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Antioxidant and deodorizing activities of phenolic components in chestnut inner shell extracts

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ABSTRACT

Phenolic compounds were extracted from chestnut inner shell using aqueous alcohols and alkaline solutions (50% ethanol, 50% methanol, 1% NaOH, and 2% NaOH) at different temperatures (25–90 °C). The phenolic composition, antioxidant activity, and deodorizing activity of the extracts were evaluated for comparison. Total phenolic content and antioxidant activity were increased as the extraction temperature increased. However, the phenolic composition and antioxidant activity were significantly different among the extracts prepared under different conditions. Aqueous ethanol (50%, v/w) was most effective in extracting the total phenolics, resulting in the highest DPPH radical scavenging activity. The alkaline solutions appeared more effective in extracting specific phenolics, such as tannins and flavonoids, than aqueous alcohols. Moreover, the alkaline extracts exhibited the higher activities in deodorizing *trans*-2-nonenal and methyl mercaptan than the alcoholic extracts. The deodorizing activity of chestnut inner shell extracts (CISE) were positively correlated to the residual contents of tannins and flavonoids (R^2 = 0.85–0.94, and 0.81–0.93, respectively).

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1. Introduction

Various bio-wastes from the forest and agricultural industries have been of interest because of their high levels of residual nutraceutical components. The re-use of the wastes as value-added materials is, not only cost-saving, but also environmentally beneficial. Chestnut (*Castanea crenata*) is a beech family plant natively grown in the northern hemisphere regions. The nuts contain hard hull and inner thin skin, both of which are about 10% of the whole weight of chestnuts (Hwang et al., 2001), and typically removed to obtain the edible flesh. Those hull and inner skin obtained in the peeling process are used mainly as fuel (Vázquez et al., 2012). According to Hwang et al. (2001), tannin content in the inner and outer shells were significantly different depending on chestnut varieties, which were in the ranges of 7.83–71.42% and 0.31–2.04%, respectively.

The phenolic compounds and antioxidant activity of those by-products from chestnut have been examined in recent years (Vázquez et al., 2008, 2009, 2012). Vázquez et al. (2008) reported that the total phenolic content in the chestnut *(C. sativa)* shell extracts was in a range between 26.2 and 59.7 g GAE/100 g extract, which was significantly higher than that in the eucalyptus bark extracts (11.9–22.3 g GAE/100 g extract). In addition, a positive correlation between the total phenolic contents and antioxidant activity was observed. Another study by the same research group (Vázquez et al., 2012) was carried out to investigate the influence of extraction conditions on the antioxidant activity of chestnut bur extracts. According to the results from a response surface methodology, total phenolic content in the chestnut bur extract reached the maximum when aqueous methanol and ethanol solutions were used: 36.32 g and 26.11 g GAE/100 g extract, respectively.

Likewise, total content and composition of the phenolics in extracts depend on the solvents and extraction conditions (Dai and Mumper, 2010). Among the organic solvents, methanol in mixture with water has been generally more efficient than acetone for the extraction of low molecular weight polyphenols, whereas aqueous acetone is better for the extraction of high molecular weight flavonoids (Prior et al., 2001; Guyot et al., 2001; Labarbe et al., 1999). Typically, mild heating helps the extraction of phenolics (Spigno et al., 2007), but excessive heating over 70 °C was reported to induce the degradation of certain phenolic compounds including anthocyanin (Havlikova and Mikova, 1985). Therefore, both the solvents and physical conditions for the extraction of phenolic compounds should be carefully considered for the extraction efficiency and stability of phenolic compounds.

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Many studies (Yasuda and Arakawa, 1995; Cabrera et al., 2006; Lodhia et al., 2008) revealed that polyphenols and their derivatives exhibited deodorizing activity. For instance, (−)-epigallocatechin gallate (EGCg), the main phenolic constituent in green tea, showed a substantial deodorizing activity against methyl mercaptan (CH_3SH), which is a major odor component in human halitosis (Tonzetich and Richer, 1964). It may be attributed to the addition of a methylthiol group to the ortho-quinone generated by atmospheric oxygen (Yasuda and Arakawa, 1995).

It is well-known that tannins from green tea or persimmon are effective in removing body odor, detected mostly for the elderly people (Yasuda and Arakawa, 1995; Lodhia et al., 2008), and soaps containing the tannins are also commercialized by several companies in USA and Japan. Personal body odor is affected by various environmental and internal factors, such as gender, age and eating habits (Yamazaki et al., 2010). Among the odor substances, *trans*-2-nonenal is known to be generated with aging, and characterized as the key component of the body odor in the elderly people (Haze et al., 2001).

Chestnut inner shell contains a large quantity of polyphenols, especially condensed tannins (Hwang et al., 2001). No study has been reported on the deodorizing activity of the chestnut inner shell. In this study, phenolic extracts were prepared from chestnut inner shell with different solvents and different conditions, and antioxidant and deodorizing activities of the extracts were investigated. Not only the total phenolic content (TPC), but also the condensed tannin content (CTC) and total flavonoid content (TFC) of the extracts were compared. For analysis of deodorizing activity, both *trans*-2-nonenal and methyl mercaptan were tested as odor substances. Additionally, the correlation between the phenolic composition and deodorizing activity of the chestnut inner shell extract (CISE) was examined.

