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Original Research Article

Bioactive compounds in different hazelnut varieties and their skins

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ABSTRACT

Bioactive profiles of hazelnut skins belonging to fourteen hazelnut varieties were identified. Concentration of phenolic compounds, flavonoids and phenolic acids in soluble free, conjugated soluble and insoluble bound fractions together with their total concentrations were presented. In addition, tocopherol content and total antioxidant capacity of hazelnuts and their skins were revealed. Concentration of total phenolic compounds ranged between 51.9 and 203.1 mg gallic acid equivalent/g of skin among varieties, which is in accordance with the total antioxidant capacity. Total flavonoid content was almost 60% of the total phenolic compounds. Flavonoids and phenolic acids were found to be concentrated mostly in the conjugated soluble fraction. Tocopherol contents of hazelnut skins ranged from 226 to 593 μg/g, and α-tocopherol was the most abundant. Total antioxidant capacity was between 309 and 1375 μmol Trolox equivalent/g of hazelnut skins, which is more than 100 times higher than for hazelnuts without the skins.

1. Introduction

Hazelnut (*Corylus avellana* L.), which belongs to the family of Betulaceae, is widely consumed (Pelvan et al., 2012). Turkey is the main hazelnut producer, with annual production of 549 000 tonnes, which is almost 65% of the total world production (FAO, 2013). There are 18 hazelnut varieties: Acı, Cavcava, Çakıldak, Foşa, Ham, İncekara, Kalınkara, Kan, Karafındık, Kargalak, Kuş, Mincane, Palaz, Sivri, Tombul, Uzun Musa, Yassı Badem, Yuvarlak Badem, grown in Turkey (Pelvan et al., 2012). Among these varieties, Tombul contributes to 25–30% of Turkey's total production (Alasalvar et al., 2003).

The skin of hazelnut may be consumed directly intact with kernel or it could be easily removed after roasting. Hazelnut skin constitutes 2.5% of the total weight of the hazelnut (Alasalvar et al., 2009) and the skin may be considered an important constituent because of its distinctive phenolic profile and high antioxidant activity (Contini et al., 2008; Pelvan et al., 2012; Özdemir et al., 2014). Improvement in colon metabolism, decreasing in total and LDL-cholesterol, triacylglycerol and non-esterified fatty acids in blood serum could be attributed to the high phenolic content, phytosterols and dietary fibre in hazelnut skins (Caimari et al., 2015). Therefore, consumption of hazelnut with its brown skin is better from the point view of health. On the other hand, because brown skin removed after roasting is a major by-product of the hazelnut industry, its utilization is considered to be necessary. For example, defatted fine hazelnut skin particles were added to coffee before and after brewing to improve the physiologically positive effects of coffee phytochemicals (Contini et al., 2012). Five percent (w/w) of hazelnut skin was also used for fortification of fresh egg pasta (Zeppa et al., 2015) and bread (Anil, 2007) to increase antioxidant activity and dietary fibre content. Additionally, hazelnut skin showed prebiotic activity, allowing the growth of *Lactobacillus crispatus* P17613 and *Lactobacillus plantarum* P17630. Even 0.01% (w/v) of hazelnut skin acted as a cryoprotective ingredient during lyophilization, making it promising for application in probiotic-containing foods and nutraceuticals (Montella et al., 2013). Defatted hazelnut skin powder obtained after high shear homogenization was not only a good source of dietary fibre, antioxidant compounds and phenolic compounds but was also rich in colouring agents.

Previous works investigated the total phenolic content, antioxidant activity and condensed tannins (Locatelli et al., 2010; Monagas et al., 2009; Alasalvar et al., 2009), dimeric B type procyanidins (Monagas et al., 2009; Esatbeyoglu et al., 2014), monomeric and oligomeric flavan-3-ol contents (Monagas et al., 2009; Del Rio et al., 2011) of roasted hazelnut skins. Shahidi et al. (2007) compared individual phenolic acids, radical-scavenging

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activity and inhibition of human LDL cholesterol in extracts of whole hazelnut, roasted hazelnut skin and other by-products. Contents of free, bound and total phenolic acids, total phenolic content and antioxidant activity of natural and roasted commercial hazelnut varieties were reported (Pelvan et al., 2012). Despite the fact that hazelnut skins may be a good dietary source, an overall screening of bioactive compounds and their distribution in hazelnut skin is lacking in the literature.

The objective of this study is to reveal the bioactive profile of natural hazelnut skins that belong to fourteen varieties. For that reason, analyses of phenolics and flavonoids (free soluble, conjugated soluble, bound), and phenolic acids (free soluble, conjugated soluble, bound) have been performed. In addition to that, total antioxidant activity and tocopherol profile of both hazelnuts and their skins have been measured.

2. Materials and methods

2.1. Chemicals and consumables

Methanol, ethanol, acetonitrile, acetone, *n*-hexane, ethyl acetate, isopropanol, hexane and water were all HPLC-grade and were obtained from Sigma Aldrich (Steinheim, Germany). Standards of gallic acid, ferulic acid, catechin, potassium peroxy disulphate, ABTS (2,2′-azino-bis(3-ethyl-benzothiazoline-6-sulfonic acid)), Folin–Ciocalteu reagent, α-tocopherol (≥96%), β-tocopherol (≥96%), γ-tocopherol (≥96%), δ-tocopherol (≥90%) were also obtained from Sigma Aldrich (Steinheim, Germany). Formic acid (98%), hydrochloric acid (37%), sodium hydroxide, sodium carbonate, sodium nitrite, aluminium chloride and diethyl ether were purchased from Merck Co. (Darmstadt, Germany). Trolox [6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid] was purchased from Fluka Chemie AG (Buchs, Switzerland). Syringe filters (nylon, 0.45 μm) were supplied from Waters (Milford, MA).

