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# The intensity of the cluster drop affects the bioactive compounds and fatty acid composition in hazelnuts

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**SUMMARY:** This study was conducted to determine how the intensity of the cluster drop effects nut traits, bioactive compounds, and fatty acid composition in Tombul, Palaz and Kalınkara hazelnut cultivars. The cluster drop significantly affected bioactive compounds and fatty acid composition while it did not affect the traits of the nuts. As cluster drop intensity increased, nut traits and bioactive compounds in all cultivars increased. Strong cluster drop intensity determined the highest total phenolics, total flavonoids, and antioxidant activity. Except for the Kalınkara cultivar, a low amount of linoleic acid was detected while high amounts of oleic and stearic acid were determined in slight cluster drop intensity. As cluster drop intensity increased, palmitic acid increased. Principal component analysis showed that the slight and intermediate drop intensity were generally associated with kernel length, oleic, linoleic, stearic, palmitoleic, 11-eicosenoic and arachidic acids. In contrast, strong intensity was associated with nut and kernel weight, kernel ratio, kernel width, kernel thickness, kernel size, bioactive compounds, and palmitic acid. As a result, the bioactive compounds and fatty acid composition, which are important for human health, was significantly affected by cluster drop intensity.

***KEYWORDS:*** *Antioxidant; Cluster drop; Hazelnut; Nut traits; Oleic acid; Phenolics.*

**RESUMEN:** ***Influencia de la intensidad de la caída del racimo sobre los compuestos bioactivos y la composición de ácidos grasos en la avellana.*** El estudio se realizó para determinar el efecto de la intensidad de la caída de los racimos en las características de las avellanas, los compuestos bioactivos y la composición de ácidos grasos en cultivares de avellanas Tombul, Palaz y Kalınkara. La caída del racimo afectó significativamente a la composición de bioactivos y ácidos grasos, mientras que no afectó a las características de la avellana. A medida que aumentaba la intensidad de la caída de los racimos, aumentaban los compuestos bioactivos en todos los cultivares. La fuerte intensidad de caída de los racimos determinó que los fenoles totales, los flavonoides totales y la actividad antioxidante fueran más altos. Excepto para el cultivar Kalınkara, con un bajo contenido de ácido linoleico, un alto contenido de los ácidos oleico y esteárico se determinó en una ligera intensidad de caída de racimos. A medida que aumentaba la intensidad de la caída de los racimos, aumentaba el ácido palmítico. El análisis de componentes principales mostró que la intensidad de caída leve e intermedia generalmente se agrupaba con la longitud del grano, los ácidos oleico, linoleico, esteárico, palmitoleico, 11-eicosenoico y araquídico. En contraste, la intensidad fuerte se agrupó con el peso de la avellana y el grano, la proporción del grano, el ancho del grano, el grosor del grano, el tamaño del grano, los compuestos bioactivos y el ácido palmítico. Como resultado, la composición de compuestos bioactivos y ácidos grasos, que es eficaz para la salud humana, se vio significativamente afectada por la intensidad de la caída del grupo.

***PALABRAS CLAVE:*** *Ácido oleico; Antioxidante; Avellana; Caída de racimo; Fenoles; Rasgos de la avellana.*

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## 1. INTRODUCTION

Hazelnut constitutes a significant part of the daily diet in developed and developing countries as well as being widely used in the confectionery, ice cream, baking, and chocolate industries. Hazelnut is rich in dietary fiber, lipids, fatty acids, micro-macro mineral elements, vitamins (Balta *et al.,* 2006; Alasalvar *et al.,* 2010; Turan, 2019), phytosterols and phytostanols, squalene, and phenolic compounds (Yılmaz *et al.,* 2019; Di Nunzio, 2019). It stands out as an antioxidant source in preventing diseases such as cardiovascular, neurodegenerative, inflammatory, colon cancer, and type-2 diabetes (Di Nunzio, 2019; Yılmaz *et al.,* 2019). Considering that the consumption of fruits with rich nutritional content is recommended to increase the body's resistance to pandemic diseases such as Covid-19 (Muscogiuri *et al*., 2020), hazelnut fruit stands out as a significant source of nutrients.

The primary factors affecting nut quality and bioactive contents in hazelnuts are genetic structure, ecology, climate, fertilization, pruning, harvest time, and diseases and pests (Balta *et al.,* 2006; Cristofori *et al.,* 2015; Turan, 2019). Irrigation is another significant factor which affects the yield and quality characteristics of hazelnuts. In recent years, the drought caused by global climate change has negatively affected the yield and quality of hazelnuts, like many other fruit species. Water availability was reported by many researchers as a factor that directly affects the yield and quality of hazelnuts (Bignami *et al.,* 2011; Bostan and Tonkaz, 2013).

When the climate data belonging to recent decades are examined, it is estimated that the temperature will increase by 1.5 °C on average (Arora, 2019), while precipitation will decrease by about 30% (Lorite *et al.,* 2018) worldwide until 2030. Hazelnut growing in Turkey generally takes place on sloping lands with no irrigation, thus precipitation provides the water requirement for the plants. Therefore, obtaining a high yield and quality product strictly depends on sufficient and regular precipitation. In the case of a water deficit, a significant cluster drop occurs, resulting in a decrease in yield and quality (Bignami *et al.,* 2011; Milosevic and Milosevic, 2012; Bostan and Tonkaz, 2013).

The cluster drop in hazelnut is a phenomenon that continues from the fruit set to ripening (Milosevic and Milosevic, 2012). The cluster drop intensity changes depending on ecological factors, variety, pollen source, incompatibility, cultural practices, diseases and pests, as well as water deficit (Bignami *et al.,* 2011; Bostan and Tonkaz, 2013). Milosevic and Milosevic (2012) reported the occurrence of nut cluster drop density at three different levels: slight (< 10%), intermediate (10-20%), and strong (> 20%).

To date, many studies have been conducted to determine the effects of factors such as cultivar (Köksal *et al.,* 2006; Alasalvar *et al.,* 2010), genotype, echography, cultural practices (Balta *et al.,* 2006; Yılmaz *et al.,* 2019; Balik, 2021), harvest time, maturity level (Cristofori *et al.,* 2015), storage, drying methods (Turan, 2019), altitude, and direction (Balta *et al.,* 2018) on the bioactive compounds and fatty acids in hazelnuts. However, there is no study in the literature on the changes in bioactive compounds and fatty acid composition depending on the cluster drop intensity in hazelnuts.

The main aim of this study is to determine the changes in nut traits, bioactive compounds, and fatty acid composition of Turkish hazelnut cultivars (Tombul, Palaz and Kalınkara) depending on the cluster drop intensity.

