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ARTICLE

# Comparative Flavan-3-ol Profile and Antioxidant Capacity of Roasted Peanut, Hazelnut, and Almond Skins

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In the present study, the flavan-3-ol composition and antioxidant capacity of roasted skins obtained from the industrial processing of three commonly consumed tree nuts (i.e., peanuts, hazelnuts, and almonds), as well as fractions containing low and high molecular weight (LMW and HMW) flavan-3-ols, were studied with the aim of assessing their potential as a source of flavonoids. Roasted peanut and hazelnut skins presented similar total phenolic contents, much higher than that of almond skins, but their flavan-3-ol profiles, as determined by LC-ESI-MS and MALDI-TOF MS, differed considerably. Peanut skins were low in monomeric flavan-3-ols (19%) in comparison to hazelnut (90%) and almond (89%) skins. On the other hand, polymeric flavan-3-ols in peanut and almond skins occurred as both A- and B-type proanthocyanidins, but in peanuts the A forms (up to DP12) were predominant, whereas in almonds the B forms (up to DP8) were more abundant. In contrast, hazelnuts were mainly constituted by B-type proanthocyanidins (up to DP9). The antioxidant capacity as determined by various methods (i.e., total antioxidant capacity, ORAC, DPPH test, and reducing power) was higher for whole extracts from roasted hazelnut and peanut skins than for almond skins; however, the antioxidant capacities of the HMW fraction of the three types of nut skins were equivalent despite their different compositions and DPs. Nevertheless, the large variation in flavan-3-ol concentration, structural composition, type of interflavan linkage, and DP found among the three types of nut skins suggests large difference in their expected in vivo biological activities.

**KEYWORDS: Nut skins; flavan-3-ols; procyanidins; propelargonidins; prodelphinidins; antioxidant capacity; LC-ESI-MS; MALDI-TOF MS**

## INTRODUCTION

Flavan-3-ols are abundant flavonoids in the human diet (*1*), and they are widely distributed in the plant kingdom, occurring as monomers and as oligomeric and polymeric forms (also called proanthocyanidins) (*2*). The most common structural monomeric units of proanthocyanidins in plant foods are (epi)afzelechin, (epi)catechin, and (epi)gallocatechin. Some of these units could also be esterified with other molecules such as glucose or gallic acid. Procyanidins are exclusively constituted by (epi)catechin, whereas propelargonidins and prodelphinidins contain (epi)afzelechin and (epi)gallocatechin, respectively, and are usually mixed with procyanidins. In relation to the interflavanic bond nature, B-type procyanidins are those in which monomers are linked through the C-4 position of the top unit and the C-6 or C-8 positions of the terminal unit, the C4−C8 isomers being more abundant than the C4−C6 ones. On the other hand, A-type procyanidins contain an additional ether type bond between the C-2 position of the top unit and the hydroxyl group at C-5 or C-7 of the lower unit.

Flavan-3-ols exhibit antioxidant, anticarcinogenic, cardioprotective, antimicrobial, and neuroprotective activities (*3*). However, the biological properties of flavan-3-ols in vivo are greatly dependent on their bioavailability, which at the same time is influenced by their chemical structure features [i.e., degree of polymerization (DP) and structural composition] (*1*). In recent years, a large body of epidemiological evidence has accumulated linking the consumption of flavonoid-rich foods with a reduced risk cardiovascualar disease (*4*, *5*). Recognition of these properties has attracted an enormous interest in the evaluation of unexploited natural sources of polyphenols for the elaboration of functional ingredients that could be used in the formulation of botanical and nutritional supplements. Nuts have been proved to be a source of antioxidants such as vitamin E, polyphenols, and other phytochemicals such as phytosterols and carotenoids (*6*, *7*). In nuts, the total polyphenol content accounts for 34−2052 mg/100 g (*8*) and the antioxidant capacity ranges from 204 to 5095 μmol of Trolox equivalents (TE)/serving (*6*). Dietary intake

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of nut polyphenols in the Spanish diet has been estimated to range from 102 to 121 mg/person/day (*9*). Evidence from observational and clinical studies on nut intake indicates their beneficial effects on cholesterol levels (*10*), inflammatory markers (*11*), endothelial function (*12*), type-2 diabetes (*13*), and antioxidant capacity and lipid peroxidation (*14*). Phenolic compounds in nuts are mainly located in the skin or testa (*15−17*), which is usually removed by blanching or roasting for the use of the kernel in the bakery and confectionary industry. Nut skins and other byproducts derived from the processing of nuts have traditionally been used for livestock feed and as raw material for energy production, but in the past few years, several studies have confirmed that they are an inexpensive valuable source of natural antioxidants for nutraceutical and pharmaceutical applications (*18−23*). Although the total polyphenol contents have been reported for a wide range of nuts (*8*, *24*), information concerning the individual phenolic composition of both non-flavonoid and flavonoid compounds of nuts is still scarce. This information would contribute to the understanding of the bioavailability of nut polyphenols, a priority area of research in the determination of health effects derived from nut consumption.

Recent studies revealed that roasting significantly enhanced the polyphenol content and antioxidant capacity of almond skin extracts in comparison to blanching and oven-drying (*25*), probably due to degradation reaction of polymerized polyphenols (*26*), hydrolysis of glycosylated flavonoids (*27*), and decomposition of aglycones (*28*), among others. To assess the potential of different nut skins as a rich source flavonoids, the detailed flavan-3-ol composition of roasted skins obtained from the industrial processing of three commonly consumed tree nuts (i.e., peanuts, hazelnuts, and almonds), as well as the antioxidant capacity of fractions containing low and high molecular weight flavan-3-ols was studied. Individual monomeric and oligomeric flavan-3-ols were determined by LC-ESI-MS, and the proanthocyanidin profile was assessed by MALDI-TOF MS analysis. Finally, the antioxidant capacity of nut skin extracts was assayed by four different methodologies (total antioxidant capacity, ORAC, DPPH test, and reducing power).

## MATERIALS AND METHODS

**Commercial Standards and Reagents.** Potassium ferricyanide and trichloroacetic acid were acquired from the P.O.Ch. Co. (Gliwice, Poland); Folin−Ciocalteu reagent, Sephadex LH-20, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, (+)-catechin, (−)-epicatechin, procyanidin B3, and disodium fluorescein were purchased from Sigma (St. Louis, MO); procyanidin B2 was from Extrasynthèse (Genay, France); and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and 2,2′-azobis(2-methylpropionamidine) dihydrochloride were from Aldrich (St. Louis, MO).

