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Araştırma
(Research)

The Effect of Kernel Size on Bioactive Compounds in Kalınkara Hazelnut Cultivar

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Abstract

Objective: This study was carried out to determine the effect of kernel size on the change of bioactive compounds in Kalınkara hazelnut cultivar.

Materials and Methods: This study was conducted on the Kalınkara hazelnut cultivar grown in a producer's orchard in Ulubey (Ordu) district. The study was designed according to the randomized plot design with three replications and three ocak in each replication. Harvested nuts were classified according to kernel size as "small", "medium" and "large". Then, total phenolics, total flavonoids, and antioxidant activity were determined. In addition, correlation coefficients were calculated, and principal component analyses were performed to determine the relationships between the examined features.

Results: In the study, it was observed that kernel size was effective on bioactive compounds in Kalınkara hazelnut cultivar. According to the research findings, the total phenolics was determined between 759 mg 100 g^{-1} (medium) and 819 mg 100 g^{-1} (large), the total flavonoids was determined between 8.2 mg 100 g^{-1} (medium) and 8.7 mg 100 g^{-1} (small), and total antioxidant activity was determined between 0.46 mmol 100 g^{-1} (medium) and 0.60 mmol 100 g^{-1} (large) according to the FRAP assay, while it was determined between 1.83 mmol 100 g^{-1} (medium) and 1.92 mmol 100 g^{-1} (small) according to the DPPH assay. In addition, a positive correlation was determined between the total phenolics and antioxidant activity. Accordingly, while the correlation coefficient between total phenolics and DPPH assay was r=0.921***, it was r=0.982*** with FRAP assay.

Conclusion: As a result of the research, it was determined that the total flavonoids, and antioxidant activity according to the DPPH test were higher in small kernels. In comparison, total phenolics, and antioxidant activity according to the FRAP assay were higher in large kernels.

Keywords: Antioxidant, Corelation, *Corylus avellana*, PCA, Total phenolics.

Kalınkara Fındık Çeşidinde İç Meyve İriliğinin Biyoaktif Bileşikler Üzerine Etkisi

Öz

Amaç: Bu çalışma Kalınkara fındık çeşidinde meyve iriliğinin biyoaktif bileşiklerin değişimi üzerine etkisini belirlemek amacıyla yapılmıştır.

Materyal ve Yöntem: Bu çalışma Ulubey (Ordu) ilçesinde bir üretici bahçesinde yetiştirilen Kalınkara fındık çeşidi üzerinde yürütülmüştür. Çalışma tedadüf parselleri deneme desenine göre 3 tekerrürlü, her tekerrürde 3 ocak olacak şekilde dizayn edilmiştir. Hasat edilen meyveler "küçük", "orta" ve "büyük" olarak iç meyve iriliğine göre sınıflandırılmıştır. Ardından toplam fenolik, toplam flavonoid ve antioksidan aktivitesi belirlenmiştir. Ayrıca incelen özellikler arasındaki ilişkileri belirlemek amacıyla korelasyon katsayıları hesaplanmış ve temel bileşen analizleri gerçekleştirilmiştir.

Araştırma Bulguları: Çalışmada Kalınkara fındık çeşidinde meyve iriliğinin biyoaktif bileşikler üzerine etkisi önemli bulunmuştur. Araştırma bulgularına göre, toplam fenolik içeriği 759 mg 100 g^{-1} (orta) - 819 mg 100 g^{-1} (büyük), toplam flavonoid içeriği 8.2 mg 100 g^{-1} (orta) and 8.7 mg 100 g^{-1} (küçük), antioksidan aktivitesi FRAP testine göre 0.46 mmol 100 g^{-1} (orta) - 0.60 mmol 100 g^{-1} (büyük), DPPH testine göre 1.83 mmol 100 g^{-1} (orta) - 1.92 mmol 100 g^{-1} (küçük) arasında belirlenmiştir. Ayrıca, incelenen özelliklerden toplam fenolik ve antioksidan kapasitesi arasında pozitif bir korelasyon tespit

edilmiştir. Buna göre toplam fenolik ile DPPH arasındaki korelasyon katsayısı r=0.921*** olarak belirlenirken, FRAP ile r=0.982*** olarak tespit edilmiştir.

