

# Profiling fatty acids, sterol, tocopherol and bioactive properties in roasted hazelnuts and their skin oil grown under organic and conventional cultivation systems

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This study aimed to compare the fatty acids, sterol, tocopherol, phenolic substance concentrations and antioxidant activities of the skins of roasted hazelnuts grown with organic and conventional systems. In addition, lipid profiles of kernels were determined. Natural hazelnuts were separated from their skin by roasting at the temperature (140°C) and time (30 min.) commonly used in the industry. According to the study results, organic hazelnut skin (OS) had higher phenolic substance (294.58 versus 251.37 mg GAE/g) and antioxidant activity (546.72 versus 450.6 mg TE/g for CUPRAC, 498.78 versus 390.06 mg TEAC/g for ABTS) than that of conventional. OS contained lower amounts of unsaturated fatty acids (UFA), total sterol and total tocopherol than that of conventional ones. On the other hand, there was a skin like trend among the kernels, except that no difference in tocopherol accumulation was observed. Regardless of the production system, the skin was found to be a source of phytosterols and tocopherols more than 10 times greater than the kernel. In conclusion, OS, which are by-products for the organic hazelnut industry, have the potential to be used for nutritional enrichment in organic food formulations due to its high phytochemical content.

**Keywords:** hazelnut skin oil, lipid characteristic, organic nut, conventional farming, bioactive compounds, antioxidant properties, roasting

## 1. INTRODUCTION

Hazelnut is one of the most popular nuts worldwide due to its pleasant aroma, rich nutritional content, fat-soluble bioactive substances, phenolics and phytochemicals [1]. Although hazelnut is consumed naturally (with skin), consumers mostly prefer the roasted (without skin) form because it is more aromatic. Therefore, roasting is the most used processing method in the hazelnut industry. With the roasting process, the skin, which is defined as the brown layer that completely covers the kernel and which constitutes 2.5% of the fruit weight, is separated [2]. It is known that phytochemicals on the outer layers of fruits and vegetables protect against oxidative stress, therefore many bioactive substances such as phenolic compounds are concentrated in the outer parts, and this is also true for hazelnuts [3]. For these reasons, hazelnut skin is very rich in fat-soluble bioactive substances and phenolics [4]. These compounds have protective properties against the damaging effects of free radicals and are known to decrease the risks of some illnesses like some types of cancer, coronary heart disease and type-2 diabetes [1]. The skin produced as a result of the roasting is the most considerable by-product of the hazelnut processing industry. It is thought that this by-product, which is extremely helpful in terms of nutrient content, should be evaluated and studies are being carried out on it. For example, it has been determined that hazelnut skin added to the formulation at 1-3% rates improves the cooking properties and antioxidant properties of chicken burgers, and it has

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been emphasised that it can be used successfully as natural ingredients in the production of burgers [5]. Roasted hazelnut skin was used for nutritional enrichment in the production of fresh egg pasta [6], and it was shown that the membrane could be used as an alternative antioxidant source.

Organic foods are associated with superior nutritional properties and non-contaminated sustainable farming practices by consumers, so the demand for fresh and processed organic foods is constantly increasing [7]. Due to the increasing needs, organic hazelnut cultivation has doubled in the last five years, reaching 21,500 tons, and meeting 3.5% of all hazelnut production. This rate is 1.5% when all foods are averaged [8, 9]. This data shows us that the demand for organic hazelnuts is more than twice the average of all organic foods.

All components used in the formulation of processed organic foods must be organic. Even though organic hazelnut skins can be used in organic food formulations for many different purposes such as nutrient enrichment, antioxidant, phenolic substance source, colouring, an overall screening of the lipid and bioactive properties of organic hazelnut skins is lacking in literature. Besides, although phenolic substance, flavonoids and antioxidant capacity studies in conventional-natural hazelnut skins [2, 10] and polyphenol and tocopherol studies in conventional-roasted Polish hazelnut skins [11] were carried out, no study was found in the literature on the fatty acid, tocopherol and sterol profile of conventional-roasted first quality Turkish hazelnut skins. The primer purpose of this research is to characterise the lipid and bioactive properties of roasted hazelnut skins cultivation by organic and conventional methods. For this aim, fatty acid, sterol, tocopherol profiles, total carotenoid, phenolics, flavonoids and antioxidant activity analyses were performed on hazelnut skin samples. In addition, lipid characteristics of roasted hazelnuts were also measured.

## 2. MATERIALS AND METHODS

### 2.1. COLLECTION AND ROASTING OF HAZELNUT SAMPLES

Organic hazelnut samples were obtained from farmers producing organic hazelnuts within the extent of the Organic Agriculture Project realized under the title of community certification by Keşap Chamber of Agriculture (Keşap, Giresun, Turkey). Conventional hazelnuts were obtained from growers engaged in conventional production from the same region. Organic nut samples were obtained from orchards inspected by the international certification company (ECAS Company, Antalya, TURKEY). Giresun Quality (First Quality) hazelnuts harvested in 2020 were used as research material. Samples randomly selected from orchards were picked in the second week of August, after their outer green husks turned yellow

and the moisture decreased to 30%. The drying process was carried out for 3 days between 09:00 and 20:00 at room temperature conditions (average temperature 24.7°C). The samples were laid on a 5 × 5 m jute cover in the concrete ground and mixed 5 times a day during the drying period. After 20:00 in the evening, each group was gathered in the centre and covered with nylon to prevent moisture entering from outside. At the end of the drying period, the humidity decreased in all samples below 6% and the samples were kept at -18°C until the day of analysis. The hazelnuts, which were dried and brought to the laboratory, were mixed equally and a single sample was obtained for roasting. After the hazelnuts were separated from their shells by hand, they were roasted in a conventional oven (Ecocell, Germany) for the time and temperature frequently used in the industry (140°C - 30 min). Roasting treatment had three replicates and all analytical measurements were performed in duplicate. Roasted nuts were stored at -18°C until the day of analysis.