2.2. Hazelnut and hazelnut skin

Fourteen Turkish hazelnut (*C. avellana* L.) varieties (Acı, Çakıldak, Foşa, İncekara, Kalınkara, Kan, Kargalak, Kuş, Palaz, Sivri, Tombul, Uzun Musa, Yassı Badem, Yuvarlak Badem), grown in the orchard of the Hazelnut Research Station in Giresun on the north-east coast of Turkey, were used for the analysis. Hazelnuts were left on the trees until the first week of August 2013; they were collected by hand when their green leafy covers became pale and moisture content decreased to 30%. They were then left in the sun until the moisture content became 6% for about 3–5 days. Pale leafy covers of hazelnuts were removed and three separate bags from each variety, each of them containing 2 kg hazelnuts, were immediately sent for analysis. Until analysis, hazelnuts were stored in their shells at −20 °C. Before analysis, hazelnut shells were cracked and hazelnut skin was removed manually by using a blade. About 2.5 g of hazelnut skin were obtained by scraping 100 g of hazelnut from each bag. Then, skin samples were finely ground using a coffee mill. Three independent hazelnut and hazelnut skin samples ($n = 3$) were analyzed with two analytical measurements.

2.3. Separation of phenolic fractions with alkaline hydrolysis

Soluble free, soluble conjugated and insoluble bound phenolic compounds were extracted as described by Moore et al. (2005). First, 0.25 g of hazelnut skin were extracted with 4 mL of methanol/acetone/water (7:7:6, v/v/v) and centrifuged at 6000 × *g* for 3 min. Extraction with 4 mL of methanol/acetone/water (7:7:6, v/v/v) was repeated 5 times and supernatants of each step were collected and combined in a test tube (extract A). Pellet obtained after this extraction was kept for the analysis of insoluble bound phenolic compounds.

A quantity of 10 mL of the extract A was transferred to another tube and 10 mL of 4 N NaOH were added to it, in order to release soluble conjugated phenolic compounds (extract B). A quantity of 7 mL of 4 N NaOH was added to the pellet simultaneously to release insoluble bound phenolic compounds (extract C). Then, both tubes were left for 4 h in a shaker at the room temperature.

After then, 5 mL of extract from A, B and C were transferred to test tubes and the pH was adjusted to 2 in all tubes, by using 6 N HCl. A quantity of 5 mL of diethyl ether:ethyl acetate (1:1, v/v) was added into these tubes, vortexed for 2 min and centrifuged at 6000 × *g* for 2 min. The same extraction procedure with 5 mL of diethyl ether:ethyl acetate (1:1, v/v) was repeated four times. Supernatants were collected at each step and extracts were combined. Then, 5 mL of the combined extract were evaporated under an N_2 stream at 30 °C to complete dryness. After then, phenolic compounds were redissolved in 1.5 mL of methanol and kept at −20 °C until the analyses were performed. As extract B contained both soluble free and soluble conjugated phenolic compounds, soluble conjugated phenolic compounds were calculated by subtracting the amount of soluble free phenolic compounds obtained from the analysis of extract A.

2.4. Analysis of total phenolic content

Total phenolic content analyses were performed according to the Folin–Ciocalteu method (Hoff and Singleton, 1977). First, 25 μL of appropriate extracts were transferred into a test tube and 0.8 mL of 0.2 N Folin–Ciocalteu reagent were added to it. After 5 min of reaction time, the solution was neutralized with 0.8 mL of 20% Na_2CO_3 (w/v). The mixture was kept in the dark for 2 h until the characteristic blue colour was observed. After centrifugation at 6000 × *g* for 4 min, absorbance of the supernatant was measured against methanol at 765 nm. Calibration curve was built with different concentrations of gallic acid dissolved in methanol and results were given as mg gallic acid equivalent (GAE) per g of hazelnut skin.

2.5. Analysis of individual phenolic acids

Chromatographic analyses were performed on an Agilent 1200 HPLC system consisting of a diode array detector, quaternary pump, autosampler, and column oven (Agilent Technologies, Waldbronn, Germany). Before analysis, extracts were filtered through a 0.45-μm nylon syringe filters and taken into vials. Phenolic acids were separated on a Waters Atlantis C18 column (250 mm × 4.6 mm id., 5 μm; Waters Corp., Milford, MA) by using 1% formic acid in water (A) and 1% formic acid in acetonitrile (B) at a flow rate of 1 mL/min with the following gradient programme: linear gradient elution from 10 to 20% B, 0–10 min; linear gradient elution from 20 to 40% B, 10–20 min; linear gradient elution from 40 to 10% B, 20–25 min and isocratic elution of 10% B, 25–30 min. The column temperature was 30 °C and injection volume was 10 μL. Analyses were performed by using diode array detector at 280 nm. Calibration curves were built for each of the compounds identified in the samples and phenolic acids were expressed as mg per g of hazelnut skin.

2.6. Analysis of total flavonoid content

Total flavonoid content was determined according to the method described by Zhishen et al. (1999). First, 100 μL of appropriate extract were mixed with 50 μL 5% $NaNO_2$. After waiting for 6 min of reaction time, 500 μL of 10% $AlCl_3$ were added to the mixture to form aluminium-flavonoid complex. After 7 min,