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Polyphenolic Composition of Hazelnut Skin

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ABSTRACT: Skins from different hazelnut samples were characterized for total polyphenol content, total antioxidant capacity (TAC), and their content in specific polyphenolic compounds. The main polyphenolic subclass, identified and quantified by means of HPLC−MS/MS, comprised monomeric and oligomeric flavan-3-ols, which accounted for more than 95% of total polyphenols. Flavonols and dihydrochalcones were 3.5% while phenolic acids were less than 1% of the total identified phenolics. The TAC values of the skin samples ranged between 0.6 and 2.2 mol of reduced iron/kg of sample, which is about 3 times the TAC of whole walnuts, 7−8 times that of dark chocolate, 10 times that of espresso coffee, and 25 times that of blackberries. By describing the profile of polyphenols present in hazelnut skins, this study provides the basis to further investigate the potential health effects of hazelnut byproduct.

KEYWORDS: flavan-3-ols, hazelnut skin, phenolic compounds, total antioxidant capacity, total polyphenol content

INTRODUCTION

Edible tree nuts include an array of hard-shelled dry fruits from deciduous trees, such as almonds, Brazil nuts, cashew nuts, hazelnuts, macadamias, pecans, pine nuts, pistachios, and walnuts, which have been consumed since ancient times as an important source of energy. Epidemiological studies show a remarkable cardioprotective effect associated with increased nut consumption, consistent in studies across a wide range of different nut intakes and across subpopulations differing in gender, age, geographical location, and occupation,[1] to the point that tree nuts are nowadays considered a qualifying component of healthy diets.[2] The reasons for the supposed benefits of a diet rich in tree nuts has been attributed to their mono- and polyunsaturated fatty acid content,[3] their high level of dietary fiber,[4] and, more recently, the presence of a number of bioactive molecules in the kernel and skin ranging from tocopherols to arginine and to polyphenols,[5] which might exert positive cardiovascular effects such as low-density lipoprotein (LDL) protection from oxidation or enhanced endothelial function.[5]

In particular, the antioxidant capacity of various nuts and their byproducts has been widely investigated, and several works have acknowledged that nut byproducts are especially rich sources of natural phenolic compounds with potential bioactivity.[6−9] For example, in walnuts, most of the polyphenolic compounds are located in the skin and less than 10% is retained in the kernel when the skin is removed. In most other cases, a significant portion of nut phenolics is located in the skin as well.[7]

Hazelnut (*Corylus avellana* L.), which belongs to the family Betulaceae, is one of the most popular tree nuts consumed worldwide, ranking second in tree nut production after almond. Turkey, specifically the Black Sea region, is the world's leading hazelnut-producing area, contributing >75% to the global production, although other important producing areas include Georgia, Spain, Italy, and Chile. Hazelnuts are typically consumed whole (raw, with skin, or roasted, without skin) or used as ingredient in a variety of processed foods, especially in bakery and confectionery products. The hazelnut skin, hazelnut hard shell, and hazelnut green leafy cover, as well as the hazelnut tree leaf, are byproducts of roasting, cracking, shelling/hulling, and harvesting processes, respectively,[6] and are now investigated for their composition in the attempt to add economic value to waste from the hazelnut industry. Previous work has shown the hazelnut kernel to be a source of monomeric flavan-3-ols, B-type procyanidins, and prodelphinidins.[10,11] Other works describe the content of some phenolic acids in hazelnut skin,[6,12] and recent work by Monagas et al. [13] investigated its flavan-3-ol composition (monomers, oligomers, and polymers). However, a complete screening of the phenolic content of hazelnut skin is still lacking despite the fact that unpeeled hazelnuts may represent an important dietary source of this class of compounds. Therefore, the aim of this work was the qualitative and quantitative characterization of skin polyphenols in different samples of *C. avellana* L., originating from the major hazelnut-growing areas of the world.

MATERIALS AND METHODS

Commercial Standards and Reagents. (+)-Catechin, (−)-epicatechin, (−)-epicatechin 3-*O*-gallate, gallic acid, protocatechuic acid, quercetin, rutin, syringic acid, coumaric acid, D-(−)-quinic acid, (+)-gallocatechin, and (−)-epigallocatechin were purchased from Sigma-Aldrich Co. (St. Louis, MO). Procyanidin B1 and B2, quercetin 3-*O*-rhamnoside, and phloretin 2′-*O*-glucoside were purchased from Extrasynthese (Genay Cedex, France). All solvents and reagents were purchased from Carlo Erba Reagents (Milano, Italy).