**Nut Skins.** Hazelnut (var. Giresun from Turkey), peanut (mixtures of Chinese varieties), and almond (mixtures of Spanish and American varieties) skins were kindly supplied by La Morella Nuts (Tarragona, Spain). Whole nuts were subjected to roasting (30 min at 145 °C) in an industrial continuous-working oven where the skins were separated from the roasted kernels. The roasted skins were then milled in a Janke & Kunkel mill (Ika Labortechnik, Wilmington, NC) to a particle size of < 50 μm. To determine the total phenolic content as well as the oxygen radical absorbance capacity (ORAC) of the nut skins, ground skins (0.05 g) were extracted with 10 mL of methanol/HCl (1000:1, v/v) by sonication for 5 min followed by an extra 15 min resting period. The mixture was then centrifuged (3024*g*, 5 min, 5 °C). The supernatant was collected and finally filtered (0.45 μm) before analysis.

**Preparation of Whole Phenolic Extracts and Phenolic Fractions from Nut Skins.** Phenolic compounds were extracted from the ground skins using 80% (v/v) acetone at a solid to solvent ratio of 1:10 (w/v), at 50 °C for 30 min (*29*). Extraction was carried out in flasks using a shaking water bath (Elpan 357, Wrocław, Poland). The extraction was repeated twice, supernatants were combined, and acetone was evaporated under vacuum at 40 °C in a rotary evaporator; the remaining water solution was removed by freeze-drying. The prepared extracts were stored at −20 °C until used.

Separation of the whole extract into a low molecular weight phenolic (LMW) fraction and a high molecular weight (HMW) phenolic fraction was achieved according to the method described by Strumeyer and Malin (*30*). A 2 g portion of the whole extract was suspended in 20 mL of 95% (v/v) ethanol and applied onto a chromatographic column (5 × 40 cm) packed with Sephadex LH-20 and equilibrated with 95% (v/v) ethanol. LMW compounds were eluted from the column using 1 L of 95% (v/v) ethanol. HMW compounds were obtained by washing the column with 500 mL of 50% (v/v) acetone. Organic solvents were evaporated, and water from the eluant was removed by freeze-drying. The fractions were stored at −20 °C until used.

The whole extracts and their corresponding fractions (LMW and HMW) were dissolved in methanol for the determination of total polyphenol and tannin contents, and antioxidant capacity [total antioxidant capacity, oxygen-radical absorbance capacity (ORAC), DPPH test, and reducing power]. For LC and MALDI-TOF analyses, they were dissolved in methanol/$H_2O$ (50:50, v/v) and filtered (0.45 μm) before analysis.

**Determination of Total Polyphenols and Condensed Tannins.** The method of Singleton and Rossi (*31*) was used for determining total polyphenols in the nut skins, the whole phenolic extracts, and the fractions (LMW and HMW). The results were expressed as milligrams of gallic acid equivalents (GAE) per gram of sample. The content of condensed tannins in the same samples was determined using the modified vanillin assay (*32*). The results were expressed as absorbance units at 500 nm per gram ($A_{500}$/g). All of the analyses were performed in triplicate.

**Total Antioxidant Capacity.** The total antioxidant capacity of the whole phenolic extract and the fractions (LMW and HMW) was determined according to the Trolox equivalent antioxidant capacity (TEAC) assay described by Re et al. (*33*) and was expressed as millimoles of Trolox equivalents (TE) per gram of sample. All analyses were performed in triplicate.

**ORAC.** The ORAC value of the nut skins and their whole phenolic extracts and fractions (LMW and HMW) was determined using fluorescein as a fluorescence probe (*34*). Briefly, the reaction was carried out at 37 °C in 75 mM phosphate buffer (pH 7.4), and the final assay mixture (200 μL) contained fluorescein (70 nM), 2,2′-azobis(2-methylpropionamidine) dihydrochloride (12 mM), and antioxidant [Trolox (1−8 μM) or sample (at different concentrations)]. The plate was automatically shaken before the first reading, and the fluorescence was recorded every minute for 98 min. A Polarstar Galaxy plate reader (BMG Labtechnologies GmbH, Offenburg, Germany) with 485-P excitation and 520-P emission filters was used. The equipment was controlled by Fluostar Galaxy software version (4.11−0) for fluorescence measurement. Black 96-well untreated microplates (Nunc, Rosklide, Denmark) were used. 2,2′-Azobis(2-methylpropionamidine) dihydrochloride and Trolox solutions were prepared daily, and fluorescein was diluted from a stock solution (1.17 mM) in 75 mM phosphate buffer (pH 7.4).

All reaction mixtures were prepared in duplicate, and at least three independent runs were performed for each sample. Fluorescence measurements were normalized to the curve of the blank (no antioxidant). From the normalized curves, the area under the fluorescence decay curve (AUC) was calculated as

$$\mathrm{AUC} = 1 + \sum_{i=1}^{i=98} f_i/f_0$$

where $f_0$ is the initial fluorescence reading at 0 min and $f_i$ is the fluorescence reading at time *i*. The net AUC corresponding to a sample was calculated as follows:

$$\text{net AUC} = \mathrm{AUC}_{\text{antioxidant}} - \mathrm{AUC}_{\text{blank}}$$

The regression equation between net AUC and antioxidant concentration was calculated. The ORAC value was calculated by dividing the slope of the latter equation by the slope of the Trolox line obtained for the same assay. Final ORAC values were expressed as millimoles of TE equivalents per gram of sample.

**DPPH Test.** The DPPH test was carried out as described by Amarowicz et al. (*35*). A 0.1 mL methanolic solution containing between 0.5 and 2.5 mg of the whole phenolic extract or LMW fraction and between 0.02 and 0.1 mg of HMW fraction was mixed with 2 mL of water and then added to a methanolic solution of DPPH$^{\bullet}$ (1 mM, 0.25 mL). The mixture was vortexed for 1 min and left to stand at room temperature for 20 min, and then the absorbance of the solution was read at 517 nm. Results were expressed as the content of extract or its fractions (milligrams per assay) versus absorbance at 517 nm. From the graph, $EC_{50}$ was read as milligrams of extract or its fractions required for scavenging the initial DPPH radical by 50%. All analyses were performed in triplicate.

**Reducing Power.** The reducing power of phenolics was determined as described by Oyaizu (*36*). A suspension of the whole extract as well as of the LMW and HMW fractions in 1 mL of distilled water was mixed with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1% (w/v) potassium ferricyanide. The mixture was incubated at 50 °C for 20 min. Following this, 2.5 mL of 10% (w/v) trichloroacetic acid was added, and the mixture was then centrifuged at 1750*g* for 10 min. A 2.5 mL aliquot of the upper layer was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% (w/v) $FeCl_3$; the absorbance of the mixture was read at 700 nm. Results were expressed as the content of extract or its fractions (milligrams per assay) versus absorbance at 700 nm and as millimoles of ascorbic acid equivalents (AAE) per gram of extract or its fraction. All analyses were performed in triplicate.

