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Extraction, identification, and quantification of antioxidant phenolics from hazelnut (*Corylus avellana* L.) shells

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ABSTRACT

Hazelnut shells are the major byproduct of the hazelnut industry. The objectives of this study were to optimize the conditions for extracting phenolics and to identify and quantify the phenolics in hazelnut shells. Preliminary optimization showed that a high recovery of phenolics could be achieved with shell particle size less than 0.5 mm when extracted with acetone at 50 °C. Response surface experiments showed that a 10 g/l liquid to solid ratio, 58% acetone, and 12 h extraction time yielded the highest amount of phenolics. Twenty-seven phenolic compounds were identified in hazelnut shells by mass spectrometry. Coumaroylquinic acid, epicatechin gallate, quercetin, and six other phenolics were identified in hazelnut shells for the first time. The most abundant phenolics in hazelnut shells were catechin, epicatechin gallate, and gallic acid, as quantified by high performance liquid chromatography (HPLC). These results can be useful for the development of industrial extraction processes of natural antioxidants from hazelnut shells.

1. Introduction

Hazelnuts (*Corylus avellana* L.) originated in the Mediterranean region and are now an important commercial crop in many countries. World production of hazelnuts achieved 488,110 metric tons (kernel basis) in 2015 and the production has increased by 50% in the past decade (INC, 2009, 2016). Turkey is the largest hazelnut producer in the world, producing 70% of the world's total. The United States (U.S.A.) produced approximately 2% of the total (INC, 2016). In the U.S.A., 99% of hazelnuts are grown in the state of Oregon, and "Barcelona" is the dominant cultivar grown in the U.S.A. (Beyer, Grishina, Bardina, Grishin, & Sampson, 2002).

Hazelnut kernels are rich in unsaturated fatty acids, essential amino acids, dietary fibres, vitamins, and minerals (Köksal, Artik, Şimşek, & Güneş, 2006). Due to their nutritious quality and unique flavour, they are widely used in dairy, bakery, coffee, spreads, confectionery products, and salads (Ozdemir & Akinci, 2004). Only 10% of hazelnuts are purchased as in-shell nuts and 90% of hazelnuts are used for industrial purposes as shelled nuts (Stévigny, Rolle, Valentini, & Zeppa, 2007). Hazelnut shells represent more than 50% of the total nut weight and they are the major byproduct in hazelnut industry production (Caglar & Aydinli, 2009). Hazelnut shells are composed of about 30% hemicelluloses, 27% celluloses, and 43% lignin, so they are mainly utilized as a low-value heat source (Demirbaş, 1999). Conversions of hazelnut shells into useful chemicals, such as methanol (Güllü & Demirbaş, 2001), hemicellulosic sugars (Arslan, Takaç, & Eken-Saraçoğlu, 2012), reducing sugar (Uzuner & Cekmecelioglu, 2014), and furfural (Demirbas, 2006) have been reported. Recently some efforts have been made to utilize hazelnut shells as a low cost raw material for phenolic compound extraction (Contini, Baccelloni, Massantini, & Anelli, 2008; Shahidi, Alasalvar, & Liyana-Pathirana, 2007; Xu, Sismour, Parry, Hanna, & Li, 2012).

Phenolic compounds are the primary bioactive components in plants. They have a wide range of health benefits, mainly due to their antioxidant properties, such as reactive oxygen species scavenging and inhibition, electrophile scavenging and metal chelation (Randhir, Lin, & Shetty, 2004). Phenolic compounds also exhibit pharmacological properties, such as anti-carcinogenic, anti-inflammatory, and anti-mutagenic effects, and anti-proliferative potential (Kaliora, Kogiannou, Kefalas, Papassideri, & Kalogeropoulos, 2014). Currently, many synthetic antioxidants are being used to retard the oxidation process, particularly in food systems. However, application of synthetic antioxidants in food products are of concern and are strictly regulated, due

