hsu, H.-F. Flavonoid content of several vegetables and their antioxidant activity. *J. Sci. Food Agric.* **2000**, *80*, 561−566.
(12) Prior, R. L.; Wu, X.; Schaich, K. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *J. Agric. Food Chem.* **2005**, *53*, 4290−4302.
(13) Amarowicz, R.; Naczk, M.; Zadernowski, R.; Shahidi, F. Antioxidant activity of condensed tannins of beach pea, canola hulls, evening primrose, and faba bean. *J. Food Lipids* **2000**, *7*, 195−205.

(14) Niki, E. Antioxidant activity: Are we measuring it correctly? *Nutrition* **2002**, *18*, 524−525.
(15) Siriwardhana, S. S. K. W.; Shahidi, F. Antiradical activity of extracts of almond and its by-products. *J. Am. Oil Chem. Soc.* **2002**, *79*, 903−908.
(16) Amarowicz, R.; Pegg, R. B.; Rahimi-Moghaddam, P.; Barl, B.; Weil, J. A. Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. *Food Chem.* **2004**, *84*, 551−562.
(17) Decker, E. A.; Warner, K.; Richards, M. P.; Shahidi, F. Measuring antioxidant effectiveness in food. *J. Agric. Food Chem.* **2005**, *53*, 4303−4310.
(18) Amarowicz, R.; Piskuła, M.; Honke, J.; Rudnicka, B.; Troszyńska, A.; Kozłowska, H. Extraction of phenolic compounds from lentil (*Lens culinaris*) with various solvents. *Pol. J. Food Nutr. Sci.* **1995**, *45*, 53−62.
(19) Moure, A.; Franco, D.; Sineiro, J.; Domínguez, H.; Núñez, M. J.; Lema, J. M. Antioxidant activity of extracts from *Gevuina avellana* and *Rosa rubiginosa* defatted seeds. *Food Res. Int.* **2001**, *34*, 103−109.
(20) Shahidi, F.; Desilva, C.; Amarowicz, R. Antioxidant activity of extracts of defatted seeds of niger (*Guizotia abyssinica*). *J. Am. Oil Chem. Soc.* **2003**, *80*, 443−450.
(21) Sang, S.; Cheng, X.; Fu, H.-Y.; Shieh, D.-E.; Bai, N.; Lapsley, K.; Stark, R. E.; Rosen, R. T.; Ho, C.-T. New type of sesquiterpene lactone from almond hulls (*Prunus amygdalus* Batsch). *Tetrahedron Lett.* **2002**, *43*, 2547−2549.
(22) Takeoka, G. R.; Dao, L. T. Antioxidant constituents of almond [*Prunus dulcis* (Mill.) D.A. Webb] hulls. *J. Agric. Food Chem.* **2003**, *51*, 496−501.
(23) Pinelo, M.; Rubilar, M.; Sineiro, J.; Núñez, M. J. Extraction of antioxidant phenolics from almond hulls (*Prunus amygdalus*) and pine sawdust (*Pinus pinaster*). *Food Chem.* **2004**, *85*, 267−273.
(24) Goli, A. H.; Barzegar, M.; Sahari, M. A. Antioxidant activity and total phenolic compounds of pistachio (*Pistachia vera*) hull extracts. *Food Chem.* **2005**, *92*, 521−525.
(25) Yen, G.-C.; Duh, P.-D. Antioxidative properties of methanolic extracts from peanut hulls. *J. Am. Oil Chem. Soc.* **1993**, *70*, 383−386.
(26) Duh, P.-D.; Yen, G.-C. Changes in antioxidant activity and components of methanolic extracts of peanut hulls irradiated with ultraviolet light. *Food Chem.* **1995**, *54*, 127−131.
(27) Yurttas, H. C.; Schafer, H. W.; Warthesen, J. J. Antioxidant activity of nontocopherol hazelnut (*Corylus spp.)* phenolics. *J. Food Sci.* **2000**, *65*, 276−280.
(28) Wu, X.; Beecher, G. R.; Holden, J. M.; Haytowitz, D. B.; Gebhardt, S. E.; Prior, R. L. Lipophilic and hydrophilic antioxidant capacities of common foods in the United States. *J. Agric. Food Chem.* **2004**, *52*, 4026−4037.
(29) Senter, S. D.; Horvat, R. J.; Forbus, W. R. Comparative GLC-MS analysis of phenolic acids of selected tree nuts. *J. Food Sci.* **1983**, *48*, 798−799, 824.
(30) Amarowicz, R.; Troszyńska, A.; Baryłko-Pikielna, N.; Shahidi, F. Polyphenolic extracts from legume seeds: Correlations between total antioxidant activity, total phenolics content, tannins content and astringency. *J. Food Lipids* **2004**, *11*, 278−286.
(31) Singleton, V. L.; Rossi, J. A. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic.* **1965**, *16*, 144−158.