2. Materials and methods

2.1. Materials

Fresh chestnut inner shell flour was purchased from a local company (Jayeon-Maeul, Seoul, Korea), and then stored in a freezer (−80 °C) prior to use. The chestnut inner shell was isolated by chestnut shell powders by sieving: the outer shell was passed through the sieve whereas the inner shell remained on the sieve.

The reagents and standards used in this study: Folin–Ciocalteu's phenol reagent (2N), gallic acid (99.1%), vanillin (99%), (+)-catechin hydrate (≥98%), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox) (97%), *trans*-2-nonenal (97%), methyl mercaptan (≥98%) and epicatechin gallate (≥98%) were purchased from Sigma–Aldrich Chemical Company (St. Louis, MO, USA). All other chemicals used for the analyses were purchased from Duksan Chemicals (Seoul, Korea).

2.2. Preparation of chestnut inner shell extract (CISE)

Chestnut inner shell flour (1 g, dry basis) was dispersed in different solvents (10 mL) including distilled water, 50% (v/v) aqueous ethanol, 50% (v/v) aqueous methanol, 1% (w/v) aqueous NaOH, and 2% (w/v) aqueous NaOH. The dispersion was magnetically stirred for 30 min at different temperatures in a water-bath (25 °C, 60 °C, or 90 °C). All samples were extracted in a water-bath and the evaporation of alcohol from the solvents was prevented by using a condenser. The aqueous methanol and ethanol solutions started boiling at the temperature of 77 °C and 82 °C, respectively when heated in water-bath (90 °C). After 30 min of extraction, the dispersion was centrifuged at 2600 × *g* for 15 min at room temperature, and the supernatant was collected. For the alkaline extracts (1% and 2% NaOH), neutralization by adding 3 M HCl solution was additionally done with re-centrifugation. The extracts were collected as the supernatants were evaporated by using a rotary vacuum evaporator (A-1000S, EYELA, Japan) at 60 °C to syrup, which was then freeze-dried and re-dissolved in methanol (10 mL) for the analysis of condensed tannins and flavonoids. For the analysis of deodorizing activity, the syrup was re-dispersed in distilled water instead of methanol to prevent the solvent effect. Total yield of extracts was expressed as solid weight percentage (wt%) of the freeze-dried extracts to the raw material (Zhang et al., 2007).

2.3. Total phenolic content (TPC)

Total phenolic content in the extracts was measured by a colorimetric assay based on procedures described by Singleton and Rossi (1965) with some modifications. An aliquot of the extracts (200 μL) was mixed with a diluted Folin–Ciocalteu's phenol reagent (800 μL). After 10 min, 7.5% (w/v) sodium carbonate solution (2 mL) was added to the mixture and incubated in the dark for 90 min. The absorbance at 725 nm was measured using an UV–vis spectrometer (BIOMATE 3S, Thermo Scientific, MA). To calculate TPC, gallic acid solutions at different concentrations (100–500 μg/mL) were used as standards. When NaOH was used for extraction, the alkalinity itself affected the absorbance during the analysis, especially when the phenolic content in the sample was low. So accurate measurement was difficult for the low phenolic content in the NaOH extracts prepared at 25 °C.

2.4. Condensed tannin content (CTC)

Condensed tannin content in the extracts was determined by the vanillin/HCl method of Broadhust and Jones (1978) with some modifications. An aliquot of the extracts (1 mL) was added to the vanillin reagent (2 mL), which had been prepared by dissolving vanillin in methanol (0.5%, w/v). Sequentially, an aqueous HCl solution (2 mL, 4%, v/v) was added to the mixture, and then the mixture was incubated in the dark. The absorbance was measured at 500 nm after 20 min of incubation. To calculate CTC, (+)-catechin standards at different concentrations (500–3000 μg/mL) were used.

2.5. Total flavonoid content (TFC)

Total flavonoid content was determined by a method described by Zhishen et al. (1999) with some modifications. An aliquot of the extracts (250 μL) was mixed with distilled water (1.25 mL) and 5% (w/v) $NaNO_2$ solution (75 μL). After stirring for 5 min, 10% (w/v) $AlCl_3$ solution (150 μL) was added. After 6 min of stirring, 1 M NaOH solution (500 μL) and distilled water (275 μL) were successively added, and then the absorbance was measured at 510 nm. To calculate TFC, (+)-catechin standards at different concentrations (100–400 μg/mL) were used.

2.6. DPPH radical scavenging activity

Antioxidant capacity of CISE was determined by measuring the DPPH radical scavenging activity. An aliquot of the extracts (0.5 mL) was mixed with DPPH solution in ethanol (0.5 mL, 0.1 mM). The reaction mixture was shaken vigorously and allowed to stand in the dark for 30 min at room temperature, after which the absorbance at 517 nm was measured. To calculate the DPPH radical scavenging activity, trolox standards at different concentrations (0–0.08 mM) were used.

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