Journal of Food Science and Technology (Iran)

Homepage:www.fsct.modares.ir

Scientific Research

Investigation of the Chemical Composition, Aflatoxin Level, and Phenolic Profile of Hazelnut Skin

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ARTICLE INFO

Article History:

Received: 2025/11/10
Accepted: 2025/12/20

Keywords:

Hazelnut skin,

Proximate composition,

Phenolic profile,

Aflatoxin,

HPLC

DOI: 10.48311/fsct.2025.117604.82926

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ABSTRACT

Hazelnut skin is a thin brown coating surrounding the kernel that separates during roasting and is usually discarded as a by-product. However, it is rich in dietary fiber and bioactive compounds that vary by cultivar and region. In this study, the chemical composition and aflatoxin levels of hazelnut skins from Kocaeli and Ordu provinces in Türkiye were analyzed, with phenolic contents evaluated only for the Ordu sample. In the Kocaeli sample, dry matter, ash, fat, and protein contents were 91.07%, 2.14%, 12.94%, and 8.72%, respectively; whereas for the Ordu sample they were 95.38%, 1.90%, 26.55%, and 15.03%. Phenolic and lipid functional groups were indicated by infrared analysis. The phenolic profile showed that isoquercitrin (436.69 µg/g), catechin (270.77 µg/g), and quinic acid (231.64 µg/g) were the major compounds, while rutin (49.09 µg/g), hesperidin (20.46 µg/g), and quercetin (13.87 µg/g) were detected at moderate levels, including some low-abundance phenolics rarely reported in hazelnut skins. Total aflatoxin in the Ordu sample was below the applicable EU maximum level (10 µg/kg) for the relevant nut product category, whereas the Kocaeli sample was close to this limit (9.99 µg/kg). These findings suggest that the Ordu sample contained higher fat, protein, and selected phenolics, supporting its potential use as a functional ingredient. Improved drying and monitoring practices may help reduce aflatoxin G_2 contamination. Further studies are required to confirm bioavailability and safety under different conditions.

1. Introduction

Hazelnut (*Corylus avellana L.)* is a commercially valuable crop and a significant agricultural product in the Black Sea region of Türkiye contributing to 64% of global production [1]. Hazelnut skin (testa) is a thin brown layer surrounding the hazelnut kernel, which is detached during roasting, and mostly utilized as animal feed or fuel [2, 3]. However, hazelnut skins are rich in dietary fiber, phenolic acids (gallic, caffeic, quinic), flavonoids (catechin, quercetin, rutin), proanthocyanidins, and tocopherols, all of which are associated with reported antioxidant, antimicrobial, and anticancer properties [4, 5].

Studies show that the incorporation of hazelnut skins into processed foods such as bread, biscuits, sausages, and crackers enhance both nutritional and functional quality by increasing fiber content and bioactive potential; however, thermal processing, particularly roasting, can reduce total phenolic content and antioxidant activity. Recently, hazelnut skins have gained attention as a useful natural ingredient in the development of functional foods, with improved technological properties of baked goods, meat emulsions, and fortified beverages [2, 5, 6].

The chemical composition of hazelnuts and their by-products (inner membrane, hard shell, green shell, leaves, skin) is strongly dependent on cultivar, geographic origin, and post-harvest practices [7, 8]. Among the eighteen hazelnut varieties cultivated in Türkiye, Palaz and Çakıldak, predominantly grown in Ordu province, are particularly valued for their nutritional attributes [9]. Previous studies reported that hazelnut skins from Ordu exhibit relatively high proximate composition and antioxidant capacity [10, 11], while similar high values were also reported for Giresun skins [12]. Meanwhile, Kocaeli represents a relatively new hazelnut-growing area, with younger orchards and increasing yield potential, but limited information exists regarding the composition and safety of its by-products. While some research has examined hazelnut kernels [13] and the technological contributions of hazelnut skins to food products [2], these studies did not focus on regional differences and systematic comparisons of regional hazelnut skins. The study by Ceran Güraslan [15] on the Akçakoca sample, together with earlier reports on cultivar and regional differences in hazelnut skins [10, 7, 8, 11, 14] is limited, particularly with respect to advanced phenolic profiling.