## 2.2. METHODS

### 2.2.1 Moisture content and oil extraction

Shelled hazelnuts used in the analysis were hand-cracked and separated from their shells. The moisture content of hazelnuts and their skins were calculated by determining the weight loss resulting from keeping the samples in an oven heated at 103°C for approximately 4 hours. Oil extraction was performed with a test scale screw press device with a 3 kW regulatable speed. The screw turning speed was adjusted at 60 rpm to extract 80% of the total oil [7].

### 2.2.2 Determination of fatty acids

The fatty acid profile of the hazelnut oil was defined after fatty acid methylation according to the method ISO 12966-2 [12]. Briefly, 0.1 g of nut oil extracted by pressing was placed into a tube. The oil was shaken by adding 5 mL of heptane as solvent and 0.5 mL of 2 N potassium hydroxide solution in methanol. The solvent in the resulting mixture was removed with anhydrous sodium sulphate. After the prepared mixtures were held for 1 minute, samples were analysed using gas chromatography-flame ionisation detector (GC-FID) (Perkin Elmer, Autosystem GLX, Shelton, USA) equipped with SP™ - 2560 [100 m × 0.25 mm × 0.2 µm (Supelco, Bellefonte, USA)] column. Data were evaluated with Total Chrome Navigator and explained as % fatty acid.

### 2.2.3 Indices

Oleic acid/linoleic acid ratio (O/L) and iodine value (IV) [13] rates were calculated according to the equations below.

$$O/L = \text{oleic acid/linoleic acid} \quad (1)$$

$$IV = (\text{palmitoleic acid} \times 1.901) + (\text{oleic acid} \times 0.899) + (\text{linoleic acid} \times 1.814) + (\text{linolenic acid} \times 2.737) \quad (2)$$

### 2.2.4 Determination of sterol profile

The sterol profile of the nut oils was performed by according to the procedure reported by Demirtas et al. [14]. First of all,  $\alpha$ -cholestanol solution used as internal standard was prepared at a concentration of 1000 mg/L. 1 ml of this solution was taken and 0.5 g was mixed with hazelnut oil extracted by pressing method. The mixture was taken into a screw capped tube and saponification reaction was carried out with potassium hydroxide solution prepared in 10 mL methanol at 80°C for 60 minutes. The mixture was then extracted 3 times in succession using 5 mL of n-hexane solvent. The resulting extract solution was treated with nitrogen gas until the volume was below 10 mL. After the mixture was extracted 3 times using 5 mL of distilled water, the volume of the organic phase was made up to 10 mL with n-hexane. The water in the mixture was evaporated with anhydrous sodium sulphate. 0.5 mL of the obtained extract was placed into a vial and 250  $\mu$ L of bis (trimethylsilyl) trifluoroacetamide / trimethylchlorosilane (4:1, v/v) and 250  $\mu$ L of pure pyridine were joined and derivatized at 60°C for 15 minutes. The resulting mixtures were studied with GC-FID and SE-54 (5%-phenyl-1%-vinylmethylpolysiloxane), 30 m  $\times$  0.32 mm  $\times$  0.25  $\mu$ m (Agilent, Santa Clara, CA, USA) column. Data were evaluated with Total Chrome Navigator and explained as mg/100 g oil.

### 2.2.5 Determination of tocol profile

Tocol isomers of oils samples extracted from hazelnut kernels and their skin was performed according to the method reported by Demirtas et al. [14]. In summary, 1 g of hazelnut oil extracted by pressing method was supplemented into a tube and then 25 mL of heptane was added, and the mixture was shaken for 10 minutes. The resulting extract was filtered using 0.45  $\mu$ m pore size syringe filter before injecting into HPLC (High Performance Liquid Chromatography). Analysis of samples was carried out using HPLC (Agilent Series 1100, Waldbronn, Germany) with a fluorescence detector and normal phase column (5 $\mu$ m LiCrosorb Si60 25 cm  $\times$  4.6 mm i.d., HiChrom Ltd., Theale, UK). Results with Chemstation were explained as mean values  $\pm$  standard deviation as mg/100 g oil.

### 2.2.6 Spectrophotometric assays

#### 2.2.6.1 Extraction of bioactive substances

For extraction, 3 g of each nut sample was weighed. It was sonicated for 30 minutes at 45°C using an ultrasonic water bath (Kudos, Shanghai Kudos Ultrasonic Instrument Co., Ltd., China) with 10 mL of 80% ethyl alcohol. After centrifugation, the supernatant was taken to another tube and 10 mL of 80% ethyl alcohol was added to the remaining pulp and sonicated at 45°C for 15 minutes. After centrifugation, the supernatant was separated and 10 ml of 80% ethyl alcohol was added to the remaining pulp and

sonicated for 5 minutes at 45°C. The supernatants were combined and made up to 25 ml with 80% ethanol and used in total phenolic, total flavonoid and antioxidant activity analyses.

