Phenolic compounds and antioxidant activity of Brazil nut (Bertholletia excelsa)

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ABSTRACT

Brazil nuts were shelled and separated as kernel and brown skin; whole nuts were also used. Soluble phenolics from each portion as well as the whole nut were extracted using 70% acetone under reflux conditions. Insoluble-bound phenolics were subsequently extracted into diethyl ether–ethyl acetate mixture (1:1, v/v) after alkaline hydrolysis. Both soluble and insoluble-bound phenolic extracts were separately examined for their total phenolics content; antioxidant activities were evaluated by trolox equivalent antioxidant capacity (TEAC), 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical and hydroxyl radical scavenging activities using electron paramagnetic resonance (EPR), reducing power, and oxygen radical scavenging capacity (ORAC). Soluble phenolics in brown skin was 1236.07 as compared to 406.83 in kernel and 519.11 mg/100 g in whole nut. Bound phenolics content of brown skin was also 86- and 19-folds higher than kernel and whole nut, respectively. Similarly extracts from the brown skin exhibited the highest antioxidant activity. Free- and bound phenolics were identified and quantified; these included nine phenolic acids and flavonoids and their derivatives (gallic acid, gallocatechin, protocatechuic acid, catechin, vanillic acid, taxifolin, myricetin, ellagic acid, and quercetin). However, some phenolics were present only in the bound form. Furthermore, the phenolics were dominant in the brown skin.

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1. Introduction

One of the most unexpected and novel findings in nutritional epidemiology relates to nut consumption and protection against ischemic heart disease, a leading cause of death in men and postmenopausal women worldwide (Sabaté, 1999). A diet rich in fruits, vegetables, nuts and minimally refined cereals is associated with a lower risk of chronic degenerative diseases. Since oxidative stress is common in chronic degenerative diseases, dietary antioxidants in plant sources may provide a beneficial effect. Antioxidants are known to protect cells against the damaging effects of reactive oxygen species (ROS) such as singlet oxygen, superoxide and hydroxyl radicals, among others. Nuts have been identified as being a rich source of antioxidants (Halvorsen et al., 2002; Wu et al., 2004). Phytochemicals, including phenolics (tannins, ellagic acid and curcumin), flavonoids (luteolin, quercetin, myricetin, kaempferol and resveratrol), isoflavones (genistein and daidzein), terpenes, and tocols are present in different nuts (Bravo, 1998; Kris-Etherton et al., 2002). Phenolic compounds have attracted considerable attention in the past few years due to their many potential health benefits. They have been demonstrated to render antibacterial, antiviral, ant carcinogenic, anti-inflammatory and vasodilatory actions (Breinholt, 1999; Duthie et al., 2000; Shahidi & Naczk, 1995, 2004).

Phenolic compounds in nuts are present in the free, esterified or bound forms. Phenolic acids may form both ester and ether linkages owing to their bifunctional nature through...
reactions involving their carboxylic and hydroxyl groups, respectively, which allows phenolic acids to form cross-links with cell wall macromolecules (Yu et al., 2001). Bound phenolics may be released by alkali, acid or enzymatic treatment of samples before extraction (Andreasen et al., 2001a; Krygier et al., 1982a,b). In mammalian intestine, microbial esterase present can release hydroxyxynamic acids bound to plant cell wall into the lumen and free acids and their metabolites can be absorbed into the circulatory system (Andreasen et al., 2001b). Moreover, the unabsorbed phytochemicals may act extracellularly in the protection of the gastrointestinal tract suggesting a potential role in colorectal cancer prevention (Larsson et al., 2005). Naczk and Shahidi (1989) took into account the bound phenolics content while determining the total phenolic content of canola meal; the contribution of bound phenolics to the total amount was about 20%.

Plant phenolics serve as antioxidants by virtue of the hydrogen-donating properties of their phenolic hydroxyl groups (Lindsay and Astley, 2002), as well as by donating electrons to stop free radical chain reactions (Bajpai et al., 2005). Almonds contain flavonoids including catechins, flavonols and flavonones in both aglycone and glycoside forms (Sang et al., 2002; Wijeratne et al., 2006). Hazelnuts contain different phenolic acids such as gallic acid, caffeic acid, p-coumaric acid, ferulic acid and sinapic acid in both free and esterified forms (Shahidi et al., 2007) while walnuts contain a wide variety of phenolics such as tocopherols and non-flavonoids such as ellagitannins (Anderson et al., 2001). Peanuts and pistachios have several flavonoids and are rich in resveratrol (Lou et al., 2001), while alkylphenols exist in abundance in cashew nuts (Trevisan et al., 2006). Among the tree nuts, Brazil nut kernel and by-products are least studied with respect to their antioxidant activity and phenolic constituents. Brazil nuts (Bertholletia excelsa) are produced by a large tropical forest tree in the family Lecythidaceae that grows throughout the Amazon Basin of South America. It is an angular nut with a very hard shell; the kernel is very white and covered with dark brown skin. Brazil nut skin and hard shell are by-products of cracking and shelling process. However, these by-products can serve as potential sources of natural antioxidants and functional food ingredients. The nuts are nutrient-dense sources of protein, fibre, selenium, magnesium, phosphorous, and thiamine. Brazil nut flesh consists of 70% lipid and 17% protein. There is also a substantial amount of niacin, vitamin E, vitamin B6, calcium, iron, potassium, zinc, copper, arginine and flavonoids and it is the richest natural source of selenium at 126 ppm in the cake (Chunhieng et al., 2004). There have been studies showing the direct connection between selenium levels and prostate cancer (Ip and Lisk, 1994; Klein et al., 2003), thus indicating that Brazil nut could serve as a major and new nutraceutical ingredient.

Although there are a considerable number of descriptive literatures available on the ecology and phenology of the Brazil nut tree and the biochemical composition of its nut (Chunhieng et al., 2004; Collinson et al., 2000; Mori, 1992), little has been documented about the phenolic content and antioxidant activity of Brazil nut kernel and its by-products. Thus, the aim of this study was to gain further insight into the phenolic profile of the Brazil nut extracts from kernel, whole nut and brown skin and to evaluate their antioxidant and free radical scavenging activities, thus establishing them as a potential natural source of antioxidants which can potentially be used as functional food ingredients. The importance of the brown skin on the antioxidant activity of the nut has also been investigated which will help better understand the antioxidant activity of Brazil nuts with or without their brown skin.