Although hazelnut skin is traditionally considered a by-product of low economic value, its high content of phenolics and dietary fiber positions it as a potential raw material for functional food, cosmetic, and pharmaceutical industries [3, 5]. Therefore, the present study investigated hazelnut skin from Ordu and Kocaeli provinces in terms of proximate composition and aflatoxin contamination, with detailed UHPLC-MS/MS phenolic profiling conducted for the Ordu sample. The samples were chosen to illustrate the composition of hazelnut skin, rather than to provide statistical regional comparisons. Accordingly, this study was designed to test the hypothesis that hazelnut skin possesses high proximate and phenolic contents and low aflatoxin levels, supporting its potential use as a functional food ingredient. From an industrial perspective, hazelnut skin may serve as a natural antioxidant and fiber-rich ingredient for bakery and meat products, supporting by-product upcycling and sustainability.

2. Materials and Methods

2.1. Materials and Sample Preparation

In this study, hazelnut skin samples were

collected in March 2024 from two industrial processors, Yiğit Fındık (Kocaeli, Türkiye) and Gürsoy Fındık A.Ş. (Ordu, Türkiye). Each region was represented by a single industrial lot, and the study was designed as an exploratory/descriptive assessment rather than a statistically representative regional survey. Due to bulk industrial processing and mixed sourcing of raw material, cultivar-specific information could not be determined. The nuts were dried and shelled, and roasted under routine industrial conditions (approximately 150 °C for 30 min); therefore, roasting intensity was not experimentally varied or controlled as an independent factor in this study. The processors indicated that routine warehouse storage was applied before roasting, although detailed temperature and humidity data were not recorded. The skins were separated from the kernels by airflow. In the laboratory, the skins were ground using a bench-top grinder (Kiwi KSPG-4812) and stored at -18 °C in the dark until analysis.

2.2. Chemicals and reagents

All solvents and reagents (n-hexane, methanol, acetonitrile, formic acid, sulfuric acid, sodium hydroxide, boric acid, catalyst tablets) were of analytical grade and purchased from Merck (Darmstadt, Germany). Aflatoxin standards (B_1, B_2, G_1, G_2) and immunoaffinity columns were obtained from Merck and R-Biopharm (Darmstadt, Germany). Phenolic standards (gallic acid, catechin, isoquercitrin, rutin, quercetin, hesperidin, etc.) were supplied by Merck, while deuterated internal standards (Rutin-D3, Quercetin-D3, Ferulic acid-D3) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.3. Proximate composition (dry matter, ash, fat, protein)

Dry matter was determined by the constant weight method at 105 °C. Ash was determined by combustion in a muffle furnace at 550 °C. Fat content was determined by Soxhlet extraction (n-hexane solvent). Protein content was determined using the Kjeldahl method (Kjeldahltherm and Gerhardt Vapodest 30s, Germany), and results were calculated using a conversion factor of N × 6.25 [16].

2.4. Aflatoxin analysis by High Performance Liquid Chromatography with Fluorescence Detection (HPLC-FLD)

Aflatoxins B_1, B_2, G_1 and G_2 were determined by HPLC-FLD (Shimadzu, Japan) after extraction with 70% methanol and immunoaffinity column (IAC) clean-up, as described in [17, 18]. Separation was performed on a C18 column (4.6 × 250 mm, 5 µm) with post-column electrochemical bromination (KOBRA cell; Ex 362 nm, Em 425 nm); injection volume was 50 µL. Quantification used matrix-matched calibration ($r^2 \geq 0.995$). Non-detects (ND) were defined as concentrations below the matrix limit of quantification (LOQ); the matrix LOQ was 0.74 µg/kg per analyte. Method performance information including matrix LOQ, calibration linearity, and injection repeatability is provided (matrix LOQ is reported in this section, while chromatographic verification is shown in Figures 1-2); AOAC-based procedures with IAC clean-up were followed [17, 18].

2.5. Structural characterization by Fourier Transform Infrared Spectroscopy (FTIR)

The functional groups of hazelnut skin samples were characterized using an FTIR spectrophotometer (Agilent, Cary series, ATR mode, USA) in the range of 4000-400 cm^{-1} with a resolution of 1 cm^{-1}. Measurements were performed in Attenuated Total Reflectance (ATR) mode [19].