Sonuç: Araştırma sonucunda, küçük irilikteki meyvelerde toplam flavonoid içeriği ve DPPH testine göre antioksidan aktivitesinin, büyük irilikteki meyvelerde ise toplam fenolik ve FRAP testine göre antioksidan aktivitesinin yüksek olduğu belirlenmiştir.

Anahtar kelimeler: Antioksidan, Korelasyon, *Corylus avellana*, PCA, Toplam fenolik

Introduction

Hazelnut, one of the most important tree nut species grown in the world, is botanically included in the order Fagales and genus *Corylus* (Botta et al., 2019). Türkiye is the homeland of this nut species and covers its natural distribution areas. Ecological conditions are suitable for hazelnut cultivation in many parts of our country, especially in the Eastern Black Sea region. Our species and variety richness and the suitability of ecological conditions allow this product to be grown intensively in our country (İslam, 2018). With this situation, Türkiye meets 63.5% of the world's hazelnut production in 2021 (FAO, 2023). In particular, a significant part of our production is considered an export product and provides substantial income to the country's economy (Bozoğlu et al., 2019).

Hazelnut is a type of nut with a wide range of uses. Consumers frequently prefer it as a natural or roasted snack, consumed with admiration. In addition, it is widely used in the chocolate industry, biscuits, confectionery, pastry products and coffee (Kırca et al., 2018; Alalwan et al., 2022).

Due to the differences in hazelnuts regarding table consumption or consumer habits, developing varieties suitable for these preferences in the future is shown among the important breeding goals (İslam, 2019). Therefore, knowing the nutritional values of hazelnut, which is a product preferred by people all over the world, is essential for the evaluation of the product. It is known that nuts, including hazelnuts, are generally among the foods in healthy diet programs as raw and processed and have high nutritional properties (Alalwan et al., 2022). It is especially rich in macro and micronutrients like fat, protein, carbohydrates, minerals, vitamins, and fat-soluble bioactive (Alasalvar and Bolling, 2015, İslam, 2018).

Plant-derived products contain phytochemicals and phenolic compounds showing antioxidant and antiradical activity in their structure. Therefore, it is reported that such components reduce the harmful effects of free radicals that cause negative effects and help prevent problems such as cancer, heart disease, and atherosclerosis (Shadidi et al., 2007; Solar et al., 2009; Solar et al., 2022).

In this context, especially considering the positive effects on human health, detailed investigation and revealing of the factors affecting bioactive components will contribute to the determination of the consumption patterns of these products. Yılmaz et al. (2019) reported that significant relationships between kernel size and bioactive compounds in hazelnut. As a matter of fact, they were stated that the bioactive contents of the fruits increased as the kernel size decreased. Thus, unmarketable hazelnuts for the consumption of hazelnut kernels can be considered as additives in different processing industries and these products' nutritional and positive properties on human health can be preserved. In this context, this study was carried out to reveal the effects of kernel size on the biochemical properties of Kalınkara hazelnut cultivar.

Material and Methods

Material

This study was carried out in Ulubey (Ordu) district (40°58'37"N, 37°34'20"E) in 2021. The research used the nuts of the Kalınkara hazelnut cultivar as plant material. Kalınkara cultivar is highly adaptable to different climatic and soil conditions. The nut is round; the shell is matte, gray-brown. The kernel is dull white with a thick, rough and dark-colored skin attached. Although it has a low commercial value since it is a cultivar with a high shrivelled kernel, twin kernel ratio and low yield level, it can find a place in the orchards due to its resistance to diseases, late spring frosts, and high pollen. It is also a rich cultivar in terms of oil content (Köksal, 2018; Anonymous, 2023). In the orchard where the study was conducted, the plants were designed as "ocak" with 4x3 m rows. A total of 400 grams of nitrogen-phosphorus-potassium (NPK) compound fertilizer and 500 grams of nitrogen (N) were applied to each 'Ocak' annually. Chemical control was implemented for diseases and pests. Weed control was conducted twice a year, and pruning was performed in the winter season.

