Impact of different harvest times on fatty acid profile, sterol, tocopherol and bioactive properties of hazelnut oil

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SUMMARY: This study was carried out to determine the effects of different harvest times of hazelnuts on their lipid profiles and bioactive compound accumulations. Tombul hazelnut cultivar was harvested at four different harvest stages, namely in PH (pre-harvest time), EH (early harvest time), NH (normal harvest time) and LH (late harvest time). According to the results of the study, oil accumulation continued up to NH but did not further increase into LH (from 49.58 to 58.54 mg/100 g). Oxidative stability indices changed positively due to decreased poly-unsaturated fatty acids (PUFA) from 9.87 to 7.70% in LH. The highest total sterol amount was reached in LH (122.32 mg/100 g). Although the change in the tocopherol content in the oil was irregular with the progression of the harvest time, its amount in the nuts increased continuously. Total carotenoid, phenolic, flavonoid, and antioxidant activity (DPPH and ABTS) peaked in EH and decreased to a minimum in LH.

KEYWORDS: *Antioxidant activity; Fatty acid profile; Hazelnut oil; Oxidative stability; Sterol; Tocopherol.*

RESUMEN: ***Impacto de diferentes épocas de cosecha en el perfil de ácidos grasos, esteroles, tocoferoles y propiedades bioactivas del aceite de avellana.*** Este estudio se llevó a cabo para determinar los efectos de diferentes épocas de cosecha de avellanas sobre los perfiles de lípidos y las acumulaciones de compuestos bioactivos. El cultivar de avellana Tombul se cosechó en cuatro épocas diferentes en el orden: PH (época previa a la cosecha), EH (época de cosecha temprana), NH (época de cosecha normal) y LH (época de cosecha tardía) con referencia al NH. Según los resultados del estudio, la acumulación de aceite continuó hasta NH, pero no aumentó más en LH (de 49,58 a 58,54 mg/100 g). Los índices de estabilidad oxidativa cambiaron positivamente debido a la disminución de los ácidos grasos poliinsaturados (AGPI) del 9,87 % al 7,70 % en la LH. La cantidad total de esteroles más alta se alcanzó en la LH (122,32 mg/100 g). Aunque el cambio en el contenido de tocoferoles en el aceite fue irregular con el avance del tiempo de cosecha, su cantidad en la nuez aumentó continuamente. La actividad total de carotenoides, fenólicos, flavonoides y antioxidantes (DPPH y ABTS) alcanzó su punto máximo en EH y disminuyó a un mínimo en LH.

PALABRAS CLAVE: *Aceite de avellana; Actividad antioxidante; Estabilidad oxidativa; Esteroles; Perfil de ácidos grasos; Tocoferoles.*

1. INTRODUCTION

In recent years, nuts and their beneficial effects on human health have attracted great attention by researchers. Their special oil profiles and their polyphenolic compounds underlie the beneficial impacts of nut consumption as determined from epidemiological research and nutrition trials (Ciemniewska-Żytkiewicz *et al*., 2015a). Together with walnuts, hazelnuts constitute approximately 60% of the dried nut production in Europe (Pycia *et al*., 2019). The hazelnut is a member of the *Betulaceae* family and is one of the most popular nuts worldwide due to its pleasant taste, nutrients, mono and polyunsaturated fatty acids, fat-soluble bioactive substances such as phytosterol, tocopherol, and polyphenols (Karaosmanoğlu and Üstün, 2019).

The chemical compositions of nuts, which are important for human nutrition, change during nut development, as reported in the literature (Cristofori *et al*., 2015; Ilyasoglu 2015; Gama *et al*., 2020). For example, Seyhan *et al.* (2007) reported that the oil ratio increased from 48.10 to 59.83% in the Tombul hazelnut variety between the early harvest and normal harvest time, the protein and ash ratios decreased, and the oleic acid ratio increased from 74.60 to 80.64, while the linoleic and palmitic acid ratios decreased. Ilyasoğlu (2015) noted that there were significant changes in sterol ratios with the progression of harvest time, and the total amount of sterols, which was 293 mg/100 g in EH, decreased to 147 mg/100 g in NH. Ciemniewska-Zytkiewicz *et al*. (2015a) reported that MUFA increased (22.02-79.17%), PUFA decreased (30.09-10.28%) and SFA remained unchanged with maturation in Polish hazelnuts. In the same study, it was stated that total tocopherol increased from 22.48 mg/100 g to 38.24 mg/100 g and alpha-tocopherol was dominant at all stages. Again, in Polish hazelnut cultivars, Pycia *et al*. (2019) reported a decrease in polyphenol and antioxidant activity with the progression of harvest time.