2. Materials and methods

2.1. Materials

Whole Brazil nuts were purchased from Sobey’s grocery store in St. John’s, NL, Canada; they were shelled and separated as whole nuts, kernel and brown skin in the laboratory. Samples were prepared as described in a later section.

Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was purchased from Acros Organics (Fair Lawn, NJ). Reagents sodium carbonate, hexane, methanol, acetone and ethanol were purchased from Fisher Scientific Co. (Nepean, ON, Canada). Ferrous sulphate, mono- and dibasic sodium phosphate, 2,2’-azobis (2-methylpropionamidine) dihydrochloride (AAPH), 2,2’-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), Folin and Ciocalteau’s phenol reagent, hydrogen peroxide, ferric chloride, mono- and dibasic sodium and potassium phosphates, ascorbic acid, 5,5-dimethyl-1-pyrroline-N-oxide (DMPO), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and standards for HPLC analyses, namely gallic acid, galocatechin, catechin, vanillic acid, protocatechuic acid, taxifolin, ellagic acid and quercetin were purchased from Sigma-Aldrich Canada Ltd. (Oakville, ON).

2.2. Preparation of crude phenolic extracts

Whole Brazil nut, kernel and brown skin were collected separately and ground in a coffee grinder (Model CBGS series, Black and Decker Canada Inc., Brockville, ON) for 5 min and then defatted by blending with hexane (1:5 w/v, 5 min × 3) in a Waring blender (Model 33BL73, Waring Products Division, Dynamics Corp. of America, New Hartford, CT) at an ambient temperature of 22 °C. The resulting mixture was filtered through a Whatman #1 filter paper under suction in a Buchner funnel. Defatted samples were air dried for 12 h and stored in vacuum packaged polyethylene pouches at –20 °C until used for analysis. Phenolic compounds present in the defatted samples were extracted using 80% ethanol, 80% methanol and 70% acetone (6 g sample/100 ml of solvent) under reflux condition in a thermostated water bath at 60 °C for 40 min. Preliminary studies were conducted using the three solvents mentioned above and the best solvent was subsequently used for all further studies. The resulting slurries were centrifuged at 4000 g (ICE Centra MS, International Equipment Co., Needham Heights, MA) for 5 min and the supernatants collected. The residue was re-extracted under the same conditions, the supernatants combined and desolvated under vacuum at 40 °C and the resulting slurry which contained soluble phenolics was lyophilized for 72 h at −48 °C and 46 × 10⁻³ mbar (Freezone 6, model 77530, Labanco Co., Kansas City, MO). The freeze dried portion represented the soluble phenolics and was stored at –20 °C until used for analysis, within a two months period.
The bound phenolic acids in the residue obtained after separating the supernatant were isolated according to the procedure described by Krygier et al. (1982a). The extracted bound phenolics were dissolved in methanol and stored at −20 °C until used.

2.3. Total soluble and bound phenolics content (TPC)

Lyophilized soluble and bound phenolics extracts of Brazil nut were dissolved in methanol separately and diluted appropriately. The total soluble and bound phenolics contents were determined according to a modified version of the procedure described by Singleton and Rossi (1965) using the Folin and Ciocalteu’s phenol reagent (Wijeratne et al., 2006). The content of total soluble and bound phenolics was determined using a standard curve prepared for gallic acid and expressed as mg gallic acid equivalents (GAE)/g defatted meal or 100 g sample.

2.4. Total antioxidant capacity by trolox equivalent antioxidant capacity (TEAC) assay

TEAC assay is based on scavenging of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonate) radical anion (ABTS\(^{−}\)) and was determined according to the method described by Van den Berg et al. (1999) and modified by Siriwardhana and Shahidi (2002). All solutions were prepared in a 0.1 M phosphate buffer (pH 7.4) solution containing 0.15 M sodium chloride (PBS). A solution of ABTS radical anion (ABTS\(^{−}\)) was prepared by mixing 2.5 mM AAPH with 2.0 mM ABTS\(^{−}\) stock solution at 1:1 (v/v) ratio. The solution was heated for 12 min at 60 °C, protected from light, and stored at room temperature. To measure the antioxidant capacity, soluble and bound phenolics extracts of Brazil nut were dissolved in PBS solution and diluted so as to fit in the range of the assay values. A standard curve was prepared by measuring the reduction in absorbance of the ABTS\(^{−}\) solution at different concentrations of trolox over a period of 6 min. To determine the TEAC values for the Brazil nut extracts, 40 μL of the samples was mixed with 1.96 mL of ABTS\(^{−}\) solution. Absorbance of the above mixture was measured at 734 nm after 6 min because the extracts needed a minimum of 6 min in order to complete the reaction. The decrease in absorption at 734 nm after 6 min of addition of a test compound was used for calculating TEAC values. Blank measurements of ABTS\(^{−}\) stock solution were also made. TEAC values were expressed as micromoles of trolox equivalents (TE) per gram of defatted material. TEAC values were determined as follows:

\[
\Delta A_{\text{Troxol}} = A_{\text{Troxol solution after 6 min}} - A_{\text{blank after 6 min}}
\]

\[
\Delta A_{\text{extract}} = A_{\text{extract after 6 min}} - A_{\text{blank after 6 min}}
\]

\[
\Delta A_{\text{Troxol}} = m \times [\text{Troxol}]
\]

\[
\text{TEAC}_{\text{extract}} = \frac{(\Delta A_{\text{extract}}/m) \times d}{\text{TEAC}_0}
\]

Where, \(\Delta A\) is reduction of absorbance, \(A\) is absorbance at a given time, \(m\) is slope of the standard curve, [Troxol] is concentration of trolox, \(d\) is dilution factor.

2.5. DPPH radical scavenging activity using electron paramagnetic resonance (EPR)

The DPPH radical scavenging activity was determined using EPR spectroscopy according to a modified version of the procedure described by Madhujith and Shahidi (2006). Two milliliters of 0.125 mM solution of DPPH in methanol were added to 500 μL of appropriately diluted soluble and bound phenolics extracts in methanol. Catechin, which is generally used as a reference antioxidant, was also analyzed in this work. Contents were mixed well, and after 10 min, the mixture was passed through a capillary tube, which guides the sample through the sample cavity of a Bruker E-scan EPR spectrometer (Bruker Biospin Co., Billercia, MA). The parameters of the EPR spectrometer were set as follows: 5.02 × 10^2 receiver gain, 1.86 G modulation amplitude, 2.621 s sweep time, 8 scans, 100,000 G sweep width, 3495.258 G centre field, 5.12 ms time constant, 9.795 GHz microwave frequency, 86.00 kHz modulation frequency and 1.86 G modulation amplitude. DPPH radical scavenging capacities of the extracts were calculated using the following equation:

\[
\text{DPPH radical scavenging capacity} (\%) = 100 - \frac{\text{EPR signal intensity of extracts}}{\text{EPR signal intensity of control}} \times 100
\]

From the standard curve plotted for the DPPH radical scavenging capacity of catechin, the scavenging activity of soluble and bound phenolics was determined and expressed as μmol of catechin equivalents (eq)/g defatted meal.

