#### **ORIGINAL ARTICLE**



# Orchard-Based Variations in Oil Content, Fatty Acid Composition, and Bioactive Compounds in 'Tombul' and 'Palaz' Hazelnut (*Corylus avellana* L.) Cultivars

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#### Abstract

Hazelnuts, which have high nutritional value, are an important source of fat, fatty acids, phenolics, and antioxidants. In this study, variations in the oil and protein contents, fatty acid compositions, and bioactive compounds of organic hazelnuts ('Tombul' and 'Palaz' cvs.) grown in seven different organic certified hazelnut orchards established with the ocak (multi-stemmed bush) planting system in Samsun (Türkiye) were evaluated. Except for protein, oleic acid, and linolenic acid, orchard-based variation in other traits investigated was significant. The effect of orchards on these traits varied depending on the cultivar. According to orchards, the oil content in the 'Tombul' cultivar varied between 61.1 and 64.4%, protein content between 14.3 and 15.3%, oleic acid content between 82.09 and 82.98%, linoleic acid between 7.49 and 8.44%, total phenolics between 5.51 and 15.92 mg g<sup>-1</sup>, total flavonoids between 4.86 and 23.20 mg 100 g<sup>-1</sup>, and antioxidant activity between 10.33–36.98 mmol kg<sup>-1</sup> and 4.74–28.62 mmol kg<sup>-1</sup> (according to FRAP and DPPH assays, respectively). 'Palaz' cultivar had a range of 59.41–62.92% for oil content, 13.22–15.51% for protein content, 82.05–83.15% for oleic acid content, 6.66–7.89% for linoleic acid, 4.65–9.15 mg g<sup>-1</sup> for total phenolics, 4.45–7.56 mg 100 g<sup>-1</sup> for the total flavonoids, and 9.17–23.58 mmol kg<sup>-1</sup> and 3.54–15.50 mmol kg<sup>-1</sup> for antioxidant activity (according to FRAP and DPPH assays, respectively). Principal component analysis (PCA) revealed that orchards in both cultivars were clustered at different points on the PCA plane and were associated with different traits. The findings showed that orchard-based variations in protein, oil, fatty acid compositions, and bioactive compounds in hazelnuts are significant.

Keywords Organic hazelnut · Oil · Fatty acids · Phenolics · Antioxidant activity

## Introduction

Hazelnut (Corylus avellana L.) is the most cultivated nut after almond and walnut. It is widely grown in temper-

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ate-climatic regions of the northern hemisphere around the world. In recent years, efforts have been made to increase its cultivation in the southern hemisphere, particularly in Chile, Australia, and South Africa (Silvestri et al. 2021). Approximately 1 million tons of hazelnuts are produced worldwide on 1 million hectares of land. Türkiye is the world's largest hazelnut producer and a leading exporter of hazelnuts. Italy, the United States, Azerbaijan, Chile, and Georgia are other important countries (FAOSTAT 2023). In addition to conventional production, efforts in Türkiye to expand organic hazelnut cultivation and increase production continue. On 14,262 ha of land in Türkiye, 6982 farmers grow organic hazelnuts. Türkiye's organic hazelnut production is approximately 35 thousand tons, with production increasing approximately four times in the last 10 years. Samsun province leads in terms of organic hazelnut production, followed by Ordu, Zonguldak, Düzce, and Artvin (MAF 2023).

In Türkiye, hazelnut is widely grown in the Black Sea Region, where ecological conditions are suitable. Hazelnut is traditionally grown in multi-stemmed bushes in the region. 'Tombul,' 'Palaz,' 'Çakıldak,' 'Mincane,' and 'Foşa' are economically important hazelnut cultivars (Karadeniz et al. 2009). Among these cultivars, 'Tombul' is a cultivar with a round nut shape, high fat and protein content, deliciousness, industrial suitability, and is known as prime class-quality (Giresun quality). 'Palaz' is a round fruitshaped cultivar with a delicious, large, plump kernel that is suitable for industrial use and is known as second quality (Levant quality) (Alasalvar et al. 2009).

Hazelnut is a popular nut enjoyed by people. In addition to being consumed raw or roasted, hazelnuts are used in industry by being processed into various products such as ice cream, chocolate, confectionery, and paste (Silvestri et al. 2021).

Hazelnuts play an important role in human nutrition and diet because they contain protein, fat, fatty acids, phenolics, antioxidants, mineral elements, and vitamins. Hazelnut kernels are considered as an important source of fat, containing about 60% fat (Balta et al. 2006; Silvestri et al. 2021). Hazelnuts are rich in fatty acids and have high levels of unsaturated fatty acids, which have an essential role in human health. Monounsaturated fatty acids (linoleic, linolenic) are both considered heart-healthy fatty acids. These fatty acids also mitigate the negative effects of hypertension by lowering the blood cholesterol levels (Yücesan et al. 2010).