2.6. Determination of phenolic compounds by Ultra-High Performance Liquid Chromatography-Tandem Mass Spectrometry (UHPLC-MS/MS)

Phenolic compounds in the Ordu hazelnut skin sample were analyzed using a UHPLC-MS/MS system (Shimadzu LCMS-8040,

Japan). A total of 53 phenolic compounds were screened qualitatively and quantitatively following the tested method of Yılmaz (2020). The method demonstrated high sensitivity and reliability, with correlation values (r^2) >0.995, LOD and LOQ values <20 µg/L, and linearity in the range 0.05-50 mg/L. Rutin-D3, quercetin-D3, and ferulic acid-D3 were used as deuterated internal standards. Validation was carried out through calibration curves and precision (RSD%) determinations, and the results were consistent with previous reports [21, 20]. Compound-specific LOD/LOQ values and validation parameters (linearity, RSD%) are provided in Supplementary Table S2. Despite existing studies on hazelnut skins, comparative data integrating regional composition, targeted phenolic profiling, and aflatoxin occurrence remain limited.

2.7. Statistical Analysis

Analyses were performed on one independent lot from each region (Ordu, Kocaeli). Proximate composition was measured in triplicate. Because each province was represented by one industrial lot (no regional replication), inferential statistics (e.g., hypothesis testing between regions) were not applied; results are reported as descriptive mean ± SD to avoid overinterpretation.

3. Results and Discussion

3.1. Dry Matter and Ash Content

Dry matter was observed to be higher in the Ordu sample than in the Kocaeli sample, while ash contents were comparable (Table 1). Our findings of dry matter (91.07-95.38%) and ash (1.90-2.14%) contents were similar to previous reports [13, 3]. These results are consistent with hazelnut skins containing significant mineral compounds and show the effect of cultivar differences and growing conditions on their chemical composition. Accordingly, hazelnut skins, with their favorable dry matter and ash contents, may be considered as a mineral-rich dietary fiber source for incorporation into functional foods and bakery products [2].

Table 1. Dry matter and ash contents of hazelnut skin samples

Sample	Dry Matter (%)	Ash (%)
Kocaeli	91.07 ± 0.06	2.14 ± 0.06
Ordu	95.38 ± 0.01	1.90 ± 0.02

Note: Values are mean ± SD (n = 3).

3.2. Fat and Protein Content

As shown in Table 2, the Ordu sample appeared to contain relatively higher fat (26.55%) and protein (15.03%) content. These findings are consistent with previous reports on Black Sea hazelnut skins [8, 10]. However, the Kocaeli sample showed lower fat (12.94%) and protein (8.72%) levels, likely reflecting differences in cultivars and orchard characteristics in this relatively new production region. Similar to our observations, Alasalvar et al. [7] and Taş and Gökmen [10] reported that genetic and environmental factors strongly influence the proximate composition of nut by-products. Given the geographical proximity of Akçakoca to Kocaeli, the results of Ceran Güraslan [15] provide a useful comparison, suggesting that Kocaeli skin had protein content comparable to Akçakoca, consistent

with other reports on cultivar and regional differences in hazelnut skins [10, 7, 8, 11].

Table 2. Fat and protein contents of hazelnut skin samples

Sample	Fat (%)	Protein (%)
Kocaeli	12.94 ± 0.08	8.72 ± 0.01
Ordu	26.55 ± 0.29	15.03 ± 0.05

Note: Values are mean ± SD (n = 3).

The results are consistent with regional differences in the nutritional properties of hazelnut skins. Previous studies have reported similar regional differences in hazelnuts and their by-products, emphasizing that altitude and microclimate conditions lead to changes, particularly in lipid and protein levels [11, 5, 12, 14].

3.3. Aflatoxin Analysis

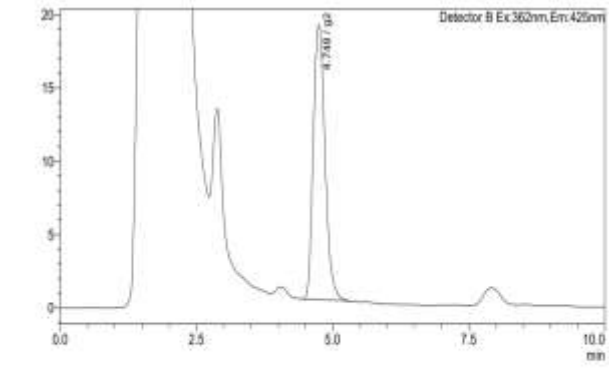
Aflatoxins (B_1, B_2, G_1, G_2) were analyzed in hazelnut skins from Kocaeli and Ordu. B_1, B_2, and G_1 were ND (<LOQ 0.74 µg/kg); G_2 was the only quantifiable aflatoxin (Table 3; Figures 1-2). Duplicate injections were consistent. Total aflatoxin was G_2 with levels of 9.99 ± 0.04 µg/kg in Kocaeli (just below the 10 µg/kg EU limit) and was lower in Ordu (8.04 ± 0.02 µg/kg) [22]. The applicable EU maximum level for total aflatoxins for the relevant nut product category is 10 µg/kg [22]; thus, the Kocaeli lot (9.99 µg/kg) is practically at the regulatory threshold and indicates a need for stricter post-harvest control.