Abbreviations: ANOVA, analyses of variance; Cs, concentration of solvent; DAD, diode array detector; DPPH, 2,2-diphenyl-1-picryl hydrazyl; DRSC, DPPH radical-scavenging capacity; FRAP, ferric reducing antioxidant power; GAE, gallic acid equivalents; HPLC, high performance liquid chromatography; MRM, multiple reaction monitoring; MS/MS, tandem mass spectrometry; *m/z*, mass-to-charge ratio; Ps, particle size; S/L, solid to liquid ratio; T, extraction temperature; t, extraction time; TE, trolox equivalents; TPC, total phenolic content

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to potential health hazards (Park, Jung, Nam, Shahidi, & Kim, 2001). Consequently, the utilization of natural phenolic antioxidants, as alternatives, has raised considerable interest among food scientists, manufacturers and consumers.

Although hazelnut shells are rich in phenolic compounds, very little is known about the extraction and composition of phenolic compounds from hazelnut shells. The objectives of this research were to investigate the optimum conditions for extracting phenolic compounds, using response surface experiments, and to characterize the phenolic composition in hazelnut shell extracts.

2. Materials and methods

2.1. Raw materials

2.1.1. Preparation of hazelnut shell powders

"Lewis" cultivar hazelnuts were harvested and de-husked from Hazelnut Hill Farm in Corvallis, Oregon in the fall of 2012. "Lewis" is a hazelnut cultivar developed by Oregon State University in 1997. "Lewis" has a higher yield efficiency and a smaller tree size than "Barcelona" cultivar (Mehlenbacher, Azarenko, Smith, & McCluskey, 2000).

Hazelnut shells were first ground to pass through a 10-mesh (2 mm) sieve, using a Wiley mill (Standard Model No. 3, Arthur H. Thomas Co., U.S.A.). The first half of the screened materials was further ground in a burr mill (Mil-Rite grain mill, Retsel Corp., U.S.A.) to pass through a 35-mesh (0.5 mm) sieve. The particle size of hazelnut shells grinding, by both Wiley and burr mills, was considered as smaller than 0.5 mm. The second half of the screened materials was sifted to pass through 18-mesh (1.0 mm) and 35-mesh (0.5 mm) sieves, sequentially. By grinding with a Wiley mill and sifting, the particle sizes of the 3 fractions of hazelnut shells were 1–2 mm, 0.5–1 mm, and < 0.5 mm. The weights of different fractions of hazelnut shells were recorded. The ground samples were stored at -20 °C for further analysis.

2.1.2. Proximate composition of hazelnut shells

Proximate composition, including moisture, ash, protein, fat, and carbohydrate contents was determined on hazelnut shell powders. Moisture, ash, protein and fat contents were analyzed, following the standard methods (AOAC, 2000). Crude fat was determined by using an extraction unit (HT1043, Soxtec, U.S.A.) with hexane as the extraction solvent. Crude protein was analyzed with a nitrogen analyzer unit (Leco FP-528, Leco Corporation 3000, U.S.A.) with 6.25 as nitrogen to protein conversion factor. Carbohydrate content was calculated by subtracting contents of other compositions from 100%. Analyses were performed in triplicate. Data were reported as a percentage of the wet weight of the hazelnut shell powder.

2.2. Chemicals

Folin-Ciocalteu reagent, 2,4,6-tri (2-pyridyl)-1,3,5-triazine (TPTZ), 2,2-diphenyl-1-picryl hydrazyl (DPPH), trolox, ferulic acid, gallic acid, coumaric acid, 3-O-caffeoylquinic acid, taxifolin, catechin and epicatechin were purchased from Sigma-Aldrich (U.S.A.). Protocatechuic acid, phlorizin, quercitrin, sodium carbonate, ferric chloride hexahydrate, acetone, ethanol, and methanol were purchased from Fisher Scientific (U.S.A.). Quercetin, kaempferol, and epicatechin gallate were obtained from Cayman Chemical (U.S.A.). Myricitrin was purchased from VWR (U.S.A.).