#### 2.2.6.2 Determination of total phenolics, total flavonoids and antioxidant activities (ABTS and CUPRAC methods)

Biochemical properties were determined in hazelnut kernel and hazelnut skins. Total phenolic, total flavonoids and antioxidant activity (according to ABTS and CUPRAC assays) were detected as biochemical properties. All measurements were made using a spectrophotometer (Shimadzu, Japan). The total amount of phenolic substances was performed with the Folin-Ciocalteu reagent according to the procedure reported by Mayda et al. [15]. Total flavonoids were detected according to the method described by Mayda et al. [15]. Total phenolics as gallic acid equivalents (mg GAE/g) and total amount of flavonoids was explained as mg catechin equivalent (mg CE/g). Antioxidant activity was measured using the ABTS [15] and CUPRAC [16] assays and explained as Trolox equivalent antioxidant capacity (TAC) per g (mg TEAC/g) and mg Trolox equivalent (mg TE/g), respectively.

#### 2.2.6.3 Extraction and determination of total carotenoids

For carotenoid analysis, 250 mg of each sample was weighed and crushed in a mortar with the help of liquid nitrogen. The homogenates were transferred to 15 mL centrifuge tubes, 5 mL of pure methyl alcohol was added, and sonicated for 15 minutes in an ultrasonic homogenizer (Bandelin MS72, Berlin, Germany). After sonication, it was kept at +4°C for 1 night and then filtered and analysed. The absorbances of the extracts were read by the spectrophotometer at 663 nm, 645 nm and 440.5 nm. Firstly, the amounts of chlorophyll a and chlorophyll b, and then the total amount of carotenoids were calculated using the formulas below [17].

$$\text{Chlorophyll a} = (12.7 \times A_{663} - 2.69 \times A_{645}) \times \frac{v}{(1000 \times w)}$$

$$\text{Chlorophyll b} = (22.9 \times A_{645} - 4.68 \times A_{663}) \times \frac{v}{(1000 \times w)}$$

$$\text{Total carotenoid} = 46.95 \times (A_{440.5} - 0.268 \times \text{chlorophyll a} + b)$$

w: weight by grams for extracted;

v: final size of extracted;

A: absorbance.

### 2.3. STATISTICAL EVALUATION

A one-way analysis of variance (ANOVA) and the Pearson correlation test were carried out on software, R version 4.1.1. Principal components analysis (PCA) has performed by using JMP version 16 and two principal components were extracted. The results were explained as mean values  $\pm$  standard deviation (n = 3).

### 3. RESULTS AND DISCUSSION

#### 3.1. FATTY ACID PROFILE

Moisture and oil content of organic roasted hazelnut (OH), conventional roasted hazelnut (CH) organic roasted hazelnut skin (OS) and conventional roasted hazelnut skin (CS) are given in Table I. While there was no effect of the production system on moisture in both skin and kernel, it was observed that organic samples contained more oil (OH, CH, OS, CS, respectively, 57.30, 56.63, 11.61, 10.58%) ( $P < 0.05$ ). In addition, the oil rate in the skin was approximately 5 times lower than in the kernel in both production systems. Tunçil [18] (57.90%) in kernel, Ivanovic et al. [10] (12.03%) and Tunçil [18] (17.80%) in the skin reported similar oil ratios.

Fatty acid profiles of kernel and hazelnut skins are presented in Table I. Eleven different fatty acids were detected in OH and CHs. While the major fatty acid in the kernel was oleic acid, it was followed by linoleic, palmitic and stearic acids, the ratio of other fatty acids remained below 0.2%. Similar rankings were found by Amaral et al. [1] in Portuguese hazelnuts. Except for stearic acid, other major fatty acids (oleic, linoleic and palmitic acid) were determined to be

influenced by the production system ( $P < 0.05$ ). The production system had a statistically significant effect on only palmitoleic acid, one of the minor fatty acids. In OH, lower unsaturated fatty acids (UFA) from lower oleic and linoleic fatty acids and higher SFA from higher palmitic acid were detected. Again, due to the lower oleic acid content of OH, the PUFA (polyunsaturated fatty acids) rate was found to be lower.

In the hazelnut skin, 14 different fatty acids were determined as three more than the kernel (pentadecanoic, lignoceric, lauric acid). As in fruit, fatty acids other than oleic, linoleic, palmitic and stearic acids, which are the dominant fatty acids, remained below 0.2%. However, there were significant variations in the fatty acid profile of the hazelnut skin compared to the kernel. Regardless the production system, it was observed that the oleic acid level of the skin was found at about 10% less than the kernel, and the linoleic acid was more than twice as high. It was also seen that the amount of palmitic acid was slightly higher, while the other fatty acids were at equal levels. Consistent with our data, Özdemir et al. [4] reported that oleic acid is the major fatty acid in hazelnut skin (75.12%), followed by linoleic (16.0%), palmitic (6.8%) and stearic acid (1.2%). Although Özyurt et al. [19]

**Table I** - Moisture, fatty acid composition (%) and oxidative stability indices of oils extracted from organic and conventional roasted hazelnut and their skins