2.6. Hydroxyl radical scavenging capacity using EPR

Hydroxyl radical was generated via Fe (II)-catalyzed Fenton reaction and spin-trapped with DMPO. The resultant DMPO-OH adduct was detected using a Bruker E-scan EPR (Bruker Biospin Co., Billercia, MA). Brazil nut extracts were dissolved in deionized water and appropriately diluted. Catechin was used as a reference antioxidant. Extracts (100 μL) were mixed with 100 μL of 10 mM H₂O₂, 200 μL of 17.6 mM DMPO, and 100 μL of 0.1 mM FeSO₄. After 1 min, the EPR spectrum of the mixture was recorded at 5.02 × 10^2 receiver gain, 1.86 G modulation amplitude, 2.621 s sweep time, 8 scans, 100,000 G sweep width, 3495.258 G centre field, 5.12 ms time constant, 9.795 GHz microwave frequency, 86.00 kHz modulation frequency, and 1.86 G modulation amplitude. Hydroxyl radical scavenging capacities of the extracts were calculated and expressed as mg catechin eq/g defatted meal, using the following equation.

\[
\text{Hydroxyl radical scavenging capacity} (\%) = 100 - \frac{\text{EPR signal intensity of extracts}}{\text{EPR signal intensity of control}} \times 100
\]

2.7. Reducing power

The reducing power of the extracts was determined as described by Amarowicz et al. (2002). A standard curve for ascorbic acid was constructed and results were expressed as μmol of ascorbic acid eq/g defatted meal.

2.8. Oxygen radical absorbance capacity (ORAC)

The ORAC assay was carried out in accordance with the method proposed by Madhujith and Shahidi (2007) with slight modifications. ORAC₅T was determined using a Fluostar
OptiMate plate reader (BMG Labtech, Durham, NC) equipped with an incubator and two injector pumps with fluorescein as the probe and AAPH as the radical generator. The reaction was carried out in 75 mM phosphate buffer (pH 7.0) medium using a 96-well Costar 2650 black plate (Nepean, ON, Canada). To each well, 20 μL of soluble or bound phenolic extracts were added followed by 200 μL of fluorescein (0.11 μM in PBS). The mixture was incubated for 15 min at 37°C in the built-in incubator of the FluoStar Optima plate reader. The microplate reader was programmed to inject 75 μL of AAPH (17.2 mg/mL in PBS) into each well, yielding a final well volume of 295 μL. The plate was shaken for 4 s after each addition at a 4 mm shaking width. Fluorescence was determined and recorded every minute for 25 cycles (cycle time, 210 s). Results were calculated using the differences in the area under the fluorescein decay curve between the blank and the sample and expressed as μmol TE/g of defatted material. TE was calculated using a standard curve prepared with trolox. A blank was also run using only phosphate buffer and fluorescein. Excitation filter for an excitation wavelength of 485 nm and emission filter for an emission wavelength of 520 nm were used.

### 2.9. Extraction, identification and quantification of phenolic compounds

Free and bound phenolic compounds were extracted, identified and quantified using high performance liquid chromatography with photodiode array detection (HPLC-DAD) and electrospray ionization mass spectrometry (HPLC-ESI-MS/MS) according to the method of Alasalvar et al. (2006). Phenolic compounds were analyzed using an Agilent 1100 Series HPLC system (Agilent Technologies, Palo Alto, CA) equipped with a quaternary pump (model G1311A), a degasser (model G1379A), an autosampler (automatic liquid sampler, ASL, model G1329A) and a diode array detector (model G1315B DAD) linked to an HP-ChemStation data handling system. Twenty-five microlitres of the sample extracts were automatically injected into a precolumn Supelcosil™ LC-18 column (250 × 4.6 mm inner diameter, 5-μm particles, Supelco, Bellefonte, PA) at room temperature. A gradient profile using two solvents was applied at room temperature (Wijeratne et al., 2006), with solvent A: 5% aqueous formic acid and solvent B: acetonitrile/methanol (5:95, v/v) and a flow rate of 0.8 mL/min. The gradient was as follows: 0 min – 15% B; 7 min – 31% B; 14 min – 47% B; 21 min – 63% B; 25 min – 85% B; 32 min – 95% B. The wavelengths of the diode array detector (DAD) were set at 260, 280 and 320 nm for monitoring phenolic compounds. Tentatively identified phenolic compounds were quantified on the basis of their peak areas and comparison with a calibration curve obtained with the corresponding standards.

For HPLC–MS analysis, an Agilent 1100 SL LC/MSD ion trap mass spectrometer (Agilent Technologies, Palo Alto, CA) was connected to the Agilent 1100 HPLC system via an ESI interface in the negative ion detection mode. The MS revealed the negative molecular ions; MS–MS broke down the most abundant one with dependent collision-induced dissociation. The selected values for spray chamber parameters were as follows: capillary potential, 3500 V; gas temperature, 350°C; drying gas flow, 13 L min⁻¹; nebulizer pressure, 414 kPa. For full scan MS analysis, the spectra were recorded in the range of m/z 50–700. Identification of compounds by HPLC–MS analysis was carried out by comparing retention times and mass spectra of the unknown peaks to those of the standards.

### 2.10. Statistical analysis

Statistical analysis was performed using SigmaStat version 10.0 (Jandel Corp., San Rafael, CA). Results were subjected to ANOVA, and differences between means were located using Tukey’s test. Significance was determined at the p < 0.05 level. Results were expressed as mean ± SD (n = 3) for each extract.

