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RESEARCH ARTICLE

# Phytochemical composition and antioxidant activity of some superior walnut (*Juglans regia* L.) genotypes

**Zahra Davarkhah · Mehdi Hosseinifarahi · Mohsen Radi · Sedigheh Ghoilpour · Ali Khadivi**

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**Abstract** Walnut (*Juglans regia* L.) is the most widespread nut tree in the world, and its production has increased worldwide in the last decade. In the present study, the phytochemical composition and antioxidant activity of 14 superior walnut genotypes in different parts of Kohgiluyeh and Boyer-Ahmad province, Iran were studied. Traits, such as oil, fatty acid profile, antioxidant activity, kernel and oil color, mineral elements, and phytochemical properties were assessed. The results showed that different genotypes have significant differences in terms of investigated traits. The average percentage of kernel oil in different genotypes varied from 36.72 to 63.69%, percentage of protein varied from 13.15 to 21.73%, the flavonoid content varied from 18.67 to 45.79 mg/100 g, the total phenol varied from 76.24 to 932.7 mg/100 g, antioxidant activity varied from 11.25 to 35.49%, and ash varied from 1.49 to 2.14%. The predominant fatty acid was linoleic acid ranging from 50.06 to 68.03%, followed by oleic acid (15.63–35.03%), palmitic acid (8.64–11.93%), linolenic acid (2.2–2.75%), and stearic acid (1.11–2.74%). The highest oil percentage belonged to genotype Setangan-1, and the highest total phenol content was observed in genotypes Setangan-1 and Kowkhdan-2. The highest antioxidant activity was related to genotype Kowkhdan-2, and the highest protein and ash contents were related to genotype Deliraj-2. The genotype Delirej-1 had the highest linoleic acid (68.07%), while the genotype Kowkhdan-2 had the lowest amount (50.06%). The highest content of phosphorus (P), potassium (K), sodium (Na), and iron (Fe) was obtained in genotypes Vezg-2 and Delirej-1, respectively. Hence, genotypes Setangan-1, Kowkhdan-2, Delirej-1, Delirej-2, and Vezg-1 are recommended to be used in walnut breeding programs.

**Keywords** Kernel color · Oil · Phosphorus · Potassium · Phenol content

Z. Davarkhah · M. Hosseinifarahi (✉)
Department of Horticultural Science, Yasouj Branch, Islamic Azad University, Yasouj, Iran
e-mail: mehdi.hosseinifarahi@iau.ac.ir

M. Hosseinifarahi · M. Radi
Sustainable Agriculture and Food Security Research Group, Yasouj Branch, Islamic Azad University, Yasouj, Iran

M. Radi
Department of Food Science, Yasouj Branch, Islamic Azad University, Yasouj, Iran

S. Ghoilpour
Department of Chemistry, Yasouj Branch, Islamic Azad University, Yasouj, Iran

A. Khadivi (✉)
Department of Horticultural Sciences, Faculty of Agriculture and Natural Resources, Arak University, Arak 38156-8-8349, Iran
e-mail: a-khadivi@araku.ac.ir

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## Introduction

Since ancient times, the Persian walnut (*Juglans regia* L.) as an important nut species has been cultivated both for its timber and edible nut (Khadivi et al. 2019). Walnut is the most widespread nut tree in the world, and its production has increased worldwide in the last decade. Walnut kernel contains a lot of oil and protein, which are vital for human nutrition. As a result, it is considered an important and planned tree for the human diet and is classified in the list of superior trees of FAO (Shah et al 2018). Nutritiously, walnuts are known for having a high amount of fats, proteins, vitamins, and minerals. Also, walnuts have high amounts of phytochemical and biochemical compounds, such as flavonoids, sterols, phenolic acids, and related polyphenols (Cerda et al 2005). Walnut kernels are rich in antioxidant compounds and have properties and a valuable role in regulating blood fat, cleaning the vessels to prevent blood clots, and regulating the body's immune system. Also, due to the presence of minerals and essential amino acids, it has a vital role in activating the activity of enzymes and living metabolisms and improving human health (Wang et al., 2020).

