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How does harvest time affect the major fatty acids and bioactive compounds in hazelnut cultivars (*Corylus avellana* L.)?

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SUMMARY: This study was conducted to investigate the effects of harvest time on the protein, oil, fatty acids and bioactive compounds in hazelnut cultivars (*Corylus avellana* L. cvs. 'Tombul', 'Palaz', 'Çakıldak', 'Okay 28' and 'Allahverdi'). The harvest was carried out at 7 different periods with weekly intervals from 20 July to 31 August. As the harvest time progressed, increases and decreases were detected in protein, oil, fatty acids and bioactive compounds. The highest oil content was measured in the H5 and H6 harvest periods. The highest content was determined in H3 for oleic acid. Higher total phenolics, total flavonoids and antioxidant activity were obtained in the first 3 harvest periods than in the other periods. The present findings revealed that the protein, fatty acids and bioactive compounds in hazelnut cultivars may differ according to the harvest time. The results obtained will provide clearer ideas to both the industry and the producers about the optimum harvest time for the intended use of these cultivars.

KEYWORDS: *Antioxidant; Corylus avellana; Fatty acids; Oleic acid; Phenolics; Protein.*

RESUMEN*: ¿Cómo afecta el tiempo de cosecha a los principales ácidos grasos y compuestos bioactivos de los cultivares de avellana (*Corylus avellana *L.)?* Este estudio se realizó principalmente para investigar los efectos del tiempo de cosecha sobre las proteínas, el aceite, los ácidos grasos y los compuestos bioactivos de cultivares de avellana (*Corylus avellana* L. cvs. 'Tombul', 'Palaz', 'Çakıldak', 'OK 28' y 'Allahverdi'). La cosecha se realizó en 7 periodos diferentes con intervalos semanales del 20 de julio al 31 de agosto. A medida que avanzó la época de cosecha se detectaron aumentos y disminuciones en proteínas, aceite, ácidos grasos y compuestos bioactivos. El mayor contenido de aceite se encontró en los períodos de cosecha H5 y H6. El contenido más alto para el ácido oleico se encontró en H3. Los mayores fenólicos totales, flavonoides totales y actividad antioxidante se obtuvieron en los primeros 3 períodos de cosecha en comparación con los otros períodos. Los hallazgos actuales revelaron que las proteínas, los ácidos grasos y los compuestos bioactivos de los cultivares de avellana pueden diferir según el momento de la cosecha. Los resultados obtenidos proporcionarán ideas más claras tanto a la industria como a los productores sobre el momento óptimo de cosecha para el uso previsto de estos cultivares.

PALABRAS CLAVE: *Antioxidante; Corylus avellana; Ácidos grasos; Ácido oleico; Fenólicos; Proteínas.*

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1. INTRODUCTION

The hazelnut is a popular type of fruit which consumers love and consume frequently. More than 90% of the world's produced hazelnuts are used in the confectionery, chocolate and ice cream industries. Hazelnuts are processed into many kinds of products, including roasted, chopped, sliced, flour, puree, chocolate and paste (Silvestri *et al.,* 2021).

Hazelnuts are rich in dietary fiber, macro-micro elements, vitamins, carbohydrates, proteins, fats, fatty acids, phytosterols, phenolic compounds and antioxidants and are an important part of many countries' healthy nutrition and diets (Karaosmanoglu and Ustun, 2022). It can prevent or delay the neutralization of free radicals and lipid oxidation due to the protein, fatty acids and phenolic compounds it contains and their antioxidant activities. Thus, hazelnuts play an important role in the treatment and prevention of diseases. The monounsaturated and polyunsaturated fatty acids in hazelnuts play an important role in lowering cholesterol levels, and the risk of heart disease as well as in alleviating the negative effects of hypertension (Chang *et al.,* 2016; Wani *et al.,* 2020).

Phenolic compounds influence several sensory properties of fruits, including taste, color, aroma, flavor and odor (Haminiuk *et al.,* 2012). Oils and fatty acids largely designate the quality of a nut species such as hazelnuts (Piscopo *et al.,* 2010). Various factors including genetic structure, ecological conditions, technical and cultural practices (irrigation, fertilization, pruning, etc.), diseases and pests, drying and storage conditions and harvest time affect these components in hazelnuts (Balta *et al.,* 2006; Pycia *et al.,* 2020; Balık, 2021).

It is critical to determine the optimal harvest time in order to minimize quality losses in commercially-grown varieties and provide higher-quality products to consumers. Early or late harvests cause significant yield and quality losses in fruit species (Cristofori *et al.,* 2015). The protein, oil, fatty acids, sugars and minerals in hazelnuts are known to be affected by harvest time and such compounds change with the progress of harvest (Seyhan *et al.,* 2007; Cristofori *et al.,* 2015; Ilyasoglu, 2016). Indeed, Pycia *et al.* (2020) noted that the antioxidant activity of hazelnuts decreased with the progress of ripening. Furthermore, the phytochemical content in nuts such as almonds (Piscopo *et al.,* 2010), walnuts (Wei *et al.,* 2022), pistachios (Kelebek *et al.,* 2020) and pecans (Bouali *et al.,* 2013) were reported to vary with harvest time.

The majority of the research on the effect of harvest time on hazelnut kernel quality has focused on changes in protein, oil and fatty acids (Seyhan *et al.,* 2007; Cristofori *et al.,* 2015; Ilyasoglu, 2016).