Bioactive compounds play an important role in disease prevention and health promotion. It is important to consume fruits that are rich in phenolics and antioxidants. Phenolics are compounds that possess antioxidant properties (Potì et al. 2019). Antioxidants, through reactive oxygen species, reduce the risk of diseases caused by oxidative stress (Valko et al. 2007). Hazelnuts, known as an important source of phenolics and antioxidants, are a natural source of antioxidant compounds (Delgado et al. 2010). Hazelnut kernels are high in phenolic compounds such as catechin, epicatechin, epicatechin gallate, and gallic acid. Phenolic compounds are known to reduce the risk of diabetes, cancer, and heart disease, and show anti-inflammatory, antiallergenic, antimicrobial, and antioxidant properties (Lorenzo and Munekata 2016; Wani et al. 2020).

Several noteworthy studies have been undertaken in recent years, examining the bioactive compounds and fatty acids present in hazelnuts (Krol and Gantner 2020; Yaman et al. 2023). These studies focus mostly on the effects of various factors such as cultivar, climatic conditions, crop load, harvest time, cultural practices, drying and storage conditions, diseases, and pests. However, no research has been conducted on orchard-based change on bioactive compounds and fatty acid composition in hazelnuts. The studies on this aspect have focused on nut characteristics (Karadeniz and Islam 1999; Bostan and Islam 1999), and nut characteristics have shown significant orchard-based variation. The main study question for this study was: What are the orchard-based variations in oil content, fatty acid composition, and bioactive compounds of 'Tombul' and 'Palaz' hazelnut cultivars? This study hypothesized that orchard conditions would have a significant effect on the oil content, fatty acid composition, and bioactive compounds of 'Tombul' and 'Palaz' hazelnut cultivars. The primary objective of the study was to determine the orchard-based variations in oil and protein content, fatty acid composition, and bioactive compounds in organic hazelnuts ('Tombul' and 'Palaz' cvs.).

# **Materials and Methods**

#### **Study Area**

Çakmak Dam Basin in the district of Çarşamba (Samsun, Türkiye) (41° 08′ 07″ N 36° 38′ 45″ E) constitutes an important share in the organic hazelnut production of Samsun province. In the research area with an altitude of about 20 m, the average annual temperature is about 14–15 °C. The coldest month is February, and the warmest month is August. Annual rainfall average is about 1000 mm and relative humidity is about 70%. In some years, especially in February and March, late spring frosts occur in the basin (TSMS 2023).

#### Plant Material

The study was conducted in seven different organic certified hazelnut ('Tombul' and 'Palaz' cvs.) orchards established with an ocak (multi-stemmed bush) planting system in Çakmak Dam Basin located in the district of Çarşamba, Samsun province, Türkiye. The hazelnut orchards had completed their fourth year of transition to organic agriculture. The planting spacings of ocaks were  $6.5 \times 3.5 \text{ m}$  (T1),  $6.5 \times$ 3.5 m (T2),  $6.5 \times 3.5 \text{ m}$  (T3),  $6.5 \times 5.5 \text{ m}$  (T4),  $4.5 \times 5 \text{ m}$  (T5),  $4.5 \times 5 \text{ m}$  (T6), and  $5 \times 5 \text{ m}$  (T7) for 'Tombul' cultivar, and 5×3m (P1), 5×5m (P2), 5×5m (P3), 5×5m (P4), 6×5m (P5),  $6 \times 5 \text{ m}$  (P6), and  $6 \times 5 \text{ m}$  (P7) for 'Palaz' cultivar. The stem ages of ocaks in the orchards were around 18-20 years. Hazelnut plants were not irrigated additionally except for rain. At the beginning of spring, 5kg of organic farmyard manure was applied per ocak. At the end of May, mid-June, and late June, organic liquid foliar fertilizer Quatro Soil (0.2 L/da) was applied. Weed control in the orchards was carried out meticulously with an automatic scythe. Nut samples were collected at harvest, removed from their husks, and dried naturally.

#### Protein and Oil Content (%)

Protein content of hazelnut kernel was determined by the *Kjeldahl* method and calculated as total N×6.25 (Venkatachalam and Sathe 2006). For oil content, 5 g hazelnut kernel powder was weighed into soxhlet cartridges. Then samples treated with *n*-hexane 4h at 130 °C by using soxhlet extraction apparatuses. Results were expressed as percentage of dry matter (Firestone 1997). The obtained oil sample was placed into the dark bottle and kept at -20 °C until fatty acids analysis.