## 2. MATERIALS AND METHODS

### 2.1. Plant materials

This research was carried out at commercial orchards (40°54'38.6"N latitude, 37°48'19.3"E longitude, 245 m altitude) in the Ordu province on the prominent hazelnut cultivars of Turkey, which are Tombul, Palaz, and Kalınkara, in two consecutive growing seasons, 2019 and 2020.

Trial orchards were established as multi-stemmed (7-9 stems per system) training systems and planted at distances between 4 m × 3 m and within rows. Standard cultural practices, such as fertilization, pruning and weed control were performed regularly, except for irrigation. During the research, branch thinning was carried out in the winter period and suckering was carried out twice during the vegetation period. Chemical control was carried out against the diseases and pests of nut weevil (*Curculio nucum*), green shield bug (*Palomena prasina*), and powdery mildew (*Erysiphe corylacearum*). Weeding was performed twice a year before harvest. A total of 250 g $NH_4H_2PO_4$ (monoammonium phosphate), 100 g $K_2SO_4$ (potassium sulphate), and 500 g N (nitrogen) were supplied per system. In addition, foliar fertilizer was applied twice a year. There weren't any nutrient deficiency symptoms in the leaf or fruit during the growing season.

At harvest time (10-15 August), all clusters on the plants were harvested, separated from husks, and dried naturally (under sunlight) until the moisture content decreased to 6%. No rainfall was observed during drying and the weather conditions were as follows: the temperature was 23.9 °C and 23.8 °C, precipitation was 0 mm and 0 mm, and hours of sunshine were 10.9 h and 10.7 h in 2019 and 2020, respectively (TSMS, 2021). The samples were stored in ambient conditions (at 22-24 °C and 70-80% RH) until analysis.

The precipitation and temperature values of the study area are presented in Figure 1 (TSMS, 2021).

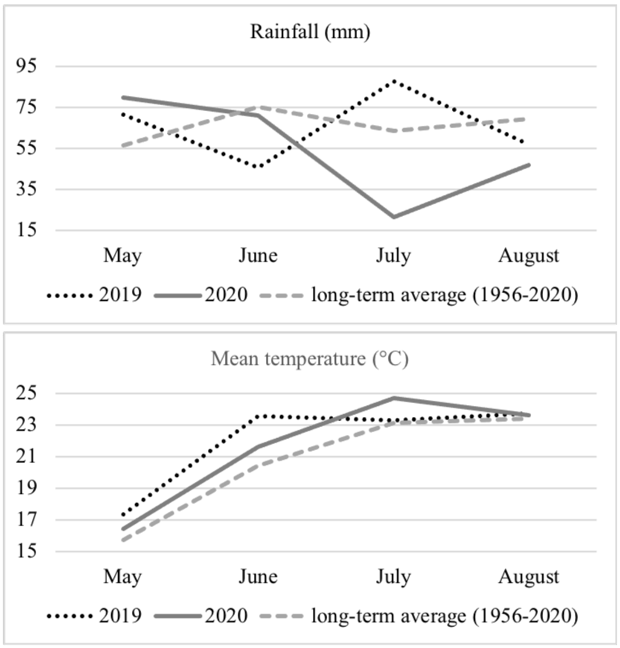


Figure 1. Rainfall (mm) and mean temperature (° C) between May and August

## 2.2 Experimental design

Fifty plants were marked for each of Tombul, Palaz and Kalınkara hazelnut cultivars as a result of observations made for many years in orchards with the same conditions, altitude and direction. Cluster drop was recorded in the marked plants after the fruit set (beginning of June). In light of the observations, it was determined that cluster drop in the orchards was different. In orchards where the cluster drop was observed, 15 plants were selected (total of 45 per cultivar) in 3 replicates for each cultivar to monitor this phenomenon.

## 2.3. Cumulative drop ratio

The cumulative drop ratio (%) was calculated by counting the dropped clusters at 20-day intervals from the fruit set (beginning of June) to harvest (beginning of August), 4 times in total. The equation of '∑dropped clusters/total clusters x 100' was used in the calculation. Then, the sampling was made in 15 plants with slight (< 10%), intermediate (10-20%) and strong (> 20%) drop density (Table 1) according to the classification by Milosevic and Milosevic (2012) in each cultivar.

Table 1. Cumulative cluster drop ratio (%) of investigated hazelnut cultivars (two-year average)

| Cultivars | Cluster drop intensity | 26 June | 16 July | 05 August |
|---|---|---|---|---|
| | Slight | 1.9±0.35 | 5.9±1.27 | 6.8±1.18 |
| Tombul | Intermediate | 4.8±0.87 | 12.0±2.65 | 15.7±2.19 |
| | Strong | 10.2±1.84 | 26.5±6.37 | 33.7±4.71 |
| | Slight | 3.6±0.71 | 7.1±1.43 | 8.9±1.34 |
| Palaz | Intermediate | 5.0±0.90 | 15.0±3.30 | 17.5±2.45 |
| | Strong | 12.0±2.16 | 24.0±5.28 | 32.0±6.08 |
| | Slight | 2.6±0.47 | 6.5±1.49 | 7.8±1.17 |
| Kalınkara | Intermediate | 5.7±1.03 | 14.3±3.14 | 18.6±2.60 |
| | Strong | 8.9±1.60 | 24.4±5.38 | 30.0±4.20 |

Values are mean ± standard deviation (n=3)

## 2.4. Nut traits

Fifty nuts were used for nut and kernel traits in each treatment. Nut weight (g) and kernel weight (g) were measured with digital balance (Radwag, AS/220/C/2, Poland) to an accuracy of 0.01 g. Shell thickness (mm) and kernel dimension (mm) (width, thickness and length) were measured with a digital caliper (Mitutoyo, CD-15CP, Japan) to an accuracy of 0.01 mm. The kernel ratio (%) was calculated by the equation of 'kernel weight/nut weight × 100' as previously reported. Kernel size (mm) was calculated as the geometric mean of kernel size (width, thickness and length) (Balta *et al.,* 2018).

## 2.5. Bioactive compounds

Bioactive compounds were determined as total phenolics, total flavonoids and antioxidant activity (FRAP and DPPH assays). Bioactive compounds were detected in defatted hazelnut samples. The defatting process was performed according to the Soxhlet extraction method (Firestone, 1997).

For the detection of bioactive compounds, 1 g defatted hazelnut sample was accurately weighed and extracted with 10 ml methanol. The obtained solution was centrifuged in a cooler-type device for 30 min at 12,000 rpm, at 4 °C. The resultant filtrate was used for determining the total phenolics, total flavonoids and antioxidant activity.