In Turkey, hazelnuts reach harvest maturity in August. However, in very large and sloping lands, harvesting is usually done by hand. For this reason, a large number of hazelnut workers are needed during the harvest season and the inability to meet the need causes early or late harvest. In addition, producers dealing with other works other than hazelnut and climatic conditions are other reasons for early or late harvest. The progression of harvest time can cause various chemical changes in nuts. For example, Kazantzis *et al*. (2003) reported that more lipid oxidation occurred and oil quality deteriorated in late harvested almonds compared to early harvested almonds. In another study conducted on Macadamia nuts, it was reported that late harvest shortened the shelf-life of the nuts (Gama *et al*., 2020). On the other hand, in *Torreya granndis* nuts, late harvest was reported to increase dry matter content, oil content and nutritional quality (Wang *et al*., 2021). In addition, the effects of early and normal harvest time on fatty acid composition in Italian (Cristofori *et al*., 2015), Polish (Ciemniewska-Zytkiewicz *et al*. 2015a) and Turkish (İlyasoğlu, 2016) hazelnuts, tocopherol content in Polish hazelnuts (Ciemniewska-Zytkiewicz *et al*. 2015a; Pycia *et al*., 2020), sterol composition in Turkish hazelnuts (Ilyasoglu, 2015) and antioxidant activity in Polish hazelnut cultivars (Pycia *et al*., 2020) were investigated. Despite these data reported on hazelnuts and other nuts, so far no study has been found in the literature on the effect of late harvest time of hazelnuts on oil composition and bioactive properties. The main purpose of our study is to reveal the effect of different harvest times on fatty acids, sterol, tocopherol composition, total carotenoid, total phenolic, total flavonoid and antioxidant activities (DPPH and ABTS). In addition, no study has been found in the literature examining the changes in tocopherol content, composition and carotenoid amount during nut development in Turkish hazelnut cultivars. Another aim of our study is to eliminate this deficiency.

2. MATERIALS AND METHODS

2.1. Nut samples

The Tombul hazelnut, which is the most important Turkish hazelnut cultivar, was chosen as the research material. Hazelnut samples were harvested by hand from three orchards in the Batlama valley, Akköy (40°51'38.52"N, 38°18'58.69"E), Seyitköy (40°51'40.40"N, 38°19'09.72"E) and Alınca (40°51'59.55"N, 38°19'00.26"E) villages of Giresun (Turkey). Sampling was harvested from each orchard according to the "Z" pattern for homogeneous and random sample selection. Sample trees were marked and nuts were collected from the same trees in determined harvest times. Sampling was carried out in four stages with reference to the reguler harvest

time in Turkey (second half of August): PH, 14 July; EH, 1 August; NH, 18 August and LH, 6 September. Harvested hazelnuts were separated from their green husks, placed in plastic bags and kept at -18 °C. The drying process was carried out for 3 days between 09:00 a.m. and 08:00 p.m. in ambient conditions (average temperature 24.7 °C). The samples were laid on a 5x5 m jute cover on the concrete ground and mixed 5 times a day during the drying period. After 8:00 p.m. in the evening, each group was gathered in the middle, and they were covered with a nylon cover to prevent moisture transfer from outside. At the end of the drying period, the humidity in all samples decreased below 6% and the samples were kept at -18 °C until the day of analysis.

2.2. Reagents and standards

Unless otherwise stated, all chemicals used were from Sigma-Aldrich-Fluka Co. Ltd. (Prolab, Turkey). Potassium hydroxide (KOH) and anhydrous sodium sulfate (Na_2SO_4) from Carlo Erba (Italy), N,O-bis (trimethylsilyl) trifluoroacetamide, trimethyl chlorosilane, 5α-cholasten-3-β-ol, tocopherol from Merck (Germany) and the standard blend of methyl esters of fatty acids were obtained from Supelco (USA).

2.3. Oil extraction

The shelled hazelnuts used in the analysis were hand-cracked (3 kg) and separated from their shells. Oil extraction was performed with a test-scale screw-press device with a 3-kW regulatable speed electric engine. The screw turning speed was adjusted to 60 rpm to extract 80% of the total oil (Demirtas *et al.*, 2013).

2.4. Determination of fatty acids

The fatty acid profile of the nuts was defined according to the method reported by Demirtas *et al.* (2013). The fatty acids were determined on a gas chromatography-flame ionization detector (GC-FID) (Perkin Elmer, Autosystem GLX, USA) equipped with a SP®-2560 (100 m x 0.25 mm x 0.2 μm, Supelco, USA) column. GC-FID operating conditions: carrier gas, helium; flow rate 0.5 mL/min; injection temperature, 280 °C; detector temperature, 260 °C; the oven temperature program was adjusted to keep the initial temperature at 120 °C for 2 minutes, increase it to 220 °C at 5 °C/min and hold it at this temperature for 10 minutes. Data were evaluated with Total Chrom Navigator and explained as % fatty acid.

2.5. Oxidative stability and health indices

The oleic acid/linoleic acid ratio (O/L), iodine value (IV) (Belviso *et al.*, 2017), atherogenic (AI) and thrombogenic (TI) indices (Ulbricht and Southgate 1991), hypocholesterolemic-to-Hypercholesterolemic ratio (h/H) and the peroxide index (PI) (Hanczakowska *et al.*, 2015) values were calculated according to the equations below:

$$\text{O/L} = \text{oleic acid/linoleic acid} \tag{1}$$

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

$$\text{PI} = (\% \text{ monoenoic} \times 0.025) + (\% \text{ dienoic} \times 1) + (\% \text{ trienoic} \times 2) + (\% \text{ tetraenoic} \times 4) + (\% \text{ pentaenoic} \times 6) + (\% \text{ hexaenoic} \times 8) \tag{3}$$

$$\text{AI} = (C12:0 + 4 \times C14:0 + C16:0)/(\sum MUFA + \sum FA\omega 6 + \sum FA\omega 3) \tag{4}$$

$$\text{TI} = (C14:0 + C16:0 + C18:0)/((0.5 \times \sum MUFA) + (0.5 \times \sum FA\omega 6) + (3 \times FA\omega 3)$$