#### Fatty Acid Composition (%)

Fatty acid methyl esters were prepared from oil of hazelnut kernel for analysis in gas chromatography (GC) by modifying the method of Altun et al. (2013). Volumes of 4 mL hexane and 1 mL potassium methylate (%25) were added to 0.1g of hazelnut oil and shaken for 30s. Then, 0.5 mL H<sub>2</sub>SO<sub>4</sub> (%25) was added to prepared mixture. Finally, the upper hexane was taken into vials and kept at -20 °C for fatty acids analysis. Fatty acids composition of the samples was analyzed using GC (GC 2010 Plus, Shimadzu, Kyota, Japan), a flame ionization detector (FID) and a capillary column (0.25 mm×0.2 µm, 100 m, Teknokroma TR-CN100). The carrier gas was helium. The flow rate was 1 mL/min. The column temperature was 140 °C for 5 min, then increased to 240 °C at 25 min, and held for 20 min. Detector and injector temperatures were 250 °C. The split ratio was 1:100. Fatty acid peaks were detected by comparison to the retention time of reference standards (Supelco, Merk, Germany). The results were expressed as percentage of the peak area of fatty acids.

#### Total Phenolics Content (mg g<sup>-1</sup>)

Total phenolics content was determined by using Folin–Ciocalteu assay. Volumes of 3.7 mL distilled water,  $100 \mu$ L Folin–Ciocalteu reagent, and  $300 \mu$ L Na<sub>2</sub>CO<sub>3</sub> were added to  $300 \mu$ L supernatant. The mixture was then vortexed and incubated for 2 h at room temperature. The total phenolics of samples were determined using by spectrophotometer (Shimadzu) at 760 nm. Obtained absorbance values were calculated by using gallic acid curve and expressed as milligrams of total phenolics content per g of sample (Beyhan et al. 2010).

### Total Flavonoid Content (mg g<sup>-1</sup>)

Total flavonoid content was determined by modifying the assay of Chang et al. (2002). A total of 3.8 mL methanol,  $100 \mu$ L ammonium nitrate, and  $100 \mu$ L ammonium acetate were added to  $500 \mu$ L supernatant. The mixture was then

vortexed and incubated for 40 min at room temperature. The total phenolics of samples were determined by spectrophotometer (Shimadzu) at 415 nm. Obtained absorbance values were calculated by using quercetin curve and expressed as milligrams of total flavonoid content per 100 g of sample.

#### Antioxidant Activity (mmol kg<sup>-1</sup>)

The ferric reducing ability of plasma (FRAP) test: antioxidant activity was determined by modified the assay of Benzie and Strain (1996). Volumes of  $1220\,\mu$ L NaH<sub>2</sub>PO<sub>4</sub> and  $1250\,\mu$ L potassium ferric cyanide were added to  $30\,\mu$ L supernatant. Then, the mixture was vortexed and incubated for  $30\,\text{min}$  at  $50\,^{\circ}$ C. After incubation,  $1250\,\mu$ L trichloroacetic acid and  $250\,\mu$ L FeCl<sub>3</sub> was added to the mixture. The total antioxidant activity of samples was determined using by spectrophotometer (Shimadzu) at 700 nm. Obtained absorbance values were calculated by using Trolox curve and expressed as mmol Trolox equivalents per kg of sample.

The 1,1-diphenyl-2-picryl-hydrazil (DPPH) assay: Antioxidant activity of sample was measured by DPPH using the method of Blois (1958). An amount of 0.5 ml of 0.1 mM ethanolic solution of DPPH-was added to 3.0 ml of all the supernatant or standard antioxidants solution (50–500 $\mu$ g mL<sup>-1</sup>) in water. The total antioxidant activity of samples was determined using by spectrophotometer (Shimadzu) at 517 nm. Obtained absorbance values were calculated by using Trolox curve and expressed as mmol Trolox equivalents per kg of sample.

## **Statistical Analysis**

The study was established in a completely randomized design. The treatments consisted of three replications. All analyses were performed three times for each replicate. Data were analyzed with ANOVA using JMP 10 (SAS Institute Inc., Cary, North Carolina, USA) software. Differences between means were determined with LSD multiplecomparison test at p < 0.05. Biplot analysis were performed based on oil, protein, fatty acid composition, and bioactive compounds of investigated hazelnut cultivars.

## **Results and Discussion**

#### **Protein and Oil Content**

While the effect of orchards on the protein content of the 'Tombul' cultivar was not significant, it was significant on the oil content (p < 0.05). Protein content varied between 14.3% (T4) and 15.4% (T2) depending on the orchards. The highest oil content was determined in T4 with 64.4%, followed by T6 (63.4%) and T3 (62.7%) orchards. The lowest

Orchards	Protein content (%)	Oil content (%)			
T1	15.3 a <sup>z</sup>	62.4 c			
T2	15.4 a	61.1 d			
Т3	14.7 a	62.7 bc			
T4	14.3 a	61.4 d			
T5	14.8 a	64.4 a			
Т6	14.9 a	63.4 b			
Τ7	15.3 a	62.5 c			
Min	14.3	61.1			
Max	15.3	64.4			
Mean	14.9	62.5			
LSD (0.05)	1.21	0.81			
Significance	NS	***			