Parameters	Roasted hazelnut		Hazelnut skin	
	Organic	Conventional	Organic	Conventional
Moisture	2.10±0.04a	2.21±0.04a	6.36±0.10a	6.92±0.06a
Total oil	57.30±0.06a	56.63±0.36b	11.61±0.07a	10.58±0.01b
Pentadecanoic acid	nd	nd	0.01±0.00a	0.01±0.00a
Palmitic acid	5.54±0.01a	4.81±0.01b	8.74±0.001a	6.43±0.07b
Palmitoleic acid	0.14±0.00a	0.11±0.01b	0.11±0.00a	0.09±0.00a
Heptadecanoic acid	0.04±0.00a	0.04±0.00a	0.03±0.00a	0.04±0.00a
Stearic acid	2.30±0.00a	2.34±0.00a	1.34±0.00b	1.66±0.02a
Oleic acid	84.70±0.03b	85.34±0.01a	75.53±0.01b	76.54±0.48a
Linoleic acid	6.86±0.11b	6.94±0.01a	13.54±0.04b	14.55±0.11a
α-Linolenic	0.06±0.00a	0.06±0.00a	0.10±0.01b	0.13±0.00a
Arachidic acid	0.11±0.00a	0.10±0.01a	0.11±0.00a	0.15±0.00a
Eicosenoic acid	0.11±0.00a	0.12±0.00a	0.09±0.00a	0.14±0.00a
Behenic acid	0.01±0.00a	0.01±0.00a	0.04±0.00a	0.06±0.00a
Miristic acid	0.02±0.00a	0.02±0.00a	0.05±0.01a	0.04±0.00a
Lignoceric acid	nd	nd	0.06±0.00a	0.01±0.00b
Lauric acid	nd	nd	0.02±0.00a	0.03±0.00a
SFA	8.02±0.01a	7.32±0.02b	10.40±0.01a	8.52±0.09b
MUFA	84.95±0.03b	85.57±0.01a	75.73±0.01b	76.77±0.48a
PUFA	6.92±0.01b	7.00±0.01a	13.64±0.01b	14.68±0.11a
UFA	91.87±0.03b	92.56±0.01a	89.37±0.01b	91.45±0.58a
Indices				
UFA/SFA	11.46±0.02b	12.65±0.03a	8.59±0.02b	10.73±0.10a
O/L	12.35±0.01a	12.30±0.02a	5.58±0.00a	5.26±0.01b
IV	88.85±0.02b	89.51±0.01a	92.68±0.01b	95.37±0.62a

All datas are expressed as means ± standard deviation (n = 3). Different letters in the same row indicate statistically significant differences between cultivation systems by one-way ANOVA test at  $P < 0.05$ . nd: not detected. SFA: saturated fatty acids, MUFA: mono unsaturated fatty acids, PUFA: poly unsaturated fatty acids, UFA: unsaturated fatty acids, O/L: oleic acid/linoleic acid, IV: iodine value.

**Table II** - Sterol composition (mg/100g) of oils extracted from organic and conventional roasted hazelnut and their skins

Sterols	Roasted hazelnut		Hazelnut skin	
	Organic	Conventional	Organic	Conventional
Campesterol	3.99±0.05b	4.77±0.21a	22.32±0.49b	37.88±1.77a
Stigmasterol	0.68±0.07b	0.94±0.17a	35.97±0.89b	73.86±0.31a
Δ7-stigmastenol	1.93±0.17a	1.96±0.11a	6.74±0.09b	10.30±1.18a
β-sitosterol	74.09±0.96b	86.47±0.05a	646.48±15.80b	897.29±13.90a
Sitostanol	1.63±0.46a	1.69±0.09a	126.88±0.30b	189.30±1.47a
Δ5-avenasterol	3.15±0.53a	3.44±0.37a	134.54±0.64b	200.60±0.31a
Δ7-avenasterol	0.38±0.07b	0.59±0.03a	3.99±0.85a	5.58±0.83a
Total sterol	85.80±0.96b	99.91±0.16a	968.60±6.26b	1415.60±13.10a

All datas are expressed as means ± standard deviation (n = 3). Different letters in the same row indicate statistically significant differences between cultivation systems by one-way ANOVA test at P<0.05.

made a similar ranking, they reported the amount of oleic acid slightly higher than the results of this work (80.52%).

While it was seen that the production system affected all the dominant fatty acids in the skin, only α-linoleic and lignoceric acids were found to affect the minor fatty acids (P<0.05). Higher levels of oleic (76.54-75.53%, respectively), linoleic (14.55-13.54%) and stearic acid (1.66-1.34%) and lower levels of palmitic acid (6.43-8.74%) accumulated in CS than OS. As a result, it was observed that there was more UFA in CS compared to OS (91.45-89.37%, respectively), as in kernel. Despite the lower stearic acid content of OS, it was determined that the SFA level was higher due to the higher palmitic acid content. Again, it was observed that the MUFA level was high owing to the excess quantity of oleic acid in the conventional samples. The fatty acid composition is affected by variety, harvest time, drying process, harvest year, geographical origin, environmental conditions, preservation and processing conditions [20]. As all other conditions are the same, greater oil accumulation and difference in fatty acid profile in organic samples may be due to differences in the cultivation system.

### 3.2. OXIDATIVE STABILITY EVALUATION

Due to the change caused by the production system in the composition of fatty acids, significant differences have emerged in the degree of unsaturation of lipids and, accordingly, in their susceptibility to oxidation. Various methods exist for determining the reactivity and susceptibility to oxidation of lipids are discussed below. Low UFA/SFA rate is thought to be a long shelf life in hazelnuts [21]. It was observed that the UFA/SFA ratio of organic samples was lower in both kernel and skin (OH, CH, OS, CS, 11.46, 12.65, 8.59, 10.75, respectively) (P<0.05). It is particularly interesting that OS has a much lower UFA/SFA ratio compared to others. Göncüoğlu Taş and Gökmen [22] reported the UFA/SFA ratio as 9.2, and Ghirardello et al. [23] as 12.03, which is comparable to our study results. The oleic/linoleic (O/L) rate is a considerable standard for evaluating kernel qualification, and a higher O/L value means better oxidative stability [13,

24]. While the O/L values of OS and CS were determined as 5.58 and 5.26, respectively, it was determined that the difference was significant and but there was no difference in kernel. The iodine value (IV) is a indicator of the grade of unsaturation of oils. High IV is a marker that oils are more reactive, less stable, and more sensitive to rancidity and oxidation [13]. It was determined that conventional samples had higher IV values in both kernel and skin (OH, CH, OS, CS, 88.85, 89.51, 92.68, 95.37, respectively) (P<0.05). In kernel, Karaosmanoğlu and Üstün [24] (89.81-94.40) and Belviso et al. [13] (89.84-86.94) reported comparable results with our study.