(MUFA: mono-unsaturated fatty acids, FAω6: omega-6 fatty acids, FAω3: omega-3 fatty acids) $\tag{5}$

$$\text{h/H} = (C18:1 + C18:2 + C18:3 + C20:4 + C20:5 + C22:6)/(C14:0 + C16:0) \tag{6}$$

2.6. Determination of sterol composition

The sterol profile of the nut oils was determined according to the procedure reported by Demirtas *et al.* (2013). Sterols were determined with a GC-FID (Perkin Elmer, Autosystem GLX, USA) and SE-54 (5%-phenyl-1%-vinylmethylpolysiloxane), 30 m x 0.32 mm x 0.25 μm (Agilent, USA) column. GC-FID operating conditions: carrier gas, helium; flow rate 0.8 mL/min; injection temperature, 280 °C; detector temperature, 300 °C; the oven temperature program was adjusted so that the initial temperature was held at 60 °C for 2 minutes, increased to 220 °C at 40 °C/min, held for 1 minute, increased to 310 °C at 5 °C/min and remained at this temperature for 30 minutes. Individual sterols without standards were identified using relative retention time (RRT) of 5α-cholastan-3β-ol. Datas were evaluated with TotalChrom Navigator and expressed as mg/100 g oil.

2.7. Determination of tocol composition

Tocol isomers of the oil samples extracted from kernels with pellicle were determined according the method reported by Demirtas *et al*. (2013). The contents in tocopherols were calculated using external standards, and measured by a HPLC (Agilent Series 1100, Germany) with fluorescence detector and normal phase column (5μm LiCrosorb Si60 25 cm x 4.6 mm i.d., HiChrom, UK). Chromatographic separation was performed with an isocratic tetrahydrofuran/n-heptane (3.8%, v/v) carrier at a flow rate of 1 mL/min. The column temperature was held at 40 °C. The wavelength of the detector was set at 270 nm for excitation and 310 nm for emission. The results with Chemstation were expressed as mean values ± standard deviation as mg/100 g oil.

2.8. Spectrophotometric analyses

2.8.1. Extract preparation of phenols, flavonoids and antioxidant activity assays

Phenolic compounds were extracted according to the method reported by Karaosmanoğlu (2022). 250 g hazelnut sample and a methanol/water (75/10) solution was used for extraction. The extract obtained as a result of the process was used in the total phenolic and flavenoid contents, and antioxidant capacity experiments.

2.8.2. Determination of total phenolic compounds, total flavonoids and antioxidant activities (DPPH and ABTS methods)

Biochemical properties were measured in nut kernels. Total phenolic compounds, total flavonoids and antioxidant activity were determined as biochemical properties and these analyses were performed according to the method described by Karaosmanoğlu (2022). All measurements were performed using a spectrophotometer (Shimadzu, Japan). Total phenolic compounds were determined using the Folin-Ciocalteu reagent. Total phenols were expressed as gallic acid equivalents (mg GAE/100 g) and total flavonoids were expressed as mg catechin equivalents (mg CE/100 g). Antioxidant activity was measured using the DPPH (1,1-diphenyl-2-picrylhydrazyl) and ABTS [2,2-azinobis (3-ethylbenzoth iazoline-6-sulfonic acid) diammonium salt] tests and stated as Trolox equivalents per 100 g (mgTE/100 g).

2.8.3. Extract preparation and determination of total carotenoid content

For the carotenoid analysis, extraction was done with the help of methyl alcohol and ultrasonic homogenizer (Bandelin MS72, Germany). The absorbance of the extracts was 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 (Amira, 2011).

$$Chlorophyll\ a = (12.7 x A_{663} - 2.69 x A_{645}) \\ x \frac{v}{1000 x w} \quad (7)$$

$$Chlorophyll\ b = (22.9 x A_{645} - 4.68 x A_{663}) \\ x \frac{v}{1000 x w} \quad (8)$$

$$Total\ carotenoid = 46.95 x (A_{440.5} - 0.268 \\ x\ chlorophyll\ a + b) \quad (9)$$

W: weight by grams for extracted; v: final size of extracted; A: absorbance.

2.9. Statistical analysis

Analyses were conducted using JMP (pro-16) statistical software. One-way ANOVA followed by Tukey's post-hoc test was used to compare the means of the study sets. A p-value of less than 0.05 was considered statistically significant. Results were expressed as means ± standart deviation (n=3) for each determination. Principal component analysis (PCA) was also performed using JMP (pro-16) software.