### *2.5.1. Total phenolics*

The total phenolic content was determined using the Folin-Ciocalteu reagent (Merck, Germany). The prepared samples were measured at a wavelength of 760 nm in a spectrophotometer (Shimadzu, Japan). The results were expressed as mg·100 $g^{-1}$ as gallic acid equivalent (GAE) (Ozturk *et al.,* 2018).

### *2.5.2. Total flavonoids*

Total flavonoid content was determined according to the method of Ozturk *et al.* (2018). Absorbance values were determined in a spectrophotometer (Shimadzu, Japan) at a wavelength of 415 nm. The results were expressed as mg·100 $g^{-1}$ in terms of quercetin equivalents (QE).

### *2.5.3. Antioxidant activity (FRAP and DPPH assays)*

The antioxidant activity was measured according to FRAP [ferric ions ($Fe^{+3}$) reducing antioxidant power assay] (Benzie and Strain, 1996) and DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) assays (Blois, 1958). Prepared samples were measured in a spectrophotometer (Shimadzu, Japan) at 700 nm for the FRAP assay and at 517 nm for the DPPH assay. The results were expressed as mmol 100 $g^{-1}$ in terms of trolox equivalents (TE).

## 2.6. Fatty acid composition

0.1 g of hazelnut oil was accurately weighed in a test tube and 1 mL potassium methylate and 4 mL hexane were added. The resultant mixture was shaken for 30 seconds and 0.5 mL $H_2SO_4$ were added. The resultant supernatants were diluted with hexane and filtered through a 0.45 µm filter. A GC (gas chromatography system) (Shimadzu, Kyoto, Japan) equipped with a flame ionization detector (FID) and capillary column (0.25 mm × 0.20 µm, 100 m) was used to analyze samples for their fatty acid compositions. The column temperature was programmed as follows: held at 140 °C for 5 min, raised to 240 °C at a rate of 4 °C/min and held at 240 °C for 15 min. The injector and detector temperatures were 250 °C. Nitrogen was used as carrier gas. At a flow rate of 3 mL/min. The injection volume was 1 mL with a split ratio of 1:100. Fatty acid peaks were identified based on standard FAMEs (fatty acid methyl esters) by comparing retention times. Results were expressed as percentages of relative areas of identified fatty acids (Sengul, 2019). The obtained fatty acid composition was used to calculate the fatty acids in terms of: saturated fatty acid (SFA) (palmitic, stearic and arachidic), monounsaturated fatty acid (MUFA) (palmitoleic, oleic and 11-eicosenoic) and polyunsaturated fatty acid (PUFA) (linoleic and linolenic).

## 2.7. Statistical analysis

The data were subjected to ANOVA by using SPSS 23.0 (SPSS Inc. Chicago, USA) software. Differences among means were determined with the LSD multiple-comparison test at $p < 0.05$. PCA (Principal Components Analysis) and component biplot analysis were performed using JMP 10 (trial) software.

# 3. RESULTS AND DISCUSSION

## 3.1. Nut traits

Nut weight, kernel weight, kernel ratio and shell thickness are significant quality characteristics in hazelnuts (Balta *et al.,* 2018). A high kernel ratio is a desirable characteristic for the hazelnut industry. Small and medium-sized nuts are important for the confectionery industry while large nuts are suitable for in-shell marketing. In addition, a thinner shell is a desired characteristic for in-shell marketing (Guler and Balta, 2020). The effect of the cluster drop intensity on nut weight, kernel weight, kernel ratio and shell thickness in the hazelnut cultivars was insignificant ($p > 0.05$). However, an increase in cluster drop intensity caused an increment in nut weight, kernel weight and kernel ratio in all cultivars. The highest nut and

Table 2. Nut weight, kernel weight, kernel ratio shell thickness and kernel size according to intensity of cluster drop in different hazelnut cultivars

| Cultivars | Cluster drop intensity | Nut weight (g) | Kernel weight (g) | Kernel ratio (%) | Shell thickness (mm) | Kernel width (mm) | Kernel thickness (mm) | Kernel length (mm) | Kernel size (mm) |
|---|---|---|---|---|---|---|---|---|---|
| | Slight | 1.75±0.10 a | 0.92±0.05 a | 52.3±0.94 a | 1.02±0.04 a | 12.53±0.30 a | 11.01±0.79 a | 14.75±0.13 a | 12.67±0.28 a |
| Tombul | Intermediate | 1.77±0.15 a | 0.93±0.09 a | 52.4±0.64 a | 0.95±0.06 a | 12.26±0.28 a | 11.73±0.54 a | 14.49±0.30 a | 12.77±0.30 a |
| | Strong | 1.83±0.07 a | 0.96±0.04 a | 52.5±0.35 a | 1.00±0.05 a | 12.57±0.15 a | 11.86±0.08 a | 14.27±0.27 a | 12.86±0.02 a |
| Significance | | ns | ns | ns | ns | ns | ns | ns | ns |
| LSD (0.05) | | 0.22 | 0.12 | 1.40 | 0.10 | 0.50 | 1.10 | 0.48 | 0.48 |
| | Slight | 1.82±0.02 a | 0.92±0.03 a | 50.3±1.12 a | 1.13±0.13 a | 14.17±0.21 a | 12.03±0.87 a | 12.70±0.38 a | 12.93±0.22 a |
| Palaz | Intermediate | 1.87±0.10 a | 0.94±0.06 a | 50.5±0.82 a | 1.06±0.05 a | 14.44±0.28 a | 12.50±0.31 a | 12.17±0.31 a | 13.00±0.17 a |
| | Strong | 1.92±0.03 a | 1.00±0.02 a | 52.0±0.63 a | 1.05±0.06 a | 14.55±0.21 a | 12.72±0.27 a | 12.01±0.48 a | 13.10±0.13 a |
| Significance | | ns | ns | ns | ns | ns | ns | ns | ns |
| LSD (0.05) | | 0.12 | 0.08 | 1.76 | 0.18 | 0.48 | 1.11 | 0.80 | 0.35 |
| | Slight | 1.97±0.12 a | 1.01±0.09 a | 51.3±2.56 a | 1.10±0.13 a | 12.08±0.60 a | 11.50±0.44 a | 16.70±0.72 a | 13.24±0.41 a |
| Kalınkara | Intermediate | 1.99±0.10 a | 1.06±0.09 a | 53.1±1.79 a | 1.23±0.14 a | 12.53±0.37 a | 11.86±0.20 a | 16.53±0.30 a | 13.49±0.26 a |
| | Strong | 2.11±0.05 a | 1.15±0.06 a | 54.7±1.57 a | 1.11±0.07 a | 13.03±0.82 a | 11.62±0.53 a | 16.43±0.73 a | 13.54±0.24 a |
| Significance | | ns | ns | ns | ns | ns | ns | ns | ns |
| LSD (0.05) | | 0.19 | 0.16 | 4.04 | 0.24 | 1.24 | 0.83 | 1.24 | 0.63 |