Hazelnut Skins. Skins from hazelnuts harvested in nine different regions were analyzed in this study. Four samples came from the Black Sea region of Turkey (Akçakoca, Giresun, Ordu, and Trabzon) and were all "multicultivar" samples. Four samples came from Italy (Tonda di

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Giffoni and Mortarella from Campania, Tonda Gentile delle Langhe from Piedmont, Tonda Gentile Romana from Lazio) and were all monocultivar samples. One was a monocultivar sample of Tonda Gentile delle Langhe harvested in Chile. Hazelnut skins were kindly supplied by Soremartec Italia Srl (Alba, CN, Italy). Hazelnuts were stored at 4 °C for 2 months and were subsequently roasted in hot-air laboratory roasters working at an average temperature of 160 °C for 35 min, causing the dehydration of the skin that was removed by air blasting. The skins were immediately sealed in 250 g vacuum aluminum bags and analyzed within 2 weeks. Individual samples were provided in triplicate.

Extraction of Polyphenols from Hazelnut Skins. Two different extraction procedures were applied: formic acid/water and methanol/water. For the extraction with 1% aqueous formic acid, hazelnut skins were pulverized with a blender (La Moulinette, Moulinex, Ecully Cedex, France), and 500 mg was weighed into 10 mL glass tubes. After addition of 5 mL of aqueous formic acid, skins were extracted for 30 min in a sonic bath. The tubes were then heated at 70 °C for 1 h in a Dubnoff bath at 20 strokes/min. At the end of the extraction procedure, the tubes were centrifuged for 10 min at 3500 rpm (2000*g*), the supernatant was removed, and the residual pellet of skins underwent a second complete extraction procedure under the same conditions. Pooled aqueous extracts were filtered through a 0.45 μm nylon filter and stored at −80 °C until analysis. For the methanolic extract, 5 mL of methanol/H_2O (75:25, v/v) was added to 500 mg of ground skins in 10 mL glass tubes. The tubes underwent sonic bath extraction for 15 min, immediately followed by 15 min of vortex mixing. This procedure was repeated twice to give a final extraction time of 1 h. The methanolic extracts were centrifuged for 10 min at 3500 rpm (2000*g*) and the filtered supernatants stored at −80 °C prior to analysis.

HPLC−ESI-MS/MS Analysis. Skin extracts were analyzed using a Water 2695 Alliance separation module equipped with a Micromass Quattro Micro Api mass spectrometer fitted with an electrospray interface (ESI) (Waters, Milford, MA). Separations were performed using a Waters Atlantis dC18 3 μm (2.1 × 150 mm) reversed-phase column (Waters), with the mobile phase pumped at a flow rate of 0.17 mL/min. For both the aqueous and methanolic extracts, an MS scan analysis was carried out for a preliminary investigation of the polyphenol content. The ESI source performed analysis on the samples in the negative ionization mode. The source temperature was 120 °C, the desolvation temperature was 350 °C, the capillary voltage was 2.8 kV, and the cone voltage was 35 V. The mobile phases were 1% aqueous formic acid (A) and acetonitrile (B) for all the applied chromatographic separations. The gradient was 0−35% B over 40 min.

On the basis of the MS scan data obtained, three multiple reaction monitoring (MRM) methods were set up for final identification and quantification of phenolic acids and flavonoids. The MRM method for monomeric flavan-3-ols and proanthocyanidins used capillary and cone voltages of 3.20 kV and 35 V, respectively (optimized by infusing a pure chemical of procyanidin B2), while the collision energy was set at 30 eV. For these analytes, the gradient was 5−30% B over 30 min. The second MRM method was set up for identification of phenolic acids. The method was optimized by infusing a pure solution of protocatechuic acid, with capillary and cone voltages set at 2.50 kV and 25 V, respectively, and collision energy of 20 eV. The gradient was 0−15% B over 30 min. The third MRM method was developed for flavonols and dihydrochalcone identification. In this case, the tuning of mass spectrometry was optimized through a direct infusion of quercetin standard, with capillary and cone voltages set at 2.80 kV and 35 V, respectively, and a collision energy of 30 eV. In this case, the gradient was 20−40% B over 10 min. For all MRM methods, the ESI source operated in negative mode with a temperature of 120 °C, desolvation temperature of 350 °C, desolvation gas (N_2) at 750 L/h, cone gas (N_2) at 50 L/h, and argon as the collision gas.