Table 3. Aflatoxin levels (µg/kg) in hazelnut skin samples

Aflatoxin type	Kocaeli Sample (µg/kg)	Ordu Sample (µg/kg)
B_1	ND	ND
B_2	ND	ND
G_1	ND	ND
G_2	9.99 ± 0.04	8.04 ± 0.02
Total	9.99 ± 0.04	8.04 ± 0.02

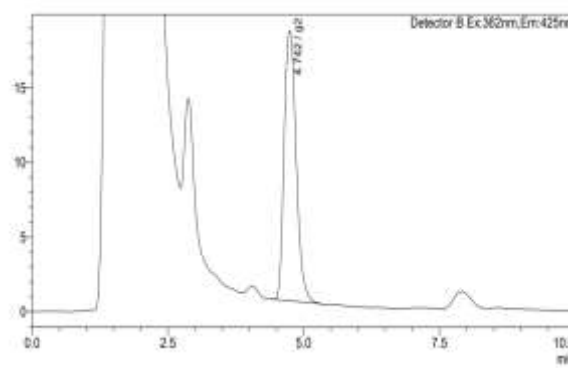
Note: Values are mean ± SD of duplicate injections from the same extract (n = 2). ND (not detected) = below matrix LOQ (0.74 µg/kg). Total aflatoxin = sum of B_1, B_2, G_1, and G_2.

Figure 1. HPLC–FLD chromatograms of aflatoxins in hazelnut skin from Kocaeli (left: first replicate; right: second replicate).



<Quantitative Results>
Detector B

ID#	Name	Ret. Time	Area	Height	Conc.	Unit
1	g2	4.748	280978	18729	10.023	ppb
2	g1	--	--	--	--	ppb
3	b2	--	--	--	--	ppb
4	b1	--	--	--	--	ppb
Total			280978	18729		

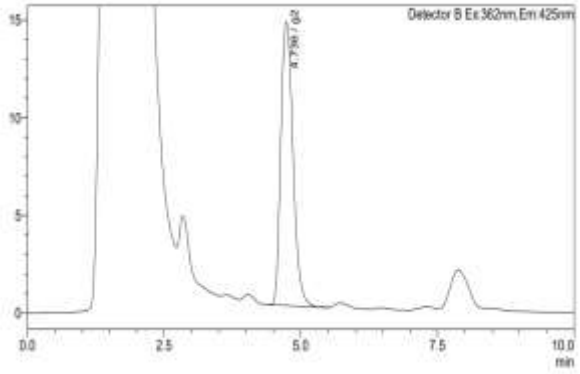


<Quantitative Results>
Detector B

ID#	Name	Ret. Time	Area	Height	Conc.	Unit
1	g2	4.742	279418	18134	9.966	ppb
2	g1	--	--	--	--	ppb
3	b2	--	--	--	--	ppb
4	b1	--	--	--	--	ppb
Total			279418	18134		

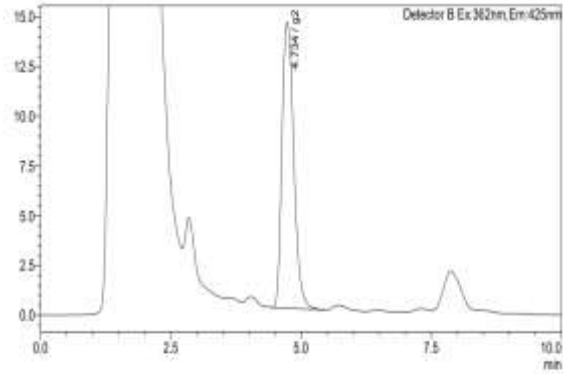
Note: A distinct G_2 peak is observed; B_1, B_2 and G_1 are < LOQ (0.74 µg/kg). Total aflatoxin equals G_2 (9.99 ± 0.04 µg/kg).