The differences among mean values shown on the same line with the same letter are not significant ($p < 0.05$). Differences were determined using the LSD test. * significant at $p < 0.05$, ** significant at $p < 0.01$, *** significant at $p < 0.001$ and ns: not significant. n= 300 for the nut and kernel traits (three replicates × five plants for each replicate × twenty nuts)

kernel weights were detected for the Kalınkara cultivar, while the lowest nut and kernel weights were found for the Tombul cultivar. The Palaz cultivar had intermediate nut and kernel weight. The Kalınkara cultivar had the highest kernel ratio, while Palaz cultivar had the lowest kernel ratio. The lowest and highest shell thickness were measured in the Tombul and Kalınkara cultivars, respectively (Table 2). Milosevic and Milosevic (2012) reported that in Tonda Gentile Romana, Nocchione and Istarski Duguljasti hazelnut cultivars, as the cluster drop intensity increased, the nut weight, kernel weight, kernel ratio and shell thickness also increased. However, they reported that the increase in these traits was not statistically significant. In previous studies, the highest nut weight and kernel weight were reported for the Kalınkara cultivar, and the lowest for the Tombul cultivar. On the contrary, the highest kernel ratio and thinnest shell was recorded for the Tombul cultivar (Balik *et al.,* 2016).

The effect of cluster drop intensity on kernel dimensions was insignificant in all cultivars ($p > 0.05$). Although not statistically significant, the kernel size of all cultivars increased with the increment in cluster drop intensity. The Kalınkara cultivar had the largest kernel size, followed by the Palaz and Tombul cultivars. The kernel size of all cultivars was above 12.5 mm (Table 2), meaning that they were all suitable for marketing (Yılmaz *et al.,* 2019). Milosevic and Milosevic (2012) reported that in Tonda Gentile Romana, Nocchione and Istarski Duguljasti hazelnut cultivars, as the cluster drop intensity increased, the kernel size increased. However, they reported that the increase in nut size was not statistically significant. In addition, Balik *et al.* (2016) reported the highest kernel size for the Palaz cultivar, followed by Kalınkara and Tombul cultivars.

The results were similar to reports on the same cultivars by different researchers in terms of nut weight, kernel weight, kernel ratio, shell thickness and kernel size. It has been reported that the nut and kernel traits of hazelnuts can be also be influenced by ecological conditions, and cultural and technical practices (Balta *et al.,* 2018; Guler and Balta, 2020; Bak and Karadeniz, 2021).

### 3.2. Bioactive compounds

Phenolic compounds play a significant role in reducing the risks of disease in human beings. The antioxidant properties of phenolic compounds are effective against many pathological problems associated with oxidative stress damage. In addition, bioactive compounds in plants have anti-inflammatory, antiulcer, antiallergic, antimicrobial, antithrombotic, antiatherogenic and anticarcinogenic effects (Di Nunzio, 2019). Ecological conditions, cultivar (Yılmaz *et al.,* 2019), maturity level, and cultural practices (Cristofori *et al.,* 2015) affect the bioactive compounds in hazelnuts. In addition, stressors such as drought, low and high temperature, pathogenic attack, and exposure to ultraviolet light cause an increase in bioactive compounds (Naikoo *et al.,* 2019).

In this study, total phenolics were significantly affected by the cluster drop intensity in all hazelnut cultivars ($p < 0.05$). However, the difference between slight and intermediate cluster drop intensity in Tombul and Kalınkara cultivars was insignificant in terms of total phenolic content. Total phenolics increased with the increment in cluster drop intensity in all cultivars. The highest total phenolic content was determined for the Palaz cultivar, followed by the Kalınkara and the Tombul cultivars (Table 3). The highest total phenolic content was reported for the Palaz cultivar by Balik (2021), while it was determined for the Tombul cultivar by Karaosmanoglu and Ustun (2021). Balık (2021) detected the lowest total phenolic content in the Kalınkara cultivar.

Flavonoids are a significant group of polyphenols with antioxidant properties (Di Nunzio, 2019). The total flavonoid content in all cultivars was significantly affected by cluster drop intensity ($p < 0.05$). The total flavonoid content increased with an increase in cluster drop intensity except for the Tombul cultivar. However, the difference between the slight and intermediate drop intensity in terms of total flavonoid content in the Kalınkara cultivar was insignificant. The Palaz cultivar had the highest total flavonoids, while the Tombul cultivar had the lowest total flavonoids (Table 3). Balık (2021) reported the highest total flavonoid content in the Tombul cultivar (34.0 mg·100 $g^{-1}$), followed by Palaz (13.2 mg·100 $g^{-1}$) and Kalınkara (12.6 mg·100 $g^{-1}$) cultivars.

Antioxidants are effective against the formation of the free radicals in the body, preventing the occurrence and progression of oxidative stress-induced diseases. The hazelnut is a natural source of antioxidants (Contini *et al.,* 2011), with high antioxidant activity. According to the FRAP assay, cluster drop intensity affected the antioxidant activity of all cul-

Table 3. Total phenolics, total flavonoids and antioxidant activity (FRAP and DPPH) according to intensity of cluster drop in different hazelnut cultivars

| **Cultivars** | **Cluster drop intensity** | **Total phenolics (mg GAE·100 $g^{-1}$)** | **Total flavonoids (mg QE·100 $g^{-1}$)** | **FRAP (mmol TE·100 $g^{-1}$)** | **DPPH (mmol TE·100 $g^{-1}$)** |
|---|---|---|---|---|---|
| | Slight | 43.5±0.10 b | 4.4±0.09 b | 0.35±0.02 b | 1.35±0.04 b |
| Tombul | Intermediate | 44.6±0.93 b | 3.9±0.15 c | 0.39±0.02 b | 1.50±0.01 a |
| | Strong | 62.6±1.38 a | 5.8±0.21 a | 0.67±0.03 a | 1.51±0.01 a |
| Significance | | *** | *** | *** | *** |
| LSD (0.05) | | 1.92 | 0.31 | 0.05 | 0.05 |
| | Slight | 87.1±0.59 c | 5.6±0.34 c | 0.99±0.04 c | 1.64±0.01 b |
| Palaz | Intermediate | 136.3±1.77 b | 14.1±0.58 b | 1.72±0.03 b | 1.64±0.01 b |
| | Strong | 193.4±2.95 a | 17.8±0.55 a | 2.86±0.07 a | 1.90±0.06 a |
| Significance | | *** | *** | *** | *** |
| LSD (0.05) | | 4.02 | 1.00 | 0.09 | 0.07 |
| | Slight | 78.3±1.13 b | 7.5±0.34 b | 0.56±0.03 b | 1.64±0.06 b |
| Kalınkara | Intermediate | 80.0±0.79 b | 7.8±0.06 b | 0.58±0.02 b | 1.90±0.01 a |
| | Strong | 109.8±0.88 a | 11.5±0.55 a | 1.20±0.03 a | 1.91±0.01 a |
| Significance | | *** | *** | *** | *** |
| LSD (0.05) | | 1.82 | 0.76 | 0.05 | 0.08 |