### 3. Results and discussion

#### 3.1. Selection of solvent for preparation of crude phenolic extracts

Preliminary studies conducted to determine the best solvent among 80% ethanol or methanol and 70% acetone showed that the highest yield of extract from Brazil nut kernel was obtained when 70% acetone was used (15.60 g/100 g defatted meal), followed by 80% ethanol (14.56 g/100 g defatted meal) and 80% methanol (12.44 g/100 g defatted meal) (Table 1). The total soluble phenolic contents of methanolic, ethanolic and acetonic extracts were 11.99 ± 0.52, 13.67 ± 0.44 and 14.73 ± 0.71 mg GAE/g defatted meal, respectively (Table 1). The corresponding values on the basis of 100 g kernel are also given in Table 1. The acetonic extracts had significantly (p < 0.05) higher soluble phenolics content than methanolic and ethanolic extracts (Table 1). The higher extract yield using acetone as extraction solvent also resulted in a higher soluble phenolics content. Similarly, Brazil nut brown skin was extracted with 80% methanol and 70% acetone. It was found that the yield of extract was higher with acetone. The total soluble phenolics content of the acetonic extract was also significantly (p < 0.05) higher than in methanolic extract (Table 1). DPPH radical scavenging capacity of

### Table 1 – Yield and total soluble phenolics in different solvent extracts of Brazil nut kernel and brown skin.

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Yield 2</th>
<th>Total soluble phenolics in defatted meal 3</th>
<th>Total soluble phenolics in 100 g sample 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kernel Methanol extract</td>
<td>12.44</td>
<td>11.99 ± 0.52 b</td>
<td>331.39 ± 21.51 a</td>
</tr>
<tr>
<td>Ethanol extract</td>
<td>14.56</td>
<td>13.67 ± 0.44 b</td>
<td>377.42 ± 18.11 a</td>
</tr>
<tr>
<td>Acetone extract</td>
<td>15.60</td>
<td>14.73 ± 0.71 c</td>
<td>406.83 ± 29.28 b</td>
</tr>
<tr>
<td>Brown skin Methanol extract</td>
<td>9.85</td>
<td>17.15 ± 0.62 d</td>
<td>1140.35 ± 81.83 c</td>
</tr>
<tr>
<td>Acetone extract</td>
<td>11.39</td>
<td>18.60 ± 0.64 a</td>
<td>1236.07 ± 63.84 b</td>
</tr>
</tbody>
</table>

1 Data are expressed as means ± SD (n = 3). Means ± SD followed by the same letter within a column are not significantly different (p > 0.05).

2 Yield of soluble phenolics extract, expressed as g/100 g defatted meal.

3-4 Total soluble phenolics content, expressed as mg GAE/g defatted meal and mg GAE/100 g sample, respectively.
the three soluble phenolics extracts of Brazil nut kernel was also determined using the EPR method. DPPH radical has widely been used in model systems to investigate the scavenging activities of antioxidative compounds. All three extracts exhibited a strong antioxidant activity against DPPH radical in a concentration-dependent manner. The IC50 value is defined as the amount of extract (milligrams per milliliter) required to lower the initial DPPH radical concentration by 50%, and this was extrapolated from the dose-dependent curves. The IC50 values obtained for methanolic, ethanolic and acetonic extracts using EPR were 0.921 ± 0.050, 0.904 ± 0.072 and 0.919 ± 0.048 mg/mL, respectively. There was no significant (p > 0.05) difference between the samples. The reducing power of the acetic acid and methanolic extracts of brown skin was also determined and found to be 59.20 ± 1.19 and 22.60 ± 0.23 μmol of ascorbic acid equivalents/g defatted meal, respectively; the values for the acetic acid extract being significantly (p < 0.05) higher than those for the methanolic extract. Based on these preliminary studies, acetone was selected as the extraction solvent for the preparation of crude phenolic extracts in all subsequent studies.

A similar study to determine the best solvent for extraction of phenolic compounds from lentil seeds was carried out by Amarowicz et al. (1995) using 80% acetone, methanol and ethanol at 80 °C. Acetone extracted markedly greater amounts of phenolic compounds compared with methanol or ethanol systems. TLC analysis of the acetonic extract also revealed the presence of tannins of higher molecular weight which were not found in ethanolic and methanolic extracts, thus clearly indicating that acetone is able to extract condensed tannins (proanthocyanidins) and hence the higher yield of total phenolics in acetic acid in the present study. Jaroszyńska (2003) studied the influence of several solvents, namely diethyl ether, acetone, methanol, chloroform and water on the recovery of phenolic compounds during extraction from plant materials and noted that the highest recovery was achieved with methanol and acetone. Similarly, a higher concentration of total phenolics in almond seeds using 80% (v/v) acetone extract was obtained (Amarowicz et al., 2005). However, 80% ethanol appeared to be the best extraction solvent in other studies carried out for almonds (Wijeratne et al., 2006) and hazelnuts (Shahidi et al., 2007).

3.2. Yield and total soluble and bound phenolics content of Brazil nut extracts

The yields of total soluble phenolics extract from Brazil nut kernel, whole nut and brown skin were 15.60, 16.36 and 11.39 g/100 g defatted meal, respectively (Table 2). The soluble phenolics content of the brown skin was found to be significantly (p < 0.05) higher (1236.07 mg GAE/100 g) than that of the whole nut (519.11 mg GAE/100 g) and kernel (406.83 mg GAE/100 g). Similarly, bound phenolics content as mg GAE/100 g of kernel was 46.2 and 123.1 mg GAE/100 g nuts, respectively, which is significantly (p < 0.05) higher than that of the methanolic extract. Studies related to the bound phenolics in wheat, also showed that the bound phenolics than the kernel or whole nut. Studies related to the bound phenolics in wheat, also showed that the bound phenolics content of hard wheat bran was 5- and 34-times higher than those in hard whole wheat and hard wheat flour, respectively (Liyana-Pathirana & Shahidi, 2006). This clearly demonstrates that the bound phenolics are concentrated in the outermost parts of the seed or nut and validates the higher levels of bound phenolics obtained in the Brazil nut brown skin in comparison to the kernel or whole nut in the present study.