Figure 2. HPLC-FLD chromatogram of aflatoxins in hazelnut skin extract from Ordu (left: first replicate; right: second replicate).



<Quantitative Results>
Detector B

ID#	Name	Ret. Time	Area	Height	Conc.	Unit
1	g2	4.736	226110	14531	8.031	ppb
2	g1	--	--	--	--	ppb
3	b2	--	--	--	--	ppb
4	b1	--	--	--	--	ppb
Total			226110	14531		



<Quantitative Results>
Detector B

ID#	Name	Ret. Time	Area	Height	Conc.	Unit
1	g2	4.734	226678	14386	8.052	ppb
2	g1	--	--	--	--	ppb
3	b2	--	--	--	--	ppb
4	b1	--	--	--	--	ppb
Total			226678	14386		

Note: Lower G_2 peak intensity compared with Kocaeli. B_1, B_2 and G_1 are < LOQ (0.74 µg/kg). Total aflatoxin equals G_2 (8.04 ± 0.02 µg/kg).

Most available literature on aflatoxin contamination focuses on hazelnut kernels and processed products, while data

specifically addressing hazelnut skins remain limited. Previous studies have demonstrated that aflatoxin B_1 is the most common and hazardous contaminant in hazelnuts, whereas other forms are typically present at low or non-detectable levels [23, 24]. For instance, Şen and Civil [25] reported aflatoxins in all 202 hazelnut paste samples analyzed (0.17–12.96 µg/kg), with 1.98% exceeding legal limits. Similarly, Baltacı et al. [26] detected contamination in 98.7% of 3188 hazelnut samples (0.02–78.98 µg/kg), although only 1.3% exceeded the permissible threshold. In another study, Samimi et al. [27] identified aflatoxin B_1 in 30 hazelnut samples imported to Iran (3.15–8.13 µg/kg; mean 4.20 µg/kg), with one sample above the national limit.

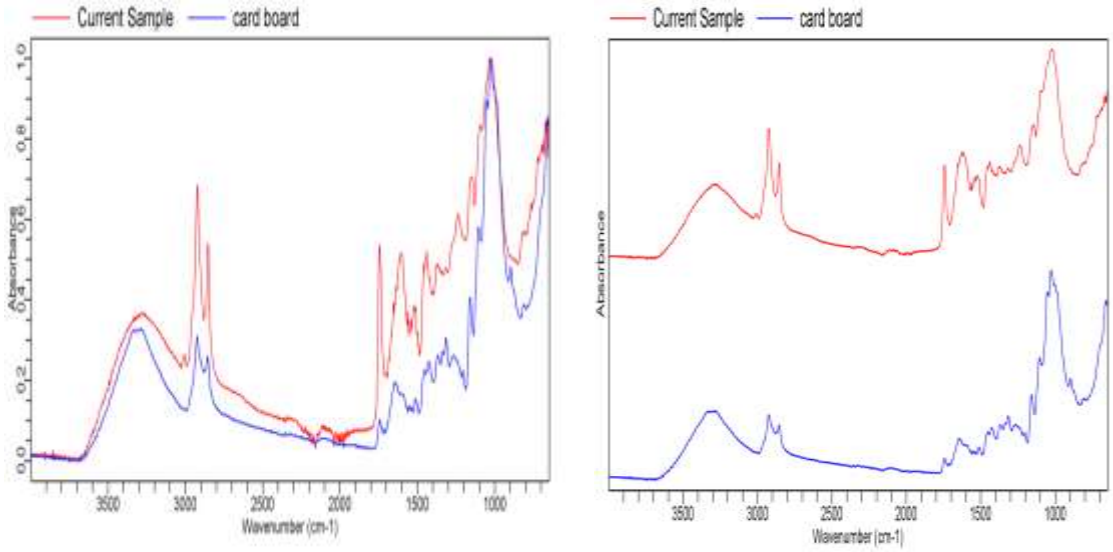
In the present study, finding only G_2 is unusual and should be verified in future tests. Possible contamination sources include inadequate drying, moisture re-absorption during storage, and insufficient segregation of contaminated lots. Although the fungal species were not identified in the present work, *Aspergillus* spp. are commonly implicated in aflatoxin formation in nuts; thus, future studies should include mycological identification alongside toxin profiling. Aflatoxin G_2 is less toxic than B_1 but still poses a food safety risk [28]. This pattern may reflect differences in drying and storage; targeted verification is recommended. Comparable observations have been made in studies highlighting the influence of drying and storage on aflatoxin formation [23, 28, 24]. The relatively high levels observed in the Kocaeli sample indicate the need for improved drying protocols and the use of new control methods.

