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Analytical Methods

Total antioxidant activity of hazelnut skin (Nocciola Piemonte PGI): Impact of different roasting conditions

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ABSTRACT

The thin brown perisperm (skin) that envelops hazelnut kernels is usually removed after roasting process, leading a phenolic-rich by-product. Principal aim of this work was to characterise the total antioxidant activity of phenolic extracts obtained from roasted "Nocciola Piemonte PGI" hazelnuts skin. Different extraction solvents (methanol, acidified methanol, ethanol, acidified ethanol, and acetone/water) and different protocols (cold solvent-assisted extraction and semi-automated Soxhlet extraction) were employed. The influence of different roasting degree (180 °C/10 min and 180 °C/20 min) was also investigated. DPPH· and $ABTS^{\cdot+}$ radical-scavenging methods, ferrous ions chelation activity and inhibition of lipid peroxidation investigated in this study demonstrated significant antioxidant properties for hazelnut skin phenolics. The main mechanism involved appeared the antiradical activity, strictly related to the total phenolic content ($r = -0.8798$ and -0.8285 for DPPH· and $ABTS^{\cdot+}$ assays, respectively). The acidification of extraction solvents led to a significant decrease of antiradical activity, whilst the different roasting conditions significantly influenced the chelation activity and the inhibition of lipid peroxidation, showing higher effectiveness for high-roasted hazelnut skin extracts. Conversely, the direct measure of the antioxidant capacity of defatted hazelnut skins revealed higher $ABTS^{\cdot+}$ scavenging properties for medium-roasted sample.

1. Introduction

In food science, antioxidants are very important in that they act preventing lipid oxidation in food and decreasing the adverse effects of reactive species (ROS: reactive oxygen species; RNS: reactive nitrogen species) on normal physiological functions in humans (Huang, Ou, & Prior, 2005).

Antioxidant synthetically obtained, like BHA (butylated hydroxyanisole) and BHT (butylated hydroxytoluene), are largely used in food industry and are included in human diet. However, in recent years the use of natural antioxidants has been promoted because of concerns regarding the safety of synthetic ones. Dietary components, including polyphenols, carotenoids and vitamins C and E, are considered effective antioxidants useful in the prevention of oxidative stress and related diseases (Kaur & Kapoor, 2001; Moure et al., 2001).

Widely distributed in the plant kingdom and abundant in our diet, polyphenols are among the most studied about classes of antioxidants. Phenolics are the products of secondary metabolism in plants, providing essential functions in the reproduction and the growth of the plants, acting as defense mechanisms against pathogens, parasites, and predators, as well as contributing to the colour of plants (Liu, 2004). In addition to their roles in plants, several epidemiological and clinical researches demonstrated that phenolic antioxidants occurring in cereals, fruits and vegetables are principal contributing factors for the decreased incidence of several chronical and degenerative diseases (Shahidi, 2000).

For all these reasons, in last few years several studies have been conduced in order to investigate the antioxidant activity of phytoextracts obtained from vegetable sources. Particularly, agricultural and industrial residues are considered as very attractive sources of natural antioxidants (Moure et al., 2001). By-products of grape (*Vitis vinifera* L.) processing, such as seeds and peels, are the most studied and promising antioxidants sources (Shi, Yu, Pohorly, & Kakuda, 2003). The extraction and antioxidant activity of phenolic compounds from other residual materials such as apple peel (Kim et al., 2005), apple pomace (Lu & Foo, 2000), sweet orange peel (Anagnostopoulou, Kefalas, Kokkalou, Assimopoulou, & Papageorgiou, 2005), blanched artichoke and artichoke blanching waters (Llorach, Espín, Tomás-Barberán, & Ferreres, 2002), leaves and stems of cauliflowers (Llorach, Espín, Tomás-Barberán, & Ferreres, 2003), olive mill waste (Mulinacci et al., 2005), cocoa by-products (Arlorio et al., 2008; Azizah, Ruslawati Nik, & Swee Tee, 1999) and nut hulls (peanut, cashew nut, hazelnut, almond, pistachios,

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Chilean hazelnut, etc.) (Goli, Barzegar, & Sahari, 2005; Kamath & Rajini, 2007; Moure et al., 2000; Shahidi, Alasalvar, & Liyana-Pathirana, 2007; Wijeratne, Abou-Zaid, & Shahidi, 2006; Yu, Ahmedna, & Goktepe, 2005) have been also investigated. The presence of polyphenols in outer layers (skins, peels, and hulls) of fruits, vegetables and seeds (nuts) may offer protection against oxidative stress: it is known that hulls play the major role in the defense of the plant seeds and, together with bran fractions, concentrate most phenols and tannins (Shahidi & Naczk, 1995). Moreover, polyphenols play an important role in the astringent taste, causing typical long-lasting puckering, shrinking, rough, and drying sensation in the oral cavity (Stark, Bareuther, & Hofmann, 2005).