There are no previously reported studies on how the protein, fatty acid composition, total phenolics, total flavonoids and antioxidant activity of Turkish hazelnut varieties varied with harvest time. The main objective in this study was to determine the effect of harvest time on the fatty acid composition and bioactive compounds in new ('Okay 28' and 'Allahverdi') and commonly grown Turkish hazelnut cultivars. It was hypothesized that harvest time would have a significant effect on these components, and this study is the first to investigate how harvest time affects protein, oil, major fatty acids and bioactive compounds in new Turkish hazelnut cultivars. Prospective outcomes will provide significant contributions to both food industry and hazelnut growers about the optimum harvest time of these cultivars.

2. MATERIALS AND METHODS

2.1. Plant materials

This study was conducted in 2016 and 2017 on the experimental fields of the Hazelnut Research Institute (Giresun, Türkiye) (40° 54' 31'' N and 38° 21' 09'' E, 5 m altitude). Plant materials consisted of 22-year-old 'Tombul', 'Palaz', 'Çakıldak', 'Allahverdi' and 'Okay 28' hazelnut cultivars (*Corylus avellana* L.). Hazelnut plants were planted in the 'Ocak' system at 3-m row spacing and 3-m plant spacing in each row. Cultural practices (fertilization, irrigation, pruning, etc.) were performed at regular intervals. The soil in the experimental area consisted of clay-loam in texture with a pH of 4.78 and organic matter content of 4.58%. Based on soil analysis results, commercial fertilizer containing 20% nitrogen, 22% phosphorus, 15% potassium, 2.0% magnesium oxide, 0.5% zinc and 0.3% boron was applied to each 'Ocak' (5 plants in each Ocak) during the second week of February. About 1 kg of fertilizer was applied to each 'Ocak'. In the second week of May, 0.5 kg of 26% calcium ammonium nitrate fertilizer was applied per 'Ocak'. Throughout the experiments, pest (powdery mildew, nut weevil and green

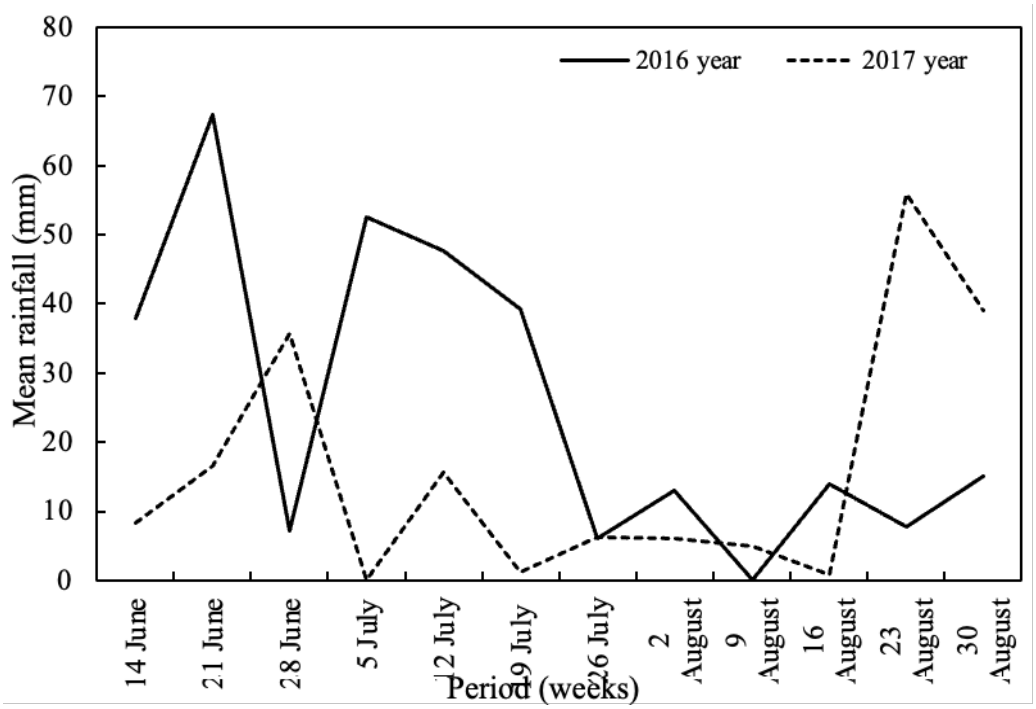

Figure 1. Mean temperature (°C) and rainfall values for the 2016 and 2017 seasons

stink bugs, etc.) and disease control, pruning and weed control were carried out at regular intervals. Figure 1 shows the climate data recorded throughout the experiments.

2.2. Experimental design

The experiments were conducted in a randomized-plot experimental design with three replicates for each cultivar and three 'Ocaks' (5 plants in each Ocak) in each replicate. Present cultivars were manually harvested at 7 weekly intervals (H1, H2, H3, H4, H5, H6 and H7) from complete kernel growth to complete shell-fill (in all hazelnut cultivars, first harvest was performed July 20 in 2016 and July 24 in 2017). Harvested fruits were manually separated from the husks and sun-dried on a concrete floor until the moisture content dropped to 6%. About 1 kg of whole nuts was used in each replicate from each cultivar for the analyses of each harvest period. The hazelnut shells were cracked and stored at -18 °C until analysis.

2.3. Protein

Protein content was determined according to the Kjeldahl method and the amount of nitrogen was calculated from the amount of ammonia. The results were expressed in percentage (%) (Venkatachalam and Sathe, 2006).