3.4. FTIR Analysis

The FTIR spectra of hazelnut skins in Kocaeli and Ordu showed characteristic absorption bands at ~3300 cm^{-1} (O-H), ~2900 cm^{-1} (C-H), ~1750 cm^{-1} (C=O), and ~1600 cm^{-1} (C=C) (Figure 3).

The observed bands show the presence of hydroxyl, aliphatic, carbonyl, and aromatic groups, indicating a phenolic-rich composition and cell-wall polysaccharides. Similarly, Košťálová and Hromádková [19] observed absorption bands at 1732 cm^{-1}, 1602 cm^{-1}, and 1200-950 cm^{-1} in roasted hazelnut skins, primarily associated with esterified pectin and phenolic compounds. Pelvan et al. [8] also reported that roasted hazelnut skins are rich in phenolic constituents and have strong antioxidant activity, which aligns with the molecular markers identified in the present FTIR analysis. Moreover, FTIR characterization of hazelnut protein-phenolic complexes showed structural modifications associated with phenolic interactions [29].

Figure 3. FTIR spectra of hazelnut skin sample from Kocaeli (left) and Ordu (right).



Note: Main absorption bands indicate hydroxyl (~3300 cm^{-1}), aliphatic (~2900 cm^{-1}), carbonyl (~1750 cm^{-1}), and aromatic (~1600 cm^{-1}) groups.

3.5. Phenolic Compounds Analysis

Phenolic profiling was performed only for the Ordu hazelnut skin sample, as the Kocaeli lot was near the EU aflatoxin limit (Section 3.3) and the study was preliminary in scope. Of the 53 targeted phenolics, 17 were quantified in the Ordu sample (Table 4), while the complete screening results, including ND (<LOQ), are provided in Supplementary Table S1. Isoquercitrin (436.69 µg/g), catechin (270.77 µg/g), and quinic acid (231.64 µg/g) were the main compounds, appearing as high-intensity peaks in the chromatogram between 15 and 25 min (Figure 4). Rutin (49.09 µg/g), protocatechuic acid (27.70 µg/g), hesperidin (20.46 µg/g), quercetin (13.87 µg/g), and nicotiflorin (12.73 µg/g) were detected at moderate levels, while astragalin, chlorogenic acid, protocatechuic aldehyde, salicylic acid, and luteolin occurred at lower concentrations. In contrast, apigenin, naringenin, acacetin, and amentoflavone were present in trace amounts, showing a wide variety of phenolics. To our knowledge, low-abundance compounds such as nicotiflorin, acacetin, and amentoflavone may be rare in hazelnut skin and require confirmation with authentic standards and MS/MS spectral matching. Although non-targeted high-resolution MS platforms, such as UHPLC coupled with quadrupole time-of-flight mass spectrometry (UHPLC-QTOF), can facilitate the discovery of unknown phenolic compounds, the targeted UHPLC-MS/MS approach applied in this study enabled the reliable quantification of 53

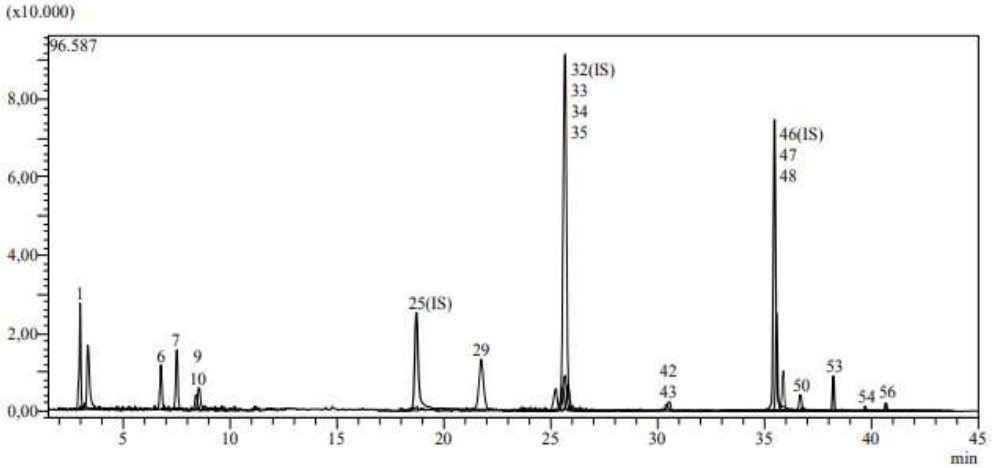
predefined analytes. Non-targeted profiling is therefore suggested for future studies.