**Analysis of Phenolic Compounds by High-Performance Liquid Chromatography (LC-DAD-Fluorescence and LC-DAD/ESI-MS).** A Waters (Milford, MA) liquid chromatography system equipped with a 600-MS controller, a 717Plus autosampler, a 996 photodiode array detector (DAD), and a fluorescence detector coupled to Waters Empower (version 5.0) for data acquisition and processing was used. Separation was performed on a 150 × 4.6 mm i.d., 4 μm reversed-phase ACE 3 C18 (Advanced Chromatography Technologies, Aberdeen, Scotland) column at room temperature. A gradient consisting of solvent A (water/acetic acid, 98:2, v/v) and solvent B (water/acetonitrile/acetic acid, 73:25:2, v/v/v) was applied at a flow rate of 1.0 mL/min as follows (*17*): 0−80% B linear, from 0 to 55 min; 80−90% B linear, from 55 to 57 min; 90% B, isocratic from 57 to 70 min; 90−95% B, linear from 70 to 80 min; 95−100% B, from 80 to 90 min; followed by washing (methanol) and re-equilibration of the column from 90 to 120 min. A 75 μL volume sample was injected into the column. The detection conditions were as follows: 210−360 nm (DAD); 280 and 310 nm for the emission and excitation filters, respectively (fluorescence detector). Flavan-3-ols were quantified by their fluorescence response. Due to the lack of commercial standards, oligomeric flavanols were quantified using the (−)-epicatechin calibration curve with the exception of procyanidin dimer B2, which was quantified using its own calibration curve.

In addition to their UV spectra, the identification of phenolic compounds was also carried out by mass spectrometry coupled to LC. A Hewlett-Packard series 1100 (Palo Alto, CA) chromatography system equipped with a diode array detector (DAD) and a quadrupole mass spectrometer (Hewlett-Packard series 1100 MSD) with an electrospray interface was used. Separation conditions were the same as described above except for the flow rate, which was set to 0.7 mL/min. The ESI source parameters were as follows: drying gas ($N_2$) flow and temperature, 10 L/min and 350 °C, respectively; nebulizer pressure, 55 psi; and capillary voltage, 4000 V. Mass spectra were obtained using in-source collision-induced dissociation mass spectrometry (CID MS), scanning negative ions from *m*/*z* 100 to 2000 using the following fragmentation program: from *m*/*z* 0 to 200 (150 V) and from *m*/*z* 200 to 2000 (300 V).

**MALDI-TOF Spectra.** Samples (1 μL) were mixed with 4 μL of the matrix consisting of 2,5-dihydroxybenzoic acid (gentisic acid) (Buchs, Switzerland) at a concentration of 20 mg/mL in water. Then, 1 μL of this solution was spotted onto a flat stainless steel sample plate and dried in air. MALDI-TOF measurements were performed using a Voyager DE-PRO mass spectrometer (Applied Biosystems, Foster City, CA) equipped with a pulsed nitrogen laser (λ = 337 nm, 10 ns pulse width, and 20 Hz frequency) and a delayed extraction ion source. Ions generated by the laser desorption were introduced into the flight tube (1.3 m flight path) with an acceleration voltage of 25 kV, 94% grid voltage, a 0.05% ion guide wire voltage, and a delay time of 100 ns in the linear positive ion mode. The low mass gate was turned on at 290 u to prevent the saturation of the detector by ions resulting from the matrix. Mass spectra were collected by averaging the signals of at least 600 laser shots over the *m*/*z* range of 300−6000. To obtain a more representative spectrum and to improve peak resolution and signal-to-noise ratio, the final mass spectrum for each sample was the accumulated one from five acquisitions in different locations across the sample spot (3000 shots in final spectrum). To determine if all signals in the mass spectra were sodium adducts, 1 μL of 15 mM sodium chloride was added to the sample spot. No decrease, disappearance, or increase of signals was detected, so all peaks should be $[M + Na]^+$ ions and the differences of 16 u are due to the different numbers of hydroxyl groups in the substances and not to $[M+K]^+$ species (*37*). Angiotensin I and bovine insulin from Calibration Mixture 2 (Sequazyme Peptide Mass Standards Kit; Applied Biosystems) were used for external mass calibration.

**Table 1.** Total Polyphenols and Antioxidant Capacity (ORAC Value) in the Methanol/HCl (1000:1, v/v) Extracts from Roasted Nut Skins

| | total polyphenols (mg of GAE/g of skin) | ORAC value (mmol of TE/g of skin) |
|---|---|---|
| peanuts | 73.9 ± 1.7 | 2.13 ± 0.03 |
| hazelnuts | 107 ± 12 | 3.05 ± 0.06 |
| almonds | 22.8 ± 0.5 | 1.07 ± 0.08 |

## RESULTS AND DISCUSSION

**Total Polyphenol Content and Antioxidant Capacity of the Different Roasted Nut Skins.** Methanol/HCl (1000:1, v/v) extraction of roasted nut skins was first carried out to perform a preliminary screening of the total polyhenol content and antioxidant capacity of the peanut, hazelnut, and almond skins (**Table 1**). Hazelnut skins presented the highest polyphenol content, followed very closely by peanut and finally by almond skins, which presented much lower contents (21 and 31% lower than hazelnut and peanut skins, respectively). ORAC values showed the same trend as the total polyphenol content, ranging from 1.07 mmol of TE/g of skin (for almonds) to 3.05 mmol of TE/g of skin (for hazelnuts) (**Table 1**). These comparative results among the three nut skins seem to be consistent with the results reported by Kornsteiner et al. (*8*), who found a higher total polyphenol content for whole peanuts (skins included) than for almond (skins included). On the other hand, Alasalvar et al. (*38*) found that an 80% acetone extract from whole hazelnut kernels contained a higher amount of total polyphenols than the same extract from almond seeds (*18*). Similar results were found by Yang et al. (*24*), although in this study whole peanuts presented a slightly higher total phenolic content than whole hazelnuts. Besides their differences in polyphenol content, major differences have been reported among nuts in relation to the distribution of free and bond phenolics. In hazelnuts, 93% phenolics were found in bond form, followed by almonds (61%) and peanuts (45%) (*24*). With regard to the antioxidant capacity, our results are also in agreement with those of Amarowicz et al. (*39*), who found a higher antioxidant capacity for whole hazelnuts than for whole almonds as determined by various methods (i.e., total antioxidant capacity, DPPH, β-carotene−linoleate emulsion, and reducing power).