2.4. Oil

Oil content was determined according to the Soxhlet extraction method. The results obtained were expressed in percentage (%) (Firestone, 1997).

2.5. Composition of fatty acids

Fatty acid methyl esters (FAMEs) were prepared for gas chromatography (GC) analysis from the total oil content of hazelnuts using a modified version of the protocol outlined below. First, 1 mL of oil was added to a tube, followed by 2 mL of H_2SO_4 (dissolved in 10% Methanol). After incubating for 40 minutes at 57 °C at 140 rpm, the mixture was cooled to room temperature. Then, 1 mL of 2.0% $NaHCO_3$ was added and vortexed, 1 mL of hexane was added and the mixture was shaken for one minute. Finally, the FAME-containing upper hexane layer was transferred to a new tube and stored at -20 °C for GC analysis. The Shimadzu GC-20A (Kyoto, Japan) GC with a flame-ionization detector was used to analyze samples filtered through a 0.2 m nylon membrane. For analysis, a Stabilwax DA column (0.25 mm x 0.25 m 60 m) was used. The carrier gas was nitrogen

and the flow rate was 3 mL/min. The initial temperature was set at 100 °C, held for four minutes and then raised to 245 °C (20 °C/min) and held there for 40 minutes. After that, the temperature was raised to 250 °C for five minutes. At 250 °C, a split injection (1:20) was performed. Peaks of fatty acids were defined using reference standards by comparing retention times. Results are expressed in a relative percentage of fatty acids and processed using the GC manufacturer's "GC Solution" program.

2.6. Sample preparation for bioactive analyses

Total phenolics, total flavonoids and antioxidant activity (according to DPPH and FRAP) were determined in defatted kernel samples. Oil extraction from the kernel samples was performed using the Soxhlet method. About 1 g defatted kernel samples was weighed on a precise balance (±0.01 g) and 10 mL methanol were added. The prepared solution was kept at +4 °C for two days. The solution was then centrifuged for 4 min at 1200 rpm.

2.6.1. Total phenolics

Total phenolics were determined by using Folin-Ciocalteu's chemical according to the method reported by Karakaya *et al.* (2023). 500 L of the stock solution were taken out, then 4.2 mL of distilled water, 100 µL of Folin-Ciocalteu's reagent, and 2% sodium carbonate (Na_2CO_3) were added. The prepared solution was measured at 760 nm in a spectrophotometer (Shimadzu UV-1280, Tokyo, Japan). The results were expressed in g gallic acid equivalent (GAE)·kg^{-1} dry weight (dw).

2.6.2. Total flavonoids

Total flavonoids were determined using the method described by Karakaya *et al.* (2023). 500 L of the stock solution were taken, then 3.8 mL of methanol, 0.1 mL of 10% $AlCl_3.6H_2O$, and 10% CH_3COOK were added. A spectrophotometer was used to measure the samples at a wavelength of 415 nm. The results were expressed in g quercetin equivalent (QE)·kg^{-1} dw.

2.6.3. Antioxidant activity

DPPH assay. DPPH was determined according to the modified method described by Blois *et al.* (1958). 2700 µL of ethyl alcohol and 1 mL of 0.26 mM DPPH (1,1-diphenyl-2-picryl-hydrazil) solution were added to 300 µL of fruit extract. The prepared samples were measured at 517 nm in the spectrophotometer. The results were expressed in mmol Trolox equivalent (TE)·kg^{-1} dw.

FRAP assay. FRAP was detected according to the modified method of Benzie and Strain (1996). 150 µL were taken from the stock solution, then 1.1 mL of phosphate buffer and 1.25 mL of potassium ferric cyanide were added. The solution was then supplemented with 1.25 mL of TCA and 0.25 mL of $FeCl_3.6H_2O$. The samples were measured at a wavelength of 700 nm in the spectrophotometer. The results were expressed in mmol Trolox equivalent (TE)·kg^{-1} dw.

2.7. Statistical analysis

Data normality was checked with the use of Kolmogorov- Smirnov's test. Variance homogeneity was checked with the use of Levene's test. Descriptive statistics were calculated. Experimental data were subjected to variance analysis. Significant means were compared with the use of Tukey's multiple comparison tests ($p \leq 0.05$). Statistical analyses were performed with the use of Minitab® 17 Statistical Software (Minitab Inc., State College, PA, USA).

3. RESULTS AND DISCUSSION

3.1. Protein content

The protein contents should be known in order to effectively time the application of nitrogen fertilizers (Wei and Zhai, 2010). Protein content significantly varied with harvest time ($p < 0.05$). Protein content fluctuated in all cultivars depending on harvest time. Except for 'Okay 28' (16.68%), in other cultivars, the highest protein content was determined in the H1 harvest time (16.71% in 'Tombul,' 16.88% in 'Palaz,' 18.66% in 'Çakıldak,' and 17.60% in 'Allahverdi'). It was also detected in the H5 in 'Okay 28'. Protein content decreased in general at the final harvest time (H7) as compared to the first harvest time (H1) (Table 1). Ilyasoglu (2015) and Seyhan *et al.* (2007) reported that the protein contents in 'Tombul', 'Palaz', 'Badem' and 'Sivri' cultivars decreased as the harvest time progressed. Cristofori *et al.* (2015), on the other hand, stated that the protein content in the 'Tonda Gentile Romana' cultivar fluctuated as the harvest time progressed. A decrease