(32) Price, M. L.; Scoyoc, S. V.; Butler, L. G. A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. *J. Agric. Food Chem.* **1978**, *26*, 1214−1218.
(33) van den Berg, R.; Haenen, G. R. M. M.; van den Berg, H.; Bast, A. Applicability of an improved Trolox equivalent antioxidant capacity (TEAC) assay for evaluation of antioxidant capacity measurements of mixtures. *Food Chem.* **1999**, *66*, 511−517.
(34) Liyana-Pathirana, C. M.; Shahidi, F. Antioxidant activity of commercial soft and hard wheat (*Triticum aestivum* L.) as affected by gastric pH conditions. *J. Agric. Food Chem.* **2005**, *53*, 2433−2440.
(35) Amarowicz, R.; Weidner, S. Content of phenolic acids in rye caryopses determined using DAD-HPLC method. *Czech. J. Food Sci.* **2001**, *19*, 201−205.
(36) Miller, H. E. A simplified method for the evaluation of antioxidants. *J. Am. Oil Chem. Soc.* **1971**, *48*, 91.
(37) Wanasundara, U.; Amarowicz, R.; Shahidi, F. Isolation and identification of an antioxidative component in canola meal. *J. Agric. Food Chem.* **1994**, *42*, 1285−1290.
(38) Amarowicz, R.; Karamać, M.; Weidner, S.; Abe, S.; Shahidi, F. Antioxidant activity of wheat caryopses and embryos extracts. *J. Food Lipids* **2002**, *9*, 201−210.
(39) Oyaizu, M. Studies on products of browning reaction-antioxidative activities of products of browning reaction prepared from glucosamine. *Jpn. J. Nutr.* **1986**, *44*, 307−315.
(40) Amarowicz, R.; Troszyńska, A.; Shahidi, F. Antioxidant activity of almond seed extract and its fractions. *J. Food Lipids* **2005**, *12*, 344−358.
(41) Madhujith, T.; Shahidi, F. Antioxidant potential of pea beans (*Phaseolus vulgaris* L.). *J. Food Sci.* **2005**, *70*, S85−S90.
(42) Shahidi, F.; Liyana-Pathirana, C. M.; Wall, D. S. Antioxidant activity of white and black sesame seeds and their hull fractions. *Food Chem.* **2006** (in press).
(43) Naczk, M.; Amarowicz, R.; Zadernowski, R.; Pegg, R. B.; Shahidi, F. Antioxidant activity of crude phenolic extracts from wild blueberry leaves. *Pol. J. Food Nutr. Sci.* **2003**, *12/13*, 166−169.
(44) Chung, K.-T.; Wong, T. Y.; Wei, C.-I. Y.; Huang, Y.-W. Y.; Lin, Y. Y. Tannins and human health: A Review. *Crit. Rev. Food Sci. Nutr.* **1998**, *38*, 421−464.
(45) Venkatachalam, M.; Monaghan, E. K.; Kshirsagar, H. H.; Sathe, S. K. Chemical composition of edible tree nuts. Presented at the Institute of Food Technologists Annual Meeting and Food Expo, Las Vegas, NV, July 12−16, 2004; Paper 67C-22.
(46) Hagerman, A. E.; Riedl, K. M.; Alexander Jones, G.; Sovik, K. N.; Ritchard, N. T.; Hartzfeld, P. W.; Riechel, T. L. High molecular weight plant polyphenolics (tannins) as biological antioxidants. *J. Agric. Food Chem.* **1998**, *46*, 1887−1892.
(47) Cuvelier, M. E.; Richard, H.; Berset, C. Comparison of the antioxidant activity of some acid-phenols: Structure-activity relationship. *Biosci., Biotechnol., Biochem.* **1992**, *56*, 324−325.
(48) Sang, S.; Lapsley, K.; Rosen, R. T.; Ho, C.-T. New prenylated benzoic acid and other constituents from almond hulls (*Prunus amygdalus* Batsch). *J. Agric. Food Chem.* **2002**, *50*, 607−609.
(49) Takeoka, G.; Dao, L.; Teranishi, R.; Wong, R.; Flessa, S.; Harden, L.; Edwards, R. Identification of three triterpenoids in almond hulls. *J. Agric. Food Chem.* **2000**, *48*, 3437−3439.
(50) Karamać, M.; Amarowicz, R.; Weidner, S.; Abe, S.; Shahidi, F. Antioxidant activity of rye caryopses and embryos extracts. *Czech. J. Food Sci.* **2002**, *20*, 209−214.
(51) Yen, W. J.; Chen, B. H. Isolation of xanthophylls from Taiwanese orange peels and their effects on the oxidation stability of soybean oil. *Food Chem.* **1995**, *53*, 417−425.
(52) Amarowicz, R.; Naczk, M.; Shahidi, F. Antioxidant activity of crude tannins of canola and rapeseed hulls. *J. Am. Oil Chem. Soc.* **2000**, *77*, 957−961.

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