 Table 1
 Protein and oil content (%) variation in 'Tombul' hazelnut cultivar based on orchards

NS Not significant

<sup>z</sup>The differences between mean values shown on the same line with the same letter is not significant (p < 0.05)

\*\*\*Significant at p < 0.001

oil content was detected in T2 with 61.1% (Table 1). An average of 15.65% protein and 55.55% oil was reported in the 'Tombul' cultivar grown organically in different regions of Türkiye (Karaosmanoglu and Ustun 2022). Karaosmanoglu (2022) determined that the 'Tombul' cultivar grown organically in different producer orchards in Giresun (Türkiye) ecological conditions contained an average of 12.45% protein and 57.49% oil. Furthermore, the protein and oil contents of the conventionally grown 'Tombul' cultivar were determined between 19.92% and 60.55% in Çarşamba district (Türkiye) by Çetin et al. (2020), between 16.35% and 64.8% in Giresun (Türkiye) by Balık (2021) and as 12.83–18.08%, and as 62.82–67.96% in Ordu (Türkiye) by Bak and Karadeniz (2021), respectively.

Table 2Protein and oil content (%) variation in 'Palaz' hazelnutcultivar based on orchards

Orchards	Protein content (%)	Oil content (%)
P1	14.71 ab <sup>z</sup>	62.72 b
P2	14.68 ab	59.41 d
P3	14.08 ab	61.12 c
P4	13.88 b	61.72 c
P5	14.08 ab	62.92 ab
P6	13.22 b	63.78 a
P7	15.51 a	59.88 d
Min	13.22	59.41
Max	15.51	62.92
Mean	14.30	61.65
LSD (0.05)	1.51	0.95
Significance	*	***

<sup>z</sup>The differences between mean values shown on the same line with the same letter is not significant (p < 0.05)

\*Significant at *p*<0.05, \*\*\*significant at *p*<0.001

In 'Palaz' cultivar, protein and oil contents varied significantly according to orchards (p<0.05). Protein content was determined between 13.51% (P6) and 15.31% (P7). The highest oil content was found in P6 with 63.78%. This was followed by P5 (62.92%) and P1 (62.72%) orchards. The lowest was determined in P2 with 59.41% (Table 2). Karaosmanoglu and Ustun (2022) reported an average of 53.95% oil and 15.44% protein in the 'Palaz' cultivar grown organically in different regions in Türkiye. Protein and oil contents in the conventionally grown 'Palaz' cultivar have been reported to be 10.64–14.15% and 60.88–66.56% in Ordu province (Bak and Karadeniz 2021), 13.80% and 64.60% in Giresun ecological conditions (Balık 2021), and 15.31% and 61.75% in Çarşamba district (Çetin et al. 2020), respectively.

In the present study, in both cultivars, the variation in oil content according to orchards was significant. A notable alteration in protein content was only determined the 'Palaz' cultivar. Karaosmanoglu and Ustun (2022) found that the protein and oil contents of the 'Tombul' and 'Palaz' cultivars, both cultivated organically and conventionally, remained unaffected by the different growing conditions. Several researchers reported similar findings (Koç and Bostan 2010; Turan et al. 2010). Moreover, the results regarding the protein and oil content in the 'Palaz' cultivar were in consistent with the reference values provided by the researchers; however, the data for the 'Tombul' cultivar exhibited disparities. The variations in protein and oil content can be ascribed to environmental conditions, geographical location, cultural practices, and the ripeness of the fruit (Cristofori et al. 2015; Yılmaz et al. 2019).

## **Fatty Acid Composition**

Hazelnut oil is predominantly composed of monounsaturated fatty acids. Subsequently, there are polyunsaturated and saturated fatty acids. The most abundant monounsaturated fatty acid in hazelnuts is oleic acid. This is followed by linoleic acid, a polyunsaturated fatty acid. The ratio of these fatty acid is crucial in determining the kernel quality of hazelnut, as well as their economic and nutritional value. A high oleic acid content indicates a longer shelf life and high oxidative stability. Polyunsaturated fatty acid-rich oils are also known to have potential health benefits (Wang et al. 2018; Gama et al. 2018).