3. RESULTS AND DISCUSSION

3.1. Variations in oil content at different harvest times

The amount of oil in hazelnuts collected at four different harvest times are summarized in Table 1. Oil in the kernel increased from PH to NH, from 49.58 mg/100 g to 58.54 mg/100 g (P < 0.01), but did not change in LH. The most substantial increase was between PH and EH, with a variation of about 15%. Seyhan *et al*. (2007) reported the oil content in Tombul hazelnut as 48.10, 59.83 mg/100 g in EH and NH, respectively, in line with our results. Ciemniewska-Zytkiewicz *et al*. (2015a) studied Polish hazelnuts, and Cristofori *et al*. (2015) studied Italian hazelnuts and reported that the oil content in ha-

TABLE 1. Oil content (mg/100g), fatty acid composition (%), oxidative stability and healthy indices of hazelnuts at different harvest times

	PH	EH	NH	LH	
Oil	49.58±0.01 c	56.67±0.01 b	58.54±0.17 a	58.19±0.15 a	**
Palmitic	4.71±0.01 c	4.63±0.01 d	4.80±0.01 b	4.99±0.01 a	**
Palmitoleic	0.11±0.00 b	0.10±0.00 c	0.11±0.00 b	0.12±0.00 a	*
Heptadecanoic	0.04±0.00	0.04±0.00	0.04±0.00	0.04±0.00	ns
Stearic	2.44±0.01 c	2.92±0.01 b	3.04±0.01 a	3.05±0.01 a	**
Oleic	82.41±0.01 c	82.99±0.01 b	82.68±0.02 b	83.55±0.11 a	**
Elaidic	0.02±0.00	0.02±0.00	0.02±0.00	0.02±0.00	ns
Linoleic	9.80±0.01 a	8.85±0.01 b	8.88±0.01 b	7.65±0.01 d	**
α- linolenic	0.07±0.00 a	0.06±0.00 b	0.05±0.00 b	0.05±0.00 b	**
Arachidic	0.12±0.00 a	0.10±0.00 c	0.11±0.00 bc	0.12±0.00 ab	**
Eicosenoic	0.14±0.00	0.15±0.01	0.15±0.01	0.15±0.01	ns
Behenic	0.01±0.00	0.01±0.00	0.01±0.00	0.01±0.00	ns
Miristic	0.02±0.00	0.02±0.00	0.03±0.00	0.03±0.00	ns
SFA	7.34±0.01 d	7.72±0.01 c	8.03±0.01 b	8.24±0.01 a	**
MUFA	82.68±0.01 c	83.26±0.01 b	82.81±0.02 c	83.84±0.10 a	**
PUFA	9.87± 0.01 a	8.91±0.01 b	8.93±0.01 b	7.70±0.01 c	**
UFA	92.56±0.01 a	92.17±0.01 b	91.74±0.01 c	91.54±0.10 c	**
Oxidative stability indices					
UFA/SFA	12.60±0.01 a	11.93±0.02 b	11.42±0.01 c	11.10±0.02 d	**
O/L	8.40±0.01 d	9.37±0.01 b	9.30±0.01 c	10.91±0.01 a	**
IV	92.27±0.01 a	91.02±0.01 b	90.78±0.01 c	89.36±0.09 d	**
PI	12.01±0.01 a	11.05±0.01 b	11.05±0.01 b	9.85±0.01 c	**
Healthy indices					
AI	0.05±0.00 b	0.05±0.00 b	0.05±0.00 b	0.06±0.00 a	**
TI	0.15±0.00 d	0.16±0.00 c	0.17±0.00 b	0.18±0.00 a	**
h/H	19.50±0.03 b	19.75±0.03 a	18.97±0.01 c	18.18±0.05 d	**

All values are presented as means ± standard deviation (n= 3). The different superscript letters (a-d) in the same row indicate statistically significant difference according to the Tukey test. * significant at P < 0.05, ** significant at P < 0.01, ns: not significant. 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, PI- peroxidisability index, AI- atherogenic index, TI- thrombogenicity index, h/H- hypocholesterolemic to Hypercholesterolemic ratio. PH- pre-harvest time, EH- early harvest time, NH- normal harvest time, LH- late harvest time

zelnuts increased as nut ripening progressed. In our study, it was observed that late harvest stage did not increase oil accumulation in the nuts. Similarly, Kazantzis *et al*. (2003) found that late harvest did not change the oil content in the nuts in a study conducted on almonds. This can be explained by the cessation of the activity of the biosynthesis pathway that forms triglycerides with maturation.

3.2. Variations in fatty acid composition at different harvest times

The variations in the fatty acid profile of nuts harvested in different stages are presented in Table 1.

The predominant fatty acid was oleic acid at all harvest times, followed by linoleic, palmitic and stearic acids. Reporting the same fatty acid order, Cristofori *et al*. (2015) reported the major fatty acid, oleic acid, at the level of 80-83%, with stearic and palmitic saturated fatty acids below 10%. Although there is no knowledge on the impact of LH on the fatty acid profile of hazelnuts, Seyhan *et al*. (2007) and İlyasoğlu (2016) reported a similar ranking between early harvest and normal harvest periods. Minor fatty acids ranged from PH to LH at 0.54 to 0.42%.

It has been observed that the amount of oleic acid increased as maturation progressed, and the

ratio, which was 82.41% in PH, reached the highest level at 83.55% in LH (P < 0.01). In contrast to oleic acid, the ratio of linoleic acid decreased from 9.80 to 7.65% as maturation progressed (P < 0.01). They showed an opposite pattern of accumulation of oleic and linoleic acids during nut development (R = -0.948), which is in close agreement with data reported by Ciemniewska-Zytkiewicz et al. (2015a) and İlyasoğlu (2016). In addition, with the progression of maturation, on average, a slight decrease in linoleic acid was emphasized by Cristofori et al. (2015), despite the increase in oleic acid in Italian hazelnut cultivars. The negative correlation between oleic and linoleic acid accumulation can be explained by the decrease in linoleic acid synthesis due to the slowdown in the activity of the Δ12-desaturase enzyme, which catalyzes the synthesis of linoleic acid from oleic acid, because of seasonal temperature increase (Bouali et al., 2013). In addition, lipoxygenase activity may have caused a decrease in the content of linoleic acid (İlyasoğlu, 2016). The slowdown in linoleic acid synthesis was accompanied by α-linoleic acid, which may be associated with the slowing of the Δ6-desaturase enzyme activity due to temperature increase. Unlike α-linoleic acid, palmitoleic acid increased slightly, but other minor fatty acids were not affected by harvest time (P > 0.05). The palmitic acid ratio fluctuated between harvest times, while stearic acid increased from PH (2.44%) to NH (3.04%) but remained the same in LH.