The differences among mean values shown on the same line with the same letter are not significant ($p < 0.05$). Differences were determined using the LSD test. * significant at $p < 0.05$, ** significant at $p < 0.01$, *** significant at $p < 0.001$ and ns: not significant. n= 9 for the bioactive compounds (three replicates × three different measurements for each replicate) tivars ($p < 0.05$). However, the difference between the slight and intermediate drop intensity in terms of antioxidant activity in Tombul and Kalınkara cultivars was insignificant. Antioxidant activity increased with the increase in cluster drop intensity for all cultivars. The highest antioxidant activity was determined for the Palaz cultivar, followed by Kalınkara and Tombul cultivars (Table 3).

The antioxidant activity determined by the DPPH assay was affected by the cluster drop intensity for all cultivars ($p < 0.05$). However, the difference between the intermediate and strong cluster drop intensity in Tombul and Kalınkara cultivars as well as slight and intermediate cluster drop intensity in the Palaz cultivar was insignificant in terms of the antioxidant activity. The increase in cluster drop intensity increased the antioxidant activity for all cultivars. The Kalınkara cultivar had the highest antioxidant activity, while Tombul cultivar had the lowest (Table 3).

In previous studies, according to FRAP and DPPH assays, the highest antioxidant activity was reported for the Tombul cultivar (1.22 mmol·100 $g^{-1}$ and 0.25 mmol·100 $g^{-1}$, respectively); while the lowest antioxidant activity was found for the Kalınkara cultivar (2.05 mmol·100 $g^{-1}$ and 0.24 mmol·100 $g^{-1}$, respectively). In the Palaz cultivar, antioxidant activity was recorded as 1.28 mmol·100 $g^{-1}$ and 0.24 mmol·100 $g^{-1}$, respectively (Balik, 2021).

The results for the bioactive compounds were generally similar to those previously reported for the same cultivars (Balik, 2021; Karaosmanoglu and Ustun, 2021). However, there were also some differences between this study and previous ones. The differences were thought to be due to ecological conditions (Yılmaz *et al.,* 2019; Balik, 2021), maturity level (Cristofori *et al.,* 2015), and cultural practices (Yılmaz *et al.,* 2019). In addition, as a general phenomenon, the increase in cluster drop intensity increased bioactive compounds in all cultivars. Similarly, higher total phenolics and antioxidant activity were reported in fruits of date palms with drops (Othmani *et al.,* 2020). Total phenolics, total flavonoids and antioxidant activity were reported to be higher in nuts of plants which were exposed to drought stress (Bignami *et al.,* 2011; Shahi *et al.,* 2020). This phenomenon is related to the production of the reactive oxygen species and the increase in secondary metabolites resulting from the defense response of plants against stress (Rejeb *et al.,* 2014). Even though the genetic structure is the primary factor in accumulating secondary metabolites in plants, the ecological factors also have a significant effect. Some climatic factors such as temperature, light and precipitation affect the accumulation of the phenolic compounds (Dumas *et al.,* 2003). Bioactive compounds in plants increase as a defense mechanism against temperature stress (Naikoo *et al.,* 2019; Shahi *et al.,* 2020). In the present study, the temperature values were higher than the long-term average (mean 2.5 °C); while the precipitation values were lower (about 39%) during nut development, between May and August (Figure 1). This situation caused the high phenolic and antioxidant accumulation in plants with strong cluster drop intensity and exposure to drought stress.

### 3.3. Fatty acid compositions

The hazelnut, a rich source of fatty acids (Contini *et al.,* 2011) with significant amounts of MUFAs (Alasalvar *et al.,* 2010; Karaosmanoglu and Ustun, 2021), effectively improves cholesterol balance and triglyceride levels and reduces the risk of atherosclerosis and coronary heart disease. The major fatty acids in hazelnuts with high MUFA content is oleic acid, constituting approximately 80% of total fatty acids (Balta *et al.,* 2006; Köksal *et al.,* 2006; Alasalvar *et al.,* 2010), followed by linoleic, palmitic and stearic acid, respectively. The fatty acid composition in hazelnuts is affected by many factors, such as ecological condition, genetic structure, location, cultural practices (Balta *et al.,* 2006; Köksal *et al.,* 2006; Balik, 2021), early harvest, storage, drying methods (Turan, 2019) and maturity level (Cristofori *et al.,* 2015), as well as drought, which is one of the ecological factors that causes significant changes in fatty acid composition (Hamrouni *et al.,* 2011; Xu *et al.,* 2011).

The oleic acid contents in all cultivars were significantly affected by drop intensity ($p < 0.05$). However, the differences between slight and strong drop intensities in the Tombul cultivar, slight and intermediate drop intensities in the Palaz cultivar, and intermediate and strong drop intensities in the Kalınkara cultivar were not statistically different. The highest oleic acid was determined in the Palaz cultivar, followed by Tombul and Kalınkara cultivars (Table 4). Balik (2021) reported the highest oleic acid content in the Palaz cultivar (82.39%) and the lowest in the