3.3. Total antioxidant capacity (TAC) of extracts

The yield of Brazil nut extracts were higher than those reported in literature for hazelnut kernel (2.26 g/100 g of defatted samples), skin (10.28 g/100 g of defatted samples) and brown skin (8.00 g/100 g defatted sample) (Shahidi et al., 2007; Wijeratne et al., 2006). Wu et al. (2004) reported the total phenolic content and antioxidant activity of 100 different kinds of foods as a part of the USDA National Food and Nutrient Analysis Program. They found that the total phenolics content of Brazil nuts as 3.10 ± 0.96 mg GAE/g nuts which is similar to the results obtained for whole Brazil nut in the present study. They also showed that pistachios had the highest content of total phenolics, followed by walnuts, pecans, hazelnuts, almonds, peanuts and Brazil nut; pine nuts having the least amount. There were no studies in the literature relating to the total phenolics content and antioxidant activity of the Brazil nut kernel, whole nut and brown skin separately. However, Yang et al. (2009) reported the free and bound phenolics content of nine tree nuts including Brazil nut. The free and bound phenolics of the Brazil nut was found to be 46.2 and 123.1 mg GAE/100 g nuts, respectively, which show a different pattern from our data. The main reason may be that both agronomic and environmental factors play important roles in the phenolic composition (Tomás-Barberán et al., 2001).

Li et al. (2006) reported the bound phenolics content of walnuts and Campbell heartnut as 275 and 194 mg GAE/100 g nut. These are much higher values than those obtained for Brazil nut in the present study. Studies by Blomhoff et al. (2006) on the antioxidant activity of 11 different nuts with and without pellicle (skin) clearly showed that nuts with pellicle had higher antioxidant capacity in terms of TEAC and ferric reducing antioxidant power (FRAP). The higher antioxidant activity of the pellicle can be associated with a higher content of phenolics, and this agrees with the present results where the bound skin of Brazil nut contained higher amounts of bound phenolics than the kernel or whole nut. Studies related to the bound phenolics in wheat, also showed that the bound phenolics content of hard wheat bran was 5- and 34-times higher than those in hard whole wheat and hard wheat flour, respectively (Liyana-Pathirana & Shahidi, 2006). The total antioxidant activity, given as TEAC values of soluble phenolics extracts of Brazil nut brown skin was 18.9 and 5.2 times greater than the kernel and whole nut samples, respectively. Wu et al. (2004)
reported the total antioxidant capacity of Brazil nut as 14.2 μmol of TE/g sample which is similar to those in the present study where TEAC of soluble phenolics extract of whole Brazil nut was 11.8 μmol of TE/g whole nut. Similarly, it is also clear from Table 3 that TEAC of bound phenolics from brown skin was significantly (p < 0.05) higher than that from kernel and whole nut. TEAC value of brown skin bound phenolics was 31.8 and 8.6 times higher than kernel and whole nut bound phenolics, respectively. The higher content of bound phenolics in the brown skin could clearly explain its higher antioxidant activity when compared to the kernel or whole nut. It is also clear that the soluble phenolic extracts had higher antioxidant activity than bound phenolics, which may also be due the higher content of soluble phenolics in all the fractions of the Brazil nut. Similar results were obtained by Siriwardhana and Shahidi (2002) who evaluated the antioxidant activity of soluble phenolics extracts of almonds and its by-products and found that TEAC values of brown skin extracts and green shell cover extracts were 13 and 10 times greater than that of seed extract, respectively. Shahidi et al. (2007) also found that the TEAC values of hazelnut by-product extracts were approximately 4–5 folds greater than that of hazelnut kernel at the same extract concentration.

Pellegrini et al. (2006) have reported the TEAC values of bound phenolics from 6 nuts, namely almonds, hazelnuts, peanuts, pinenuts, pistachios and walnuts as 4.12, 5.33, 3.00, 3.15, 24.07 and 17.11 mmol trolox equivalents (TE)/kg nut. These values are higher than those obtained for bound phenolics of Brazil nuts (2.17 mmol TE/kg nut). The difference in values may be due to the existing differences in experimental conditions and hence TEAC values of the bound phenolics of Brazil nut in this study cannot be conclusively compared with those obtained for other nuts. However, Blomhoff et al. (2006) reported that the total antioxidant capacity (TAC) of Brazil nut with pellicle was 1.3 times more than those without pellicle. A significant portion of nut antioxidants is therefore located in the pellicle, in support of the earlier findings.

### 3.4. DPPH radical scavenging activity of extracts

The DPPH radical has widely been used in model systems to investigate the scavenging activities of antioxidative compounds. DPPH is more stable than hydroxyl and superoxide radicals, which makes its use advantageous in evaluating antioxidant activities (Siriwardhana & Shahidi, 2002). The soluble and bound phenolic extracts of the brown skin exhibited higher DPPH radical scavenging activity in terms of catechin equivalents than those of the kernel and whole nut, as shown in Table 3. The EPR spectra obtained for bound phenolics from kernel, whole and brown skin clearly demonstrates the difference in their DPPH radical scavenging activity (Fig. 1). Bound phenolics extracted from 180 mg defatted kernel meal; 43 mg defatted whole nut meal and 1.3 mg defatted brown skin meal showed DPPH scavenging activity of 19.65, 56.39 and 32.68%, respectively. However, it is also clear that the DPPH radical scavenging activity of the soluble phenolic extracts were better than the bound phenolic extracts in all fractions, which may be due to their relatively higher concentration in each (Table 3). The phenolic compounds present may have acted as free radical scavengers by virtue of their hydrogen-donating ability (Shahidi et al., 2007) and as soluble phenolic compounds are better assimilated in the body, the higher radical scavenging activity of the soluble phenolics in the Brazil nut may also be an indication of their better activity in vivo.

The results shown above indicate that the skin extract scavenges organic free radicals more effectively than the other Brazil nut extracts. Similar results were also obtained by Siriwardhana and Shahidi (2002) who evaluated the antiradical activity of different almond extracts and found that 210, 50 and 120 ppm of almond seed, skin and shell soluble phenolics extracts, respectively, were needed to completely scavenge DPPH radicals. Thus, almond skin was the most effective DPPH radical scavenger amongst the almond products examined. Similarly, Alasalvar et al. (2006) demonstrated that the hazelnut green leafy cover scavenged organic free radicals more effectively than seed/kernel extracts. As proposed by Blois (1958), hydrogen donation from an antioxidant involves the decolouration of DPPH radical which was observed upon the addition of higher concentrations of extracts in the present study and this confirms the hydrogen-donating ability of Brazil nut extracts.