It can be said that organic samples may be highly resistant to lipid oxidation and have more shelf life because of lower UFA/SFA, IV and higher O/L values in both kernel and skin. Regardless the production system, it can be said that the skin is more sensitive to oxidation compared to kernel due to low O/L, close levels of UFA/SFA and high PUFA/SFA, IV values. The oxidation ratios of fatty acids are almost 1:10:100:200 for stearic, oleic, linoleic and linolenic acids, respectively [25]. In relation to this, organic samples with lower linoleic acid levels are more stable to oxidative changes. In addition, this information can explain that the skin may be less durable than hazelnuts, because there was approximately twice as much linoleic acid in the skin than in the kernel.

### 3.3. STEROL PROFILE

Seven sterols identified in organic and conventional hazelnut and their skins are presented in Table II. β-sitosterol, the highest amount of sterol in roasted hazelnuts, met 86% of the total. β-sitosterol was detected at the levels of 74.09 and 86.47 mg/100g in OH and CH, respectively, and the difference was statistically significant (P<0.05). Similar to our work, Amaral et al. [1] reported that the most abundant sterol in Portuguese hazelnuts is β-sitosterol. β-sitosterol was followed by campesterol and Δ-5avenasterol, while others (stigmasterol, Δ7-stigmastenol, sitostanol, Δ7-avenasterol) comprised less than 5% of total sterol. Total sterol amount of OHs (85.80 mg/100g) was found to be significantly lower than CHs (99.91

mg/100g) ( $P < 0.05$ ). Since other sterols are not affected by the production system, the difference in total sterol may be due to the lower amount of  $\beta$ -sitosterol and campesterol in organic samples. Alasalvar et al. [25] determined total sterol and  $\beta$ -sitosterol levels in Tombul hazelnut as 113.52 and 105.48 mg/100g, consistent with our data. In a research carried out on Polish hazelnuts, 7 different phytosterol and 3 different stanols were reported, while the total sterol was found to be in the range of 130.32-152.22 mg/100g, slightly higher than our results [26].

Although  $\beta$ -sitosterol is the most abundant sterol in the skin as in kernel, its ratio in total sterol is lower (OS: 66%, CS: 63%). Total sterol was 11 times higher in OS (968.60mg/100g) and 14 times higher in CS (1415.60 mg/100g) than kernel. It is also quite interesting that sitostanol was found to be 77 and 112 times higher in OS and CS than in OH and CH (respectively). It was determined that individual sterols and total sterols, except for  $\Delta^7$ -avenasterol, which is found in small amounts in the skin, accumulate less in the organic production system compared to the other one ( $P < 0.05$ ). Individual and total sterols in hazelnuts can be affected by cultivar, geographic origin, harvest time and environmental factors [25, 27]. As the conditions listed above were the same for all samples, this work shows that the production system can also have an effect on the level of sterols.

Total sterol content of OS was 1.65, 12.96, 10.99, 52.64 times higher than the sterol contents reported by Alberici et al. [28] for corn (587 mg/100g), olive (74.7 mg/100g), rapeseed (88.1 mg/100g) and soybean oils (18.4 mg/100g), respectively. According to the results of the study, organic production was led to slight decrease the amount of sterol. However, despite this decrease, OS can be said to be a richer source of phytosterols when compared to the oils given in the above work [28]. The sterols found in plant sources have high anticancer properties in human metabolism, in addition to lowering LDL cholesterol, preventing diabetes, enhancing immunity [1]. Therefore, the inclusion of OSs in organic food formulations

may increase the potential health benefits of the food in which it is used.

### 3.4. TOCOPHEROL PROFILE

The individual and total tocopherol contents of organic and conventional hazelnuts and their skins are given in Table III. Three isomers of tocopherol have been determined and identified in hazelnut kernel. Among the tocol isomers determined in OH and CH,  $\alpha$ -tocopherol (53.89, 54.71 mg/100g, respectively) is the most abundant isomer, constituting 85 and 98% of the total, followed by  $\gamma$ -tocopherol (7.30, 3.19 mg/100g, respectively) and  $\beta$ -tocopherol (1.67-1.68 mg/100g, respectively). Stuetz et al. [29] reported the  $\alpha$ -tocopherol amount of hazelnuts as 40.06 mg/100g oil. Ciemniowska-Zytkiewicz et al. [26] determined that  $\alpha$ -tocopherol, the most higher level of tocopherol in Polish hazelnut varieties, constituted 86-90% of the total tocopherol, followed by  $\gamma$ -tocopherol (6-10%) and  $\beta$ -tocopherol (2.9-3%). While it was understood that the production system did not affect the total tocopherol amount of hazelnuts ( $P > 0.05$ ), it was observed that CHs had slightly more  $\alpha$  and  $\gamma$ -tocopherol ( $P < 0.05$ ). Even though there was no significant difference between the production systems in terms of total tocopherol content, it was determined that CHs had a higher Vitamin E effect due to higher  $\alpha$ -tocopherol content.