In the current study, oleic, linoleic, palmitic, and stearic acids were determined as major fatty acids in both cultivars, while palmitoleic, arachidic, linolenic, and cis 11-eicosenoic acids were detected in trace amounts (Tables 3 and 4). In the 'Tombul' cultivar, except oleic acid, other fatty acids differed significantly according to orchards (p < 0.05). According to the orchards, palmitic acid was determined from 5.97% (T1) to 6.71% (T4), palmitoleic

Orchards	Fatty acids (%)								
	Palmitic (C16:0)	Palmitoleic (C16:1)	Stearic (C18:0)	Oleic (C18:1)	Linoleic (C18:2)	Linolenic (C18:3)	Arachidic (C20:0)	Cis 11- eicosenoic (C20:1)	_
T1	5.97 d <sup>z</sup>	0.15 c	2.80 ab	82.30 a	8.44 a	ND	0.17 a	0.19 a	
T2	6.08 cd	0.23 a	2.89 a	82.09 a	7.50 b	ND	0.16 ab	0.20 a	
Т3	6.14 cd	0.21 ab	2.65 cd	82.38 a	8.20 a	ND	0.14 b	0.14 b	
T4	6.71 a	0.25 a	2.88 a	82.21 a	7.49 b	ND	0.18 a	0.16 ab	
Т5	6.24 c	0.19 b	2.71 bc	82.98 a	7.71 b	ND	0.13 b	0.17 ab	
Тб	6.31 bc	0.21 ab	2.60 d	82.26 a	8.31 a	ND	0.15 ab	0.16 ab	
T7	6.52 ab	0.21 ab	2.76 b	82.32 a	7.78 b	ND	0.17 a	0.17 ab	
Min	5.97	0.15	2.60	82.09	7.49	ND	0.13	0.14	
Max	6.71	0.23	2.89	82.98	8.44	ND	0.17	0.20	
Mean	6.28	0.21	2.76	82.36	7.92	ND	0.16	0.17	
LSD (0.05)	0.26	0.04	0.10	1.41	0.34	_	0.03	0.04	
Significance	***	**	***	NS	***	_	*	*	

Table 3 Fatty acid (%) variation in 'Tombul' hazelnut cultivar based on orchards

NS Not significant, ND Not determined

<sup>z</sup>The differences between mean values shown on the same line with the same letter is not significant (p < 0.05)

\*Significant at p<0.05, \*\*significant at p<0.01, \*\*\*significant at p<0.001

acid from 0.15% (T1) to 0.25% (T4), stearic acid from 2.60% (T6) to 2.89% (T2), oleic acid from 82.09% (T2) to 82.98% (T5), from linoleic acid 7.49% (T4) to 8.31% (T6), arachidic acid from 0.13% (T5) to 0.18% (T4), and cis 11-eicosenoic acid from 0.14% (T3) to 0.20% (T2) (Table 3). In the 'Tombul' cultivar grown organically in important hazelnut production areas in Türkiye, palmitic acid 5.27%, palmitoleic acid 0.17%, stearic acid 2.41%, oleic acid 81.42%, linoleic acid 10.25%, arachidic acid

0.12%, and eicosenoic acid 0.17% were found (Karaosmanoglu and Ustun 2021). In the 'Tombul' cultivar grown organically in different producer orchards under Giresun (Türkiye) ecological conditions, 5.60% palmitic acid, 0.15% palmitoleic acid, 2.20% stearic acid, 84.64% oleic acid, 6.98% linoleic acid, 0.09% arachidic acid, and 0.11% eicosenoic acid were reported (Karaosmanoglu 2022). The composition of fatty acids in the conventionally grown 'Tombul' hazelnut cultivar has been reported by vari-

 Table 4
 Fatty acid (%) variation in 'Palaz' hazelnut cultivar based on orchards

Orchards	Fatty acids (%)							
	Palmitic (C16:0)	Palmitoleic (C16:1)	Stearic (C18:0)	Oleic (C18:1)	Linoleic (C18:2)	Linolenic (C18:3)	Arachidic (C20:0)	Cis 11- eicosenoic (C20:1)
P1	7.09 a <sup>z</sup>	0.27 ab	2.66 ab	82.25 b	7.40 ab	ND	0.21 a	0.22 a
P2	6.79 b	0.28 a	2.60 bc	82.05 b	7.89 a	ND	0.17 b	0.19 ab
P3	6.77 b	0.30 a	2.62 ab	82.43 ab	7.45 ab	0.09 a	0.13 c	0.21 a
P4	6.84 b	0.31 a	2.68 a	82.93 ab	6.70 c	0.09 a	0.14 c	0.13 d
P5	6.77 b	0.23 b	2.66 ab	83.15 a	6.66 c	ND	0.18 b	0.17 bc
P6	6.88 b	0.31 a	2.62 ab	82.65 ab	6.88 c	0.08 a	0.13 c	0.19 ab
P7	6.88 b	0.28 a	2.53 c	82.62 ab	7.15 bc	0.09 a	0.15 c	0.15 cd
Min	6.84	0.23	2.53	82.05	6.66	0.08	0.13	0.13
Max	7.09	0.31	2.68	83.15	7.89	0.09	0.21	0.22
Mean	6.86	0.28	2.62	82.58	7.16	0.09	0.16	0.18
LSD (0.05)	0.18	0.04	0.07	0.90	0.51	0.03	0.02	0.03
Significance	*	*	**	*	***	NS	***	***