Method

The study was planned according to the randomized plot design with three replications and three ocak in each replication. At harvest (15-20 August), 100 clusters were collected from each ocak to represent the ocak and placed in mesh bags. Harvested nut samples were dried at room temperature (24 °C) until the moisture content decreased to 6% after they were separated from their husks (Sali et al. 2022). After the dried nut samples were cracked, the hazelnut kernel classification of TSE was modified and divided into three groups, small, medium, and large, according to their kernel size (Anonymous, 2019). Kernel size is calculated by taking the geometric mean of the kernel length, width, and thickness. Accordingly, those with kernel size between 9.0-11.0 mm were defined as 'small', between 11.01-13.0 mm as 'medium', and between 13.01-15.0 mm as 'large' (Yılmaz et al. 2019). Biochemical properties, total phenolic, total flavonoid, and antioxidant activity (according to FRAP and DPPH assays) were determined in kernels classified according to kernel size. Nuts with a good kernel were used in biochemical analyses.

Total phenolics (mg 100 g^{-1})

Total phenolics were modified from the method used by Beyhan et al. (2010). 1000 µL of the prepared stock solution was taken, and 3600 µL of distilled water, 100 µL of Folin-Ciocalteu's, and 300 µL of sodium carbonate (Na_2CO_3) were added and incubated for two h. The samples were then read at a wavelength of 760 nm. The values obtained were calculated in gallic acid equivalent (GAE) and expressed as mg 100 g^{-1}.

Total flavonoids (mg 100 g^{-1})

Total flavonoids were modified from the method used by Zhishen et al. (1999). 1000 µL of the prepared stock solution was taken, and 3300 µL of methanol, 100 µL of aluminium nitrate [Al $(NO_3)_3$], and 100 µL of ammonium acetate (NH_4CH_3COO) were added and incubated for thirty min. The samples were then read at a wavelength of 415 nm. The values obtained were calculated in quercetin equivalent (QE) and expressed as mg 100 g^{-1}.

Antioxidant activity

Antioxidant activity was determined using Ferric Ions (Fe^{+3}) Reducing Antioxidant Power Assay (FRAP) and Free Radical Scavenging Activity (DPPH) assays in the study.

FRAP assay (mmol 100 g^{-1})

Antioxidant activity (according to the FRAP assay) was modified from the method Benzie and Strain (1996) used. Accordingly, 20 µL of the prepared stock solution was taken, and 1230 µL of phosphate buffer and 1250 µL of potassium ferric cyanide were added and incubated for twenty-five minutes at 50 °C in a water bath. Then, 1250 µL of TCA and 250 µL of ferric chloride were added to the samples. The samples were then read at a wavelength of 700 nm. The values obtained were calculated in Trolox equivalent (TE) and expressed as mg 100 g^{-1}.

DPPH assay (mmol 100 g^{-1})

Antioxidant activity (according to the DPPH assay) was modified from the method Blois (1958) used. Accordingly, 30 µL of the prepared stock solution was taken, and 2970 µL of ethyl alcohol and 0.26 mM 1000 µL DPPH solution were added and incubated for thirty min. The samples were then read at a wavelength of 517 nm. The values obtained were calculated in Trolox equivalent (TE) and expressed as mg 100 g^{-1}.

Statistical analysis

The data obtained from the study were evaluated using the SPSS 23.0 (New York, USA) statistical package program. The relationship between the examined features was determined using the Pearson correlation coefficient. The differences between the means were determined using the Tukey multiple comparison method at the 5% significance level. PCA (Principal Components Analysis) and component biplot analysis were performed using JMP Pro 16 (SAS Institute, Cary, North Carolina) software.