The highest amount of SFA (saturated fatty acids) was detected in LH (8.24%) among harvest times, associated with a rise in the amount of stearic and palmitic fatty acids (P < 0.01). Although the MUFA ratio fluctuated over time, it increased from 82.68 to 83.84 between PH and LH in relation to oleic acid. Unlike MUFA, PUFA (poly-unsaturated fatty acids) was highest in PH (9.87%) and lowest in LH (7.70%). This decrease is largely associated with a drop in the content in linoleic acid with nut ripening. It has been observed that as the maturation progressed, the amount of UFA (unsaturated fatty acids) decreased up to NH but did not change in LH. In general, it has been determined that LH caused more SFA and MUFA and less PUFA accumulation than NH. Kazantzis et al. (2003) reported that late harvested nuts contained more SFA in a study conducted on almonds.

3.3. Variations in oxidative stability and healthy indices at different harvest times

Depending on the changes in the fatty acid profile of hazelnuts harvested at different harvest times, significant differences emerged in the indices of oxidation resistance and health effects (Table 1). The effect of harvest times on these indices is evaluated below. A lower UFA/SFA rate is considered to have a longer shelf-life (Alasalvar et al., 2003). With the delay in harvest time, this ratio decreased continuously and reached the lowest level at 11.10 in LH (P < 0.01). A greater O/L value means better oxidative stability (Karaosmanoğlu 2022). Although the O/L value fluctuated between harvest times, the lowest value was found in PH (8.40), the highest value in LH (10.91), and the difference was statistically significant. The increase between NH (9.30) and LH (10.91) was slightly significant. Consistent with our work, Seyhan et al. (2007) reported that the O/L ratio increased with maturation. Low IV is an indication that oils are less reactive, more stable, more resistant to rancidity and oxidation (Belviso et al., 2017). The IV, which was 92.27 in PH, showed a continuous decrease and reached the lowest value in LH (89.36). The lowest IV in LH may be related to the decrease in linoleic acid along with the delay in harvest time because the lowest linoleic acid ratio was detected in LH. We previously (Karaosmanoğlu and Üstün, 2019) reported the IV of NH hazelnuts in the range of 89.81-94.40, which is comparable to to the present study. The PI value reflects the grade of unsaturation of dietary oils and is used as an indicator of PUFA peroxidation (Hanczakowska et al., 2015). In this work, the highest PI value was detected in PH and decreased slightly with maturation.

The oxidation ratios of fatty acids are approximately 1:10:100:200 for stearic, oleic, linoleic and linolenic acids, respectively (Alasalvar et al., 2003). Therefore, LH samples with higher stearic acid levels and lower linoleic acid levels are more stable against oxidative changes. As a result, it can be said that LH hazelnuts may be more durable against lipid oxidation and have a longer shelf-life because of lower UFA/SFA, and IV, and higher O/L values. The resistance of LH to oxidation is largely associated with high SFA and low PUFA. It should not be forgotten that this situation may reduce the positive effects in terms of health, despite the potential to extend the shelf-life of hazelnuts.

AI, TI and h/H values are indices used to estimate the lipid character of foods and are calculated based on their fatty acid composition. The values for these indices indicate the quality of fats for the risk of pro-atherogenic, pro-thrombogenic and cardiovascular disease. (Hanczakowska *et al*., 2015). In order to protect cardiovascular health, AI and TI are expected to be low and h/H to be high. With the late harvest, AI and TI values slightly increased compared to NH, while h/H decreased (0.06, 0.18, 18.18, respectively) (P < 0.01). Even if the late harvest negatively changed the AI and TI values, it was 2.3 times lower for AI and 1.8 times lower for TI than the values reported by Ulbricht and Southgate (1991) for olives (0.14, 0.32, respectively).

3.4. Variations in sterol composition at different harvest times

Seven different sterols detected and quantified in hazelnut samples which were harvested at different harvest times are listed in Table 2. The prominent sterols β-sitosterol, campesterol and Δ5-avenasterol accounted for more than 93% of total sterols, while other sterols (stigmasterol, Δ7-stigmastenol, Δ7-avenasterol, Δ7-campesterol) were in small amounts. β-sitosterol was the most plentiful sterol, accounting for more than 85% of the all sterols. Similar to our results, Alasalvar *et al*. (2009) reported in Turkish hazelnuts and Amaral *et al*. (2006) in Portuguese hazelnuts, that major sterols constituted more than 90% of total sterols and β-sitosterol was the predominant sterol. The amount of total sterol (116.14 mg/100g in NH) was quite consistent with the results of Alasalvar *et al*. (2003) (113.52 mg/100g) but were higher

than those reported by Ciemniewska-Zytkiewicz *et al*. (2015b) (130.32-152.22 mg/100g). This may be due to variety, geographical location or environmental factors (Alasalvar *et al*., 2003).