Table 1. Protein and oil ratio (%) in the investigated hazelnut cultivars depending on harvest time (means of 2016 and 2017)

Cultivar/ Harvest time	Protein (%)						
	H1	H2	H3	H4	H5	H6	H7
Tombul	16.71 a[z]	14.59 de	14.09 e	16.02 b	15.49 c	15.68 bc	14.73 d
Palaz	16.88 a	14.49 d	14.19 d	15.77 bc	13.24 e	15.46 c	15.99 b
Çakıldak	18.66 a	16.52 c	17.51 b	15.85 d	18.15 a	16.41 c	16.22 cd
Okay 28	15.32 b	15.66 b	16.25 a	15.59 b	16.68 a	13.79 d	14.40 c
Allahverdi	17.60 a	17.51 a	15.26 d	14.62 e	16.75 b	15.94 c	16.75 b
	Oil (%)						
Tombul	59.73 d	64.97 b	67.05 a	61.69 c	63.41 bc	67.58 a	68.66 a
Palaz	57.90 d	66.99 ab	65.51 bc	55.84 e	68.65 a	66.74 b	64.06 c
Çakıldak	52.78 e	58.49 d	58.83 cd	66.40 a	60.49 bc	64.68 a	61.65 b
Okay 28	58.29 d	58.18 d	59.39 d	69.10 a	69.86 a	65.90 b	64.04 c
Allahverdi	56.09 e	63.68 b	63.46 b	65.03 ab	65.78 a	60.91 c	58.28 d

[z]Means indicated by the same letter in the same line do not differ according to the Tukey test ($p < 0.05$).
n= 9 for the protein and oil content (three replicates × three different measurements for each replicate)

was also reported in 'Tonda di Giffoni' and 'Nocchione' cultivars. The present findings on protein ratio comply with the findings of previous studies.

3.2. Oil percentage

The period in which hazelnut kernels have maximum oil levels is critical for detecting the optimal harvest time (Bouali *et al.,* 2013). Harvest time had a significant effect on the oil contents in the investigated hazelnut cultivars ($p < 0.05$). All cultivars had fluctuating oil contents with harvest times. The highest oil contents were determined in the H7 in 'Tombul' (68.66%), in the H4 in 'Çakıldak' (66.40%); in the H5 in 'Palaz' (68.65%), 'Okay 28' (69.86%) and 'Allahverdi' (65.78%) (Table 1). Although oil contents fluctuated with harvest times, they generally increased as maturation progressed. Reserve lipid formation at the final stage of fruit maturation resulted in high oil contents. However, due to lipids being synthesized during the early stages of kernel development in nut species and being used to form new fruit tissues, oil accumulation during the first stage of kernel development may be lower (Bouali *et al.,* 2013). Consistent with the present findings, it was reported that oil level increased during the kernel development of the cultivars 'Tombul', 'Palaz' and 'Sivri' cultivars (Ilyasoglu, 2015). Similar findings were reported for 'Tombul', 'Palaz' and 'Badem' cultivars (Seyhan *et al.*, 2007), 'Tonda Gentile Romana', 'Tonda di Giffoni' and 'Nocchione' cultivars (Cristofori *et al.,* 2015).

3.3. The composition of main fatty acids

Hazelnut oil is a good source of food which is enjoyed by consumers. Oleic acid is the main fatty acid in hazelnut oil, followed by linoleic, palmitic and stearic acids. These fatty acids account for 98-99% of the total fatty acids in hazelnuts, with other fatty acids found in trace amounts (Balta *et al*., 2006; Karaosmanoglu and Ustun, 2021; Karakaya *et al*., 2023). Human diets should have a high oleic acid content and low-density lipoprotein lowers cholesterol levels (Wani *et al.,* 2020). Several factors including variety, ecological conditions, cultural practices, and harvest time affect the fatty acids in hazelnuts (Balta *et al.,* 2006; Cristofori *et al.,* 2015; Balık, 2021).

The effects of harvest time on the oleic acid content in hazelnut cultivars were found to be significant ($p < 0.05$). The oleic acid contents in the cultivars fluctuated (in the form of an increase-decrease-increase) with harvest time. The highest oleic acid contents were determined in the H3 in the 'Tombul' (79.75%), 'Palaz' (81.87%), 'Çakıldak' (82.13%) and 'Okay 28' (82.63%) cultivars. However, it was also detected in the H4 in 'Allahverdi' (80.03%). The oleic acid contents in the 'Tombul' and 'Allahverdi' cultivars increased at the last harvest time (H7) as compared to the first harvest time

(H1). In contrast, it decreased in 'Palaz' and 'Okay 28' cultivars (Table 2). The stearic acid contents in 'Palaz' and 'Okay 28' cultivars increased, while their oleic acid contents decreased with the progress of harvest time. This can be explained by the conversion of oleic acid to stearic acid by Δ9–stearoyl-ACP from desaturase enzymes (Salas *et al.,* 2000). Again, the decrease in oleic acid content in these cultivars may be related to oleic acid conversion into linoleic acid. Temperature has a direct effect on the activity of the 9-stearoyl-ACP desaturase enzyme, which converts oleic acid into linoleic acid (Bouali *et al.,* 2013). In fact, higher linoleic acid content was determined in these cultivars at the last harvest time (H7) as compared to the first harvest time (H1). Similarly, Cristofori *et al.* (2015) reported that the effect of harvest time on oleic acid in hazelnut varies by variety. They noted an increase-decrease in oleic acid content in 'Tonda Gentile Romana' cultivar as the harvest progressed, as well as a decrease-increase in 'Tonda di Giffoni' and 'Nocchione' cultivars. Ciemniewska-Zytkiewicz *et al*. (2015) stated similar results for oleic acid content in the 'Katalonski' hazelnut cultivar. In contrast to present findings, Seyhan *et al.* (2007) and Ilyasoglu (2016) reported increasing oleic acid contents for 'Tombul', 'Palaz', 'Badem' and 'Sivri' cultivars as harvest time progressed.