2.3. Shaking bath extraction

The shaking bath extraction was carried out in a temperature-controlled incubator shaker (Innova 26, New Brunswick Scientific, U.S.A.). Each sample was macerated with 10 ml of extraction solvent in a closed 50 ml centrifuge tube. The centrifuge tubes were shaken at a constant speed (250 rpm) at a prescribed temperature. Afterwards, the extracts were centrifuged for 5 min at 5000*g*, and the residues were washed with 10 ml of distilled water and re-centrifuged twice under the same conditions. The supernatants were collected for phenolic content and antioxidant analyses. Each extraction was carried out in triplicate.

2.4. Experimental design

2.4.1. Preliminary study of the extraction conditions

Before the optimization by response surface experiment, a first set of three tests was performed to identify the relevant extraction conditions used in the formal experiment, including the particle size of the hazelnut shells, the type of solvent, and the extraction temperature. First, a two-factorial design (4 levels of particle sizes × 4 levels of extraction time) was applied to evaluate the effects of particle size and extraction time on the extraction of phenolic antioxidants. The 4 levels of particle size were 1–2 mm, 0.5–1 mm, < 0.5 mm (Wiley mill), and < 0.5 mm (Wiley and burr mills). The 4 levels of extraction time were 2, 4, 6, and 12 h. Second, another two-factorial design (3 solvents × 3 levels of concentration) was used to evaluate the effects of solvents on the extraction of phenolics. The solvents used were methanol, ethanol, and acetone. Each solvent was tested at concentrations of 20%, 50%, and 80%. Lastly, a single-factorial design was used to evaluate the influence of temperature (30, 40, and 50 °C) on the extraction. All experiments were repeated at least twice for each treatment combination.

2.4.2. Response surface experiment

A full factorial experimental design (3 × 3 × 5) was used to determine the optimum extraction condition for phenolic antioxidants. The variables were solid to liquid ratio (S/L = 10, 30, and 50 g/l), concentration of solvent (Cs = 20, 50, and 80%), and extraction time (t = 1, 2, 4, 6, and 12 h). The particle size (< 0.5 mm), type of solvent (acetone) and extraction temperature (50 °C) were kept constant. All of the experiments were repeated at least twice for each treatment combination in completely randomized designs.

2.5. Analyses of the response variables

2.5.1. Total phenolic content (TPC)

The TPC was measured according to the method of Siriwardhana and Shahidi (2002) with modification. Equal volumes (0.1 ml) of Folin-Ciocalteau reagent and diluted extract were mixed, then 1.0 ml of sodium carbonate solution (75 g/l) was added to the content. After 1 h of incubation at room temperature in the dark, 200 µl of the mixture were transferred into the designated well of a 96-well microplate. The absorbance was read at 725 nm, using a microplate reader (BioTek Instruments, U.S.A.). Gallic acid standard solutions were used for calibration. The results of TPC were expressed as mg gallic acid equivalents (GAE) /g of shell.

2.5.2. Antioxidant capacity

2.5.2.1. DPPH radical-scavenging capacity (DRSC). The DRSC values of antioxidants in the hazelnut shell extracts were evaluated, based on the method by Lu, Yuan, Zeng, and Chen (2011). The DPPH radical solution was prepared by dissolving 3.5 mg of DPPH radical in 100 ml of ethanol. Accurately, 3 ml of DPPH radical ethanolic solution were added to 0.15 ml of properly diluted hazelnut shell extract. The mixture was shaken vigorously for 1 min and left to stand at room temperature in the dark for 30 min. Absorbance was measured against the blank reagent at 517 nm (Evolution 201 UV–Visible spectrophotometer, Thermo Fisher, China). All determinations were carried out in triplicate. The percent inhibition of DPPH radical was calculated according to the equation as shown below

$$\text{Inhibition of DPPH radical } (\%) = [(A_c - A_s)/A_c] \times 100 \quad (1)$$

where A_c is the absorbance of the control solution, and A_s is the