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Phenolic profiles and antioxidant activity of Turkish Tombul hazelnut samples (natural, roasted, and roasted hazelnut skin)

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ABSTRACT

The phenolic profiles and antioxidant status of hazelnut samples [natural (raw) hazelnut, roasted hazelnut, and roasted hazelnut skin] were compared. Free and bound (ester-linked and glycoside-linked) phenolic acids were examined using liquid chromatography–tandem mass spectrometry (LC–MS/MS). Comprehensive identification of phenolics was carried out using Q-exactive hybrid quadrupole-orbitrap mass spectrometer (Q-OT-MS). Samples were also assessed for their total phenolics and antioxidant activities using three different assays. Ten free and bound phenolic acids were quantified in hazelnut samples. Roasted hazelnut skin contained the highest content of total phenolic acids, followed by natural and roasted hazelnuts. The majority of phenolic acids were present in the bound form. Using a Q-OT-MS, 22 compounds were tentatively identified, 16 of which were identified for the first time in hazelnut samples. The newly identified compounds consisted of flavonoids, phenolic acids and related compounds, hydrolysable tannins and related compounds, and other phenolics. Three antioxidant assays demonstrated similar trends that roasted hazelnut skin rendered the highest activity. The present work suggests that roasted hazelnut skin is a rich source of phenolics and can be considered as a value-added co-product for use as functional food ingredient and antioxidant.

1. Introduction

Hazelnut (*Corylus avellana* L.) is one of the most popular tree nuts worldwide due to its pleasant flavour (Alasalvar, Shahidi, & Cadwallader, 2003), nutrients (Alasalvar, Shahidi, Amaral, & Oliveira, 2009; United Sates Department of Agriculture, 2016), fat-soluble bioactives (Alasalvar, Amaral, & Shahidi, 2006; Alasalvar & Bolling, 2015; Alasalvar & Pelvan, 2011), and phenolics/phytochemicals (Alasalvar & Bolling, 2015; Alasalvar, Hoffman, & Shahidi, 2009; Chang, Alasalvar, Bolling, & Shahidi, 2016). Health claims both from Food and Drug Administration (FDA) and European Food Safety Authority (EFSA) recommend daily consumption of nuts (42.5 g FDA and 30 g EFSA), including hazelnut, for coronary heart disease risk reduction (European Food Safety Authority, 2011; Food and Drug Administration, 2003).

Hazelnut may be consumed as natural (with skin) or preferably roasted (without skin). Hazelnut skin, a rich source of fat-soluble bioactives and phenolics, is the co-product of roasting process. It represents about 2.5% (skin absorbs oil during roasting) of the total hazelnut weight and is discarded upon roasting (Alasalvar, Karamać et al., 2009; Lainas, Alasalvar, & Bolling, 2016). Although several studies have been published regarding phenolic compounds of hazelnut and its co-products (Alasalvar, Karamać, Amarowicz, & Shahidi, 2006; Alasalvar, Pelvan, & Amarowicz, 2010; Alasalvar et al., 2009a; Lainas et al., 2016; Pelvan, Alasalvar, & Uzman, 2012), a large percentage of compounds present remain unknown and need to be identified before the health-promoting properties of hazelnut products are properly elucidated. Therefore, it is of great importance to identify phenolic profiles and antioxidant activities of natural and roasted hazelnuts as well as roasted hazelnut skin. The aims of this study were to identify phenolic profiles in hazelnut samples by liquid chromatography–tandem mass spectrometry (LC–MS/MS) and Q-exactive hybrid quadrupole-orbitrap mass spectrometer (Q-OT-MS) techniques and to assess their antioxidant activities by three different *in vitro* assays.

2. Materials and methods

2.1. Samples

The premium class natural (raw) Turkish Tombul hazelnut (*Corylus avellana* L.), roasted hazelnut (free of skin), and roasted hazelnut skin (co-product of roasting process) were obtained from Giresun Commodity Exchange (Giresun, Turkey), at the beginning of the harvest

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season of 2016. The hazelnut samples were kept in a control cabinet (at 5 °C with relative humidity of 65–70%) until they were analysed.

2.2. Reagents and standards

All chemical reagents were obtained from Sigma-Aldrich-Fluka Co., Ltd. (Prolab, Istanbul, Turkey), unless otherwise stated.

2.3. Preparation of hazelnut crude extract

Hazelnut samples (natural hazelnut, roasted hazelnut, and roasted hazelnut skin) were finely ground and then defatted by mixing with hexane (1:10, w/v, for 3 min, 3 times) in a homogenizer (model DI 25 basic IKA, Staufen, Germany) at ambient temperature. Phenolic compounds were extracted from defatted meals as described by Robbins, Gong, Wells, Greenspan, and Pegg (2015). Briefly, defatted meals were mixed with an acetone/water/acetic acid mixture (70:29.5:0.5, v/v/v) at a solid to solvent ratio of 1:10 (w/v), and subsequently placed in a shaking water bath (SW 22, Julabo GmbH, Seelbach, Germany) at 50 °C for 30 min. After cooling, the supernatant was carefully decanted. The residue was re-extracted twice under the same conditions and supernatants were combined. The solvent was then removed from the combined supernatants under vacuum at 40 °C (model Büchi Anniversary Eddition, Zurich, Switzerland) and the remaining water was removed by lyophilisation for 72 h at −48 °C [Christ Epsilon 2–4 Lyo-Screen-Control (LSC), Osterode am Harz, Germany]. Finally, the prepared crude phenolic extracts were stored at −20 °C in vacuum-sealed pouches (in the dark) until they were used for further analysis. Extraction yield of each sample was recorded and used to convert the results to 100 g of hazelnut sample.