Table 1. Analytical Parameters for the Determination of Polyphenols in Hazelnut Skins: Linear Dynamic Range, LOD, and LOQ for Each Used Standard

phenolic standard	linearity (μmol/L)	LOD (μmol/L)	LOQ (μmol/L)
procyanidin B2	1.0−200.0	0.3	1.0
(+)-catechin	2.0−500.0	0.6	2.0
(-)-epicatechin 3-*O*-gallate	1.0−50.0	0.3	1.0
gallic acid	0.5−25.0	0.2	0.5
protocatechuic acid	0.5−25.0	0.2	0.5
phloretin 2′-*O*-glucoside	1.0−100.0	0.3	1.0
quercetin 3-*O*-rhamnoside	0.5−50.0	0.2	0.5
quercetin	0.5−25.0	0.2	0.5

All proanthocyanidins were quantified as procyanidin B2, whereas monomers were quantified as (+)-catechin and (−)-epicatechin 3-*O*-gallate equivalents. The phenolic acids and phloretin 2′-*O*-glucoside were quantified using calibration curves of the appropriate standard compound. Flavonol rhamnosides were quantified as quercetin 3-*O*-rhamnoside equivalents, whereas all aglycons were quantified as quercetin equivalents. The response linearity of each analysis was tested over a wide range of concentrations, and the limits of detection (LOD) and quantification (LOQ) were calculated as the concentration of each analyzed pure standard generating a peak area that was 3 or 10 times the background noise (Table 1).

Colorimetric Assays. Aqueous extracts were also analyzed for their total polyphenol content using the Folin−Ciocalteau method[14] and for their total antioxidant capacity with the ferric reducing antioxidant power (FRAP) assay.[15]

RESULTS

Flavan-3-ols identified in hazelnut skins (including isomers and epimers) are summarized in Table 2. A total of 31 flavan-3-ols were identified and quantified. The identification was based on the mass-to-charge ratio (*m*/*z*) of the molecular ion and on characteristic fragment ions. Comparison with authentic standards was performed whenever possible. Typical LC−MS/MS chromatograms of some identified compounds in aqueous and methanolic extracts are shown in Figure 1.

In detail, also with the aid of the available commercial standard, it was possible to identify (+)-catechin and (−)-epicatechin that share the same molecular ions ($[M - H]^-$) at *m*/*z* 289, (−)-epicatechin 3-*O*-gallate, with an $[M - H]^-$ equal to *m*/*z* 441, and (+)-gallocatechin and (−)-epigallocatechin, sharing the same molecular ions ($[M - H]^-$) at *m*/*z* 305. The latter compounds are not reported in Table 2 because they were present in the aqueous extract at levels below the LOQ. Among proanthocyanidins, at least nine B-type dimers of procyanidins (PCs) were identified, presenting an $[M - H]^-$ at *m*/*z* 577 and typical fragment ions at *m*/*z* 125, 287, 289, 407, 425, and 451 formed by A ring cleavage (*m*/*z* 125), interflavanic bond cleavage through the quinone methide mechanism (*m*/*z* 289 and 287), retro-Diels−Alder (RDA) cleavage and loss of a water molecule (*m*/*z* 425 and 407), and heterocyclic ring fission (*m*/*z* 451).[16−18] The procyanidins B1 and B2 were identified through comparison with authentic standards, whereas procyanidin B3 was identified on the basis of its elution between the B1 dimer and (+)-catechin, as previously reported.[19] Three procyanidin gallate dimers were identified, with $[M - H]^-$ at *m*/*z* 729, and showing the typical

Table 2. Mass Spectral Characteristics of Phenolics Identified in Aqueous Extracts of Hazelnut Skins[a]

Flavan-3-ols			
compound	type[b]	$[M - H]^-$ (m/z)	MS^2 ions (m/z)
B-type trimer	PD	881	**125**, 289, 407, 425, 577, 695
C2 trimer	PC	865	**125**, 287, 289, 407, 425, 575, 577
B-type dimer	PD	593	125, **289**, 407
B-type dimer	PD	593	125, **289**, 407
B-type dimer	PD	593	125, **289**
B-type dimer	PD	593	125, **289**, 407
B-type trimer	PD	881	125
B-type dimer	PD	593	125, **289**, 407
B-type dimer	PD	593	125, **289**, 407
B1 dimer	PC	577	125, 287, **289**, 407, 425, 451
B3 dimer	PC	577	125, 287, **289**, 407, 425, 451
B-type trimer	PC	865	**125**, 287, 289, 407, 425, 575, 577
(+)-catechin		289	137
B-type trimer	PC	865	**125**, 287, 289, 407, 425, 575, 577
B-type dimer	PD	593	289
B-type trimer	PC	865	**125**, 287, 289, 407, 425, 575, 577
B-type dimer	PC	577	125, 287, **289**, 407, 425, 451
B2 dimer	PC	577	125, 287, **289**, 407, 425, 451
B-type dimer	PD	593	125, **289**, 407
B-type dimer	PC	577	125, 287, **289**, 407, 425, 451
(−)-epicatechin		289	137
B-type dimer	PC	577	125, 287, **289**, 407, 425
B-type dimer gallate	PC	729	125, 169, **289**, 407
B-type trimer	PC	865	**125**, 287, 289, 407, 425, 575, 577
B-type dimer gallate	PC	729	125, 169, **289**, 407, 441
B-type dimer	PC	577	125, 287, **289**, 407, 425, 451
B-type dimer	PD	593	125, **289**, 407
B-type dimer	PC	577	125, **289**, 407, 425
(−)-epicatechin 3-*O*-gallate		441	**169**, 289
B-type dimer	PC	577	125, **289**, 407, 425, 451
B-type dimer gallate	PC	729	**289**, 407