The linoleic acid contents in the investigated hazelnut cultivars varied with harvest time ($p < 0.05$). The highest linoleic acid was determined in the H4 in 'Tombul' (10.95%) and 'Palaz' (11.12%); in the H2 in 'Çakıldak' (11.63%) and 'Okay 28' (8.72%); in the H1 in 'Allahverdi' (16.54%). Except for 'Allahverdi', linoleic acid content was higher in other cultivars at the last harvest time (H7) as compared to the first harvest time (H1) (Table 2). Different researchers reported that linoleic acid content decreased-increased in 'Tonda Gentile Romana' and 'Palaz' cultivars (Seyhan *et al.,* 2007; Cristofori *et al.,* 2015) as the harvest progressed; decreased in 'Tombul', 'Badem', 'Sivri' and 'Katalonski' cultivars (Seyhan *et al.,* 2007; Ciemniewska-Zytkiewicz *et al*., 2015; Ilyasoglu, 2016); and increased in 'Tonda di Giffoni' and 'Nocchione' cultivars (Cristofori *et al.,* 2015). The linoleic acid content in the present cultivars was similar to that reported for 'Tonda Gentile Romana' and 'Palaz' cultivars (Seyhan *et al.,* 2007; Cristofori *et al.,* 2015). However, it was stated that the effect of harvest time on linoleic acid content in hazelnuts may vary depending on the cultivar (Seyhan *et al.,* 2007; Cristofori *et al.,* 2015; Ilyasoglu, 2016).

While the effect of harvest time on palmitic acid was significant in 'Tombul' and 'Okay 28' cultivars, it was insignificant in the other cultivars ($p < 0.05$). Palmitic acid contents fluctuated in 'Tombul' and 'Okay 28' cultivars with harvest time. The highest palmitic acid contents were determined in the H3 in 'Tombul' (7.75%); and in the H2 in 'Okay 28' (7.82%). It increased in 'Tombul' at the last harvest (H7) as compared to the first harvest (H1); while it decreased in 'Okay 28' (Table 3). Such a decrease in 'Okay 28' cultivar can be explained by the fact that palmitic acid was

Table 2. Oleic and linoleic acid contents (%) in the investigated hazelnut cultivars depending on harvest time (means of 2016 and 2017)

Cultivars/ Harvest time	Oleic acid (%)						
	H1	**H2**	**H3**	**H4**	**H5**	**H6**	**H7**
Tombul	72.57 b[z]	79.55 a	79.75 a	78.47 a	78.31 a	79.48 a	78.88 a
Palaz	80.64 a	80.75 a	81.87 a	74.93 b	80.95 a	80.28 a	78.55 ab
Çakıldak	79.11 c	77.16 e	82.13 a	80.48 b	76.35 f	78.31 d	79.11 c
Okay 28	82.05 ab	80.43 c	82.63 a	79.84 c	79.98 c	80.98 bc	80.51 c
Allahverdi	72.83 bc	76.49 ab	76.56 ab	80.03 a	77.97 a	68.43 c	75.88 ab
	Linoleic acid (%)						
Tombul	7.35 c	8.54 bc	8.97 bc	10.95 a	9.51 ab	9.06 abc	9.66 ab
Palaz	8.30 c	7.76 c	6.73 d	11.12 a	6.82 d	8.04 c	9.36 b
Çakıldak	10.38 b	11.63 a	7.46 d	7.97 c	11.47 a	10.28 b	11.43 a
Okay 28	7.04 c	8.72 a	7.06 c	7.62 b	8.38 a	8.38 a	7.85 b
Allahverdi	16.54 a	11.77 bc	12.62 b	9.54 d	10.09 cd	15.32 a	12.49 b

[z]Means indicated by the same letter in the same line do not differ according to Tukey's test ($p < 0.05$).
n= 9 for the oleic and linoleic content (three replicates × three different measurements for each replicate)

the primary product of the fatty acid synthesis pathway and thus other fatty acids in the kernels were derived from palmitic acid (Bouali *et al.,* 2013). Furthermore, different researchers reported that the palmitic acid contents in 'Tombul', 'Palaz', 'Badem', 'Sivri', 'Tonda Gentile Romana' and 'Tonda di Giffoni' cultivars decreased as harvest time progressed (Seyhan *et al.,* 2007; Cristofori *et al.,* 2015; Ilyasoglu, 2016). In 'Nocchione' and 'Katalonski' cultivars, on the other hand, it showed a decrease-increase (Cristofori *et al.,* 2015; Ciemniewska-Zytkiewicz *et al*., 2015). In the current study, similar to results reported for 'Nocchione' and 'Katalonski' cultivars, palmitic acid content fluctuated (increase-decrease or decrease-increase) depending on cultivar as harvest time progressed.