Among walnut species, Persian walnut is the most cultivated species and the most commercially important species. In addition, walnuts have high nutritional value rich in protein, minerals, and lipids, and contain at least 70% unsaturated fatty acids. Walnut is a common nut in the diet of many countries (Anonymous, 1994). Walnut is a valuable and useful tree that uses all parts of its fruit (Fig. 1).

The walnut industry plays an important role in the world economy, and walnuts are appreciated for their nutritional composition, culinary versatility, and commercial applications (Kafkas et al. 2020). Iran is one of the main centers of origin and diversity of walnuts in the world and is, therefore, one of the main sources of walnut germplasm (Golzari et al. 2013). Therefore, research into genetic diversity, identification, and introduction of superior walnut genotypes is very important. Genetic diversity is an important issue in the development of breeding programs, genetic resource management, and selection of high-quality walnut genotypes from various regions of Iran (Khadivi et al. 2019). In a study, 14 promising walnut genotypes in Kermanshah province, Iran were evaluated based on walnut oil characteristics. The results showed that more than


**Fig. 1** Different parts of walnut

88% of the fatty acids of walnut oil are unsaturated. Linoleic (the dominant fatty acid), oleic, linolenic, palmitic, and stearic acids (1.47–4.67%) are the most important fatty acids in walnut oil (Rasouli and Ershadi 2018).

According to the FAO (2021), the amount of walnut production in the whole world was 3,500,172 tons, and Iran is the third largest producer of walnuts after China and the United States a the production of 386,976 tons (Davarkhah et al., 2023). So, the present study aimed to characterize some important genotypes of walnut in different areas of this province to find commercially and nutritionally superior quality walnuts. Accordingly, the most important phytochemical properties of 14 selected genotypes were evaluated and compared. The results of the present research are not only useful for understanding the quality of different Iranian walnut genotypes but also provide more information for researchers in the program of identifying these genotypes in terms of phytochemical compounds and their use in Iranian walnut breeding programs.

## Materials and methods

### Geographical location

The study was carried out in the year 2020 in seven areas of Kohgiluyeh and Boyer-Ahmad province, Iran, located in the southwest of the country; where the average temperature is 17 °C, the annual average rainfall is 865 mm, and 1870 m above sea level. The distributions of rainfall as well as other weather characteristics of Kohgiluyeh and Boyer-Ahmad province, Iran are given in Table 1. In each area, two genotypes and three trees were considered for each genotype. Also, 100 fruits were taken from each tree to evaluate pomological and phytochemical tests.

### Sources of the plant material

In this research, 14 superior walnut genotypes based on preliminary research and the IPGRI (International Plant Genetic Resources Institute) descriptor were identified and labeled in seven different walnut-growing areas, including Sisakht, Kowkhdan, Delirej, Shahniz, Vezg, Ganjegon, and Setangan (Figs. 2 and 3).

### The characteristics evaluated

During the harvest season, the fruits were harvested in those areas; while the harvesting was done based

**Table 1** Some climatic characteristics of Yasouj, Kohgiluyeh and Boyer-Ahmad, Iran in the 2017–18 crop season (Kohgiluyeh and Boyer-Ahmad Meteorological admistration, Iran, https://www.kbmet.ir/)

| Months of the year | Monthly rainfall (mm) | Monthly humidity (%) | | Monthly temperature (°C) | |
|---|---|---|---|---|---|
| | | Minimum humidity | Maximum humidity | Minimum temperature | Maximum temperature |
| October | 0 | 1 | 51 | 6.4 | 30.4 |
| November | 17.3 | 1 | 100 | −1.2 | 26.8 |
| December | 60.8 | 2 | 100 | −4.2 | 20 |
| January | 16.8 | 1 | 97 | −0.6 | 21.6 |
| February | 33.5 | 3 | 100 | −0.8 | 19.2 |
| March | 98.2 | 2 | 98 | 3.2 | 22.2 |
| April | 66.7 | 1 | 100 | 4.8 | 27.4 |
| May | 129.3 | 2 | 70 | 9.2 | 27 |
| June | 0 | 1 | 40 | 14 | 36.2 |
| July | 0 | 1 | 40 | 14 | 39.6 |
| August | 0 | 2 | 55 | 16.8 | 37.8 |
| September | 2.7 | 1 | 59 | 13.6 | 36.8 |