The antioxidant activity of nuts and their by-products has been previously studied. These studies have highlighted that nut by-products are rich sources of natural antioxidants and phenolic compounds. Among nuts, hazelnuts (*Corylus avellana* L.) are very interesting in that rich in phenols and, particularly, proanthocyanidins (Gu et al., 2003). Recent studies tentatively identified several phenolic acids in both hazelnut kernels (Alasalvar, Karamać, Amarowicz, & Shahidi, 2006; Yurttas, Schafer, & Warthesen, 2000) and hazelnut by-products (skin, green leafy cover, hard shell and tree leaf) (Contini, Baccelloni, Massantini, & Anelli, 2008; Shahidi et al., 2007). These works demonstrated that hazelnut skin (or perisperm, or testa) is a rich and low-cost source of natural phenolic antioxidants. More recently, Alasalvar et al. (2009) obtained two fractions from crude phenolic extracts of Turkish Tombul hazelnuts skin (low-molecular-weight phenolics and tannins, respectively), showing higher antioxidant/antiradical activity for tannin fraction, followed by the crude extract and low-molecular-weight phenolic compounds. However, the impact of different roasting conditions on both phenols extraction and antioxidant activity should also to be investigated, particularly considering the formation of Maillard products (melanoidins) during roasting.

The aim of this work was to characterise the total antioxidant activity of phenolic extracts obtained from roasted "Nocciola Piemonte PGI" hazelnuts skin, considering different approaches (free radical-scavenging activity, chelation of pro-oxidant ferrous ions, inhibition of lipid peroxidation). Different extraction protocols were employed (cold solvent-assisted extraction and semi-automated Soxhlet extraction) and the influence of different roasting processes (medium- and high-roasting degrees) was investigated. Finally, the total antioxidant capacity of hazelnut skins (defatted powders) was determined using a direct measurement protocol.

2. Materials and methods

2.1. Samples

Samples of hazelnut skins were kindly provided by Dr. Giuseppe Zeppa (University of Turin, Italy). Hazelnut skins were obtained from Italian "Nocciola Piemonte PGI" hazelnut kernels (*C. avellana* L.), namely Tonda Gentile delle Langhe cultivar, cultivated only in specific areas and according to the disciplinary of production of the protected geographical indication (PGI) "Nocciola Piemonte". Dried unshelled hazelnuts were roasted at two different conditions: 180 °C for 10 min (medium roasting, MR) and 180 °C for 20 min (high roasting, HR), and hazelnut skins were recovered after spontaneous separation from the kernels after roasting. All samples were stored under vacuum and kept in the dark at −20 °C until they were analysed.

2.2. Chemicals

All reagents and standard chemicals ((±)-catechin monohydrate, trolox, gallic acid monohydrate, caffeic acid, (−)-epicatechin, quercetin dihydrate, butylated hydroxyanisole (BHA) and disodium ethylenediaminetetraacetate dihydrate (Na_2-EDTA)) used for the determination of total phenol content and antiradical activity were purchased from Sigma–Aldrich (Milano, Italy). All chemicals and solvents were of reagent-grade level and purchased either from Sigma–Aldrich (Milano, Italy).

2.3. Proximate composition analysis

The moisture content of hazelnut skin samples was determined using a thermo-balance Sartorius MA30 (Sartorius AG, Goettingen, Germany). Total nitrogen content and total protein content (conversion factor: 6.25) were obtained according to Kjeldahl method using the Kjeltec system I (FOSS Tecator, Sweden). The ash content was determined in a muffle furnace according to AOAC (1990) procedure. Lipid fraction was extracted from ground hazelnut skins (after grinding and sieving particles size <1 mm) using a semi-automatic Soxhlet Büchi Extraction System B-811 (Büchi Labortechnik AG, Flawil, Switzerland) for 12 h, employing dichloromethane as solvent. All the results have reported as percentage on the basis of dry weight (dw).

2.4. Extraction of phenolic fraction

The extraction of phenolic fraction from high- and medium-roasted defatted hazelnut skins was performed using two different methods: (i) cold-extraction under magnetic stirring and (ii) Soxhlet extraction. For cold-extraction five different solvents were used: methanol, acidified methanol (hydrochloric acid 0.1%, v/v), ethanol, acidified ethanol (hydrochloric acid 0.1%, v/v), acetone/water 80:20, v/v; methanol was chosen as solvent for Soxhlet extraction.

2.4.1. Cold-extraction under stirring

Four grams of defatted hazelnut skins powders were extracted using 100 mL of solvent; extraction was carried out in closed Erlenmeyer flasks and under constant magnetic stirring, in the dark at room temperature (22 °C). After 1 h of stirring/extraction, the suspension was filtered (Buchner funnel) through Perfecte 2 paper filter (Superfiltro, Milan, Italy), and the solid residue was re-extracted with 50 mL of solvent for 30 min. This last step was repeated until the complete decolouration was achieved (exhaustive extraction); then, filtrates were collected. The total time required to obtain the complete extraction varied depending on the solvents employed and on the different roasting conditions (data not shown). Finally, the solvent was evaporated to dryness (vacuum, 40 °C) and dry extract was stored at −20 °C until use.

2.4.2. Soxhlet extraction

Ten grams of the defatted hazelnut skin powders were extracted with Soxhlet apparatus using methanol for 7 h. The solvent was then evaporated to dryness (vacuum, 40 °C) and the dry extract was stored at −20 °C until use.

2.5. Determination of phenolic content

The determination of total phenolic content was obtained using the classic Folin-Ciocalteu assay, as previously described in Arlorio et al. (2008). Results were expressed as catechin equivalents, through the calibration curve of (±)-catechin monohydrate. The calibration curve linearity range was 50–250 μg (r = 0.9987).

2.6. DPPH· scavenging activity

The DPPH (1,1-diphenyl-2-picrylhydrazyl) radical-scavenging assay was performed according to the method reported by Locatelli