Phenolic Acids		
compound	$[M - H]^-$ (m/z)	MS^2 ions (m/z)
(−)-D-quinic acid[c]	191	85, 111
derivate of quinic acid[c]		85, 111, 191
gallic acid	169	125
protocatechuic acid	153	109
hexose ester of syringic acid	359	197, 182
hexose ester of syringic acid	359	197, 182
pentose ester of coumaric acid	295	163, 119

[a] The fragment ions used for MRM quantification (quantifier ions) are reported in bold. [b] PC = procyanidin, and PD = prodelphinidin. [c] Nonphenolic acid.

fragmentation patterns of PC (m/z 125, 287, 289, and 407). The presence of galloylation was clear in one of them, as fragments at m/z 441 (epicatechin gallate) and 169 (gallic acid) were clearly detected. Five PC trimers were identified, characterized by an $[M - H]^-$ at m/z 865 besides typical fragment ions at m/z 125, 287, 289, 407, 425, 451, 575, and 577 formed through the same fragmentation pattern observed for PC dimers. One trimer was tentatively identified as procyanidin C2 on the bais of its elution time preceding that of procyanidin B1.[19] Furthermore, in the aqueous extract, molecules with $[M - H]^-$ at m/z 1153 with fragment ions at m/z 287, 577, and 575 were also present, indicating PC with a degree of polymerization (DP) of 4. However, these tetrameric flavanols (not indicated in Table 2) were not quantified because the relative commercial standards were unavailable and because they were not well resolved. In the aqueous skin extract, the analysis also allowed us to identify several prodelphinidins (PDs). In detail, nine isomers of B-type PD dimers were present on the basis of their $[M - H]^-$ at m/z 593 and their typical spectrometric fragmentation at m/z 125, 289, and 407. Among PD trimers, at least two isomers were present in hazelnut skins, showing an $[M - H]^-$ equal to m/z 881 and m/z 125, 289, 407, 425, 577, and 695 as MS^2 ion fragments.

Some free and bound phenolic acids were also identified, as reported in Table 2. Among free acids, gallic and protocatechuic were identified through their $[M - H]^-$ at m/z 169 and 153, respectively, through the ion fragments at m/z 125 and 109 derived from the loss of the carboxylic group (44 amu), and through comparison with authentic standards. These two hydroxybenzoic acids were previously identified in hazelnut kernels,[20] whereas only gallic acid was already identified in hazelnut byproducts such as skins and shells, green leafy covers, and tree leaves.[6] Two compounds showed an $[M - H]^-$ at m/z 359 with fragments ions at m/z 197 and 182 and were tentatively identified as two hexose esters of syringic acid. Among hydroxycinnamates, one pentose ester of coumaric acid was identified by the $[M - H]^-$ at m/z 295, with fragment ions at m/z 163 and 119, typical of coumaric acid. In fact, this monohydroxycinnamic acid has been previously detected in esterified form in byproducts of *C. avellana* L.[6,12] Quinic acid and a derivative of quinic acid were also present. Free quinic acid was identified through both the comparison of authentic standards and the analysis of the spectrometric characteristics, although for its derivative it was impossible to specify the molecular ion. Quinic acid was not quantified, as it is not included in the polyphenolic class.

The methanolic extraction was performed with the principal objective of quantifying other less polar flavonoids in skin samples. Seven flavonols and one dihydrochalcone (Table 3) were identified on the basis of their chromatographic and spectrometric behaviors and comparison with authentic standards. In detail, quercetin 3-*O*-rutinoside (rutin) was characterized by an $[M - H]^-$ at m/z 609, with a main fragment ion at m/z 301 derived from the loss of the rutinose moiety and corresponding to the aglycon quercetin. Another flavonol was identified as isorhamentin rutinoside, as it showed an $[M - H]^-$ at m/z 623 (14 amu higher than that of rutin) corresponding to one additional methyl group. Furthermore, this compound generated a fragment ion at m/z 315, indicating once again the loss of the rutinose moiety. However, both rutin and isorhamnetin rutinoside were not quantified because they were present at unquantifiable levels. Three flavonol rhamnosides were also identified in the methanolic extract. Myricetin rhamnoside was identified on the basis of an $[M - H]^-$ at m/z 463, with a main fragment ion at m/z 317 derived from the loss of rhamnose and subsequent aglycon ionization. Quercetin 3-*O*-rhamnoside (quercitrin) showing an $[M - H]^-$ at m/z 447, with a fragment ion of m/z