The effects of harvest time on the stearic acid content in the investigated hazelnut cultivars were found to be significant ($p < 0.05$). With the exception of the 'Çakıldak' cultivar, the stearic acid content in the other cultivars fluctuated with harvest time. It increased up to the H5 harvest time in the 'Çakıldak' cultivar, then decreased. The highest stearic acid contents were seen in H5 in 'Tombul' (4.91%), 'Çakıldak' (4.21%) and 'Allahverdi' (4.46%); in H7 in 'Palaz' (4.30%); in H4 in 'Okay 28' (4.72%). Except for 'Çakıldak' cultivar, the other cultivars had significantly higher stearic acid contents at the last harvest (H7) than at the first harvest (H1) (Table 3). The stearic acid contents in the different hazelnut varieties were reported to vary depending on the variety as harvest progressed (Seyhan *et al.,* 2007; Cristofori *et al.,* 2015; Ilyasoglu, 2016). Stearic acid contents were determined to increase as harvest time progressed in the 'Tombul', 'Palaz' and 'Badem' cultivars (Seyhan *et al.,* 2007); while they decreased in 'Tonda Gentile Romana' and 'Tonda di Giffoni' cultivars. It was also determined that stearic acid contents decreased-increased in 'Nocchione' (Cristofori *et al.,* 2015) and 'Katalonski' cultivars (Ciemniewska-Zytkiewicz *et al*., 2015) and increased-decreased in the 'Palaz' cultivar (Ilyasoglu, 2016).

3.4. Total phenolics and total flavonoids

Hazelnut phenolics protect kernels from oxidation and affect flavor formation in fresh hazelnuts (Delgado *et al.*, 2010). Many researchers have reported that phenolic compounds with antioxidant properties may have a positive effect on human health (Karaosmanoglu and Ustun, 2021). Hazelnuts have high antioxidant capacity (Chang *et al.*, 2016). Therefore, consumers prefer hazelnuts with high phenolic compound levels. However, phenolic compounds in hazelnut can be influenced by a variety of factors, including cultivar, environmental conditions, cultural practices and harvest time (Pycia *et al.,* 2020; Balık, 2021; Karakaya *et al.,* 2023).

The total phenolics in the present hazelnut cultivars were significantly affected by harvest time ($p < 0.05$). Except for 'Okay 28' and 'Allahverdi' cultivars, the total phenolics in the other cultivars fluctuated with harvest time and generally decreased after the H3 stage.

Table 3. Palmitic and stearic acid contents (%) in the investigated hazelnut cultivars depending on harvest time (means of 2016 and 2017)

Cultivars/ Harvest time	Palmitic acid (%)						
	H1	H2	H3	H4	H5	H6	H7
Tombul	6.83 b[z]	7.29 ab	7.75 a	7.26 ab	7.28 ab	7.33 ab	7.36 ab
Palaz	7.47 a	7.44 a	7.59 a	6.67 a	7.84 a	7.42 a	7.48 a
Çakıldak	8.00 a	7.50 a	7.35 a	7.42 a	7.66 a	7.65 a	7.26 a
Okay 28	7.48 b	7.82 a	7.32 bc	7.54 b	7.18 cd	7.13 cd	7.07 d
Allahverdi	7.26 a	7.24 a	7.23 a	7.06 a	7.25 a	6.79 a	7.33 a
	Stearic acid (%)						
Tombul	1.55 d	4.44 ab	3.49 c	3.28 c	4.91 a	3.93 bc	4.11 abc
Palaz	3.54 a	3.72 a	3.62 a	1.83 b	4.04 a	3.96 a	4.30 a
Çakıldak	2.48 e	3.27 cd	3.01 d	3.87 ab	4.21 a	3.51 bc	1.85 f
Okay 28	3.81 cd	2.99 e	2.97 e	4.72 a	4.22 bc	3.45 d	4.39 ab
Allahverdi	3.31 d	4.24 ab	3.59 cd	3.33 d	4.46 a	4.00 bc	3.99 bc

zMeans indicated by the same letter in the same line do not differ according to the Tukey test ($p < 0.05$).
n= 9 for the palmitic and stearic content (three replicates × three different measurements for each replicate)

Table 4. Total phenolic (g GAE·kg^{-1}) and total flavonoid (g QE·kg^{-1}) contents in the investigated hazelnut cultivars depending on harvest time (mean of 2016 and 2017)

Cultivars/ Harvest time	Total phenolics (g GAE·kg^{-1})						
	H1	**H2**	**H3**	**H4**	**H5**	**H6**	**H7**
Tombul	13.92 b[z]	14.74 a	13.35 c	8.71 d	3.88 e	4.06 e	2.36 f
Palaz	14.58 b	11.97 c	15.54 a	7.27 d	4.95 e	4.71 f	2.37 g
Çakıldak	20.20 b	18.63 c	20.62 a	14.36 e	16.48 d	10.19 g	12.49 f
Okay 28	21.28 a	16.89 b	14.52 c	5.40 e	14.42 c	6.75 d	3.50 f
Allahverdi	12.04 c	12.74 b	18.38 a	8.98 d	6.72 f	7.65 e	5.87 g
	Total flavonoids (g QE·kg^{-1})						
Tombul	1.65 c	3.56 a	3.16 b	1.60 c	1.15 d	0.86 e	0.80 e
Palaz	2.42 b	2.85 a	1.86 c	0.87 f	1.06 e	1.38 d	0.88 f
Çakıldak	3.24 a	2.02 b	3.15 a	2.01 b	1.84 c	1.82 c	3.16 a
Okay 28	4.91 a	2.59 b	2.38 c	1.25 e	1.02 f	1.69 d	1.19 e
Allahverdi	3.38 a	1.59 d	2.75 b	1.80 c	1.07 e	1.60 d	1.00 e