**Phenolic Content and Antioxidant Capacity of Whole Extracts and Fractions (LMW and HMW) from Roasted Nut Skins.** To study the monomeric, oligomeric, and polymeric flavan-3-ol composition of roasted skins from peanuts, hazelnuts, and almonds, an exhaustive extraction with 80% acetone was carried out. Extraction yields (g of extract obtained/100 g of skins) were 19.7% for peanuts, 22.5% for hazelnuts, and 15.3% for almonds. For the three nut skins, the whole extract was further fractionated on Sephadex LH-20 into a LMW fraction and a HMW fraction (tannin fraction). The UV spectra of the whole extract and LMW

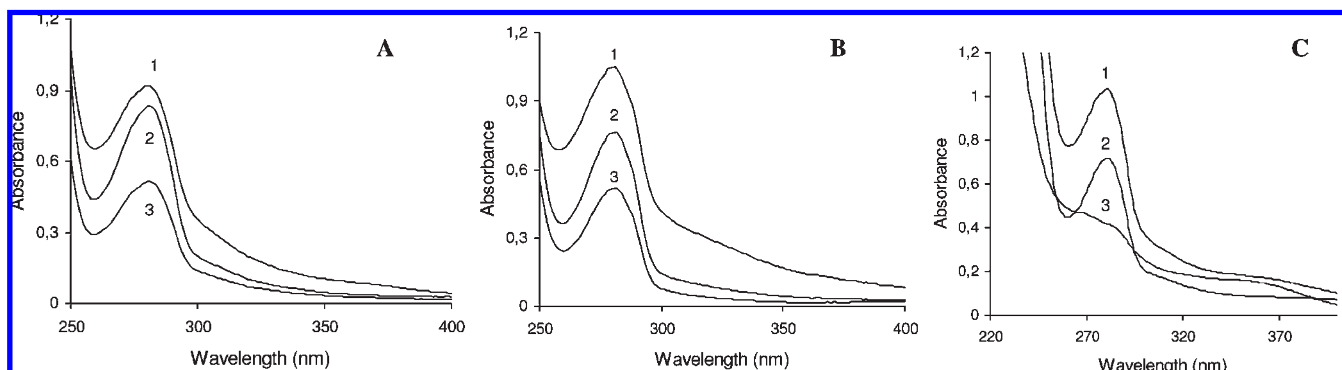


**Figure 1.** UV spectra of the whole extracts (2) and their corresponding LMW (3) and HMW (1) fractions from peanut (**A**), hazelnut (**B**), and almond (**C**) roasted skins.

**Table 2.** Phenolic Content and Antioxidant Capacity of the Whole Extracts (80% Acetone) and Their Fractions (LMW and HMW) from Roasted Nut Skins

| | total polyphenols (mg of GAE/g) | tannins by the vanillin method (absorbance$_{500}$/mg of extract) | total antioxidant activity (mmol of TE/g) | ORAC value (mmol of TE/g) | DPPH test EC$_{50}$ (mg/assay) | reducing power (mmol of AAE/g) |
|---|---|---|---|---|---|---|
| peanuts | | | | | | |
| whole extract | 371 ± 15 | 0.607 ± 0.014 | 4.07 ± 0.04 | 13.2 ± 0.3 | 0.018 | 4.321 ± 0.070 |
| LMW fraction | 235 ± 11 | 0.009 ± 0.004 | 1.24 ± 0.08 | 8.74 ± 0.81 | 0.064 | 1.126 ± 0.052 |
| HMW fraction | 506 ± 4 | 1.085 ± 0.009 | 6.41 ± 0.26 | 14.7 ± 1.0 | 0.012 | 5.859 ± 0.037 |
| hazelnuts | | | | | | |
| whole extract | 315 ± 4 | 1.100 ± 0.012 | 5.42 ± 0.30 | 14.5 ± 1.7 | 0.016 | 4.373 ± 0.077 |
| LMW fraction | 163 ± 2 | 0.008 ± 0.001 | 2.15 ± 0.08 | 10.9 ± 0.9 | 0.034 | 2.151 ± 0.040 |
| HMW fraction | 518 ± 20 | 1.502 ± 0.009 | 6.02 ± 0.18 | 19.2 ± 0.9 | 0.015 | 4.868 ± 0.085 |
| almonds | | | | | | |
| whole extract | 134 ± 1 | 0.210 ± 0.012 | 1.25 ± 0.04 | 4.03 ± 0.25 | 0.063 | 0.988 ± 0.006 |
| LMW fraction | 60.6 ± 5.9 | 0.011 ± 0.002 | 0.189 ± 0.010 | 2.01 ± 0.10 | 0.641 | 0.199 ± 0.040 |
| HMW fraction | 536 ± 13 | 0.964 ± 0.013 | 5.67 ± 0.22 | 15.5 ± 1.0 | 0.015 | 6.410 ± 0.046 |

and HMW fractions from the different nut skins are illustrated in **Figure 1**, indicating the presence of compounds with $\lambda_{max}$ = 280 nm, characteristic of many phenolic compounds, including flavan-3-ols. The total polyphenol and condensed tannin contents as well as the antioxidant capacity, determined as the total antioxidant capacity, ORAC, DPPH, and reducing powder values, of the whole extract and their fractions from the different nut skins, are presented in **Table 2**. The whole extract from peanut skins presented the highest total polyphenol content, followed very closely by the hazelnut extract and finally by the almond extract. In line with these results, Gu et al. (*40*) reported that the sum of flavan-3-ol monomers, oligomers, and polymers (> DP10) was higher for hazelnuts than for almonds. As expected, the HMW fraction was more abundant in total polyphenol and tannin contents than the whole extract and the LMW fraction for the three types of nuts. Although almond skins presented the poorest whole extract, the HMW fraction from these nut skins presented contents of total polyphenols and tannins similar to those of peanuts and hazelnuts. The results from the different antioxidant assays followed the same trend of the polyphenol content. Whole extracts from hazelnut and peanut skins presented very similar antioxidant capacities, whereas that from almond skins showed approximately 4-fold inferior total antioxidant activity and reducing powder in comparison to peanuts and hazelnut extracts. According to Amarowicz et al. (*39*), the antioxidant capacity as measured by various methods also indicated a 2.7-fold higher total antioxidant activity and a 7.6-fold higher reducing power for hazelnuts in comparison to almonds. Among the different fractions, the HMW fraction showed the highest antioxidant capacity in the three cases, ranging from 5.67 to 6.41 mmol of TE/g for total antioxidant activity, from 14.7 to 19.2 mmol of TE/g for the ORAC value, from 0.012 to 0.015 mg/assay of EC$_{50}$ value in the DPPH test, and from 4.868 to 6.410 mmol of AAE/g in the reducing power test. A significant correlation, as determined by the Pearson test, was found between the ORAC values and all methods tested: total antioxidant activity (0.921, $p < 0.01$), DPPH (−0.696, $p < 0.05$), and reducing power (0.889, $p < 0.01$).