TABLE 4. Fatty acids composition (%) according to intensity of cluster drop in different hazelnut cultivars

| Cultivars | Cluster drop intensity | Oleic | Linoleic | Palmitic | Stearic | Palmitoleic | 11-eicose-noic | Arachidic | Oleic/ Linoleic | ∑SFA | ∑PUFA | ∑MUFA |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Slight | 82.45±0.84 a | 9.13±0.08 c | 5.75±0.14 b | 2.60±0.09 a | 0.07±0.00 a | nd | nd | 9.0±0.02 a | 8.3±0.23 a | 9.1±0.08 c | 82.5 ±0.84 a |
| Tombul | Intermediate | 80.17±0.79 b | 11.42±0.12 a | 6.04±0.16 ab | 2.29±0.07 b | 0.07±0.00 a | nd | nd | 7.0±0.01 c | 8.3±0.23 a | 11.4±0.12 a | 80.2 ±0.79 b |
| | Strong | 81.40±0.82 ab | 9.91±0.09 b | 6.11±0.16 a | 2.50±0.08 a | 0.07±0.00 a | nd | nd | 8.2±0.01 b | 8.6±0.24 a | 9.9±0.09 b | 81.5±0.82 ab |
| Significance | | * | *** | * | ** | ns | - | - | *** | ns | *** | * |
| LSD (0.05) | | 1.63 | 0.20 | 0.31 | 0.16 | 0.0 | - | - | 0.02 | 0.47 | 0.20 | 1.63 |
| | Slight | 83.55±0.88 a | 9.44±0.05 b | 4.58±0.11 c | 2.08±0.07 a | 0.09±0.00 b | 0.15±0.00 a | 0.07±0.01 a | 8.8±0.05 a | 6.7±0.18 c | 9.5±0.05 b | 83.8±0.88 a |
| Palaz | Intermediate | 81.90±0.85 ab | 10.61±0.07 a | 5.22±0.14 b | 1.99±0.07 a | 0.17±0.01 a | 0.07±0.00 b | 0.02±0.01 b | 7.7±0.03 b | 7.2±0.21 b | 10.7±0.07 a | 82.1±0.86 ab |
| | Strong | 80.37±0.82 b | 10.55±0.07 a | 6.79±0.22 a | 2.08±0.07 a | 0.09±0.00 b | 0.07±0.00 b | 0.02±0.01 b | 7.6±0.03 c | 8.9±0.29 a | 10.6±0.07 a | 80.5 ±0.82 b |
| Significance | | * | *** | *** | ns | *** | *** | *** | *** | *** | *** | ** |
| LSD (0.05) | | 1.70 | 0.13 | 0.32 | 0.14 | 0.01 | 0.01 | 0.01 | 0.07 | 0.46 | 0.13 | 1.71 |
| | Slight | 77.66±1.55 b | 15.64±0.31 a | 4.38±0.22 c | 1.88±0.11 c | 0.14±0.01 a | 0.18±0.00 a | 0.11±0.00 a | 5.0±0.00 c | 6.4±0.33 b | 15.6±0.31 a | 78.0±1.57 b |
| Kalınkara | Intermediate | 78.57±1.57 ab | 13.88±0.28 b | 4.95±0.25 b | 2.60±0.16 a | 0.06±0.02 b | 0.09±0.02 b | 0.06±0.02 b | 5.7±0.00 b | 7.6±0.42 a | 13.9±0.28 b | 78.7±1.58 ab |
| | Strong | 81.39±1.63 a | 10.31±0.21 c | 6.05±0.30 a | 2.24±0.13 b | 0.05±0.01 b | 0.07±0.01 b | 0.04±0.01 b | 7.9±0.00 a | 8.3±0.45 a | 10.3±0.21 c | 81.5±1.63 a |
| Significance | | * | *** | *** | ** | *** | *** | ** | *** | ** | *** | * |
| LSD (0.05) | | 3.16 | 0.54 | 0.52 | 0.27 | 0.03 | 0.03 | 0.03 | 4.87 | 0.81 | 0.54 | 3.18 |

The differences among mean values shown on the same line with the same letter are not significant ($p < 0.05$). Differences were determined using the LSD test. * significant at $p < 0.05$, ** significant at $p < 0.01$, *** significant at $p < 0.001$ and ns: not significant; nd: not detected. n= 9 for the fatty acid composition (three replicates × three different measurements for each replicate)

Tombul cultivar (78.19%). It was reported at 76.76% in the Kalınkara cultivar. Köksal *et al.* (2006) reported the highest oleic acid content in the Kalınkara cultivar (78.9%), followed by the Tombul (77.8%) and Palaz (77.6%) cultivars.

The linoleic acid was significantly affected by cluster drop intensity in all cultivars ($p < 0.05$). Besides, the difference between intermediate and strong drop intensities in the Palaz cultivar was insignificant. The Kalınkara cultivar had the highest linoleic acid, while the Tombul cultivar had the lowest (Table 4). Similarly, the highest linoleic acid was reported for the Kalınkara cultivar by different researchers (9.13-13.41%). The lowest was recorded for the Palaz cultivar (5.91-7.56%) (Alasalvar *et al.,* 2010; Balik, 2021).

The palmitic acid content in all cultivars was significantly affected by the cluster drop intensity ($p < 0.05$). Palmitic acid increased as the cluster drop intensity increased in all cultivars. The highest palmitic acid content was determined in the Tombul cultivar followed by Palaz and Kalınkara cultivars (Table 4). Similarly, Balik (2021) reported the highest oleic acid content in the Tombul cultivar (7.71%), followed by the Palaz (7.34%) and Kalınkara (6.58%) cultivars. On the contrary, Köksal *et al.* (2006) reported the highest palmitic acid content in the Kalınkara cultivar (5.71%) and the lowest in the Palaz cultivar (4.87%).

The cluster drop intensity did not affect the stearic acid content in the Palaz cultivar; whereas the the Tombul and Kalınkara cultivars were significantly affected ($p < 0.05$). The Tombul cultivar had the highest stearic acid content, while the Palaz cultivar had the lowest (Table 4). Alasalvar *et al.* (2010) determined that the highest stearic acid content was detected in the Tombul cultivar (3.24%). The lowest was recorded for the Kalınkara cultivar (2.08%). On the contrary, Köksal *et al.* (2006) reported the highest stearic acid content in the Kalınkara cultivar (2.42%) and the lowest in the Tombul cultivar (1.75%).

The palmitoleic acid content in the Tombul cultivar was not significantly affected by the drop intensity; whereas the Palaz and Kalınkara cultivars ($p < 0.05$) were significantly affected. The highest level of palmitoleic acid was determined in the Palaz cultivar followed by Kalınkara and Tombul cultivars (Table 4).

11-eicosenoic and arachidic acid were not detected in the Tombul cultivar and were significantly altered by the cluster drop intensity in the Palaz and

Kalınkara cultivars ($p < 0.05$). The Kalınkara cultivar had the highest 11-eicosenoic acid content, whereas Tombul cultivar had the lowest 11-eicosenoic acid content. The highest arachidic acid was determined in the Kalınkara cultivar, followed by the Palaz and Tombul cultivars (Table 4). In previous studies, the highest levels of palmitoleic and arachidic acid were reported for the Palaz cultivar (0.29% and 0.18%, respectively), while the lowest was detected for the Tombul cultivar (0.16% and 0.12%, respectively). Also, the Kalınkara cultivar had the highest 11-eicosenoic acid (0.20%) content. The lowest 11-eicosenoic acid was reported for the Tombul cultivar (0.16%) (Alasalvar *et al.,* 2010).