In our study β-sitosterol was detected to be the predominant sterol at all harvest times and the same sterols were determined (Δ7-campesterol could not be detected in LH). β-sitosterol was found to be increased and Δ5-avenasterol was found to be decreased in hazelnuts with LH time (P < 0.01). Since Δ5-avenasterol is a precursor in the biosynthesis of β-sitosterol (Fernandez-Cuesta *et al*., 2013), the different enzymatic efficiency in LH may explain the negative relationship between both sterol accumulations. Similar to β-sitosterol, total sterol increased significantly (P < 0.01) in LH (122.32 mg/100 g) compared to NH (116.14 mg/100 g), but PH, EH and NH were in the same group. Harvest times did not affect the accumulation of campesterol and Δ7-avenasterol, while the changes in stigmasterol and Δ7-campesterol were irregular. There is no data in the literature on the effect of late harvest on sterol synthesis. Consistent with our results, the same major sterols were detected in a single study examining the sterol change during maturation stages, and it was reported that β-sitosterol was dominant in all maturation stages, but contrary to our results, the amount of sterols decreased as time progressed (Ilyasoglu 2015). This difference may be due to the fact that the samples were collected much earlier than the harvest maturity in the study, because the values reported ripe nuts were comparable to our results, and abiotic factors may also have affected sterol biosynthesis (Misina *et al*., 2020). Similar to our results, it

TABLE 2. Sterol composition (mg/100 g oil) of hazelnut oil at different harvest times

	PH	EH	NH	LH	
Campesterol	6.69±0.01	5.95±0.29	6.04±0.1	6.54±0.31	ns
Stigmasterol	1.31±0.03 ab	1.27±0.01 b	0.97±0.01 c	1.50±0.08 a	**
Δ7-stigmastenol	1.78±0.16 b	1.52±0.19 b	1.71±0.01 b	2.78±0.13 a	**
β-sitosterol	100.80±0.32 b	98.19±0.88 b	100.39±0.46 b	105.45±1.33 a	**
Δ5-avenasterol	4.22±0.11 b	3.66±0.31 b	5.11±0.01 a	2.80±0.07 c	**
Δ7-avenasterol	0.90±0.15	1.17±0.05	0.82±0.01	1.14±0.15	ns
Δ7-campesterol	0.83±0.01 b	1.55±0.11 a	0.86±0.01 b	nd	**
Total sterol	116.41±0.47 b	112.67±1.08 b	116.14±0.40 b	122.32±1.34 a	**

All values are presented as means ± standard deviation (n= 3). The different superscript letters (a-d) in the same row indicate statistically significant difference according to the Tukey test, * significant at P < 0.05, ** significant at P < 0.01, ns: not significant, Nd: not detected, PH: pre harvest time, EH: early harvest time, NH: normal harvest time, LH: late harvest time

has been reported that the total sterol (Yorulmaz and Konuşkan 2017) in olives increased as harvest time prolongated. The results have shown that LH led to an increase in the sterol content of the samples.

3.5. Variations in tocopherol composition and carotenoid amount at different harvest times

The compositions and amounts of tocopherol in hazelnuts harvested at varied harvest times are summarized in Table 3. In samples collected in NH, α-tocopherol (47.17 mg/100 g) was the predominant isomer, followed by γ-tocopherol (6.26 mg/100 g) and β-tocopherol (1.22 mg/100 g). α-tocopherol constituted 86% of the total tocopherol. A similar profile of tocopherols was reported by Ciemniewska-Zytkiewicz et al. (2015a). Alasalvar et al. (2009) reported the α-tocopherol (34.5 mg/100 g) and total theocopherol (46.9 mg/100 g) contents of Turkish Tombul hazelnuts, which are quite consistent with our research data.

In this research, it has been observed that α-tocopherol was predominantly homologous at harvest times, its concentration ranged from 53.59 mg/100 g (PH) to 47.17 mg/100 g (NH), and decreased as the harvest time progressed except for LH (P < 0.01). A parallel increase in the amount of total tocopherol was observed with α-tocopherol. γ-tocopherol accumulation was inversely correlated with α-tocopherol (R= -0.765). This may be due to the alteration of gamma tocopherol methyltransferase enzyme activity, which converts γ-tocopherol to α-tocopherol, depending on abiotic stress factors (Jin and Daniell 2014). While β-tocopherol decreased slight-ly between PH and EH and remained stable at other harvest times, δ-tocopherol was detected only in a low quantity at PH. Unlike our study, Ciemniewska-Zytkiewicz et al. (2015a) reported that the amount of tocopherol increased as maturation progressed. Considering that the most intense period in tocopherol biosynthesis is the first period of nut development (Bouali et al., 2013), the reason for the difference with the literature may be that the sampling started close to ripening, since the primary objective of the work was to determine the effects of late harvest. On the other hand, it is thought that the main biochemical functions of tocopherols are to protect PUFA against peroxidation (Bouali et al., 2013; Bouali et al., 2022). While the ratio of PUFA in total fatty acids was 9.87% in PH, it decreased to 7.70% in LH. Therefore, the decrease in tocopherol levels as the ripening progressed can also be attributed to the decrease in the degree of unsaturation of fatty acids. Although the amount of tocopherol in the oil decreased, the total amount of tocopherol in the nut increased due to the increase in the amount of oil over time (PH, EH, NH, LH; 30.25 mg/100 g, 31.86 mg/100 g, 31.99 mg/100 g, 32.30 mg/100 g, respectively) (R= 0.979). A similar increase was reported in Polish hazelnuts (Pycia et al., 2020).