2.4. Extraction and hydrolysis of phenolic acids

Phenolic acids were extracted according to the method described by Robbins et al. (2015), with slight modification. A total of 0.8 g of freeze-dried extract (from natural hazelnut, roasted hazelnut, and roasted hazelnut skin) was homogenized in 20 mL of acidified water (pH 2.0). Free phenolic acids were extracted with diethyl ether. The aqueous phase of first extraction was hydrolysed with 2 M NaOH (20 mL) for 4 h in N_2 flushed vials and ester-linked phenolic compounds were extracted with diethyl ether. After adjusting pH to 2 using 6 M HCl, glycoside-linked phenolic compounds were extracted with diethyl ether. Organic phase was removed under vacuum at 40 °C (model Büchi Anniversary Eddition, Zurich, Switzerland) from free, ester-linked, and glycoside-linked phenolic extracts. Finally, the residue was re-dissolved in methanol and filtered before the analysis performed both in LC–MS/MS and Q-OT-MS.

2.5. Identification and quantification of phenolic profiles

Free, ester-linked, and glycoside-linked phenolic compounds for each sample were analysed using both LC–MS/MS (AB SCIEX API 4000 QTrap, Framingham, MA, USA) and Q-OT-MS (Q-exactive hybrid quadrupole-orbitrap, Thermo Scientific, Bremen, Germany).

2.5.1. Phenolic acids of hazelnut samples by LC–MS/MS

Analysis was performed according to Del Rio, Calani, Dall'Asta, and Brighenti (2011), with slight modification. Briefly, a high-performance liquid chromatography (HPLC) system (20AD pump, DGU-20A5 degasser, CTO-2OAC column oven, CMB-20A communications bus module, and SIL-20A HT auto sampler) (Shimadzu Corporation, Kyoto, Japan) was coupled to a AB SCIEX API 4000 QTrap mass spectrometer (MS) with as electrospray ionization (ESI) interface. Samples were injected (10 μL) into the ODS, Hypersil column (250 mm × 4.6 mm, 5 μm, Thermo Scientific, Thermo Scientific, Waltham, MA, USA) with a 0.8 mL/min gradient flow (mobile phase A: 0.1% formic acid-water; mobile phase B: methanol; 0–5 min, mobile phase B concentration changed as 0–9% B; 5–16 min, 9–2% B; 16–35 min, 2–18% B; 35–50 min, 18–20% B; 50–65 min, 20–30% B; 65–80 min, 30% B. Tandem MS detection was performed using the multiple reaction monitoring (MRM) mode both in positive and negative ion modes. The optimal MRM conditions for each analyte were optimized using direct infusion. Source temperature was 550 °C, the ionization voltage was 5500 V, curtain gas was nitrogen at 20 psi, and the nebulizer gas was air at 50 psi. Scheduled MRM was used with a setting of a 90 s detection window covering the expected retention time of each analyte and the target scan time was 2 s for all compounds.

2.5.2. Phenolic profiles of hazelnut samples by Q-OT-MS

Q-OT-MS was used to tentatively identify the phenolic profiles of hazelnut samples. Samples were injected (10 μL) into the ODS, Hypersil column (250 mm × 4.6 mm, 5 μm, Thermo Scientific, Thermo Scientific, Waltham, MA, USA) with a 0.8 mL/min gradient flow (mobile phase A: 0.1% formic acid-water, mobile phase B: methanol; 0–5 min, mobile phase B concentration changed as 0–9% B; 5–16 min, 9–2% B; 16–35 min, 2–18% B; 35–50 min, 18–20% B; 50–65 min, 20–30% B; 65–80 min, 30% B. The Q-exactive hybrid quadrupole – orbitrap mass spectrometer equipped with an ESI source working in negative mode was used for the analysis. Operation parameters were as follows: ion spray voltage, 2.8 kV; capillary temperature, 300 C; capillary voltage, 35 V; tube lens voltage, 95 V; sheath gas, 19 (arbitrary units); and auxiliary gas, 7 (arbitrary units). Mass spectra was recorded a mass range between *m/z* 55–1000. Default values were used for most other acquisition parameters [Automatic gain control (AGC) target 3×10^6 ions]. XCalibur 2.2 software (Thermo Fisher Scientific, Waltham, MA, USA) was used for data processing. The tentative identification was carried out based on the library created by $[M-H]^-$ *m/z* values and fragmentation patterns of phenolic compounds reported in the literature for nuts.

2.6. Determination of total phenolics of hazelnut samples

The content of total phenolics in samples was determined using Folin–Ciocalteu's phenol reagent as described by Pelvan et al. (2012). Crude extracts of each sample were dissolved in methanol and the results were expressed as milligrams of gallic acid equivalents per gram of sample (mg of GAE/100 g of sample).

2.7. Determination of antioxidant activities of hazelnut samples

2.7.1. DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity

The method described by Pelvan Pelitli, Janiak, Amarowicz, and Alasalvar (2017) was used to assess DPPH radical scavenging activity of samples. Crude extract of each sample was dissolved in methanol and the results were given as IC_{50}, which is the amount of extract required to scavenge the initial DPPH radical by 50% and expressed as per mg of sample.

2.7.2. ABTS [2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)] radical scavenging activity

The method described by Pelvan Pelitli et al. (2017) was used to assess the ABTS radical scavenging activity of samples. Crude extract of each sample was dissolved in methanol and the results were expressed as millimoles of Trolox equivalents per gram of sample (mmol of TE/g of sample).

2.7.3. ORAC (oxygen radical absorbance capacity) activity

The ORAC activity was determined using a microplate reader (FLUOStar Omega, BMG Labtech, Ortenberg, Germany) according to ORAC assay. Crude extract of each sample was dissolved in methanol and the analysis was performed on the diluted samples as described by Pelvan et al. (2012). ORAC values were calculated using Trolox and the