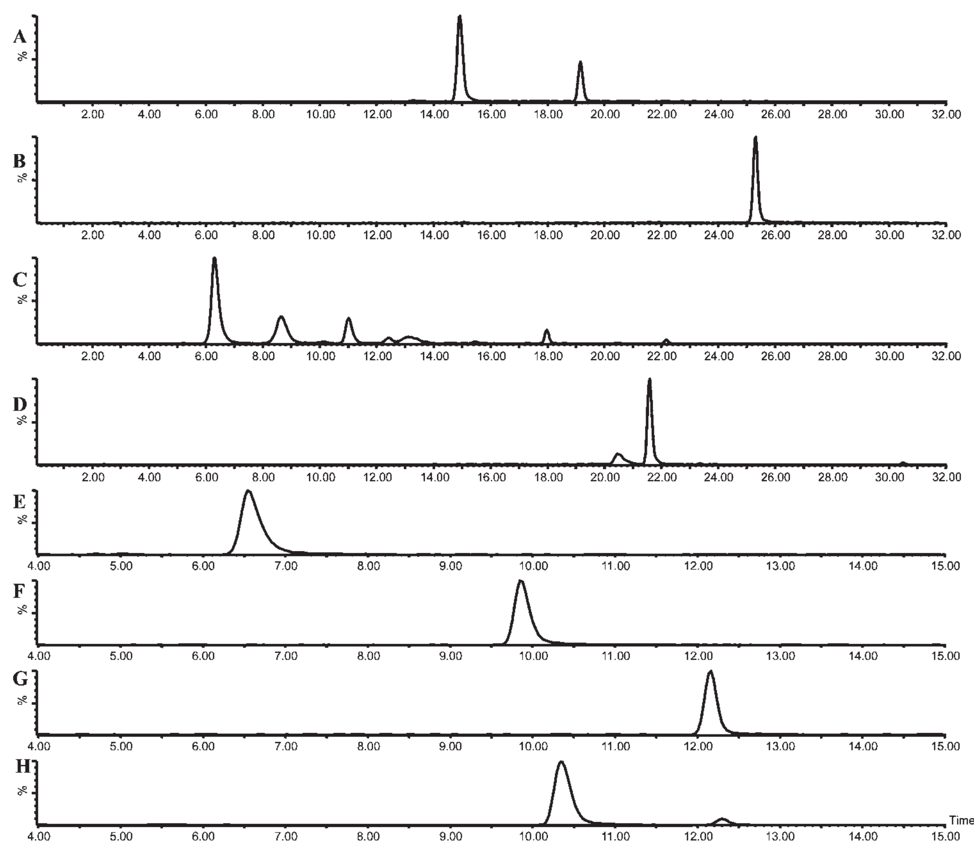

Figure 1. LC−MS/MS traces of several phenolic compounds detected in hazelnut skin extracts: (A) (+)-catechin and (−)-epicatechin; (B) epicatechin 3-*O*-gallate; (C) B-type dimers of PD; (D) B-type dimer gallates of PC; (E) myricetin rhamnoside; (F) quercetin 3-*O*-rhamnoside; (G) kaempferol rhamnoside; (H) phloretin 2′-*O*-glucoside.

Table 3. Mass Spectral Characteristics of Phenolics Identified in Methanol Extract of Hazelnut Skins

compound	$[M - H]^-$ (m/z)	MS^2 ions (m/z)
quercetin 3-*O*-rutinoside	609	301
isorhamnetin rutinoside	623	315
myricetin rhamnoside	463	317
quercetin 3-*O*-rhamnoside	447	301
kaempferol rhamnoside	431	285
phloretin 2′-*O*-glucoside	435	273
myricetin	317	151
quercetin	301	151

301 formed after the loss of rhamnose, was then confirmed by reference to an authentic standard. Kaempferol rhamnoside was identified through MS data, consisting of an $[M - H]^-$ at m/z 431 (16 amu lower than that of quercitrin) and a fragment ion at m/z 285, indicating the loss of the sugar moiety and the ionization of kaempferol. These glycosylated flavonols have already been identified in leaves and kernels of *C. avellana* L., with the exception of kaempferol rhamnoside, which appears to be present only in the leaves.[20,21] Finally, in the methanolic extract two aglycon flavonols, namely, myricetin and quercetin, were also detected. Both flavonols shared the same fragment ion at m/z 151, but the molecular signal for myricetin was at m/z 317 whereas m/z 301 was the molecular ion for quercetin. The presence of quercetin was also unambiguously established by comparison with a pure standard.