**Monomeric and Oligomeric Flavan-3-ol Composition of Whole Extracts from Roasted Nut Skins.** The LC chromatogram of monomeric and oligomeric flavan-3-ols present in the whole extracts from roasted nut skins is illustrated in **Figure 2**. The identification of flavan-3-ols was confirmed by their UV spectrum and retention time ($t_R$) in comparison to authentic standard as well as by their molecular ion and derived fragments obtained by ESI-MS in negative mode, as described by Monagas et al. (*17*). Monomeric (+)-catechin was identified in the three cases, whereas (−)-epicatechin was detected only in the peanut and almond extracts (**Table 3**). With regard to procyanidins, B-type procyanidins were detected in hazelnuts (i.e., procyanidin B3 and another unknown dimer) and in almonds (i.e., procyanidin B2). Both A- and B-type procyanidins were detected in peanuts, including B-type dimers and A-type dimers, trimers, and tetramers. These profiles seem to be consistent with previous reports. B-type procyanidins have been reported to occur in whole hazelnuts (*41*), although information concerning the individual compounds and their quantitative levels has not been described to date. A- and B-type procyanidins, propelargonidins, and prodelphinidins have been identified in almond skins after blanching and drying (*17*); however, in the present study only B-type procyanidins were detected in the whole extract from roasted almond skins. Procyanidins B2, B3, and B4 (*42*), as well as several A-type dimeric, trimeric, and tetrameric procyanidins, including proanthocyanidins A1 and A2 and some

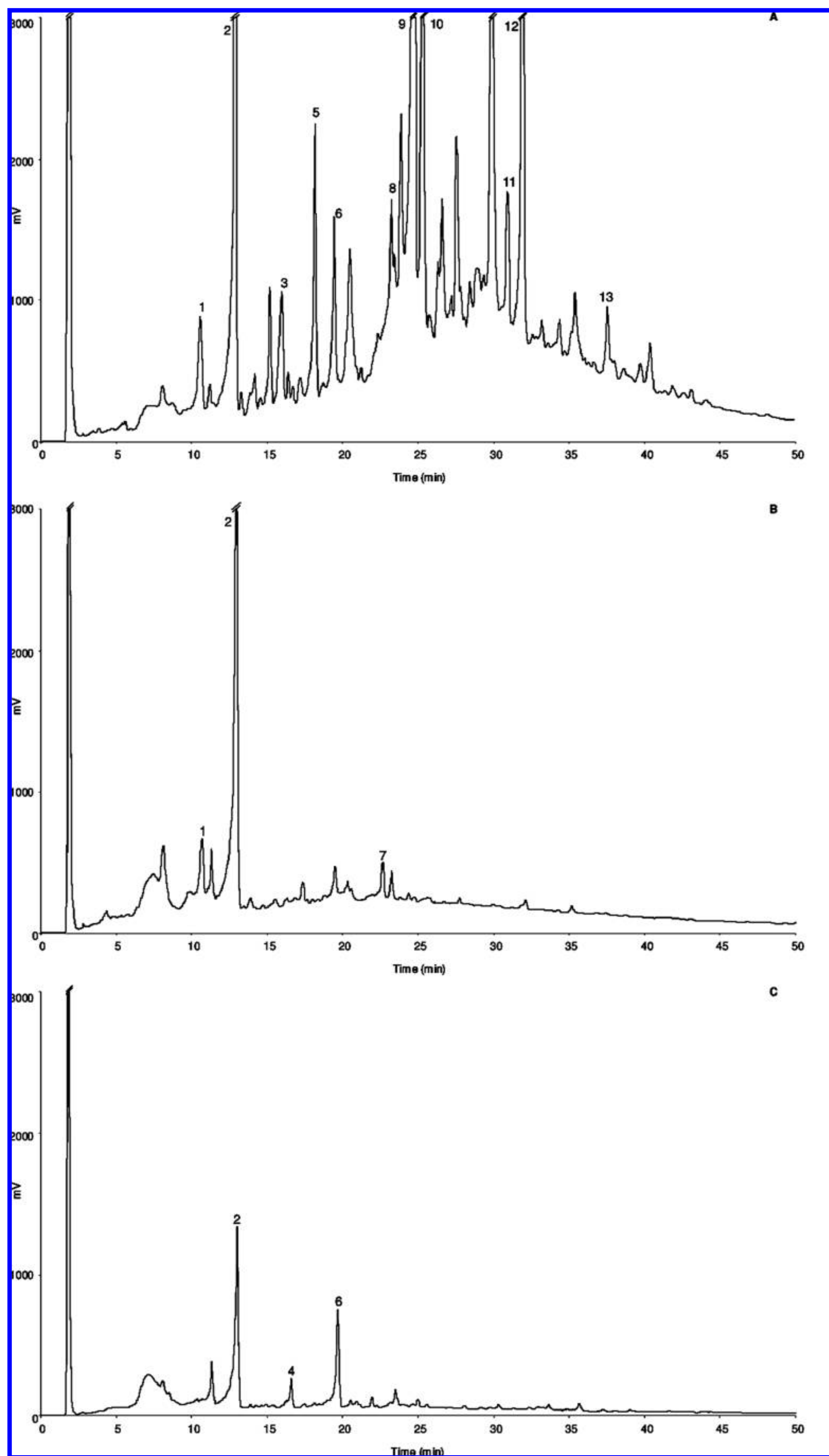


**Figure 2.** LC chromatograms (fluorescence) corresponding to whole fraction from peanut (**A**), hazelnut (**B**), and almond (**C**) roasted skins. Peaks: 1, dimer B3; 2, (+)-catechin; 3, unknown trimer A; 4, dimer B2; 5, unknown trimer A; 6, (−)-epicatechin; 7, unknown dimer B; 8, unknown dimer B; 9, unknown tetramer A; 10, unknown trimer A; 11, unknown dimer A; 12, unknown dimer A; 13, unknown dimer A.

of their regio- and stereoisomers, have been characterized in peanut skins (*42−44*).

A relative quantification of oligomeric flavan-3-ols [as (−)-epicatechin equivalents] was performed to compare the profiles of the different nut skins. The whole extract from roasted peanut skins presented the highest total content, whereas the extract from almond skins showed the poorest, in line with the total polyphenol contents described in **Table 2** . (+)-Catechin was more abundant than (−)-epicatechin in the different nut skins. However, the distribution of the different species varied among nuts studied. Total monomers accounted for 90% in hazelnut skins and 89% in almond skins, but only for 19% in peanut skins. On the other hand, the whole extract from peanut skins was characterized by a high proportion of A-type procyanidins, total dimers + trimers accounting for 40% and tetramers for 37% of the total content. Yu et al. (*26*) also found that A-type tetramers accounted for the highest proportion of total monomers + oligomers in roasted peanut skins. Although the whole extract from peanut skins largely differed in flavanol-3-ol monomeric and oligomeric composition from that of hazelnut and almond, the antioxidant activity of the former (**Table 2**) was apparently not affected by this matter and was found to be more in line with the total polyphenol content. Studies comparing the bioactivity of A- and B-type proanthocyanidins are limited. According to Shahat et al. (*45*), the antiviral activity and inhibitory activity against microsomal peroxidation were similar for A- and B-type dimers. However, in another study, protection of the lipid bilayer from disruption by Triton X-100 was higher for A-type dimers than for B2, but the contrary was observed in the case of trimers (*46*).