NS Not significant, ND Not determined

<sup>z</sup>The differences between mean values shown on the same line with the same letter is not significant (p < 0.05)

\*Significant at p<0.05, \*\* Significant at p<0.01, \*\*\*Significant at p<0.001

ous researchers. The 'Tombul' cultivar contains approximately 4.85–7.80% palmitic acid, 0.16–0.20% palmitoleic acid, 2.73–4.06% stearic acid, 76.10–82.79% oleic acid, 8.26–12.30% linoleic acid, 0.12–0.14% arachidic acid, and 0.16% cis 11-eicosenoic acid (Ozdemir et al. 2001; Alasalvar et al. 2010; Balık 2021).

In 'Palaz' cultivar, except for linolenic acid, the effect of orchards on other fatty acids was significant. According to the orchards, palmitic acid was determined from 6.77% (P3 and P5) to 7.09% (T4), palmitoleic acid from 0.27% (P1) to 0.31% (P4 and P6), stearic acid from 2.53% (P7) to 2.68% (P4), oleic acid from 82.05% (T2) to 83.15% (P5), linoleic acid from 6.66% (P5) to 7.89% (P2), linolenic acid from 0.08% (P6) to 0.09% (P3, P4 and P7), arachidic acid from 0.13% (P3 and P6) to 0.21% (P1), and cis 11eicosenoic acid from 0.13% (P4) to 0.22% (P1) (Table 4). Karaosmanoglu and Ustun (2021) noted 5.60% palmitic acid, 0.20% palmitoleic acid, 2.44% stearic acid, 81.92% oleic acid, 9.36% linoleic acid, 0.12% arachidic acid, and 0.16% eicosenoic acid in the 'Palaz' cultivar grown organically in different regions of Türkiye. The 'Palaz' hazelnut cultivar, grown conventionally, has been found to contain the following percentages of fatty acids: 6.62-8.30% palmitic acid, 0.20-0.29% palmitoleic acid, 2.63-4.15% stearic acid, 75.70-82.39% oleic acid, 5.91-12.90% linoleic acid, 0.18% arachidic acid, and 0.16% cis 11-eicosenoic acid (Ozdemir et al. 2001; Alasalvar et al. 2010; Balık 2021).

In the current study, the fatty acid contents of the 'Tombul' and 'Palaz' cultivars were generally consistent with the researchers' findings. The results revealed that the majority of fatty acids varied significantly among orchards. However, the effect of orchards on oleic acid content in the 'Tombul' cultivar and linolenic acid content in the 'Palaz' cultivar was not significant. Many researchers reported that the fatty acid composition of hazelnut kernels can be influenced by climate, geographic origin, cultural practices, harvest time, and growing season (Krol and Gantner 2020; Bostan 2020).

# Total Phenolics, Total Flavonoids, and Antioxidant Activity

Phenolics are compounds with antioxidant properties and constitute a significant part of the secondary metabolites in plants. Phenolics and flavonoids in the plants are known to be defense molecules against biotic and abiotic stress (War et al. 2012). Furthermore, phenolics play an important role in reducing disease risks by promoting human health. Antioxidants, preventing the formation of free radicals by preventing or delaying oxidation, play an important role in human health by neutralizing reactive oxygen species in the body (Forman et al. 2014; Valdes et al. 2015). Consumers have shown a growing interest in fruit species that possess elevated levels of antioxidant activity in recent years. Hazelnuts are recognized as a natural source of antioxidants, with high antioxidant activity (Delgado et al. 2010).

In the 'Tombul' cultivar, the effects of total phenolics, total flavonoids, and antioxidant activity were significant (p < 0.05). According to the orchards, the highest total phenolics were determined in T4 with 15.92 mg g<sup>-1</sup>, and the lowest in T5 with 4.96 mg g<sup>-1</sup>. Total flavonoids ranged from 4.70 mg 100 g<sup>-1</sup> (T5) to 23.20 mg 100 g<sup>-1</sup> (T4). According to the FRAP test, the highest antioxidant activity was determined in T6 (36.98 mmol kg<sup>-1</sup>), while the lowest was detected in T3 and T5 (10.33 mmol kg<sup>-1</sup> and 10.47 mmol kg<sup>-1</sup>, respectively). Similarly, according to the DPPH test, the highest antioxidant activity was detected in T6 (28.62 mmol kg<sup>-1</sup>), while the lowest was detected in T5 and T3 (4.74 mmol kg<sup>-1</sup> and 5.65 mmol kg<sup>-1</sup>, respectively).

