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Short communications

Effects of roasting on proanthocyanidin contents of Turkish Tombul hazelnut and its skin

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ABSTRACT

The effects of roasting on extractable and bound proanthocyanidins of Turkish Tombul hazelnut and its skin were studied. Natural (raw) and roasted hazelnuts as well as roasted hazelnut skin were extracted, hydrolysed, and analysed by normal-phase high performance liquid chromatography. Natural hazelnut extractable proanthocyanidins were 81% oligomers (4–9mers) and polymers (≥10mers). However, roasted hazelnut extractable proanthocyanidins were only monomers to trimers. This decrease was apparently due to skin loss during roasting. Additional proanthocyanidins of roasted hazelnut residue were recovered by alkaline hydrolysis. In contrast, natural hazelnut had proportionally less bound proanthocyanidins, mainly as dimers. Roasted hazelnut skin proanthocyanidins were 21% bound with mainly dimers, trimers, and tetramers recovered after alkaline hydrolysis. These data suggest that prior studies may have underestimated hazelnut proanthocyanidin content because a significant quantity of bound proanthocyanidins is not recoverable by conventional organic solvent extraction.

1. Introduction

Hazelnut and other tree nuts contain many bioactive and health-promoting components and are nutritionally-dense foods (Alasalvar & Bolling, 2015). Increased consumption of tree nuts reduces cardiovascular risk by lowering total cholesterol and low-density lipoprotein (LDL) cholesterol (Del Gobbo, Falk, Feldman, Lewis, & Mozaffarian, 2015). Thus, health claims or dietary guidelines from United States, Canada, and the European Union recommend increasing tree nut consumption (Alasalvar & Bolling, 2015).

Hazelnut (*Corylus avellana* L.) is consumed worldwide and the majority of its production is concentrated in the Black Sea coast of Turkey. Turkey is the world's largest producer of hazelnut, contributing around 70% of the total global production (INC, 2015). Hazelnut contains many phenolic compounds, which have significant antioxidant activity (Alasalvar et al., 2009). It is especially rich in flavan-3-ols and proanthocyanidins (Del Rio, Calani, Dall'Asta, & Brighenti, 2011). Hazelnut has the highest reported total proanthocyanidin content among tree nuts with 500 mg/100 g and also has a significant content of hydrolysable tannins (Alasalvar & Bolling, 2015). Like other nuts, hazelnut polyphenols are concentrated in its skin (Alasalvar

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et al., 2009; Del Rio et al., 2011; Fabani et al., 2013; John & Shahidi, 2010). Hazelnut skin constitutes 2.5% of the total hazelnut weight, but is often discarded during roasting.

Polyphenols can have covalent or strong non-covalent associations with plant proteins and polysaccharides (Castro-Lopez, Gomez-Plaza, Ortega-Regules, Lozada, & Bautista-Ortin, 2016; Le Bourvellec & Renard, 2012). Furthermore, nut skin polyphenols may be entrapped in its fibrous cells (Mandalari et al., 2010). Thus, a significant portion of plant polyphenols are not extractable by organic solvents, referred to as "bound" polyphenols (Arranz, Saura-Calixto, Shaha, & Kroon, 2009). Almond, hazelnut, and possibly other nuts may have a significant portion of bound polyphenols (Alasalvar et al., 2009; Mandalari et al., 2010; Xie, Roto, & Bolling, 2012). While many nuts are rich in proanthocyanidins, the composition of bound proanthocyanidins in most nuts is not well-described. Characterising bound hazelnut polyphenols could help inform food processing and health research on delivering proanthocyanidins in functional foods, characterising hazelnut polyphenol bioactivity, and determining polyphenol metabolism after hazelnut consumption. Therefore, the purpose of this study was to characterise and compare the extractable and bound proanthocyanidins in natural (raw) and roasted Turkish hazelnuts as well as roasted hazelnut skin.

2. Materials and methods

2.1. Chemicals and reagents

(+)-Catechin and procyanidin B2 were purchased from Sigma-Aldrich (St. Louis, MO, USA). Solvents for extraction and chromatography, namely hexane, acetone, methanol, hydrochloric acid, and acetic acid were from Fisher Scientific (Fairlawn, NJ, USA), while high performance liquid chromatography (HPLC)-grade dichloromethane was from Mallinckrodt Baker (Paris, KY, USA). All other reagents were purchased from Fisher Scientific (Fairlawn, NJ, USA). The premium class Giresun quality natural (sun-dried for 3–4 days at around 20–25 °C) and roasted Turkish Tombul hazelnuts (*Corylus avellana* L.) as well as roasted hazelnut skin were kindly provided by the Giresun Commodity Exchange in 2013 and stored at –20 °C until they were analysed, within 2 months.