**Table 3.** Flavan-3-ols (Micrograms of Catequin Equivalents per Gram) in the Whole Phenolic Extracts from Roasted Nut Skins

| peak | compound | $t_R$ (min) | peanuts | hazelnuts | almonds |
|---|---|---|---|---|---|
| 1 | dimer B3 | 10.6 | 136.31 | 104.62 | |
| 2 | (+)-catechin | 12.9 | 848.79 | 1168.00 | 301.68 |
| 3 | unknown trimer A | 16.0 | 142.69 | | |
| 4 | dimer B2 | 16.5 | | | 56.15 |
| 5 | unknown trimer A | 18.2 | 342.47 | | |
| 6 | (−)-epicatechin | 19.5 | 195.78 | | 135.35 |
| 7 | unknown dimer B | 22.6 | | 28.37 | |
| 8 | unknown dimer B | 23.2 | 112.14 | | |
| 9 | unknown tetramer A | 24.7 | 2102.84 | | |
| 10 | unknown trimer A | 25.3 | 844.29 | | |
| 11 | unknown dimer A | 30.9 | 126.68 | | |
| 12 | unknown dimer A | 31.9 | 727.92 | | |
| 13 | unknown dimer A | 37.5 | 60.42 | | |
| | total | | 5640.33 | 1300.99 | 493.17 |

**Proanthocyanidin Composition of HMW Fraction from Roasted Nut Skins.** The proanthocyanidin structural composition of the HMW fraction from roasted nut skins was studied by MALDI-TOF MS (**Figures 3−5**). The MALDI-TOF mass signals (sodium adducts) and their corresponding structures are summarized in **Table 4**. Assignation of MALDI-TOF mass signals to a particular proanthocyanidin structure (i.e., propelargonidins, procyanidins, and prodelphinidins) was achieved by the determination of the theoretical or calculated monoisotopic mass, according to the equation

$$[\mathrm{M{+}Na}]^+ = 290.08 \times \mathrm{CAT} + 274.08 \times \mathrm{AFZ} + 306.07 \times \mathrm{GCAT} + 152.01 \times \mathrm{GALLOYL} - 2.02 \times B - 4.04 \times A + 22.99$$

where CAT, AFZ, and GCAT are, respectively, the numbers of (epi)catechin, (epi)afzelechin, and (epi)gallocatechin units contained in the proanthocyanidin molecule, GALLOYL is the number of galloyl ester units attached to the flavan-3-ol units, and $B$ and $A$ are, respectively, the numbers of B- and A-type linkages between units.

MALDI-TOF signals corresponding to A- and B-type proanthocyanidins were detected in roasted peanut skins (**Figure 3**; **Table 4**). B-type structures were composed of (epi)catechins up to DP6. A-type structures consisted of procyanidins up to DP12 containing one and two A-type linkages. As the DP increased from DP8 to DP12, structures presented only two A-type linkages. In

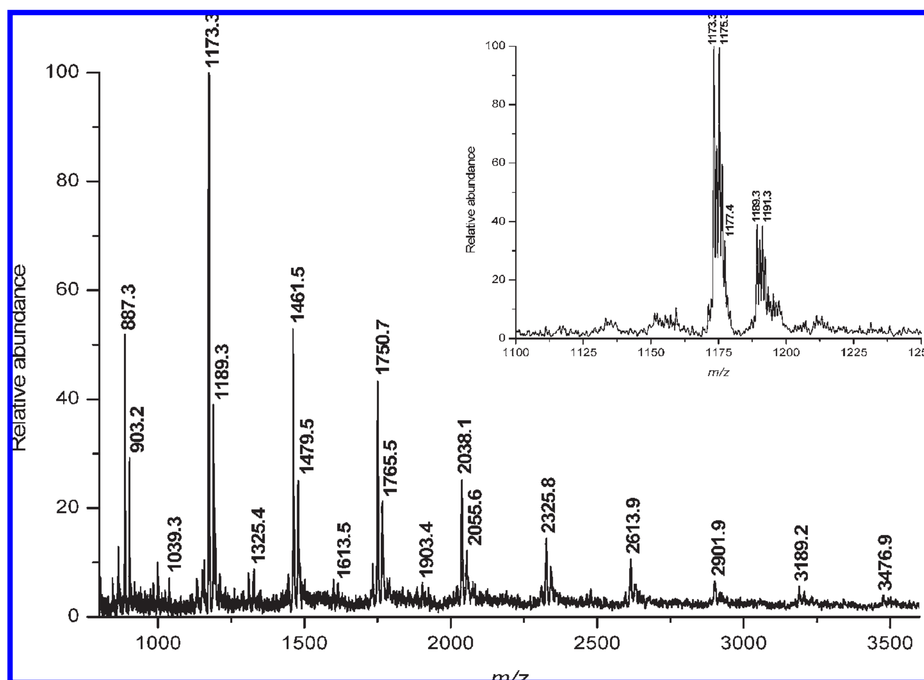


**Figure 3.** MALDI-TOF MS spectrum of the HMW fraction from roasted peanut skin extracts using 2,5-dihydroxybenzoic acid as matrix on linear positive ion mode. The inset shows an enlarged section of the DP4 series.

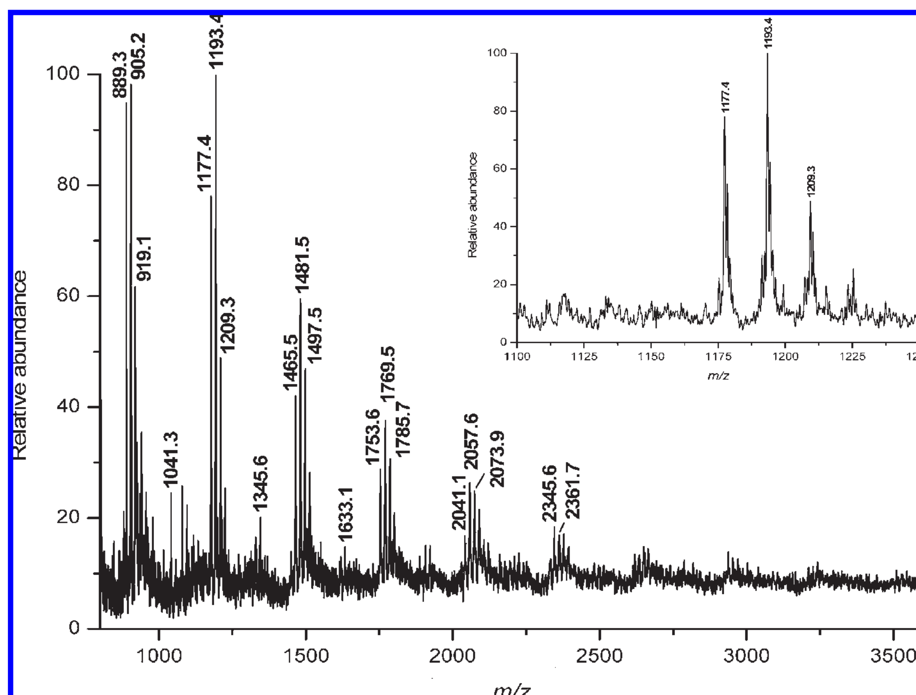


**Figure 4.** MALDI-TOF MS spectrum of the HMW fraction from roasted hazelnut skin extracts using 2,5-dihydroxybenzoic acid as matrix on linear positive ion mode. The inset shows an enlarged section of the DP4 series.