The difference in oleic/linoleic ratio depending on the cluster drop intensity was significant in all cultivars ($p < 0.05$). While the highest oleic/linoleic acid ratio was determined in the slight cluster drop intensity in the Tombul and Palaz cultivars, it was determined in the strong cluster drop intensity in the Kalınkara cultivar. Depending on the cultivars, the highest oleic/linoleic acid ratio was recorded for the Tombul cultivar, while the lowest ratio was determined in the the Kalınkara cultivar (Table 4). According to different researchers, the oleic/linoleic ratio was reported to be the highest in the Tombul cultivar and the lowest in the Kalınkara cultivar (Alasalvar *et al.*, 2010; Balik, 2021).

The saturated fatty acid (SFA) in the Tombul cultivar was not affected by drop intensity, whereas in the Palaz and Kalınkara cultivars it was significantly affected ($p < 0.05$). In all cultivars, the highest SFA values were recorded for the strong cluster drop intensity and increased as the cluster drop intensity increased. While the Tombul cultivar had the highest SFA value, the Palaz cultivar had the lowest value (Table 4). In previous studies, the highest SFA was reported for the Palaz cultivar by Karaosmanoglu and Ustun (2021), while it was determined in the Kalınkara cultivar by Balik, (2021).

The polyunsaturated fatty acid (PUFA) in all cultivars was significantly affected by drop intensity ($p < 0.05$). Whereas the highest PUFA was determined in the intermediate cluster drop intensity in Tombul and Palaz cultivars, it was determined in the slight cluster drop intensity in the Kalınkara cultivar. The highest PUFA was recorded for the Kalınkara cultivar, while the lowest was determined in the Tombul cultivar (Table 4). On the contrary, Alasalvar *et al.* (2010) determined that the highest PUFA was detected in the Palaz cultivar. The lowest level was recorded for the Kalınkara cultivar. In addition, Karaosmanoglu and Ustun (2021) reported a higher PUFA content in the Tombul cultivar than in the Palaz cultivar.

The difference in monounsaturated fatty acid (MUFA) depending on the cluster drop intensity was significant in all cultivars ($p < 0.05$). While the highest MUFA was determined in the slight cluster drop intensity in Tombul and Palaz cultivars, it was determined in the strong cluster drop intensity in the Kalınkara cultivar. Whereas the Tombul cultivar had the highest MUFA value, the Kalınkara cultivar had the lowest value (Table 4). Similarly, Alasalvar *et al.* (2010) reported the highest MUFA for the Tombul cultivar, followed by Palaz and Kalınkara cultivars. Also, Karaosmanoglu and Ustun (2021) reported higher PUFA for the Palaz cultivar than the Tombul cultivar.

The findings of the fatty acids composition in the study are generally similar to previous reports on the same cultivars by different researchers (Köksal *et al.,* 2006; Alasalvar *et al.,* 2010; Balik, 2021; Karaosmanoglu and Ustun, 2021). Fatty acid composition is affected by many factors such as the genetic structure, ecological condition, location, cultural practices (Balta *et al.,* 2006; Balik, 2021), early harvest, storage, drying methods (Turan, 2019) and maturity (Cristofori *et al.,* 2015).

Previous studies stated that the fatty acid composition changes with an increment in unsaturated fatty acid content in plants exposed to drought stress (Xu *et al.,* 2011). On the contrary, Hamrouni *et al.* (2011) reported a decrease in unsaturated fatty acid and an increase in saturated fatty acid content under drought stress. Similar results were reported in studies that determined the change in fatty acid composition due to water stress in hazelnuts (Bignami *et al.,* 2011; Bostan, 2020). Many researchers reported that drought stress stimulates a wide range of physiological and biochemical responses such as changes in fatty acid composition, wax biosynthesis and osmoprotectant synthesis in plants (Xu *et al.,* 2011). In addition, it has been reported that drought stress and fatty acid composition are related, and the unsaturated fatty acid content that increases in the adaptation process of the plant to drought stress maintains the stability and fluidity of the cellular membranes in the plant (Xu *et al.,* 2011). In the current study, the temperature values were higher (mean 2.5 °C), while the precipitation values were lower (about 39%) than the long-term average during the nut development period (Figure 1). The cluster drops resulting from this situation significantly affected the fatty acid composition of the cultivars in agreement with Xu *et al.* (2011) by having higher oleic and stearic acid and lower linoleic acid in the kernels of low cluster dropped plants except for the Kalınkara cultivar. Palmitic acid increased as the cluster drop intensity increased.

### 3.4. Principle component analysis

In the Tombul cultivar, the first two components explained 61.6% of the data. PC1 was related to nut weight, kernel weight, kernel thickness, kernel size, total phenolics and antioxidant activity (FRAP and DPPH) and explained 33.6% of the data. PC2 was mainly related to shell thickness, kernel width, total flavonoids, oleic acid, linoleic acid and stearic acid, and explained 28.0% of the data. There was a high positive relation from nut weight to kernel weight, total phenolics to total flavonoids, total phenolics to FRAP, total flavonoids to FRAP, oleic acid to stearic acid. According to the PCA results, the slight cluster drop intensity was grouped in terms of kernel length, oleic and, stearic acid. The intermediate cluster drop intensity was grouped by linoleic acid while the strong cluster drop intensity was grouped by nut weight, kernel width, kernel size, total phenolics, total flavonoids, antioxidant activity and, palmitic acid (Figure 2).

In the Palaz cultivar, the first two components explained 72.3% of the variability in the data. PC1 explained 56.6% of the data and related to nut weight, kernel weight, kernel width, kernel thickness, kernel length, kernel size, kernel ratio, total phenolics, total flavonoids, antioxidant activity (FRAP and DPPH), oleic, linoleic, palmitic, 11-eicosenoic and, arachidic acids. PC2 was defined by stearic and palmitoleic acids and explained 15.7% of the data. A significant highly positive relation was also computed from nut weight to kernel weight, total phenolics to total flavonoids, total phenolics to FRAP, total flavonoids to FRAP and arachidic acid to 11-eicosenoic acid. According to the PCA results, the slight cluster drop intensity was grouped by kernel length, oleic, arachidic and 11-eicosenoic acid. The intermediate cluster drop intensity was grouped by linolenic and palmitoleic acid and the strong cluster drop intensity was grouped by kernel weight, kernel ratio, kernel size, kernel width, total phenolics, total flavonoids, antioxidant activity and palmitic acid (Figure 3).