As seen in Table 3, harvest time had a significant effect on carotenoid concentration (P < 0.01). The amount of carotenoids, which peaked in EH (3.96 mg/g nut) showed a continuous decrease in NH (2.04 mg/g nut) and LH (1.60 mg/g nut) with the progression of harvest time. This decrease can be explained by the blockage of carotenoid production as

TABLE 3. Tocopherol composition (mg/100 g oil) and total carotenoid content (mg/g nut) of hazelnut oil at different harvest times

	PH	EH	NH	LH	
α- tocopherol	53.59±0.07 a	49.72±0.01 b	47.17±0.01 c	49.76±0.03 b	**
β- tocopherol	2.77±0.12 a	1.49±0.01 b	1.22±0.01 b	1.29±0.01 b	**
γ- tocopherol	4.59±0.01 c	5.22±0.01 b	6.26±0.01 a	4.45±0.01 d	**
δ- tocopherol	0.02±0.00	nd	nd	nd	ns
Total tocopherol	61.02±0.05 a	56.41±0.03 b	54.65±0.02 d	55.51±0.04 c	**
Vitamin E	55.16±0.04 a	50.84±0.01 b	48.28±0.01 c	50.72±0.03 b	**
Carotenoid	1.53±0.01 d	3.96±0.01 a	2.04±0.01 b	1.60±0.01 c	**

All values are presented as means ± standard deviation (n= 3). The different superscript letters (a-d) in the same row indicate statistically significant difference according to the Tukey test. * significant at P < 0.05, ** significant at P < 0.01, ns: not significant, Nd: not detected, PH: pre-harvest time, EH: early harvest time, NH: normal harvest time, LH: late harvest time, Vitamin E (expressed as α-tocopherol equivalents). The conversion factors for vitamin E activity were as follows: α-Tocopherol x 1.00, β-tocopherol x 0.40, γ-tocopherol x 0.10, δ-tocopherol x 0.01 (Alasalvar et al., 2009).

a result of the inhibition of enzymes which catalyze the oxidation steps for carotenoid synthesis with nut ripening (Bouali *et al.*, 2013). Similar to our results, in some studies conducted on pecan nuts and olives, researchers reported carotenoid loss with the progression of ripening (Bouali et al., 2013; Yorulmaz and Konuskan 2017; Baccouri *et al.*, 2008).

3.6. Variations in total phenolic compounds, flavonoids and antioxidant activities at different harvest times

The changes in the amounts of total phenolic compounds (TPC) in hazelnuts among harvest times are shown in Figure 1. TPC was determined as 251.18 mgGAE/100 g in hazelnuts collected in NH, consistent with some previous studies (Arcan and Yemenicioğlu 2009). Significant differences were detected in the TPCs of the samples among harvest times and the periods were listed as EH > NH > PH > LH

(P < 0.01). The TPC in LH (174.84 mgGAE/100 g) was observed to be 62% lower than that in NH, and 30% less than that in NH (450.71 mgGAE/100 g), where the greatest amount was detected. It is known that there is a difference between the enzymatic activities of polyphenol oxidase (POD) and peroxidase enzymes in hazelnuts at different harvest times (Seyhan *et al.*, 2007). The decrease in TPC as harvest time progressed can be explained by altered POD and peroxidase enzyme activity. A similar downward trend was reported by Pycia et al. (2020) for Polish hazelnuts and Pycia *et al.* (2019) for walnuts. In agreement with our results, Baccouri et al. (2008) associated the decreasing oleuropein concentration with progressive harvest time with the increased hydrolytic enzyme activity of olives with ripening. On the other hand, similar to TPC, the highest TFC was detected in EH (277.85 mgCE/100 g) and the lowest
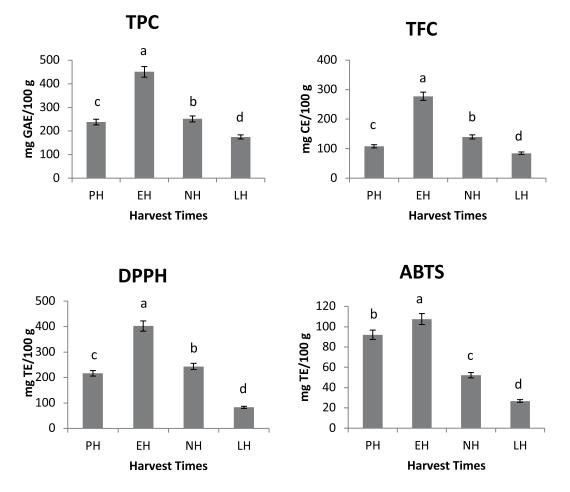

FIGURE 1. Changes in total phenolic compounds (TPC), total flavonoids (TFC) and antioxidant activities (DPPH and ABTS) of hazelnuts at different harvest times (n=3). Different lowercase letters in the same parameter indicate the statistical difference of harvest times according to the Tukey test (P <0.01). PH: pre-harvest time, EH: early harvest time, NH: normal harvest time, LH: late harvest time.

in LH (84.61 mgCE/100 g). With the late harvest, an approximately 40% decrease was observed in TFC compared to NH. Persic *et al*. (2018) reported that TFC first peaked and then decreased in Slovenian hazelnuts as the harvest time was delayed. The ratios of TFC in TPC varied from 45 to 61% among harvest times. There is a decrease in phenolic compounds as the biological processes ends with ripening and the fruit no longer needs protection from herbivores (Persic *et al*., 2018; Pycia *et al*., 2020). In this study, this information was confirmed for hazelnuts.