Table 5 Total phenolics, total flavonoids, and antioxidant activity variation in 'Tombul' hazelnut cultivar based on orchards

Orchards	Total phenolics (mg GAE/g)	Total flavonoids (mg QE/100g)	FRAP (mmol TE/kg)	DPPH (mmol TE/kg)
T1	9.84 d <sup>z</sup>	6.01 d	22.10 c	17.69 c
T2	9.90 d	5.52 d	20.04 d	14.97 d
Т3	5.51 e	4.86 e	10.33 e	5.65 f
T4	15.92 a	23.20 a	19.97 d	7.46 e
T5	4.96 e	4.70 e	10.47 e	4.74 f
Тб	14.58 b	8.30 b	36.98 a	28.62 a
T7	11.69 c	6.75 c	27.84 b	23.98 b
Min	5.51	4.86	10.33	4.74
Max	15.92	23.20	36.98	28.62
Mean	10.34	8.48	21.10	14.73
LSD (0.05)	0.66	0.56	1.71	1.03
Significance	***	***	***	***

<sup>z</sup>The differences between mean values shown on the same line with the same letter is not significant (p < 0.05)

\*\*\*Significant at p < 0.001

tively) (Table 5). In the 'Tombul' cultivar grown organically in important hazelnut production areas in Türkiye, total phenolics were reported as 3.89 mg g<sup>-1</sup>, and antioxidant activity was stated as 31.50% according to the DPPH test (Karaosmanoglu and Ustun 2021). In the 'Tombul' cultivar grown organically in different producer orchards under Giresun (Türkiye) ecological conditions, 2.05 mg g<sup>-1</sup> total phenolics, 123.7 mg 100 g<sup>-1</sup> total flavonoids, and 6.19 mmol kg<sup>-1</sup> antioxidant activity (according to DPPH assay) were determined (Karaosmanoglu 2022). In the conventionally grown 'Tombul' cultivar, total phenolics, total flavonoids, and antioxidant activity (according to FRAP and DPPH assays) were reported as 4.48 mg g<sup>-1</sup>, 34.0 mg 100 g<sup>-1</sup>, 12.18 mmol kg<sup>-1</sup>, and 2.46 mmol kg<sup>-1</sup>, respectively (Balık 2021). In a different study, total phenolics in 'Tombul' cultivar was determined as 4.32 mg g<sup>-1</sup> (Pelvan et al. 2012). In addition, Karakaya (2023) reported 4.35–6.26 mg g<sup>-1</sup> total phenolics,  $3.9-5.8 \text{ mg } 100 \text{ g}^{-1}$  total flavonoids, and  $3.5-6.7 \text{ mmol kg}^{-1}$ and 13.5-15.1 mmol kg-1 antioxidant activity (according to FRAP and DPPH assays, respectively) in the 'Tombul' cultivar.

In 'Palaz' cultivar, the effect of orchards on total phenolics and total flavonoids was significant (p < 0.05). The highest total phenolics were detected in P7 with 9.15 mg g<sup>-1</sup>, and the lowest were detected in P2 with 4.65 mg g<sup>-1</sup>. Total flavonoids were found between 4.45 mg 100 g<sup>-1</sup> (P2) and 7.56 mg 100 g<sup>-1</sup> (P4). According to the FRAP test, the highest antioxidant activity was found in P7 with 23.58 mmol kg<sup>-1</sup>, and the lowest was found in P2 with 9.17 mmol kg<sup>-1</sup>. According to the DPPH test, the highest antioxidant activity was determined in P4 with 15.50 mmol kg<sup>-1</sup>, and the lowest was determined in P2 with 3.54 mmol kg<sup>-1</sup> (Table 6). In the 'Palaz' cultivar grown organically in different hazel-nut production areas in Türkiye, 3.31 mg g<sup>-1</sup> total phenolics and 21.89% antioxidant activity (according to the

DPPH test) were determined (Karaosmanoglu and Ustun 2021). There is no study determining the total flavonoid content of the organically grown 'Palaz' cultivar. In the conventionally grown 'Palaz' cultivar, Pelvan et al. (2012) reported 7.27 mg g<sup>-1</sup> total phenolics; Balık (2021) determined 4.69 mg g<sup>-1</sup> total phenolics, 13.2 mg 100 g<sup>-1</sup> total flavonoids, 12.82 mmol kg<sup>-1</sup>, and 2.41 mmol kg<sup>-1</sup> antioxidant activity (according to FRAP and DPPH assays, respectively); Karakaya (2023) found 8.71–19.34 mg g<sup>-1</sup> total phenolics, 5.6–17.8 mg 100 g<sup>-1</sup> total flavonoids, as well as 9.9–28.6 mmol kg<sup>-1</sup> and 16.4–19.0 mmol kg<sup>-1</sup> antioxidant activity (according to FRAP and DPPH assays, respectively).