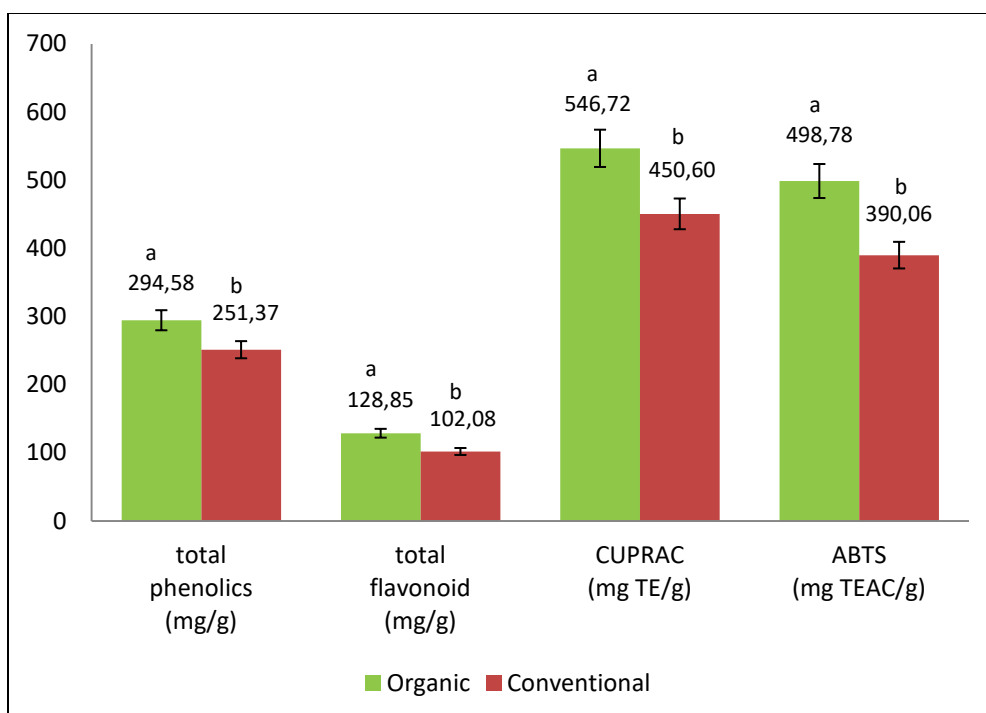
Unlike kernel,  $\alpha$ ,  $\beta$  and  $\gamma$ -tocopherol were detected in the skin, as well as  $\delta$ -tocopherol.  $\alpha$ -tocopherol (320.31, 380.65 mg/100g, respectively) was the most abundant in both OS and CS, followed by  $\gamma$ -tocopherol (282.14, 294.66 mg/100g, respectively),  $\delta$ -tocopherol (22.20, 23.27 mg/100g, respectively), and  $\beta$ -tocopherol (18.67, 22.78 mg/100g, respectively). Our results are comparable to Özdemir et al. [4]. Göncüoğlu Taş and Gökmen [2] reported that  $\alpha$ -tocopherol (16.82, 44.39 mg/100g) and total tocopherol (22.61, 59.35 mg/100g) obtained from different hazelnut cultivars were much lower than our results. In OS and CS, the ratio of  $\alpha$ -tocopherol, which is the dominant isomer, in total tocopherol decreased

**Table III** - Tocopherol composition (mg/100g), vitamin E (mg/100g) content of oils extracted and total carotenoid content (mg/g) from organic and conventional roasted hazelnut and their skins

Parameters	Roasted hazelnut		Hazelnut skin	
	Organic	Conventional	Organic	Conventional
$\alpha$ -tocopherol	53.89±0.35b	54.71±0.21a	320.31±2.15b	380.65±13.94a
$\beta$ -tocopherol	1.67±0.11a	1.68±0.07a	18.69±0.06b	22.78±0.39a
$\gamma$ -tocopherol	7.30±0.08a	3.19±0.03b	282.14±1.82b	294.66±6.70a
$\delta$ -tocopherol	nd	nd	22.20±0.09b	23.27±0.35a
Total tocopherol	62.98±0.53a	59.58±0.17a	643.34±3.92b	721.34±21.48a
Vitamin E	55.28±0.39b	55.78±0.39a	356.20±2.35b	419.45±14.78a
Total carotenoid	1.59±0.24a	2.01±0.27a	7.13±0.59a	7.33±1.03a

All datas are expressed as means  $\pm$  standard deviation ( $n = 3$ ). Different letters in the same row indicate statistically significant differences between cultivation systems by one-way ANOVA test at  $P < 0.05$ . nd: not detected.

Vitamin E (expressed as  $\alpha$ -tocopherol equivalents). The conversion factors for vitamin E activity were as follows:  $\alpha$ -Tocopherol  $\times$  1.00,  $\beta$ -tocopherol  $\times$  0.40,  $\gamma$ -tocopherol  $\times$  0.10,  $\delta$ -tocopherol  $\times$  0.01 [27].