Table 4. Phenolic composition of Ordu hazelnut skin

Phenolic compounds	Concentration (µg/g)	Phenolic compounds	Concentration (µg/g)
Isoquercitrin	436.69	Astragalin	7.57
Catechin	270.77	Chlorogenic acid	6.40
Quinic acid	231.64	Salicylic acid	1.75
Rutin	49.09	Luteolin	1.54
Protocatechuic acid	27.70	Apigenin	0.45
Hesperidin	20.46	Naringenin	0.42
Quercetin	13.87	Acacetin	0.41
Nicotiflorin	12.73	Amentoflavone	0.26
Protocatechuic aldehyde	10.41		

Note: All targeted phenolics are listed in Supplementary Table S1; compound-specific LOQs and validation parameters are in Supplementary Table S2 (n = 1).

Figure 4. UHPLC-MS/MS chromatogram of phenolic compounds analysis in Ordu hazelnut skin.



Note: Major peaks correspond to isoquercitrin (peak 34), catechin (peak 7), and quinic acid (peak 1). Axes: time (min) vs MS/MS signal (a.u.). Internal standards are labeled as IS (peaks 25, 32, 46), confirming the accuracy of quantification.

When expressed as the sum of the quantified targeted phenolics (Table 4), the Ordu sample contained approximately 109.2 mg/100 g. This value is lower than the total phenolic content (446 mg/100 g) reported by Spagnuolo et al. [30], which included high levels of catechin, procyanidin dimers, and quercetin-3-rhamnoside. The difference may reflect methodological variations, as their

study utilized Folin–Ciocalteu or broader profiling approaches, while our analysis focused on a targeted list of phenolics. Isoquercitrin and catechin, in particular, are well documented for their antioxidant and anticancer activities while rutin, hesperidin, and quercetin contribute antimicrobial, anti-inflammatory, and cardioprotective properties [4, 29]. Even at low concentrations, luteolin, apigenin, naringenin, and acacetin were detected in our analysis, consistent with earlier studies on hazelnut skins [8, 14]. These properties are similar to literature data, where hazelnut testa extracts were found to be rich in phenolics and to exhibit strong antioxidant potential [3]. A study also reported regional variations in phenolic profiles, with catechin, rutin, quercetin, and apigenin commonly identified [14]. These findings confirm that the phenolic profile of Ordu hazelnut skins is consistent with results from different regions [7, 8, 11, 14] and underline their potential application as a valuable source of phenolics for functional food development.

4. Conclusions

In this study, hazelnut skins from Ordu and Kocaeli were found to contain valuable bioactive compounds with differences observed between the two samples. Detailed phenolic profiling was performed only for the Ordu sample, where fat, protein, and phenolics such as isoquercitrin and catechin were present at higher levels. Some low-abundance compounds such as nicotiflorin, acacetin, and amentoflavone, may be rare and require confirmation. Overall, the profile indicates good antioxidant potential. Total aflatoxin content was below the regulatory maximum level in the Ordu sample, whereas the Kocaeli sample was just below the maximum level, indicating that better monitoring may be needed. The findings support the potential of Ordu hazelnut skin as a functional ingredient candidate; however, industrial implementation requires standardized sourcing, roasting standardization/monitoring, and routine contaminant monitoring. Confirmation with replicated sampling, bioavailability testing, and safety assessments under relevant storage and processing conditions is still required.

Limitations of this study include the use of a single industrial lot per province, and cultivar information was not available, which limits generalizability and precludes statistical regional comparisons. Future studies should include multi-lot sampling across different harvest years, controlled roasting conditions, and broader compositional analyses, including fatty acid profiles, minerals, tocopherols, and dietary fiber fractions. Additional work on non-targeted phenolic characterization, fungal identification, and bioavailability and toxicological assessment is also needed.

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