[z]Means indicated by the same letter in the same line do not differ according to Tukey's test ($p < 0.05$).
n= 9 for the total phenolics and total flavonoids content (three replicates × three different measurements for each replicate)

They decreased up to H5 in the 'Okay 28' cultivar and then fluctuated. They increased up to H3 in the 'Allahverdi' cultivar, then decreased. The highest total phenolics were seen in H2 in 'Tombul' (14.74 g GAE·kg^{-1}); in H3 in 'Palaz' (15.54 g GAE·kg^{-1}), 'Çakıldak' (20.62 g GAE·kg^{-1}) and 'Allahverdi' (18.38 g GAE·kg^{-1}); in H1 in 'Okay 28' (21.28 g GAE·kg^{-1}) (Table 4).

Changes in total flavonoids with harvest time were significant for all cultivars ($p < 0.05$). Except for the 'Tombul' and 'Okay 28' cultivars, the total flavonoids in the other cultivars fluctuated with harvest time. In the 'Tombul' cultivar, it decreased after H2. It decreased up to H6 in the 'Okay 28' cultivar. The highest total flavonoid contents were determined for the H2 in 'Tombul' (3.56 g QE·kg^{-1}) and 'Palaz' (2.85 g QE·kg^{-1}), and for H1 in 'Çakıldak' (3.24 g QE·kg^{-1}), 'Okay 28' (4.91 g QE·kg^{-1}) and 'Allahverdi' (3.38 g QE·kg^{-1}). Total flavonoids were generally higher at the first harvest (H1) than at the last harvest (H7), except for the 'Çakıldak' cultivar (Table 4).

Overall, total phenolics in the cultivars significantly decreased at the last harvest (H7) as compared to the first harvest (H1). This has been associated with an increase in polyphenol oxidase activity during fruit ripening (Parr and Bolwell, 2000). Again, the decrease in phenolic contents in fruits as harvest time progresses is explained by the condensation of different phenolic acids during these periods, followed by the formation of complex phenolic compounds such as tannins and lignin (Ben-Ahmed *et al.,* 2009). Furthermore, ripe fruits have lower total phenolic contents than semi-ripe fruits (Yang *et al.,* 2011). Indeed, many researchers reported the highest total phenolic contents in early-harvested walnut fruit (Wei *et al.,* 2022). Cristofori *et al.* (2015) stated that total phenolic contents fluctuated (decrease-increase) in 'Tonda Gentile Romana' and increased in 'Tonda di Giffoni' and 'Nocchione', depending on the harvest time. In terms of total phenolics, the present findings were compatible with those obtained from the 'Tonda Gentile Romana' cultivar. Similarly, total phenolics were observed to decrease as harvest progressed in walnuts (Pycia *et al.,* 2019) and pistachios (Kelebek *et al.,* 2020). However, some studies showed that the polyphenol concentration in hazelnuts increased as they ripened (Persic *et al.,* 2018). Furthermore, different researchers reported that climate conditions, variety, harvest time, biotic and abiotic stress factors may influence total the phenolics in hazelnuts (Cristofori *et al.,* 2015; Pycia *et al.,* 2020; Karakaya *et al.,* 2023).

3.5. Antioxidant activity

High antioxidant capacity of fruits is mostly linked to health benefits. Chang *et al.* (2016) reported that antioxidant capacity is related to the fruits' total phenolic contents. The antioxidant activity (both DPPH and FRAP) of the investigated hazelnut cultivars was significantly affected by harvest time ($p < 0.05$). According to the DPPH test, while the

Table 5. Antioxidant activity (mmol TE·kg^{-1}) (according to DPPH and FRAP assays) of the investigated hazelnut cultivars depending on harvest time (means of 2016 and 2017)

Cultivars/ Harvest time	DPPH (mmol TE·kg^{-1})						
	H1	H2	H3	H4	H5	H6	H7
Tombul	33.48 ab^{z}	34.18 a	32.19 b	26.97 c	15.31 de	16.13 d	13.71 e
Palaz	25.72 b	23.24 c	33.52 a	15.75 d	16.64 d	15.11 d	13.05 e
Çakıldak	35.34 a	34.17 a	33.90 a	31.71 b	28.02 c	25.74 d	20.77 e
Okay 28	35.13 a	29.79 b	28.40 b	20.00 c	12.58 e	15.76 d	12.96 e
Allahverdi	16.98 d	24.50 b	31.07 a	20.87 c	17.53 d	15.81 de	14.30 e
	FRAP (mmol TE·kg^{-1})						
Tombul	52.75 b	60.47 a	49.31 c	31.34 d	8.42 e	10.34 e	10.07 e
Palaz	52.45 a	51.36 a	48.62 b	16.12 c	14.74 cd	13.18 d	13.28 d
Çakıldak	68.81 a	71.20 a	72.71 a	56.03 b	62.68 c	37.08 d	35.26 d
Okay 28	77.16 a	72.44 b	45.70 c	18.27 d	11.67 e	20.76 d	8.74 e
Allahverdi	39.44 c	36.44 b	55.14 a	27.77 d	16.90 f	20.94 e	11.95 g