**Figure 1** - Total phenolics, total flavonoids and antioxidant activities of organic and conventional roasted hazelnut skins by different tests such as CUPRAC and ABTS. Different letters indicate of statistical difference according to one-way ANOVA test at  $P < 0.05$ .

compared to kernel (50-52%), while the ratio of  $\gamma$ -tocopherol increased (43-40%). It was determined that CSs were higher than OSs in all tocopherol isomers and accordingly in terms of total tocopherol amount and vitamin E activity ( $P < 0.05$ ). The knowledge in literature that the stressful conditions inherent in the organic cultivation system support the synthesis of bioactive compounds could not be confirmed for tocopherol and phytosterol in this study [30]. Fertilisation is an important factor affecting the biosynthesis of bioactive compounds such as carotenoids and tocopherols in fruits [25]. This fact could explain the greater tocopherol amount observed in conventionally grown hazelnut skins in this work.

Tocopherols are known as fat-soluble antioxidants found in different amounts in all vegetable oils [31]. There is strong evidence that they protect fats from oxidative degradation and show the vitamin E activity in the human body, playing an important role in the prevention of some chronic health problems such as heart disease and certain cancer types [25]. Compared to kernel, OS and CS's were 5.90, 6.95 times for  $\alpha$ -tocopherol, 11.19-13.5 times for  $\beta$ -tocopherol, 38.6-92 times for  $\gamma$ -tocopherol, 10.21-12 times for total tocopherol and 6.44-7.50 times for vitamin E effect was found to be higher. On the other hand, while it was determined that approximately 4 times more carotenoids were accumulated in the skin than in the kernel, it was determined that the production system did not affect it ( $P > 0.05$ ).

Organic and conventional hazelnut skins with high tocopherol content can be used to increase the

oxidative stability of the foods, to contribute to the antioxidant capacity and to increase the amount of tocopherols. Considering that all additives to be added to processed organic foods must be organic, OSs can be used to increase the shelf life, tocopherol and carotenoid content of many organic foods.

### 3.5. TOTAL PHENOLICS, TOTAL FLAVONOIDS AND TOTAL ANTIOXIDANT ACTIVITIES (DPPH and ABTS TESTS)

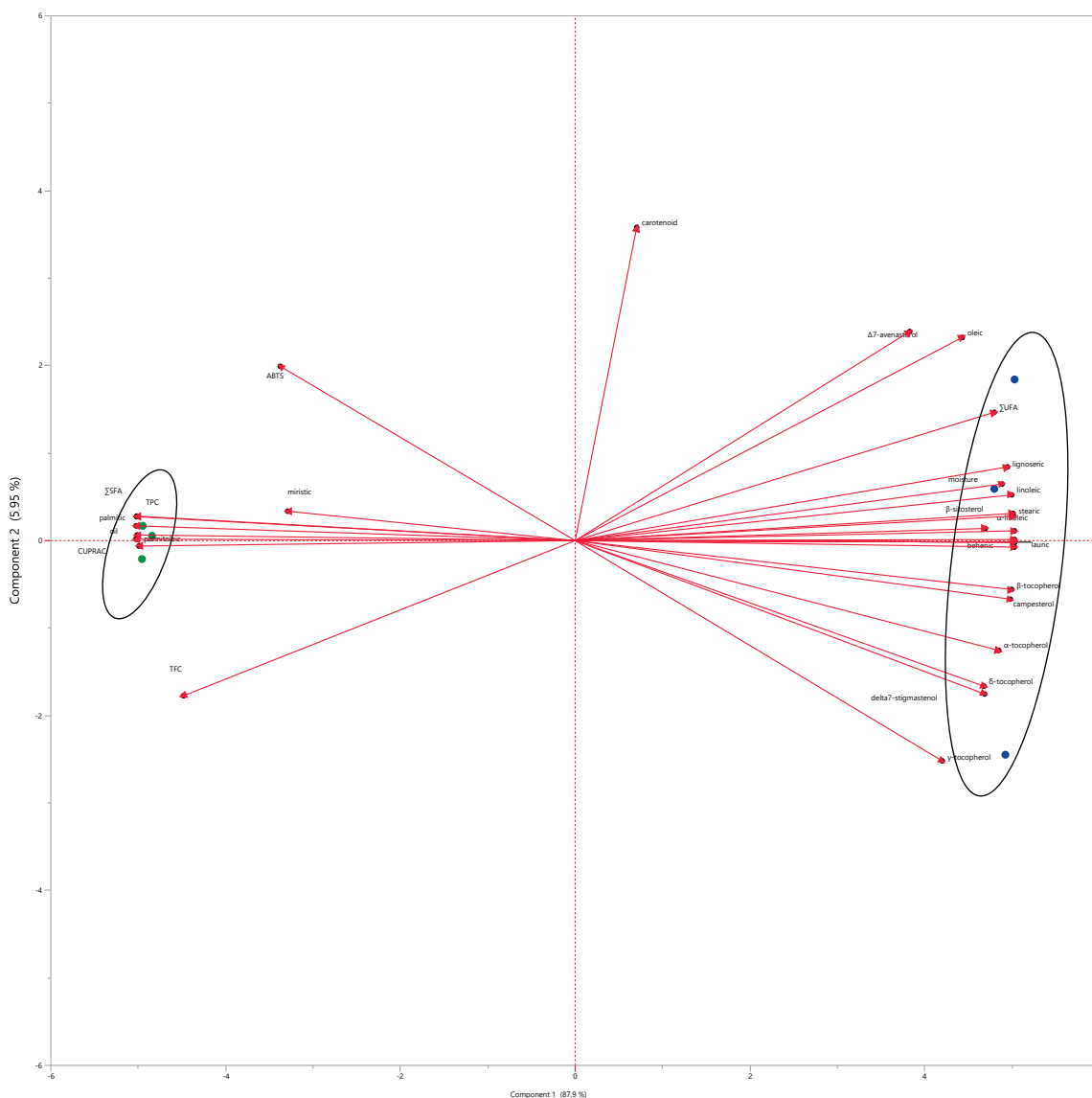
To date, the total phenolic contents (TPC) and antioxidant properties of many organic and conventional foods have been investigated and it has been reported that organic samples contain higher phenolic substances and show antioxidant activity [32, 33]. In our study, TPC of hazelnut skins were 294.58 mg GAE/g and 251.37 mg GAE/g for OS and CS, respectively (Figure 1). Based on our study data, it was understood that the production system affected the TPC of hazelnut skins and OS contained 17% more. Moreover, the total flavonoid content (TFC) of the skins was determined, and OS (128.85 mg CE/g) was found to contain 26% more TFC than CS (102.08 mg CE/g) ( $P < 0.05$ ). It is estimated that exposure of plants to aggressive and stressful conditions leads to the induction of secondary metabolic forming, resulting in greater speed and amount of synthesis of phenolic substances [30]. Furthermore, the exposure pathogenic pressure of plants in organic agriculture where chemical pesticides is not used can also lead to a rise in phenolic synthesis [33]. Due to the nature of organic agriculture, plants are exposed to stress. The