Finally, a phenolic compound belonging to the dihydrochalcone subclass, namely, phloretin 2′-*O*-glucoside (phloridzin), was also identified. In detail, this polyphenol showed an $[M - H]^-$ at m/z 435 and a main fragment ion at m/z 273 derived from the loss of the glucose moiety and resulting ionization of phloretin, as recently reported in a study focused on the phenolic content of hazelnut kernels.[20] The comparison with a pure standard confirmed the preliminary identification.

The quantitative profiles of flavan-3-ols and phenolic acids in the aqueous extracts of all samples are reported in Table 4. The quantitative profiles of other flavonoids recovered in the methanolic extract are reported in Table 5.

The total polyphenolic content (measured by the Folin−Ciocalteu assay) and the total antioxidant capacity content (measured by the FRAP assay) of the nine different samples of hazelnut skin are reported in Table 6.

■ DISCUSSION

To our knowledge, this is the most complete study describing the polyphenolic composition of hazelnut skins. Unfortunately, it was impossible to know which specific hazelnut cultivar was present in the multicultivar Turkish samples. However, at the industrial level, hazelnuts harvested outside of Italy are discriminated by their region of origin, and not by their cultivar.

The average content of total polyphenols of the nine skin samples analyzed, as measured by HPLC−MS/MS, was about 675 mg/100 g. The main polyphenolic subclass was by far the flavan-3-ols in both their monomeric and polymeric forms,

Table 4. Quantification of Phenolic Compounds in Hazelnut Skin Aqueous Extracts[a]

	flavan-3-ols								phenolic acids	
skin type	C	EC	ECG	PC dimers	PC dimer gallates	PC trimers	PD dimers	PD trimers	GA	PA
Akçakoca	178.9 ± 37.8	30.7 ± 6.2	6.1 ± 0.8	474.6 ± 17.0	23.9 ± 1.3	35.1 ± 5.3	60.8 ± 2.3	4.8 ± 0.9	1.6 ± 0.1	1.4 ± 0.0
Chile	99.9 ± 5.7	11.9 ± 1.6	0.7 ± 0.1	133.2 ± 1.4	3.4 ± 0.3	4.0 ± 0.9	25.2 ± 0.8	NQ	3.0 ± 0.1	2.4 ± 0.1
Giffoni	190.0 ± 29.2	22.3 ± 4.3	3.0 ± 0.4	277.5 ± 34.4	7.7 ± 2.3	16.0 ± 2.1	72.5 ± 11.8	4.6 ± 0.2	4.0 ± 0.0	2.2 ± 0.0
Giresun	153.1 ± 28.5	30.2 ± 1.2	4.9 ± 0.8	278.7 ± 21.7	18.5 ± 1.8	26.5 ± 5.9	48.7 ± 3.6	4.3 ± 1.1	1.9 ± 0.1	2.4 ± 0.1
Mortarella	200.9 ± 5.1	32.4 ± 1.9	4.8 ± 0.7	383.2 ± 24.7	17.0 ± 1.4	27.4 ± 3.7	76.9 ± 4.6	5.0 ± 0.4	2.6 ± 0.1	1.3 ± 0.1
Ordu	274.3 ± 11.5	30.4 ± 1.1	6.7 ± 0.2	445.2 ± 12.6	19.5 ± 0.5	35.9 ± 3.7	54.3 ± 1.2	3.6 ± 0.3	3.6 ± 0.1	3.4 ± 0.1
TGL	193.1 ± 6.8	75.9 ± 6.8	10.2 ± 0.4	341.6 ± 17.4	21.3 ± 1.2	14.7 ± 2.3	112.6 ± 7.9	5.9 ± 0.2	3.9 ± 0.6	1.8 ± 0.2
TGR	191.0 ± 28.2	60.8 ± 12.9	5.9 ± 2.6	359.7 ± 68.6	15.4 ± 2.9	17.6 ± 1.9	55.9 ± 11.0	3.1 ± 1.3	2.1 ± 0.1	1.0 ± 0.0
Trabzon	145.5 ± 5.6	25.2 ± 0.9	6.6 ± 0.1	220.5 ± 1.0	20.4 ± 0.4	17.8 ± 1.5	31.8 ± 0.2	3.6 ± 0.3	3.6 ± 0.2	2.4 ± 0.2

[a] Data expressed as mg/100 g ± SD (n = 3). C = (+)-catechin, EC = (−)-epicatechin, ECG = (−)-epicatechin 3-*O*-gallate, PC = procyanidin, PD = prodelphinidin, GA = gallic acid, PA = protocatechuic acid, NQ = not quantifiable, TGL = Tonda Gentile delle Langhe, and TGR = Tonda Gentile Romana.