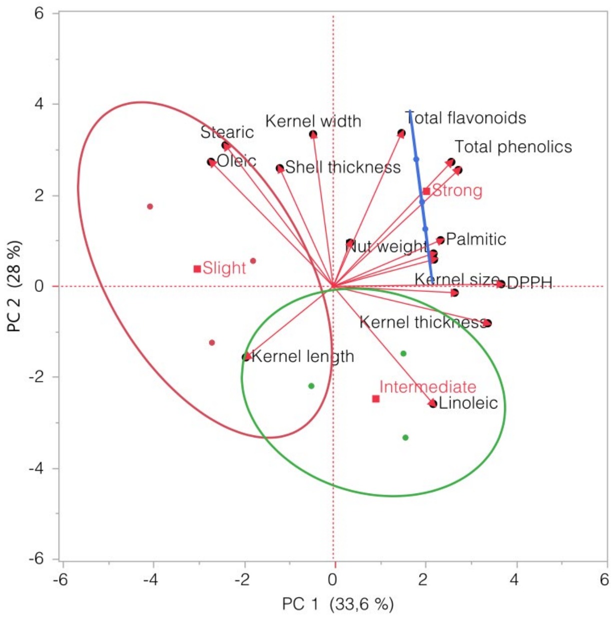


Figure 2. Relationships amongst nut traits, bioactive compounds and fatty acids composition in Tombul cultivar in terms of cluster drop intensity

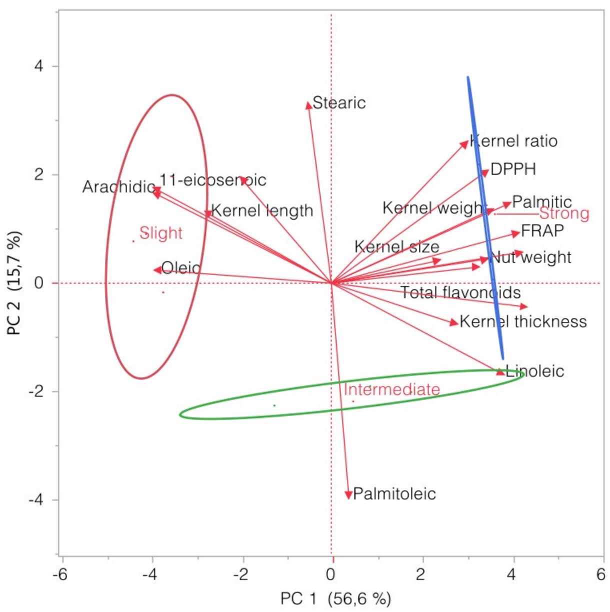


Figure 3. Relationships amongst nut traits, bioactive compounds and fatty acids composition in Palaz cultivar in terms of cluster drop intensity

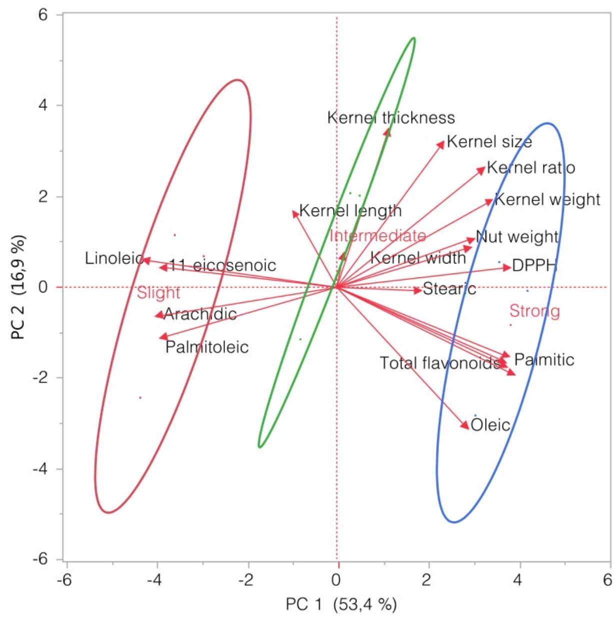


FIGURE 4. Relationships amongst nut traits, bioactive compounds and fatty acids composition in Kalınkara cultivar in terms of cluster drop intensity

In the Kalınkara cultivar, the first two components explained 70.3% of the data. PC1 was related to nut weight, kernel weight, kernel width, kernel ratio, total phenolics, total flavonoids, antioxidant activity (FRAP and DPPH), oleic, linolenic, palmitic, 11-eicosenoic, palmitoleic and arachidic acids, and explained 53.4% of the data. PC2 explained 16.9% of the data and was mainly related to kernel thickness, kernel length and kernel size. There was a highly positive relation from nut weight to kernel weight, kernel weight to kernel ratio, total phenolics to total flavonoids, total phenolics to FRAP, total flavonoids to FRAP, oleic acid to palmitic acid, 11-eicosenoic to palmitoleic and arachidic acid to palmitoleic acid. According to PCA, the slight cluster drop intensity was grouped by linoleic, palmitoleic, arachidic and, 11-eicosenoic acid. The intermediate cluster drop intensity was grouped by kernel length and thickness while the strong cluster drop intensity was grouped by kernel weight, kernel ratio, kernel width, kernel size, total phenolics, total flavonoids, antioxidant activity, oleic and palmitic acid (Figure 4).

In this study, the results from the principal component and correlation analyses supported each other. The properties in the PC1 and PC2 components of all cultivars were highly correlated with each other (Figures 2, 3, and 4). Many researchers have confirmed such a relationship in hazelnuts (Balta *et al.,* 2006; Yılmaz *et al.,* 2019, Bak and Karadeniz, 2021).

## 4. CONCLUSIONS

Nut traits were not found to be significantly affected by the cluster drop intensity in this study. However, bioactive compounds and fatty acid composition were significantly altered by the intensity of cluster drop. Bioactive compounds of the cultivars were enhanced by increasing cluster drop intensity. In addition, the severity of drop intensity affected the oleic/linoleic acid balance, and slight cluster drop intensity mostly caused higher oleic acid content, except for the Kalınkara cultivar. In general, the slight and intermediate cluster drop intensities were effective on fatty acids, whereas the strong cluster drop intensity was effective on nut traits and bioactive compounds. As a result, it has been determined that the cluster drop intensity significantly affects bioactive compounds and fatty acid composition, which is beneficial for human health. Also, the results of this study showed the possible effects of the upcoming dangers of global climate change (global warming) on hazelnuts and will be useful for future studies.

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