**Fig. 2** Map of Kohgiluyeh and Boyer-Ahmad province, Iran

on the cracking of the walnut green skin (walnut harvest index). For each genotype, 100 fruits were randomly picked from the entire tree crop. First, the fruit was weighed in the laboratory, and then the green skin was removed and the nuts were dried (drying in the shade and moving the walnuts continuously until all parts were exposed to the air and dried uniformly).

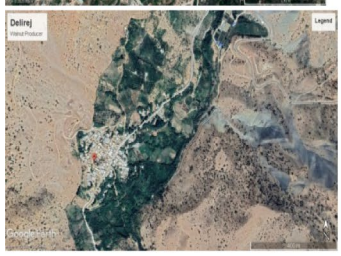
*Total phenolic content*

The total phenolic content was measured using Folin–Ciocalteu (Sigma-Aldrich, USA) colorimetric method (Singleton and Rossi 1965). Standard solutions of gallic acid were prepared with concentrations of 25, 50, 75, and 100 ppm. Then, 0.2 ml of each was transferred to the test tube and 1.8 ml of distilled water, 1 ml of 10% Folin-Siocaltio reagent solution and 2 ml of 7.5% sodium carbonate solution were added to them. It was added, then the tubes were kept for 30 min in the dark and at the temperature of the laboratory, and after that, the amount of light absorption was measured using a UV–visible spectrophotometer (Model UV2100PC) at a wavelength of 765 nm. The results were expressed as mg gallic acid per g of kernel fresh weight (mg GAE/g FW). Gallic acid

Region: Dena
Village: Sisakht
Genotype Code: C1, C2
Altitude (m): 2365
Longitude: 30° 87.200 N
Latitude: 51°.45.791 E


Region: Dena
Village: Kowkhdan
Genotype Code: KO1, KO2
Altitude (m): 2162
Longitude: 30° 83.073 N
Latitude: 51°.48.490 E


Region: Maregoon
Village: Delirej
Genotype Code: D1, D2
Altitude (m): 1626
Longitude: 31° 03.698 N
Latitude: 50°.93.020 E




Region: Maregoon
Village: Shahniz
Genotype Code: Sh1, Sh2
Altitude (m): 1964
Longitude: 30° 03.260 N
Latitude: 51°.03.744 E


Region: Boyerahmad
Village: Setangan
Genotype Code: S1, S2
Altitude (m): 2264
Longitude: 31° 03.698 N
Latitude: 51°.67.316 E


Region: Boyerahmad
Village: Ganjegon
Genotype Code: G1, G2
Altitude (m): 2203
Longitude: 30° 46.799 N
Latitude: 51°.69.984 E


Region: Boyerahmad
Village: Vezg
Genotype Code: D1, D2
Altitude (m): 2060
Longitude: 30° 54.258 N
Latitude: 51°.65.399 E


**Fig. 3** Distribution, geographic characteristics, and google earth images of the investigated genotypes in the main walnut cultivation areas of Kohgiluyeh and Boyer-Ahmad, Iran

was used as the standard for plotting the calibration curve.

*Total flavonoid content*

The total flavonoid content in the kernel was measured using the method of Grzegorczyk-Karolak et al. (2015). Standard solutions with concentrations of 10, 25, 50, and 75 ppm of quercetin were prepared in 60% methanol and 0.5 ml of these solutions were transferred to test tubes and then 0.1 ml of aluminum chloride solution 10% was added to it. Then 0.1 ml of 1 M potassium acetate solution was added to it and the amount of light absorption after 40 min at a wavelength of 415 nm was read for the flavonoid standard and the standard graph was drawn. To measure the flavonoid content, first, 0.1 ml of 10% aluminum chloride was mixed with 0.1 ml of 1 molar potassium acetate and then 2.8 ml of double distilled water was added to them. In the next step, 0.5 ml of the solution of each extract, which was mixed with 1.5 ml of 95% ethanol, was added to the mixture of aluminum chloride, potassium acetate and water. The final mixture for each extract was placed at room temperature for 30 min. Then, the absorbance of the reaction mixture was measured at 415 nm wavelength by Lambda 45-UV/Visible spectrophotometer. The total flavonoid content was

calculated and expressed as mg quercetin per g of dry weight.