Results and Discussion

The findings obtained from the study regarding the total phenolics were presented in Table 1. The variation in the total phenolics were significant depending on the kernel size of the Kalınkara hazelnut cultivar ($p<0.05$). According to the kernel size, the total phenolics were determined between 759 mg 100 g^{-1} (medium) and 819 mg 100 g^{-1} (large) in the study. In Kalınkara hazelnut cultivar, the total phenolics were determined to be 1006 mg 100 g^{-1} (Göncüoğlu-Taş and Gökmen, 2015), 436.5 mg 100 g^{-1} (Balık, 2021), 709 mg 100 g^{-1} - 1181 mg 100 g^{-1} (Karakaya, 2021), and between 783 - 1098 mg 100 g^{-1} (Karakaya, 2023). In general, the results obtained from the study in terms of total phenolics were found to be compatible with other studies. It is thought that ecological conditions and cultural practices may cause some of the differences. In addition, in this

study, while the total phenolics of the kernels in the small and large groups were higher, it was determined that the medium-sized kernels had the lowest total phenolics. Therefore, in this study, it can be said that kernel size affects the total phenolics. In the study conducted by Yılmaz et al. (2019) in Çakıldak hazelnut cultivar, similar to this study, they reported that the effect of kernel size on total phenolics was significant.

The results obtained from the study regarding the total flavonoids were presented in Table 1. The effect of kernel size on total flavonoids were insignificant ($p<0.05$). The total flavonoids were determined between 8.2 mg 100 g^{-1} (medium) and 8.7 mg 100 g^{-1} (small). In Kalınkara hazelnut cultivar, the total flavonoids were determined to be 12.59 mg 100 g^{-1} (Balık, 2021), 5.07 mg 100 g^{-1} (Göncüoğlu-Taş and Gökmen, 2015), 5.8 mg 100 g^{-1} - 10.6 mg 100 g^{-1} (Karakaya, 2021), and between 7.5 mg 100 g^{-1} - 11.5 mg 100 g^{-1} (Karakaya, 2023). It can be said that the results obtained from other studies in terms of total flavonoids were similar to this study's results. In addition, the effect of kernel size on total flavonoids were insignificant in this study. On the contrary, Yılmaz et al. (2019) reported that the effect of kernel size on total flavonoid was significant in their study with Çakıldak cultivar. It was thought that the difference seen may be due to the nuts' cultivar, ecological conditions or maturity period.

Table 1. Change of total phenolics, and total flavonoids depending on kernel sizes in Kalınkara hazelnut cultivar

Kernel sizes	Total phenolics (mg GAE 100 g^{-1})	Total flavonoids (mg QE 100 g^{-1})
Small	811 a	8.7 a
Medium	759 b	8.2 a
Large	819 a	8.3 a

The difference between the means indicated by the same letter in the column is insignificant ($p<0.05$).

In the study, antioxidant activity was determined according to DPPH and FRAP assays. The findings obtained from the study regarding the antioxidant activity were presented in Table 2. Accordingly, the effect of kernel size on antioxidant activity was found to be statistically significant in the values obtained from both FRAP and DPPH assays ($p<0.05$). Accordingly, small and large kernels were in the same group in the assays. Antioxidant activity was determined between 0.46 mmol 100 g^{-1} (medium) and 0.60 mmol 100 g^{-1} (large) according to the FRAP assay, while it was determined between 1.83 mmol 100 g^{-1} (medium) and 1.92 mmol 100 g^{-1} (small) according to the DPPH assay. In Kalınkara hazelnut cultivar, the antioxidant activity was determined to be 2.37 mmol 100 g^{-1} (according to DPPH), 2.04 mmol 100 g^{-1} (according to FRAP) (Balık, 2021), 0.46 mmol 100 g^{-1} - 1.19 mmol 100 g^{-1} (according to FRAP) and 0.60-1.37 (according to DPPH) (Karakaya, 2021), 0.56 mmol 100 g^{-1} - 1.20 mmol 100 g^{-1} (according to FRAP) and 1.64 mmol 100 g^{-1} - 1.91 mmol 100 g^{-1} (according to DPPH) (Karakaya, 2023). In this study, kernel size was effective in antioxidant activity. Similarly, Yilmaz et al. (2019) also reported that the kernel size had an effect on the antioxidant activity. In addition, as observed in the study results, the results differed according to the different antioxidant assays applied in other studies. Therefore, it is thought that the applied method, ecological conditions, cultural practices and maturity of the kernels may affect the differences observed between the studies.