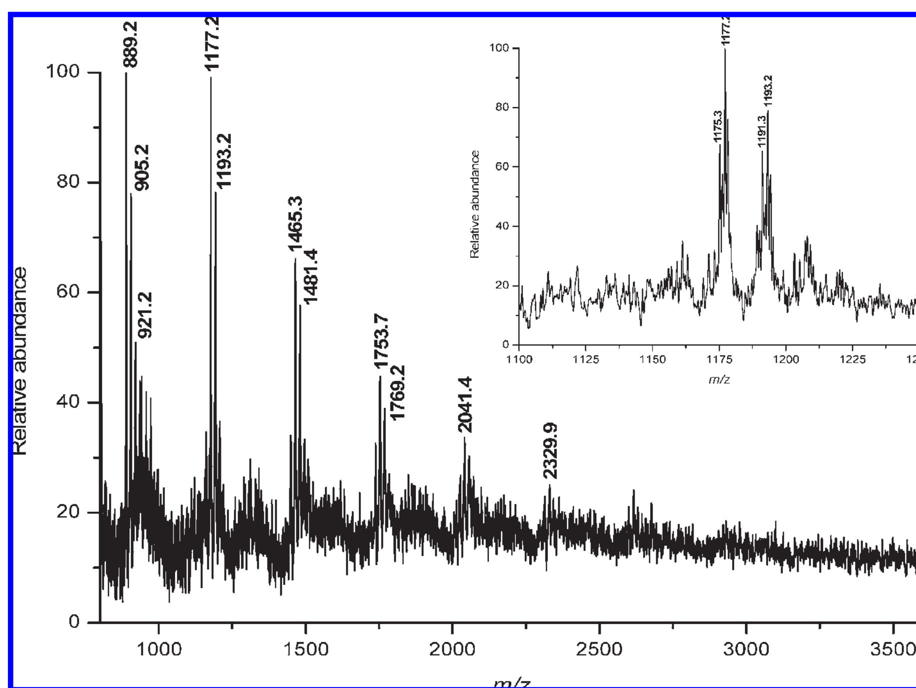


**Figure 5.** MALDI-TOF MS spectrum of the HMW fraction from roasted almond skin extracts using 2,5-dihydroxybenzoic acid as matrix on linear positive ion mode. The inset shows an enlarged section of the DP4 series.

line with our findings, Lazarus et al. (*47*) reported A-type procyanidins up to DP8 and B-type procyanidins up to DP8 in whole peanuts by normal-phase LC. However, mean DPs of 12.7 and 3.2 for B- and A-type procyanidins, respectively, have been reported for whole peanuts using thiolytic degradation (*41−44*, *47*), which seem more distant to previous results and to our findings. In addition to A- and B-type proanthocyanidins exclusively composed of (epi)catechins, weak signals corresponding to a B-type hexamer containing one galloylated unit (*m*/*z* 1903.4), as well as to A-type structures presenting one galloylated unit (up to DP 5) or one (epi)gallocatechin unit (up to DP 8) and containing up to two A-type linkages were also detected. To our knowledge there is no previous report of the occurrence of galloylated procyanidins and prodelphinidins in peanut skins, probably due to their low abundance on the samples.

Roasted skins from hazelnuts were composed of B-type procyanidins up to DP7 (**Figure 4**; **Table 4**). A trimeric procyanidin presenting one galloylated unit was also detected (*m*/*z* 1041.3). In addition, signals corresponding to procyanidin−prodelphinidin heteropolymers composed of two (epi)gallocatechin units