Table 5. Quantification of Phenolic Compounds in Hazelnut Skin Methanolic Extracts[a]

	flavonols					dihydrochalcone
skin type	quercetin	myricetin	quercetin 3-*O*-rhamnoside	myricetin rhamnoside	kaempferol rhamnoside	phloretin 2′-*O*-glucoside
Akçakoca	2.3 ± 0.3	0.2 ± 0.0	8.7 ± 0.4	0.8 ± 0.0	0.4 ± 0.0	10.6 ± 0.5
Chile	1.6 ± 0.1	0.2 ± 0.0	4.7 ± 0.3	0.5 ± 0.0	0.4 ± 0.0	8.8 ± 0.6
Giffoni	1.5 ± 0.2	0.2 ± 0.0	7.2 ± 0.5	1.0 ± 0.1	0.4 ± 0.0	19.0 ± 1.4
Giresun	1.8 ± 0.3	0.3 ± 0.1	8.1 ± 0.3	1.0 ± 0.1	0.5 ± 0.0	10.3 ± 0.3
Mortarella	1.4 ± 0.1	0.2 ± 0.0	6.6 ± 0.2	0.9 ± 0.0	0.4 ± 0.0	11.4 ± 0.9
Ordu	2.9 ± 0.2	0.2 ± 0.0	10.0 ± 0.5	0.7 ± 0.0	0.4 ± 0.0	13.3 ± 0.7
TGL	2.7 ± 0.2	0.5 ± 0.0	9.4 ± 0.4	1.7 ± 0.0	0.4 ± 0.0	17.4 ± 0.7
TGR	2.5 ± 1.5	0.2 ± 0.1	11.1 ± 0.7	0.7 ± 0.1	0.8 ± 0.0	13.6 ± 0.8
Trabzon	2.4 ± 0.6	0.2 ± 0.0	7.0 ± 0.7	0.5 ± 0.0	0.5 ± 0.0	5.7 ± 0.4

[a] Data expressed as mg/100 g ± SD (n = 3). TGL = Tonda Gentile delle Langhe, and TGR = Tonda Gentile Romana.

Table 6. Total Polyphenol Content (Folin−Ciocalteau Assay) and Total Antioxidant Capacity (FRAP Assay) in Hazelnut Skin Aqueous Extracts

sample[a]	Folin−Ciocalteau[b]	FRAP[c]
Chile	4.1 ± 0.4	660.1 ± 59.1
Trabzon	7.6 ± 0.1	1028.5 ± 60.5
Mortarella	8.2 ± 0.3	1246.5 ± 45.3
Giresun	8.6 ± 0.1	1289.6 ± 51.5
Giffoni	8.7 ± 0.3	1321.1 ± 54.4
TGR	9.0 ± 0.0	1229.9 ± 47.0
Ordu	10.9 ± 1.1	1726.8 ± 134.4
TGL	11.1 ± 0.3	1782.3 ± 37.5
Akçakoca	12.7 ± 0.4	2206.0 ± 93.6

[a] TGL = Tonda Gentile delle Langhe, and TGR = Tonda Gentile Romana. [b] Mean ± SD (n = 3), g of polyphenol/100 g of hazelnut skin. [c] Mean ± SD (n = 3), mmol of reduced Fe/kg of hazelnut skin.

accounting for more than 95% of the total polyphenols detected. Flavonols and dihydrochalcones represented an additional 3.5% while phenolic acids were responsible for less than 1% of the total identified phenolics. Among the different hazelnut samples, Ordu and Akçakoca were the richest in monomeric and oligomeric flavan-3-ols, respectively, whereas prodelphinidins reached their highest level in the Tonda Gentile delle Langhe (TGL) skin. Among monomers, catechin alone constitutes 80% of the total content on average. Ordu also provided the skin highest in catechin, whereas TGL contained the highest level of the other two identified flavan-3-ol monomers, namely, epicatechin and its gallate form. The skin sample from the Chilean hazelnut ranked the lowest for all flavan-3-ols. Ordu was also the cultivar with the highest phenolic acid content, whereas the lowest content was observed in Akçakoca.

Compared to flavan-3-ols, the flavonols and phloretin identified in the methanolic extracts showed a higher variability without clear differences among cultivars.