### 3.5. Hydroxyl radical scavenging activity of extracts

The iron-dependent decomposition of hydrogen peroxide produces hydroxyl radical, a highly reactive and biologically toxic species, via the Fenton reaction. This reductive cleavage of \( \text{H}_2\text{O}_2 \) is considered as the main source of hydroxyl radical in vivo, and is a major mechanism of biological damage (Mao et al., 1996). However, hydroxyl radicals have exceptionally short lifetime (10⁻⁹ s), which practically prevents their direct measurement (Bačić et al., 2008). To overcome this
The generated hydroxyl radicals were spin-trapped with DMPO, a relatively stable free radical that can be easily detected with EPR. The intensity of the characteristic 1:2:2:1 quarter with a hyperfine coupling constant of 14.9 G (Yen & Chen, 1995) diminished with the addition of the extract. This could be due to either scavenging of the hydroxyl radical by the extract, chelation of Fe (II) by the extract, or most likely a combination of both. The EPR spectra obtained for bound phenolics extracted from Brazil nut kernel, whole nut and brown skin are depicted in Fig. 1, showing the difference in their hydroxyl radical scavenging activity. Both soluble and bound phenolics from brown skin had the highest activity in terms of μmol of catechin eq/g defatted meal, followed by kernel and whole nut (Table 3). However, it was interesting to find that the both the soluble and bound phenolic extracts of the kernel had better hydroxyl radical scavenging activity than whole nut, which is contradictory to the results obtained in other antioxidant activity tests.

The better hydroxyl radical scavenging capacity kernel extracts may be related to its selenium content. Zhao et al. (2004) observed that protein extracts from selenium enriched mushroom species exhibited strong DNA protective effects against oxidative damage, which increased with an increase in the selenium content, indicating that selenium plays an important role in the antioxidant activities of the protein extract. The spin trapping experiment using EPR also showed that Se-enriched samples had higher activities for scavenging superoxide and hydroxyl radicals than corresponding samples devoid of selenium. Similarly, glutathione peroxidase, which is a natural antioxidant enzyme, contains selenium and helps prevent the formation of the highly destructive hydroxyl radicals (Barhoumi et al., 1997). Among foodstuff, Brazil nut has been reported to contain the highest known level of selenium (Reilly, 1999). The selenium in Brazil nuts is readily absorbed by the gastrointestinal tract and is present in the organic form as S-containing amino acids, especially as selenocysteine and selenomethionine. Addition of Brazil nuts to the diet has been shown to result in a significant increase in blood levels of selenium and of the activity of selenium-dependent enzyme glutathione peroxidase. Moreover, as explained in a later section, it has been demonstrated in the present study through HPLC analysis that citric acid is present in the kernel and whole nut bound phenolics extract, but is absent in the brown skin extract. Citric acid may be present as a selenium metal ligand and may also have a role in the hydroxyl radical scavenging activity. Thus, selenium and citric acid content may be responsible for the higher hydroxyl radical scavenging capacity of the kernel, while the phenolic constituents that may have better antioxidant activities can explain the highest scavenging activity in the brown skin.

### 3.6 Reducing power of extracts

The reducing power of soluble and bound phenolic extracts of Brazil nut kernel, whole nut and brown skin in terms of μmol of ascorbic acid equivalents/g of defatted meal is shown in Table 3. The reducing power of the brown skin soluble phenolics extract was superior and was found to be 5.8 and 2.4 times higher than those from kernel and whole nut,
Fig. 1 – Electron spin resonance spectra showing the effect of addition of bound phenolics extracts from Brazil nut kernel, whole nut and brown skin on scavenging of DPPH (A) and OH (B). Bound phenolics extracted from 180 mg defatted kernel meal (1); 43 mg defatted whole nut meal (2) and 1.3 mg defatted brown skin meal (3) showed DPPH scavenging activity of 19.65, 56.39 and 32.68%, respectively. Bound phenolics extracted from 1.8 mg defatted kernel meal (4); 1.72 mg defatted whole nut meal (5) and 1.03 mg defatted brown skin meal/ml (6) showed hydroxyl radical scavenging activity of 79.36, 50.23 and 79.62%, respectively.
respectively. The brown skin bound phenolics also exhibited reducing powers 190 and 11 times higher than those from kernel and whole nut, respectively. Similar to the other antioxidant activity assays, the soluble phenolic extracts exhibited better reducing power than the bound phenolic extracts in all samples tested, possibly due to their relatively higher content. The phenolics present in the extracts displayed a considerable reducing power, primarily due to their effect as electron donors and thereby suppressing radical chain reactions by converting free radicals to more stable products. Thus, reducing activity leads to termination of the radical chain reactions that may otherwise be very damaging (Alasalvar et al., 2006). In this assay, the yellow colour of the test solution changed to various shades of green depending on the reducing power of the extract (brown skin had the highest reducing power and showed the darkest green colour for the test solution).

In an experiment with hazelnut kernel (Hke) and hazelnut green leafy cover (HGLC), it was found that HGLC had higher reducing power than the Hke extract (Alasalvar et al., 2006). Though there are no references in the literature for the reducing power of bound phenolics in nuts, Blomhoff et al. (2006) analyzed the ferric reducing antioxidant power (FRAP) of nuts with and without the pellicle. It has clearly been demonstrated that nuts with pellicles have higher FRAP values than those without any pellicle. In the present study the phenolics in the brown skin of Brazil nut displayed a high reducing power as well.

### 3.7. Oxygen radical absorbance capacity (ORACFL)

The ORACFL assay is among the standard and widely used assays accepted for measuring the antioxidative activity of botanicals, herbs and nutraceuticals (Madhujith & Shahidi, 2007). The ORAC assay uses peroxyl radical, a biologically relevant radical source, and it combines both inhibition time and degree of inhibition into one quantity (Dávalos et al., 2004). ORAC values for soluble and bound phenolics of Brazil nut kernel, whole nut and brown skin as μmol of TE/g defatted meal are shown in Table 3. The ORAC values of soluble phenolics from kernel and whole nut were similar (p > 0.05), while the brown skin had a significantly (p < 0.05) higher value (Table 3). ORAC values of bound phenolics in kernel, whole nut and brown skin of Brazil nut were recorded as 1.36 ± 0.05, 23.43 ± 1.27 and 168.35 ± 11.27 μmol of TE/g of defatted meal, with values for brown skin significantly (p < 0.05) higher than those for kernel and whole nut. In all the samples tested, the soluble phenolics demonstrated higher ORAC values than the bound phenolics, which may be due to their relatively higher abundance and which may again indicate their better activity in vivo.