In the present study the antioxidant capacities of hazelnut samples were determined by two different methods (DPPH and ABTS) and were found to be significantly affected by the harvest times (P < 0.01)

(Figure 1). According to the results, EH was characterized by the highest antioxidant capacity (402.03 mgTE/100 g, 107.54 mgTE/100 g; DPPH, ABTS respectively). In both methods, antioxidant activity peaked in EH and then decreased continuously. Similarly, it has been reported that antioxidant activity decreased with ripening in Polish hazelnuts (Pycia *et al*., 2020) and walnuts (Pycia *et al*., 2019). Wang *et al*. (2021) have found that the antioxidant activity in *Torreya granndis* nuts decreased with ripening after the peak point. In LH compared to NH, 66 and 51% decrements were found for DPPH and ABTS, respectively. When the correlation between antioxidant capacity and TPC was evaluated, the lower antioxidant activity of LH can be explained by the decrease in
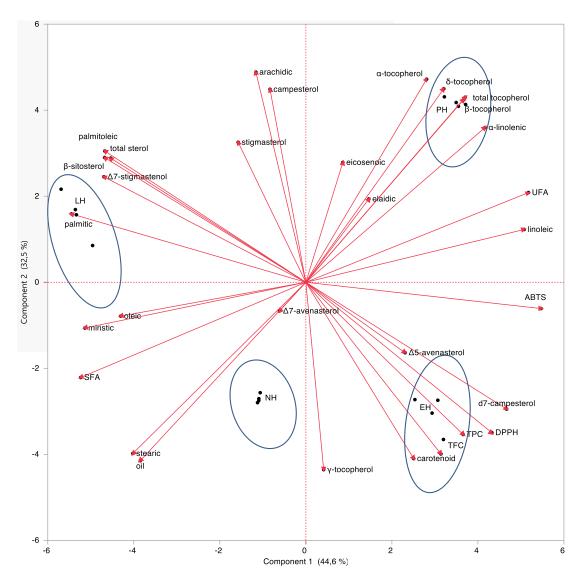

FIGURE 2. Biplot of the relationship of bioactive compounds in hazelnuts with different harvest times (n=3). PH- pre-harvest time, EH- early harvest time, NH- normal harvest time, LH- late harvest time.

total phenol content due to the progression of harvest times (r=0.960, r=0.797, DPPH and ABTS, respectively). This result is consistent with previous studies reporting that the antioxidant activity of hazelnuts is correlated with TPC (Arcan and Yemenicioğlu 2009).

3.7. Principal component analysis

Principal component analysis (PCA) was applied to get an overview of the sample variations and to reduce the initial variables to a small number of principal components. PC1 (Component 1) and PC2 (Component 2) components explained 44.6 and 32.5% of the total variability, respectively (Figure 2). The lower right quadrant contains variables that correlate quite well with each other. Here, it can be seen that TPC and TFC and two methods used to determine antioxidant capacity (DPPH and ABTS) are correlated. There is also a strong positive correlation between carotenoid and antioxidant activity. This group includes EH samples. In the left upper quadrant, there were, among others, β-sitosterol, total sterol, and palmitic acid, whose accumulation increased with LH. The positive effect of LH on sterol accumulation is clearly seen in this quadrant. In the upper right corner where the pH samples are located, all tocopherols except γ-tocopherol are included in addition to UFA. However, it should not be forgotten that this appearance is related to the accumulation of tocopherol in the oil because the amount of tocopherol in the nuts was higher in the LH samples. As a result, four different harvest times were clearly separated by PCA and ANOVA was largely confirmed.

4. CONCLUSIONS

According to the results, although there was no difference in oil accumulation between the NH and LH periods, SFA and MUFA increased while PUFA decreased. It can be thought that LH hazelnuts may be more resistant to oxidation due to lower UFA/SFA, IV, and PI, and higher O/L values related to the changes in the fatty acid profile. The highest total sterol and β-sterol levels were reached in LH, but there was no difference among the other periods. Although the amount of tocopherols in the oil showed an irregular change between harvest times, it showed a continuous increase in the nuts due to an increase in oil. On the other hand, carotenoids, TPC, TFC and antioxidant activities peaked in EH and then decreased to the low-

est level in LH with a continuous decrease. Although LH caused a loss of polyphenols, it can be thought that it can increase the resistance to lipid oxidation and extend storage stability and shelf-life. As a result, late harvesting can be recommended to preserve the kernel quality of hazelnuts, which must be stored for certain periods until they are consumed or processed. In addition, hazelnuts to be used for special food designs and pharmaceutical purposes are recommended to be harvested early due to the increase in polyphenol content and antioxidant capacity.

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