*Antioxidant activity*

Antioxidant activity was measured using 2,2-Diphenyl-1-picryl-hidrazil (DPPH) radical scavenging capacity (RSC) method. According to this method, 10 mg of DPPH powder was dissolved in 100 ml of pure methanol. To 50 µl of 1 ml concentration of extract in methanol (100 µl of extract with 900 µl of methanol), 1.5 ml of alcoholic DPPH solution and 1.5 ml of methanol were added and mixed. Also, 1.5 ml of alcoholic DPPH solution and 1.5 ml of methanol were used as control. After 30 min in the dark and ambient temperature, the light absorption of the samples was read at a wavelength of 570 nm by a spectrophotometer against TB containing the control. This test was repeated three times on each sample and the inhibition percentage of DPPH free radicals was calculated using the following formula:

$$\text{DPPH scavenging effect (\%)} = \left(A_{control} - A_{sample}/A_{control}\right) \times 100$$

where $A_{control}$ and $A_{sample}$ represent the control absorbance and the sample absorbance, respectively (Zhu et al. 2009).

*Total protein content*

Protein content was measured using the Kjeldahl method, which includes four steps: collection, digestion, distillation, and titration. The amount of crude protein was calculated, and finally, the results were expressed as the protein percentage. This method shows the amount of nitrogen in the sample and by multiplying the amount of nitrogen by 6.25 the amount of crude protein was calculated. Finally, the results in the form of protein percentage in walnut kernels were reported (Kafkas et al. 2020).

*Percentage of kernel oil*

The lipid content was determined after an extraction using a Soxhlet apparatus with petroleum ether as the solvent for 6 h and measured after the evaporation of the solvent from the extract (Golzari et al. 2013).

*Fatty acid profile*

For the determination of fatty acid profile, the method described by Yazdani et al. (2017) was performed. An Agilent gas chromatograph device (7890, USA), equipped with an FID detector, a capillary column (CPSill-88, Varian, USA, length 100 m, the internal diameter of 0.22 mm), and nitrogen as the carrier gas was used. The temperature program used to separate fatty acid components was set as follows: injection temperature 270 °C, detector temperature 260 °C, and the following temperature program was used for the column: 5 min at 190 °C, the increase in the temperature of the column was at a rate of 5 °C per minute to a temperature of 235 °C; the injection volume was 0.2 µl. Finally, the peaks obtained were identified and measured according to the standard injection of acids. Fatty acids include palmitic acid (16:0), stearic acid (C18:0), oleic acid (18:1), linoleic acid (C18:2), and linolenic acid (18:3).

*Content of mineral elements in kernel*

To prepare the extract for the measurement of minerals and nutrients, such as phosphorus (P), potassium (K), sodium (Na), iron (Fe), and zinc (Zn), the method of dry ashing method in combination with HCL was used (Yaghobifar et al. 2020). For this purpose, 4.0 g of dried walnut kernel samples were ground and poured into a porcelain crucible and then placed in an electric furnace at a temperature of 550 °C for 8 h. The ashes were soaked with a few drops of distilled water and 5 mL of 2 M hydrochloric acid. Then, the crucibles were placed in a boiling water bath until dry. Then, 5 mL of 2 M hydrochloric acid was added again and the solution was poured into a 50 mL volumetric flask using a funnel and brought to volume. The ash solutions in the flasks were passed through filter paper and the obtained extracts were used for reading the elements. The iron and zinc elements were measured using a flame atomic absorption spectrometer device (AAnalyst 200, PerkinElmer, USA). Sodium and potassium were determined by the flame photometric method (AOAC, 2006). Phosphorus was measured with a spectrophotometer (Lambda 25, Perkin Elmer, USA) at a wavelength of 430 nm and calculated based on the standard curve results (Kabas et al. 2007).