Some studies have demonstrated a linear relationship between the content of total phenolics (TP) and antioxidant

### Table 4 – Free and bound phenolics identified in Brazil nut using HPLC–ESI(−)-MS analyses.

<table>
<thead>
<tr>
<th>Tentatively identified compounds</th>
<th>Peak no.</th>
<th>Rt (min)</th>
<th>[M–H] (m/z)</th>
<th>Fragments (m/z)</th>
<th>Concentration (µg/g defatted meal)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Free phenolics</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>K&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Citric acid</td>
<td>1</td>
<td>3.2</td>
<td>191</td>
<td>111, 173</td>
<td>*</td>
</tr>
<tr>
<td>Gallocatechin</td>
<td>2</td>
<td>3.4</td>
<td>305</td>
<td>125, 179</td>
<td>–</td>
</tr>
<tr>
<td>Gallic acid</td>
<td>3</td>
<td>4.8</td>
<td>169</td>
<td>125</td>
<td>23.30</td>
</tr>
<tr>
<td>Protocatechuic acid derivative</td>
<td>4</td>
<td>6.4</td>
<td>–</td>
<td>153, 109, 124</td>
<td>–</td>
</tr>
<tr>
<td>Protocatechuic acid</td>
<td>5</td>
<td>7.3</td>
<td>153</td>
<td>109</td>
<td>–</td>
</tr>
<tr>
<td>Catechin</td>
<td>6</td>
<td>8.3</td>
<td>289</td>
<td>245, 205</td>
<td>–</td>
</tr>
<tr>
<td>Protocateualdehyde</td>
<td>7</td>
<td>9.2</td>
<td>137</td>
<td>109</td>
<td>–</td>
</tr>
<tr>
<td>Protocatechuic acid derivative</td>
<td>8</td>
<td>10.6</td>
<td>329</td>
<td>153, 109</td>
<td>–</td>
</tr>
<tr>
<td>Catechin derivative</td>
<td>9</td>
<td>11.7</td>
<td>–</td>
<td>289</td>
<td>–</td>
</tr>
<tr>
<td>Vanillic acid</td>
<td>10</td>
<td>12.3</td>
<td>167</td>
<td>123</td>
<td>0.13</td>
</tr>
<tr>
<td>Protocatechuic acid derivative</td>
<td>11</td>
<td>13.8</td>
<td>–</td>
<td>153, 109</td>
<td>–</td>
</tr>
<tr>
<td>Taxifolin</td>
<td>12</td>
<td>15.0</td>
<td>303</td>
<td>178, 285, 125</td>
<td>–</td>
</tr>
<tr>
<td>Protocatechuic acid derivative</td>
<td>13</td>
<td>16.2</td>
<td>314</td>
<td>270, 185, 153, 109</td>
<td>–</td>
</tr>
<tr>
<td>Unknown</td>
<td>θ</td>
<td>16.5</td>
<td>245</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Myricetin-3-ο-rhamnoside</td>
<td>14</td>
<td>17.8</td>
<td>463</td>
<td>317</td>
<td>–</td>
</tr>
<tr>
<td>Taxifolin derivative</td>
<td>15</td>
<td>18.4</td>
<td>–</td>
<td>303, 261</td>
<td>–</td>
</tr>
<tr>
<td>Ellagic acid derivative</td>
<td>16</td>
<td>18.8</td>
<td>447</td>
<td>301, 257, 229</td>
<td>–</td>
</tr>
<tr>
<td>Ellagic acid</td>
<td>17</td>
<td>19.4</td>
<td>301</td>
<td>257, 229</td>
<td>6.28</td>
</tr>
<tr>
<td>Gallic acid derivative</td>
<td>18</td>
<td>21.4</td>
<td>187</td>
<td>125, 169</td>
<td>–</td>
</tr>
<tr>
<td>Quercetin</td>
<td>19</td>
<td>24.0</td>
<td>301</td>
<td>179, 151</td>
<td>–</td>
</tr>
<tr>
<td>Vanillic acid derivative</td>
<td>20</td>
<td>30.1</td>
<td>329</td>
<td>167</td>
<td>*</td>
</tr>
<tr>
<td>Unknown</td>
<td>θ</td>
<td>31.2</td>
<td>265</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

*: compounds detected but concentration in sample not determined.
–: not detected.
<sup>a</sup>: Rt, retention time.
<sup>b</sup>: K, Brazil nut kernel.
<sup>c</sup>: WN, whole Brazil nut.
<sup>d</sup>: BS, Brazil nut brown skin.
capacity (Kaur & Kapoor, 2002). Wu et al. (2004) ranked raw food samples into four groups based on their ORACFL to TP ratios. This ratio in most foods fell in the range from 5 to 15. Values close to 10 indicated a strong positive linear correlation between TP and antioxidant capacity. They found no significant correlation between TP and antioxidant capacity, and concluded that measurement of TP alone may not be a good indicator of antioxidant capacity. In the present study, the ORACFL/TP ratios of soluble phenolics extracts of Brazil nut kernel, whole nut and brown skin were 5.08, 5.17 and 10.18, respectively. Samples with high antioxidant capacity tended to have higher ORACFL/TP ratios. This may be due to the presence of compounds with antioxidant activity that are not “phenolic” or some phenolic compounds being more “effective” than others or having a higher reactivity with peroxyl radicals. However, under normal reaction conditions, phenolic compounds are the predominant antioxidants in hydrophilic extracts of samples that easily transfer a hydrogen atom to the peroxyl radical (Wu et al., 2004).

3.8. HPLC analysis of free and bound phenolic compounds in Brazil nut extracts

The identity of soluble and bound phenolic compounds were ascertained using HPLC-DAD and HPLC-MS analyses and by comparison of their retention times and mass spectra with those of available standards and reports from the literature. Efficient separation of phenolics in Brazil nut samples was achieved using a reversed-phase Supelcosil™ LC-18 column. The phenolic compounds were quantified using regression equations from their respective standard curves. All other compounds tentatively identified were reported as detected but not quantified. Concentrations were expressed as μg/g defatted meal in Brazil nut kernel, whole nut or brown skin. Table 4 lists the compounds identified in the Brazil nut extracts from LC-DAD and LC–MS/MS analyses.