2.2. Defatting nut meat

Frozen hazelnut was ground in a lab mill and 4.0 g aliquots were further homogenised for 2 min in 40 mL of hexane using an Ultra-Turrax homogeniser (IKA, Wilmington, NC, USA) (Xie et al., 2012). The homogenate was centrifuged (8 min, 23 °C, and 1500*g*), and the supernatant was removed and reserved. The pellet layer was re-homogenised and re-centrifuged under the same conditions. The supernatants were combined and dried under nitrogen gas. The remaining lipid layer was weighed and placed in the freezer, along with the remaining pellet layer, (–20 °C) until further analysis.

2.3. Acetone extraction of hazelnuts and skin

Extraction was previously described by Prior and Gu (2005), with modifications as we previously reported (Xie et al., 2012). An extraction solution of acetone, water, and acetic acid (70:29.5:0.5, v/v/v) was added to defatted hazelnut samples, with 1–2 g of defatted hazelnut or 0.3 g skin per 40 mL solution. The mixtures were vortexed and sonicated at 37 °C for 10 min. Next, they were placed on a rocker for 50 min and centrifuged (15 min, 23 °C, and 1500*g*). The supernatant layer (extract) was removed and placed under nitrogen gas. The pellet was stored in the freezer (–20 °C) for re-extraction by alkaline hydrolysis. After acetone was evaporated from the extracts, they were frozen at –80 °C and lyophilised. The lyophilised extracts were stored at –20 °C until they were analysed, within 4 months.

2.4. Alkaline hydrolysis of hazelnuts and skin

Hydrolysis was based on a previously described method developed for cranberry pomace (White, Howard, & Prior, 2010) that was modified for extraction of almond proanthocyanidins (Xie et al., 2012), which we adapted to extract hazelnut proanthocyanidins. A 20 mL aliquot of 4 N sodium hydroxide was added to natural and roasted pellets or hazelnut skin from the freezer in a test tube and vortexed for 30 s. Test tubes were then placed in a water bath for 15 min at 60 °C with the caps slightly open, and further vortexed at 5 and 10 min of incubation. The samples were then cooled over ice for 15 min. Samples were neutralised with 6 N hydrochloric acid using litmus paper to ensure that neutralisation had occurred. The samples were then frozen (–20 °C) and stored for less than 2 weeks before Sephadex LH-20 chromatography.

2.5. Isolation of proanthocyanidins by Sephadex LH-20 chromatography

Proanthocyanidins were isolated by Sephadex LH-20 chromatography as previously described (Xie et al., 2012). Sephadex LH-20 powder (~4 g) was equilibrated with a solution of 30% methanol in water (v/v) and loaded into a 1 cm diameter glass column. Dried extract or hydrolysis residue (Sections 2.3 and 2.4) was reconstituted with ~5 mL of 30% methanol in water (v/v), filtered through a 0.20 μm nylon syringe filter, and loaded onto the column. The column was then washed with 50 mL of 30% methanol in water (v/v) to remove sugars and phenols. Then the column was washed with 100 mL of 70% acetone in water (v/v) to elute the proanthocyanidins. The 100 mL aqueous acetone eluate was collected and dried under a stream of nitrogen gas at 23 °C to remove acetone. The remaining water was frozen, lyophilised to a powder, and then stored at –80 °C for less than 2 months before analysis.

2.6. Normal phase HPLC analysis of proanthocyanidins

Proanthocyanidin fractions were reconstituted in 70% acetone:methanol (1:2.5, v/v) for hazelnut analysis or methanol for skin analysis and centrifuged to remove any particulates. Proanthocyanidins were resolved by normal-phase HPLC as previously described, with modifications (Taheri, Connolly, Brand, & Bolling, 2013). A Dionex U3000 (Sunnyvale, CA, USA) equipped with a temperature-controlled autosampler, column oven, diode array detector (DAD), and a 260 mm × 40 mm i.d., 5 μm Hypersil column (ThermoFisher, Bellefonte, PA, USA) was used for the separations. A 1 mL/min gradient of dichloromethane and