The bioactive compounds in both 'Tombul' and 'Palaz' cultivars varied significantly depending on orchards. The total phenolics and antioxidant activity of the 'Tombul' cultivar were generally higher than the researchers' findings, while the total flavonoids content was consistent with the reported reference values. While total phenolics and antioxidant values in the 'Palaz' cultivar were consistent with the researchers' reported reference values, total flavonoids values were low. Many studies have noted that the bioactive contents of hazelnut kernels can be influenced by growing ecology, geographic orijin, cultural practice, harvest time, and growing season (Cristofori et al. 2015; Tonkaz et al. 2019).

#### Principal Component Analysis (PCA)

In the PCA analysis performed to evaluate the relationship between the protein, oil, fatty acids, and bioactive compounds of 'Tombul' hazelnut cultivar as orchard based revealed a correlation of 65.1%. Based on the PCA results, T1 and T6 orchards, located in the first region of the PCA plane, were related to linoleic acid and protein. T2 and

Table 6 Total phenolics, total flavonoids, and antioxidant activity variation in 'Palaz' hazelnut cultivar based on orchards

Orchards	Total phenolics (mg GAE/g)	Total flavonoids (mg QE/100 g)	FRAP (mmol TE/kg)	DPPH (mmol TE/kg)
P1	6.00 d <sup>z</sup>	5.68 c	12.09 d	6.04 c
P2	4.65 e	4.45 d	9.17 e	3.54 d
P3	7.19 c	6.01 c	16.50 c	11.08 b
P4	8.64 b	7.56 a	19.93 b	15.50 a
P5	9.15 ab	6.66 b	20.91 b	11.85 b
P6	5.22 e	6.01 c	11.52 d	3.88 d
P7	9.41 a	6.25 bc	23.58 a	12.19 b
Min	4.65	4.45	9.17	3.54
Max	9.15	7.56	23.58	15.50
Mean	7.18	6.09	16.24	9.15
LSD (0.05)	0.58	0.64	1.44	1.45
Significance	***	***	***	***

<sup>z</sup>The differences between mean values shown on the same line with the same letter is not significant (p < 0.05)

\*\*\*Significant at p<0.001



Fig. 1 Biplot graph based on oil, protein, fatty acids, and bioactive compounds in the 'Tombul' cultivar. *PC* Principal component

T7 orchards located in the second region were associated with arachidic acid, stearic acid, cis 11-eicosenoic acid, total phenolics, and antioxidant activity. T3 and T5 orchards were associated with oil and oleic acid and were located in the third region. T4 orchard, located in the fourth region, was associated with palmitic acid, palmitoleic acid, and total flavonoids (Fig. 1).



Fig. 2 Biplot graph based on oil, protein, fatty acids, and bioactive compounds in the 'Palaz' cultivar. *PC* Principal component

The correlation was 54.4% in the PCA analysis performed to evaluate the relationship between the protein, oil, fatty acids, and bioactive compounds of 'Palaz' hazelnut cultivar as orchard-based. According to PCA results, P1 and P6 orchards located in the first region in the PCA plane were associated with arachidic acid, palmitic acid, and cis 11-eicosenoic. P4 and P5 orchards were related to oil, stearic acid, oleic acid, and total flavonoids, and were located in the second region. P2 and P3 orchards were located in the third region of the PCA plane and were related to linoleic acid and protein. P7 orchard, located in the fourth region, was associated with palmitoleic acid, linoleic acid, total phenolics, and antioxidant activity (Fig. 2).

# Conclusion

The study represents the first investigation on orchard-based variations of oil content, fatty acid composition, and bioactive compounds in hazelnut. An in-depth investigation was conducted on orchard-based variations of these traits. The nutritional value of hazelnut kernel depends on the quality of the oils obtained. Orchards had a significant impact on the oil content and fatty acid composition of the investigated cultivars. However, the oleic acid content of the 'Tombul' cultivar and the linolenic acid content of the 'Palaz' cultivar did not vary according to orchard. This illustrates that the effect of orchards on oleic and linolenic acids differs depending on the cultivar. Orchard-based variations of total phenolics, total flavonoids, and antioxidant activity in the cultivars investigated were significant. These findings revealed that protein, oil, fatty acids, and bioactive compounds, which are important quality parameters in hazelnuts, exhibit substantial variations based on the orchards.

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Author Contribution M. Yılmaz: investigation, methodology, conceptualization, writing. T. Kaya: investigation, conceptualization, validation, writing. O. Karakaya: investigation, methodology, formal analysis, visualization, writing. F. Balta: investigation, methodology, formal analysis, validation, conceptualization, writing, review & editing. K. Çalışkan: investigation, methodology, conceptualization, writing.

**Conflict of interest** M. Yılmaz, T. Kaya, O. Karakaya, F. Balta and K. Çalışkan declare that they have no competing interests.

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