^{z}Means indicated by the same letter in the same line do not differ according to Tukey's test ($p < 0.05$).
n= 9 for the antioxidant activity (three replicates × three different measurements for each replicate)

antioxidant activity fluctuated with the harvest time in 'Palaz', it decreased in 'Çakıldak' and 'Okay 28' from the first harvest time (H1). It decreased after H2 in 'Tombul'. It increased up to H3 in 'Allahverdi' and then decreased. The highest antioxidant activities were found in H2 in 'Tombul' (34.18 mmol TE·kg^{-1}), in H1 in 'Çakıldak' (35.34 mmol TE·kg^{-1}) and 'Okay 28' (35.13 mmol TE·kg^{-1}), in H3 in 'Palaz' (33.52 mmol TE·kg^{-1}) and 'Allahverdi' (31.07 mmol TE·kg^{-1}) (Table 5). According to the FRAP test, antioxidant activity fluctuated depending on harvest time in 'Tombul', 'Çakıldak' and 'Allahverdi', while it decreased in 'Palaz' and 'Okay 28'. The highest antioxidant activities were determined in H2 in 'Tombul' (60.47 mmol TE·kg^{-1}); in H1 in 'Palaz' (52.45 mmol TE·kg^{-1}) and 'Okay 28' (77.16 mmol TE·kg^{-1}), in H3 in 'Çakıldak' (72.71 mmol TE·kg^{-1}) and 'Allahverdi' (55.14 mmol TE·kg^{-1}) (Table 5).

According to both methods, the antioxidant activity of the cultivars decreased significantly at the last harvest time (H7) compared to the first harvest time (H1). Similarly, many researchers reported that the antioxidant activity of hazelnuts and walnuts decreased as harvest time progressed (Pycia *et al.,* 2020; Wei *et al.,* 2022). In the current study, antioxidant activity decreased as the harvest time progressed due to a decrease in total phenolics and flavonoids. Indeed, a decrease in antioxidant activity during fruit ripening may be related to a decrease in total phenolic compounds (Pycia *et al.,* 2020).

3.6. Principal component analysis (PCA)

According to PCA results, the correlation between the investigated traits was 69.1% (PC1 + PC2). Tombul, Palaz and Okay 28 cultivars were associated with oil and stearic acid and were located in the first region in the PCA plane with the H5 harvest time. The Çakıldak cultivar was related to protein

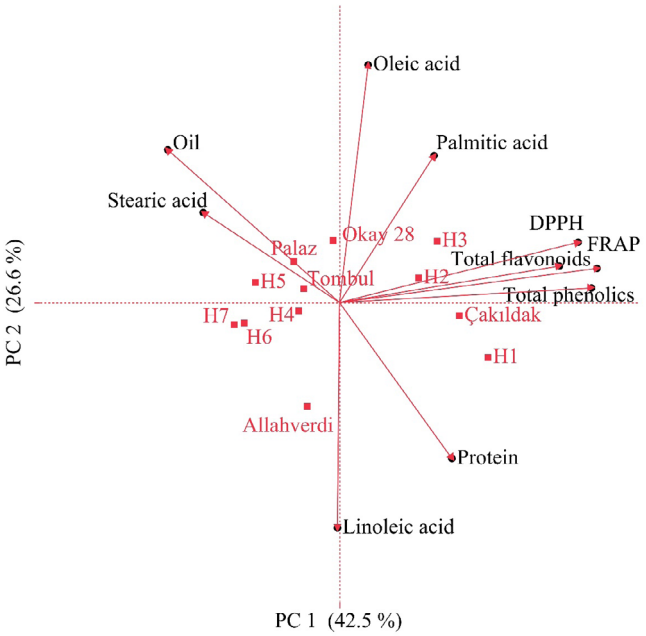

Figure 2. Relationships amongst protein oil, bioactive compounds and fatty acid composition in the investigated hazelnut cultivars based on harvest time.

and was grouped in the fourth region with the H1 harvest time. The Allahverdi cultivar was associated with linoleic acid and was located in the third region with the H4, H6, and H7 harvest times. Furthermore, bioactive compounds were clustered at the same point in the PCA plane. Protein, oil and fatty acids were also located at different points (Figure 2).

4. CONCLUSIONS

Depending on harvest time, the present cultivars yielded different results in terms of protein, oil, major fatty acids, total phenolics, total flavonoids and antioxidant activity. The investigated parameters were significantly affected by harvest time. In general, the investigated parameters fluctuated with harvest time. The oil rate increased significantly at the last harvest (H7) as compared to the first harvest (H1); while the protein contents decreased. The major fatty acids of some cultivars increased; while they decreased in others. Bioactive compounds decreased as harvest time progressed. In short, the present findings on the protein, oil, fatty acids and bioactive compounds of hazelnut cultivars depending on harvest time will provide clearer ideas for both industry and producers about the optimal harvest time for the intended use of these cultivars. The present findings are also important in terms of minimizing quality losses that may occur during the harvest and storage of hazelnuts and providing a higher quality product to the consumer.

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6. DATA AVAILABILITY

Since this article already contains all newly created data, data sharing is not applicable to it.

7. DECLARATION OF COMPETING INTEREST

The authors of this article declare that they have no financial, professional or personal conflicts of interest that could have inappropriately influenced this work.

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