The aqueous extracts, containing the flavan-3-olic fraction, were also analyzed with the colorimetric Folin−Ciocalteau assay, which measures the total polyphenol content through a chemical reduction. This approach is considered to give a rough approximation of the real polyphenol content, but is also known to take into account the high molecular weight components that are not resolved for DP > 4 with the MS/MS technique used. Hazelnut skin is a rich source of high molecular weight tannins, as already observed in a recent study reporting the presence of procyanidins with DP up to 7 and prodelphinidins with DP up to 9 in hazelnut skin samples by means of MALDI-TOF mass spectrometry.[13] Nevertheless, the level of heavy compounds is not necessarily in line with the profile of low molecular weight polyphenols in

these samples. As an example, Ordu, which was the highest in flavan-3-ol content (considering up to 3 as the degree of polymerization), was not the highest in total phenolics after the Folin−Ciocalteau assay. Akçakoca presented the highest Folin−Ciocalteau value with almost 13 g of total polyphenols in 100 g. Values such as these classify hazelnut skins as one of the highest dietary sources of polyphenols, surpassing grapeseed extracts, cocoa, tea, and red wine.[22]

The total antioxidant capacity (TAC) of each sample was also evaluated with the FRAP assay. In comparison to most of the food items recognized as being rich in antioxidants,[23,24] hazelnut skins are largely more active in reducing iron by this antioxidant assay. The FRAP values ranged between 0.6 and 2.2 mols of reduced iron/kg of sample, which is approximately 3 times the value shown by whole walnuts (the food item with the highest FRAP according to Pellegrini et al.[23]), 7−8 times the value of chocolate, 10 times the value of espresso coffee, and 25 times the antioxidant capacity of blackberries.[23,24]

All these observations indicate that hazelnut skins are an extremely rich source of several different polyphenolic compounds. Polyphenols have been linked to protection against several chronic diseases[25] with mechanisms of action possibly involving antioxidant, anti-inflammatory, and vasoactive properties.[26,27]

It has been reported that a diet with a high total antioxidant capacity, to which polyphenols are the main contributor, is associated with several health markers, including reduced inflammation,[28] increased circulating β-carotene,[29] improved vascular elasticity,[30] and increased stool weight.[31] A high TAC diet could also be protective against ischemic stroke.[32] The higher quartile of intake of dietary TAC in these studies was on the order of 25−30 mmol of reduced iron as measured by FRAP, which is the TAC that could be provided by less than 5 g of hazelnut skin as found in the present study. It must be pointed out that the effect of roasting on TAC could result in thermal degradation of naturally occurring antioxidants, but also in the formation of new Maillard reaction products having antioxidant activity.[33]

From a more polyphenol-specific point of view, several compounds, which are present in hazelnut skins at high concentrations, have been described in the literature as potentially related to health effects. Epidemiological studies suggest that proanthocyanidins could reduce the risk of cardiovascular diseases. Apart from their antioxidant activity, one of the mechanisms by which they could exert cardiovascular protection is by improving lipid homeostasis.[34] In animal studies, acute supplementation with proanthocyanidins was able to increase the oxidative capacity of skeletal muscle and brown adipose tissue mitochondria.[35] Moreover, in endothelial cells, procyanidins from different food sources have been shown to increase the formation of potent vasoprotective factors, including nitric oxide (NO) and endothelium-derived hyperpolarizing factor. Experimental and clinical studies indicate that daily intake of several procyanidin-rich foods could improve endothelial dysfunction and decrease vascular oxidative stress associated with major cardiovascular risk factors such as hypertension.[36] Oligomeric procyanidins were identified as the principal vasoactive polyphenols in red wine, present at higher concentrations in wines from areas of southwestern France and Sardinia where traditional production methods ensure that these compounds are efficiently extracted during vinification.[37] These regions also happen to be associated with increased longevity in the population. However, numerous feeding studies with animals and humans indicate that oligomeric and polymeric procyanidins are not efficiently absorbed and most pass unaltered to the large intestine where they are exposed to the colonic microbiota, yielding an array of smaller phenolic acids, which are absorbed into the circulatory system and are the best candidates for procyanidin health-related effects.[38]

In conclusion, even with the inevitable limitation linked to the impossibility of quantifying all the identified polyphenols using the exact standards, we have provided a quantitative profile of polyphenols found in the skins of hazelnuts harvested in nine different world areas. Hazelnut skins, which are generally considered a byproduct, are probably one of the richest edible sources of polyphenolic compounds. Specifically identified polyphenolic components have recently been linked to several health effects in animals and humans, and the astounding antioxidant capacity of these skins makes them a very interesting and innovative ingredient to increase the daily antioxidant intake with natural ingredients.

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ABBREVIATIONS

$[M-H]^-$, negatively charged molecular ion; DP, degree of polymerization; FRAP, Ferric reducing antioxidant power; MRM, multiple reaction monitoring; NO, nitric oxide; PC, procyanidin; PD, prodelphinidin; RDA, retro-Diels−Alder; TAC, total antioxidant capacity; TGL, Tonda Gentile delle Langhe; TGR, Tonda Gentile Romana

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