In the present study, we used LC–MS/MS in negative ionization mode to determine the conjugated forms of phenolic compounds. Electrospray ionization (ESI) is a gentle ionization method in mass spectrometry. It generates mainly deprotonated molecules [M–H]– of the compounds analyzed in the negative ion mode of ESI-MS (Gioacchini et al., 1996). When an ESI interface is connected to an HPLC system, these deprotonated molecules allow a rapid determination of the molecular mass of a compound directly after its elution from the HPLC column (Wolfender & Hostettmann, 1993). The use of HPLC–ESI-MS makes it possible to detect and quantify compounds with different mass, even if they were co-eluting in the HPLC-DAD. Figs. 2 and 3 show the LC-DAD chromatograms recorded at 260 nm for the soluble and bound phenolics, respectively, extracted from Brazil nut kernel, whole nut and brown skin.

Peak 1 (Figs. 2 and 3) with retention time (Rt) of 3.2 min was identified as citric acid. Though not a phenolic acid, it eluted as a prominent peak in the HPLC chromatogram of Brazil nut kernel and whole nut was absent in the brown skin. Citric acid was identified by comparing the data with a standard and in the LC–MS analysis it formed m/z 191 (M–H)– ion. The MS/MS spectra showed ions at m/z 111 and 173; the latter being the dehydrated form of m/z 191, in agreement with that reported by Ng et al. (2004). The presence of citric acid can be associated with the high concentration of selenium in Brazil.
There are a number of plants known as metal hyperaccumulators that accumulate heavy metal ions such as nickel, zinc, copper, cobalt and lead in unusually high concentrations in their shoots (Baker et al., 1999). It has been reported that the compounds involved in metal complexation in hyperaccumulators include organic acids (Lee et al., 1977; Sagner et al., 1998) and free histidine. Oven et al. (2001) observed an increase in citric acid when extracts of the cobalt hyperaccumulator, Crotalaria cobalticola cells were exposed to 10 mM CoCl₂ for 7 days. They suggested citric acid to be a possible cobalt ligand. Sagner et al. (1998) and Rauser (1999) also observed an increase in citric acid in metal accumulators. Brazil nuts have been reported to contain the highest known level of selenium of any foodstuff. Brazil nuts purchased in an US supermarket were found to contain an average of $36 \pm 50 \mu g/g$ of selenium, with the extraordinarily high level of $512 \mu g/g$ in an individual nut (Reilly, 1999). Though not reported earlier, the presence of citric acid in the Brazil nut samples in our study can be associated with its role as a possible metal ligand.

Peak 3 in Figs. 2 and 3 had UV₅max at 280 nm, with the HPLC retention time at 4.8 min. Although the detector sensitivity of HPLC–MS was lower for phenolic acids than those for other phenolic compounds (Tomas-Barberan et al., 2001), the m/z at 169 was detected in the ESI-MS negative mode with fragment of m/z 125. Compared with the standard, this compound was unambiguously identified as gallic acid. Similarly, peaks 2, 5, 6, 10, 12, 17 and 19 were identified as galloatechin, protocatechuic acid, catechin, vanillic acid, taxifolin, ellagic acid and quercetin, respectively, by comparison of their retention times and mass spectral data with those of the available standards.

Peaks 4, 7, 8, 11 and 13 in Figs. 2 and 3 were tentatively identified as derivatives of protocatechuic acid as they formed fragments of m/z 153, 109. Xu et al. (2007) have described the LC–MS analysis of different protocatechuic acid derivatives. Similarly, peak 9 which gave a fragment of m/z 289 was a derivative of catechin. Peak 14 produced an ion of m/z 463 with fragment of m/z 317 and was tentatively identified as myricetin-3-o-rhamnoside by comparison of its mass spectrum with those reported in MassBank.jp (2008). Peaks 15, 16, 18 and 20 which gave fragments of m/z 303, 301, 169 and 167, respectively, were identified as derivatives of taxifolin, ellagic, gallic and vanillic acids, respectively. Peaks with Rt 16.5 and 31.2 min released ions with m/z 245 and 265, respectively, but were not identified. The concentrations of the different phenolic compounds tentatively identified in the soluble and bound phenolics extract of Brazil nut kernel, whole nut and brown skin are shown in Table 4. The profiles of the free and bound phenolics showed a variation, with more number of compounds identified in the bound form. It is clear that the concentration of phenolics in the brown skin was much higher than those in the kernel and whole nut as also demonstrated earlier in this study.

To the best of our knowledge there are no reports on the phenolic compounds of Brazil nuts, though they can be found for other tree nuts. Shahidi et al. (2007) tentatively identified a total of five phenolic acids in hazelnut kernel and by-products, one of which was a hydroxylated derivative of benzoic acid (gallic acid) and four of which were cinnamic acid derivatives (caffeic acid, p-coumaric acid, ferulic acid, and sinapic acid). In addition, there were several unknown compounds in both free

Fig. 3 – HPLC chromatogram of bound phenolics extracted from Brazil nut samples (peak numbers refer to Table 4).
and esterified phenolic acids. Among the identified phenolic acids, p-coumaric acid was most abundant in hazelnut kernel, hazelnut green leafy cover, and hazelnut tree leaves, whereas gallic acid was most abundant in hazelnut skin and hazelnut hard shell, possibly implying the presence and perhaps the dominance of tannins in the latter samples. Senter et al. (1983) compared phenolic acids of nine edible tree nuts produced in the United States. The extracts from the nut samples showed great diversity in the phenolic acids present. Qualitative and quantitative differences existed among nut samples in the phenolic acids present, with gallic acid being predominant except in pine nut, almond, and hazelnut (filbert). A total of eight phenolic acids were isolated and identified among nine nuts (p-hydroxybenzoic acid, p-hydroxyphenylacetic acid, vanillic acid, protocatechuic acid, syringic acid, gallic acid, caffeic acid, and ferulic acid); some of which were also found to be present in Brazil nut in this study.

4. Conclusions

Our results clearly show that Brazil nuts contained substantial amounts of phenolic antioxidants that can effectively control oxidative stress in the body. The study also clearly shows that brown skin had significantly higher levels of antioxidant phenolic constituents and thus showed better antioxidant activities than the kernel. Though Brazil nut seemed to have a lower content of bound phenolics than those reported in the literature for other nuts, it was demonstrated to have very good antioxidant and radical scavenging activities, which are important when the health benefits of whole Brazil nut is considered. These bound phytochemicals may be released in the colon through microflora-assisted digestion and thereafter exert their health benefits. Thus, retention of maximum amount of brown skin on the kernel of Brazil nut is essential for taking advantage of their benefits. Furthermore, Brazil nut skin and hulls have great potential in development of nutraceuticals rich in antioxidants and as functional food ingredients.

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