**Table 4.** Proanthocyanidins Detected by MALDI-TOF MS in the HMW Fraction from Roasted Nut Skins

| unit | linkage | ions (*m*/*z*) calcd $[M + Na]^+$ | peanuts $[M + Na]^+$ | hazelnuts $[M + Na]^+$ | almonds $[M + Na]^+$ |
|---|---|---|---|---|---|
| **Trimers** | | | | | |
| 3 (epi)catechin | 1A, 1B | 887.2 | 887.3 | | 887.2 |
| 3 (epi)catechin | 2B | 889.1 | | 889.3 | 889.2 |
| 2 (epi)catechin, 1 (epi)gallocatechin | 1A, 1B | 903.1 | 903.2 | 903.1 | 903.2 |
| 2 (epi)catechin, 1 (epi)gallocatechin | 2B | 905.1 | | 905.2 | 905.2 |
| 1 (epi)catechin, 2 (epi)gallocatechin | 1A, 1B | 919.2 | | 919.1 | 919.0 |
| 1 (epi)catechin, 2 (epi)gallocatechin | 2B | 921.2 | | 921.2 | 921.2 |
| 3 (epi)catechin, 1 gallate | 1A, 1B | 1039.2 | 1039.3 | | |
| 3 (epi)catechin, 1 gallate | 2B | 1041.2 | | 1041.3 | |
| **Tetramers** | | | | | |
| 4 (epi)catechin | 2A, 1B | 1173.2 | 1173.3 | | |
| 4 (epi)catechin | 1A, 2B | 1175.2 | 1175.3 | | 1175.3 |
| 4 (epi)catechin | 3B | 1177.2 | 1177.4 | 1177.4 | 1177.2 |
| 3 (epi)catechin, 1 (epi)gallocatechin | 2A, 1B | 1189.2 | 1189.3 | | |
| 3 (epi)catechin, 1 (epi)gallocatechin | 1A, 2B | 1191.2 | 1191.3 | | 1191.3 |
| 3 (epi)catechin, 1 (epi)gallocatechin | 3B | 1193.2 | | 1193.4 | 1193.2 |
| 2 (epi)catechin, 2 (epi)gallocatechin | 1A, 2B | 1207.2 | | | |
| 2 (epi)catechin, 2 (epi)gallocatechin | 3B | 1209.1 | | 1209.3 | |
| 4 (epi)catechin, 1 gallate | 2A, 1B | 1325.2 | 1325.4 | | |
| 4 (epi)catechin, 1 gallate | 1A, 2B | 1327.2 | 1327.4 | | |
| 3 (epi)catechin, 1 (epi)gallocatechin, 1 gallate | 3B | 1345.3 | | 1345.6 | |
| **Pentamers** | | | | | |
| 5 (epi)catechin | 2A, 2B | 1461.2 | 1461.5 | | |
| 5 (epi)catechin | 1A, 3B | 1463.2 | 1463.4 | | 1463.3 |
| 5 (epi)catechin | 4B | 1465.2 | 1465.4 | 1465.5 | 1465.3 |
| 4 (epi)catechin, 1 (epi)gallocatechin | 2A, 2B | 1477.2 | 1477.5 | | |
| 4 (epi)catechin, 1 (epi)gallocatechin | 1A, 3B | 1479.2 | 1479.5 | | 1479.3 |
| 4 (epi)catechin, 1 (epi)gallocatechin | 4B | 1481.2 | | 1481.5 | 1481.4 |
| 3 (epi)catechin, 2 (epi)gallocatechin | 1A, 3B | 1495.2 | | | |
| 3 (epi)catechin, 2 (epi)gallocatechin | 4B | 1497.2 | | 1497.5 | |
| 5 (epi)catechin, 1 gallate | 2A, 2B | 1613.3 | 1613.5 | | |
| 5 (epi)catechin, 1 gallate | 1A, 3B | 1615.3 | 1615.4 | | |
| 4 (epi)catechin, 1 (epi)gallocatechin, 1 gallate | 4B | 1633.3 | | 1633.1 | |
| **Hexamers** | | | | | |
| 6 (epi)catechin | 2A, 3B | 1749.3 | 1750.7 | | |
| 6 (epi)catechin | 1A, 4B | 1751.4 | | | 1751.4 |
| 6 (epi)catechin | 5B | 1753.4 | | 1753.6 | 1753.7 |
| 5 (epi)catechin, 1 (epi)gallocatechin | 2A, 3B | 1765.3 | 1765.5 | | |
| 5 (epi)catechin, 1 (epi)gallocatechin | 1A, 4B | 1767.3 | 1767.5 | | 1767.0 |
| 5 (epi)catechin, 1 (epi)gallocatechin | 5B | 1769.3 | | 1769.5 | 1769.2 |
| 4 (epi)catechin, 2 (epi)gallocatechin | 5B | 1785.3 | | 1785.7 | |
| 6 (epi)catechin, 1 gallate | 5B | 1903.4 | 1903.4 | | |
| **Heptamers** | | | | | |
| 7 (epi)catechin | 1A, 5B | 2037.4 | 2038.8 | | |
| 7 (epi)catechin | 2A, 4B | 2039.4 | | | |
| 7 (epi)catechin | 6B | 2041.4 | | 2041.1 | 2041.4 |
| 6 (epi)catechin, 1 (epi)gallocatechin | 1A, 5B | 2053.4 | 2053.6 | | |
| 6 (epi)catechin, 1 (epi)gallocatechin | 2A, 4B | 2055.4 | 2055.6 | | |
| 6 (epi)catechin, 1 (epi)gallocatechin | 6B | 2057.4 | | 2057.8 | |
| 5 (epi)catechin, 2 (epi)gallocatechin | 6B | 2073.4 | | 2073.9 | |
| **Octamers** | | | | | |
| 8 (epi)catechin | 2A, 5B | 2325.5 | 2325.8 | | |
| 8 (epi)catechin | 7B | 2330.3 | | | 2329.9 |
| 7 (epi)catechin, 1 (epi)gallocatechin | 2A, 5B | 2341.4 | 2342.5 | | |
| 7 (epi)catechin, 1 (epi)gallocatechin | 1A, 6B | 2343.5 | | | |
| 7 (epi)catechin, 1 (epi)gallocatechin | 7B | 2345.5 | | 2345.5 | |
| 6 (epi)catechin, 2 (epi)gallocatechin | 7B | 2361.5 | | 2361.7 | |
| **Nonamers** | | | | | |
| 9 (epi)catechin | 2A, 6B | 2613.5 | 2613.9 | | |
| 9 (epi)catechin | 8B | 2617.9 | | | |
| 8 (epi)catechin, 1 (epi)gallocatechin | 1A, 7B | 2631.5 | 2632.1 | | |
| 8 (epi)catechin, 1 (epi)gallocatechin | 8B | 2633.5 | | | |
| 7 (epi)catechin, 2 (epi)gallocatechin | 8B | 2649.5 | | 2650.2 | |
| **Decamers** | | | | | |
| 10 (epi)catechin | 2A, 7B | 2901.6 | 2901.9 | | |
| **Undecamers** | | | | | |
| 11 (epi)catechin | 2A, 8B | 3189.6 | 3189.2 | | |
| **Dodecamers** | | | | | |
| 12 (epi)catechin | 2A, 9B | 3477.7 | 3476.9 | | |

(up to DP 9) or presenting one (epi)gallocatechin unit plus one galloylated unit (up to DP8) were also detected. These findings seem to be in agreement with a previous study based on thiolytic degradation (*41*). Although the occurrence of A-type proanthocyanidins has not been previously reported in hazelnuts, signals corresponding to trimeric heteropolymers containing one A-type linkage were also detected. To our knowledge this is the first

report of the structural composition of hazelnut skin proanthocyanidins by MALDI-TOF MS.

Application of MALDI-TOF MS to roasted almond skins revealed the presence of A- and B-type proanthocyanidins corresponding to procyanidins (up to DP6 for A-type structures and up to DP8 for B-type structures) or to procyanidin−prodelphinidin heteropolymers (up to DP6) containing up to two (epi)gallocatechin units in the case of trimers or just a single unit from DP4 to DP6 (**Figure 5**; **Table 4**). Although propelargonidins have been identified in almond skins after blanching and drying (*17*), they were not detected in the roasted almond skins studied herein.

In summary, flavan-3-ols from roasted skins from peanuts, hazelnuts, and almonds differed in concentration, structural composition, type of interflavan linkage, and DP. In terms of concentration, roasted peanut and hazelnut skins presented similar total phenolic contents, but their flavan-3-ol distributions and compositions varied considerably. Peanut skins were low in monomeric flavan-3-ols in comparison to hazelnuts. On the other hand, polymeric flavan-3-ols in peanuts occurred as both A- and B-type proanthocyanidins (up to DP12), but predominately in the A forms, whereas hazelnuts were mainly constituted by B-type proanthocyanidins (up to DP9). Finally, roasted almond skins were considerably lower in total polyphenols than peanut and hazelnut skins, and the high molecular weight polymers (up to DP8) were mainly composed of B-type proanthocyanidins. As a result of their high polyphenolic content, whole extracts from roasted skins from hazelnuts and peanuts presented higher antioxidant capacity than roasted almond skins; however, the antioxidant capacities of the HMW fractions of the three types of nut skins were equivalent despite their different compositions and DPs. Nevertheless, it is important to highlight that due to this structural diversity, the in vivo antioxidant capacities are expected to differ considerably among the different nut skins.

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