*The oil and kernel color*

The color of the oil samples was measured using a digital handheld colorimeter (CR-400, Konica-Minolta, Japan) and reported in the CIE L*a*b* color parameters; where L* represents the lightness/darkness (0: black, 100: white), a* shows red/green (positive values: red, negative values: green), and b* indicates yellow/blue (positive values: yellow, negative values: blue) color scales.

The color of the kernel samples was measured according to the method described by Afshari et al. (2012). The digital image processing method was used to measure the color parameters after capturing the image of the samples under standard light conditions (full-spectrum diffuse light) and analyzing the color parameters using Adobe Photoshop software (version 19, Adobe Inc., USA).

*Ash measurement*

AACC standard number 08–01 was used to measure the ash content of the samples. Briefly, 4 g of the powdered sample was weighed into a porcelain crucible and then burned on a flame, and ashed in an electric furnace with a temperature of 550 °C. After removing the crucible from the furnace, it was weighed again and the percentage of ash was calculated from the following equation.

$$(W2/W1) \times 100 = \text{ash percentage}$$

In this relation, W2 is the weight of the ash, and W1 is the weight of the sample.

Experimental design and data analysis

This research was conducted as a randomized complete block design with three replications and 100 fruits in each replication. Data collected were subjected to analysis of variance (ANOVA) using the statistical software MSTAT-C (Michigan State University, East Lansing, MI), and the means were compared using the DMRT test ($p<0.05$). Different letters in columns of Tables indicate significant differences between values according to the DMRT test ($p<0.05$). Principal components analysis (PCA) was performed to reduce the amount of data and focus on the main variables that justify a significant part of the variation.

## Results and discussion

Kernel oil percentage

Results showed significant differences among the studied genotypes. The highest kernel oil percentage was 63.69% (in Setangan-1 genotype) and the lowest oil percentage was 36.72% (in Delirej-2 genotype) (Table 2). It is well known that several factors such as genotype, geographical origin, altitude above sea level, temperature, soil properties, and agricultural practices can affect the nutritional value and chemical composition of walnuts (Verardo et al. 2009; Sharma et al. 2022). The percentage of oil has been reported from 57 to 72.1% (Yerlikaya et al. 2012). In a study that was conducted to evaluate the oil percentage of 33 walnut genotypes, the results showed that the lowest and highest oil percentages were observed in MKG46 and MSG15 genotypes, respectively, at the rate of 51.93 and 72.95% (Rasouli and Ershadi 2018).

The environment and region of walnut growth, genotype, and maturity of the fruit during harvesting and their interaction affect the percentage of oil and the diversity of unsaturated fatty acids. Among these factors, genotype is the main factor of variation in the amount and composition of fatty acids in walnut oil, and only minor differences have been attributed to environmental conditions and harvest season (Martínez et al. 2010).

**Table 2** The content of kernel oil of 14 top walnut genotypes

| Genotype | Oil (%) |
|---|---|
| Sisakht-1 | 44.22 ± 1.01 g |
| Sisakht-2 | 40.40 ± 0.86 h |
| Kowkhdan-1 | 52.99 ± 1.59 e |
| Kowkhdan-2 | 57.27 ± 1.91 cd |
| Delirej-1 | 39.34 ± 0.81 h |
| Delirej-2 | 36.72 ± 0.76 i |
| Shahniz-1 | 60.05 ± 2.39 b |
| Shahniz-2 | 56.41 ± 1.8 d |
| Vezg-1 | 59.48 ± 2.31 b |
| Vezg-2 | 52.66 ± 1.56 e |
| Ganjegon-1 | 54.02 ± 1.63 e |
| Ganjegon-2 | 58.29 ± 2.2 bc |
| Setangan-1 | 63.69 ± 2.65 a |
| Setangan-2 | 48.00 ± 1.32 f |