Table 2. Change of antioxidant activity (FRAP and DPPH) depending on kernel sizes in Kalınkara hazelnut cultivar

Kernel sizes	FRAP (mmol TE 100 g^{-1})	DPPH (mmol TE 100 g^{-1})
Small	0.59 a	1.92 a
Medium	0.46 b	1.83 b
Large	0.60 a	1.91 a

The difference between the means indicated by the same letter in the column is insignificant ($p<0.05$).

The data obtained from the first two components in the principal component analysis explained 100% of the total variation. The first component was associated with total phenolics, flavonoids, FRAP and DPPH, explaining 86.4% of the total variation. DPPH (0.99) and FRAP (0.97) were the most influential parameters on the first component. While the second component explained 13.6% of the variation, total flavonoids (0.65) were the most influential parameter on this component (Figure 1). The correlation (r) showing the relationship between the total phenolics, total flavonoids, and antioxidant activity values determined in Kalınkara cultivar is presented in Table 3.

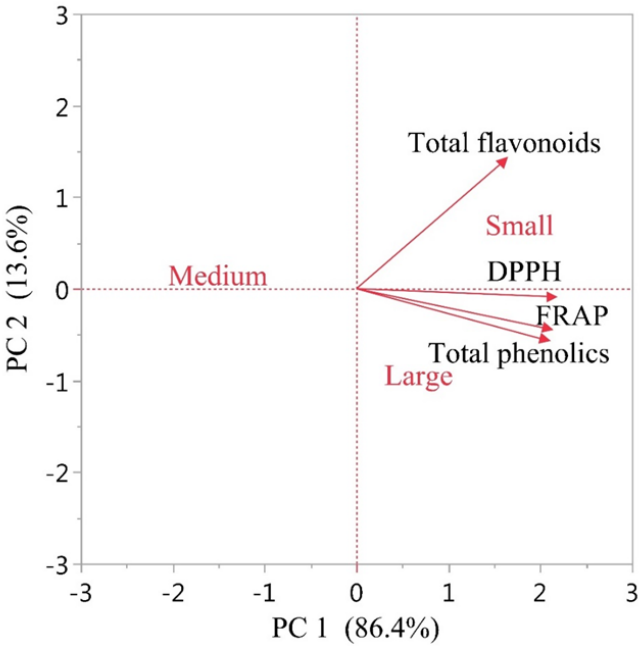

Figure 1. Biplot of the principal components (PC1and PC2) in Kalınkara cultivar based on bioactive compounds

Table 3. Correlation coefficient values of the examined traits in Kalınkara cultivar

Traits	Total phenolics	Total flavonoids	FRAP	DPPH
Total phenolics	1			
Total flavonoids	0.441^{ns}	1		
FRAP	0.982***	0.543^{ns}	1	
DPPH	0.921***	0.417^{ns}	0.873**	1

p<0.01**; p<0.001***, ns: not significant

The correlation between total phenolics and antioxidant activity was determined by DPPH and FRAP methods, and the correlation between DPPH and FRAP was statistically significant (p<0.05). The correlation between total flavonoids, antioxidant activity, and total phenolics was insignificant (p<0.05). Accordingly, in the study, it was determined that there was a positive correlation between total phenolics and antioxidant activity. The correlation coefficient was determined as r=0.921*** according to the DPPH test and r=0.982*** according to the FRAP test. In addition, a positive correlation (r=0.873**) was found between FRAP and DPPH. Similar to the study's results, studies conducted by different researchers have reported a positive relationship between the total amount of phenolic substances and antioxidant capacity in hazelnut (Yılmaz et al., 2019; Yaman et al., 2023).

Conclusion

In the study, it was determined that the total phenolic and antioxidant activity of Kalınkara hazelnut cultivar showed a significant change depending on the kernel size. Bioactive characteristics examined values were higher in small and large kernel sizes than in medium. It was determined that the total phenolics, and antioxidant activity (according to the DPPH and FRAP assays) were higher in small and large kernels. The total flavonoid was not affected by the kernel size. In addition, a positive and strong correlation was found between total phenolic and antioxidant activity. As a result, it has been determined that the products that were not suitable for the hazelnut kernel market due to the size of the nut have rich values in terms of bioactive compounds that positively affect human health. Therefore, it is thought that evaluating these products as additives in the processing industry with different processing techniques will benefit the health and economy.

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