higher level of TPC and TFC synthesis in OS may be owing to these causes. Studies in which hazelnut skin phenolics were determined have been carried out in the literature and quite different results have been reported. For example, Göncüoğlu Taş and Gökmen [2] detected TPC and TFC in Tombul hazelnut skin as 142.2 mg GAE/g and 57.0 mg CE/g, Ivanovic et al. [10] as 706.0 mg GAE/g and 477.7 mg CE/g, respectively. These differences in the literature may be related to different extraction conditions and solvents. In this study, the antioxidant activities of OS and CS were determined using CUPRAC and ABTS methods (Figure 1). The antioxidant activity of OS was found to be 21% and 28% higher than CS in the CUPRAC and ABTS method, respectively ( $P < 0.05$ ). Some scientists have determined a strong relationship between the phenolic substance concentration and antioxidant capacity in hazelnuts [34]. Our study confirmed this

information and similarly positive correlations were found between TPC and CUPRAC ( $r = 0.991$ ) and ABTS ( $r = 0.687$ ). This correlation may explain the higher antioxidant activity of OS because OS had more TPC than CS. As a result, it can be said that the use of pesticides in conventional production reduces the TPC of hazelnut skins, and accordingly, the antioxidant activity decreases. Similar to our study, Murathan et al. [32] reported that organic samples showed higher antioxidant activity in almond hull.

### 3.6. PRINCIPAL COMPONENT ANALYSIS

PCA biplot indicates both PC points of samples and loadings of variables and provided better understanding of the correlation between fatty acids, sterol, tocopherol and bioactive compounds of hazelnut skins cultivation by different methods. According to PCA results, the first two components were explained



**Figure 2** - Principal Component Analysis biplot displaying scores of roasted hazelnut skins (organic samples: green, conventional samples: blue) and loadings of variables (red vectors). SFA-saturated fatty acids, UFA-unsaturated fatty acids. TPC-total phenolics, TFC-total flavonoids, ABTS and CUPRAC-antioxidant activity.



88.6% of the data (Figure 2). Component 1 (PC1) accounted for 87.9% of the total variance, while Component 2 (PC2) accounted for 5.9%. Samples of both production methods are obviously separated from each other along PC1 on the score plot. All organic samples were collected on the negative part of PC1, while all conventional samples were on the positive part. According to PCA results, OS was grouped with TPC, TFC, antioxidant activity (ABTS, CUPRAC), SFA, palmitic acid, palmitoleic acid, oil; CS was grouped mainly with UFA, oleic acid,  $\beta$ -sitosterol and  $\alpha$ -tocopherol. A significant positive correlation was seen between TPC, CUPRAC, and ABTS. Therefore, the methods used to evaluate antioxidant activity (ABTS and CUPRAC) appear to be related to TPC. In this study, the principal component and ANOVA analysis results support each other.

#### 4. CONCLUSIONS

This is the first study to comprehensively elucidate the influence of the cultivation system on the fatty acid, sterol and tocopherol profile, oxidative stability, phytochemical content (carotenoid, TPC, TFC) and antioxidant activity of hazelnut skin. Evaluation of the main compounds of the skins obtained from hazelnuts produced under conventional and organic farming practices revealed significant differences. In general, significantly higher TPC, TFC, antioxidant activity (ABTS and CUPRAC), linoleic acid and SFA, equal levels of carotenoids, whereas lower levels of UFA, total sterol and total tocopherol were detected in organic hazelnut skins than conventional ones. Depending on the difference in fatty acid composition, it has been observed that organic hazelnut skins may be more durable to lipid oxidation and therefore have a greater shelf life. Although the production system did not affect the amount of tocopherol in the kernel, a parallel change with the skin was observed in other parameters. On the other hand, the skin had quite different fatty acid composition due to its lower oleic acid and higher linoleic acid content than the kernel. Again, the skin contained approximately 13 times sterol, 11 times tocopherol and 4 times carotenoids compared to the kernel. According to the results of this study, organic hazelnut skin is a very rich source of antioxidants, tocopherols and phytosterols. Therefore, organic hazelnut skins may be proposed as a source of phytochemicals for nutritional enrichment in processed organic food formulations and in nutraceutical applications.

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