In a study, the oil content of 14 genotypes examined in Markazi province, Iran varied between 51 and 73% (Ghasemi et al. 2010). The oil percentage reported in Argentina for Ser, Chandler, and Lara cultivars was 8.72, 5.72, and 2.72%, respectively. Also, in Turkey, the oil content of Sebin cultivar was 69.3% (Yerlikaya et al. 2012). Also, in another study, the highest amount of oil (71.2%) was reported for 'Ser' cultivar (Golzari et al. 2013), and it was between 5.75 and 65.65% in another research (Sharma and Sharma 2001). Çağlarırmak (2003) reported the average percentage of oil among the genotypes investigated in Turkey as 62.84. In addition to the genetic differences related to cultivars, other factors can also affect the amount of oil, including the geographical location and climate (Ozcan 2009). It has been reported that with the increase in height and average temperature of the growing season, the oil content of most cultivars increases (Atefi 1995).

Total phenol and total flavonoid content

The highest total phenol content was observed in Stengan-1 and Kowkhdan-2 genotypes (932.7 and 917.3 mg gallic acid/100 g, respectively), and the lowest total phenol content was observed in Deliraj-1 genotype (76.24 mg gallic acid/100 g). The genotypes of Sisherd 1, Shahneez 1, Shahneez, Stengan-2, Vezg-1, and the genotypes Kowkhdan-1, Ganjegon-1, Tozeg 2, Deliraj-2, and Ganjegon-2 genotypes did not show significant differences (Table 3).

The highest total flavonoid content (45.79 and 40.34 mg quercetin/100 g) was found in Stengan-1 and Kowkhdan-2 genotypes. The lowest flavonoid content (in the range of 18.67–22.38 mg/quercetin/100 g) was observed in Deliraj-2, Kowkhdan-1, Deliraj-1, Ganjegon-1, Tozag-1, and Shahniz-1, Ganjegon-2, and Shahniz-2 genotypes, which didn't show significant differences ($P \geq 0.05$) (Table 3).

Consuming high amounts of phenolic compounds positively affects the human body and potentially prevents diseases related to free radicals (Santos et al. 2013; Cosmulescu et al. 2014). Walnut kernel is very important for human health due to its rich content of phenolic compounds and natural antioxidants (Jahanban-Esfahlan and Amarowicz 2018).

Various factors are effective on the percentage and amount of phenolic compounds. The amount of phenolic compounds in plants is affected by various factors, such as genetic factors, environmental conditions, storage conditions, and even varies between cultivars of the same species. Also, the degree of ripening and the time of harvesting are effective on the phenol content (Rahimipanah et al 2011). The amount of phenol and antioxidant properties of plants in each region depends on many parameters such as climate, soil, altitude, and different types of plants (Mirzaei

**Table 3** Phytochemical characteristics of 14 superior walnut genotypes

| Genotype | Total phenol (mg gallic acid/100 g) | Total flavonoid (mg quercetin/100 g) | Antioxidant activity (%) | Protein (%) | Ash (%) |
|---|---|---|---|---|---|
| Sisakht-1 | 522.4 ± 7.23 bc | 26.73 ± 0.96 cd | 16.82 ± 0.78 de | 15.86 ± 0.57 f | 1.90 ± 0.13 cd |
| Sisakht-2 | 554.9 ± 7.42 b | 29.1 ± 1.01 c | 24.56 ± 0.9 c | 20.70 ± 0.79 b | 1.94 ± 0.14 c |
| Kowkhdan-1 | 252.3 ± 4.76 cd | 19.23 ± 0.7 e | 13.37 ± 0.62ef | 17.73 ± 0.69 d | 2.03 ± 0.16 b |
| Kowkhdan-2 | 917.3 ± 13.1 a | 40.34 ± 1.28 ab | 35.49 ± 1.32 a | 13.33 ± 0.45 h | 1.7 ± 0.1 f |
| Delirej-1 | 76.24 ± 2.2 d | 20.44 ± 0.72 e | 11.25 ± 0.5 f | 16.12 ± 0.6 ef | 1.49 ± 0.06 h |
| Delirej-2 | 305.3 ± 5.21 b-d | 18.67 ± 0.68 e | 14.53 ± 0.69 d-f | 21.73 ± 0.85 a | 2.14 ± 0.18 a |
| Shahniz-1 | 387.4 ± 5.7 bc | 21.3 ± 0.82 de | 16.54 ± 0.75 de | 20.22 ± 0.75 c | 1.85 ± 0.12 de |
| Shahniz-2 | 358.3 ± 5.52 bc | 22.38 ± 0.85 de | 16.65 ± 0.76 de | 17.63 ± 0.68 d | 1.4 ± 0.04 i |
| Setangan-1 | 932.7 ± 13.17 a | 45.79 ± 1.32 a | 25.54 ± 0.92 c | 16.34 ± 0.62 e | 1.51 ± 0.06 h |
| Setangan-2 | 515.6 ± 7.11bc | 35.6 ± 1.22 b | 30.84 ± 1.11 b | 14.32 ± 0.5 g | 1.61 ± 0.09 g |
| Ganjegon-1 | 259.1 ± 4.81 cd | 20.77 ± 0.76 de | 14.08 ± 0.65 d-f | 20.00 ± 0.72 c | 1.82 ± 0.12 e |
| Ganjegon-2 | 322.4 ± 5.33 b-d | 21.6 ± 0.83 de | 14.92 ± 0.7 d-f | 14.72 ± 0.52 g | 1.5 ± 0.06 h |
| Vezg-1 | 498.5 ± 7.03bc | 29.53 ± 1.12 c | 17.64 ± 0.8 d | 13.15 ± 0.43 h | 1.59 ± 0.08 g |
| Vezg-2 | 240.3 ± 4.45 cd | 21.14 ± 0.81 de | 13.37 ± 0.62 ef | 20.15 ± 0.73 c | 1.72 ± 0.1 f |

et al. 2010). It has been reported that the oil content of most walnut cultivars increases with the increase in altitude and the average temperature of the growing season (Atefi 1995). In a study, a significant difference was reported in total phenol, total flavonoid, and antioxidant activity among walnut hybrid genotypes (Kömür et al 2023). In a study, the amount of antioxidant activity of walnut genotypes grown in the Indian Kashmir Valley was reported as 82–97.1 nmol 100 $g^{-1}$. The results of this research corespounded with the findings of some researchers (Erdoğan et al 2021; Ara et al., 2023). Differences in total phenolic content may be due to genetic structure, ecological conditions, fruit ripeness, and cultivation practices (Kömür et al 2023).

Antioxidant activity

The highest antioxidant activity was observed in Kowkhdan-2 genotype (35.49%) and the lowest antioxidant activity was related to the Deliraj-1, Kowkhdan-1, and Vezg-2 (11.25, 13.37, and 13.37%, respectively, Table 3). Several reports pointed to the importance of walnut consumption due to its high antioxidant properties in preventing the occurrence of some diseases. In a study, the antioxidant properties including total phenol, total flavonoid, anthocyanin, hydrogen peroxide radicals, superoxide secretion activity, and DPPH radical scavenging activity of 27 top Iranian walnut genotypes and three foreign cultivars were evaluated and the results showed a significant difference among the examined samples (Soleimani-Sarghashk 2016). The difference in the phenolic content of different walnut cultivars has been reported by Kafkas et al. (2017).

Differences in the amount of antioxidants and phenolic compounds can be related to the differences in genotypes and the ecological conditions of the harvesting area, which affect the accumulation and storage of phenolic compounds, the amount of synthesis, or the type of phenols (Lachman et al. 2010). Temperature affects the production of phenolic and flavonoid compounds in plants (Nicoli et al. 2000). Considering that in the present study, walnut genotypes were collected from different parts of Kohgiluyeh and Boyer-Ahmad province, there is a possibility that environmental factors besides the genotype and their interaction were also effective.

Wang et al. (2020) reported that antioxidant contents in *J. regia* were higher than those in *J. sigillata*. In another study, Gao et al. (2019) found the polyphenol content in *J. regia* was 2–3 times higher than that in *J. sigillata*. The difference in the antioxidant capacity is due to the type of cultivar, maturity stage, harvest year, storage conditions, or antioxidant extraction methods, which are effective parameters related to the biosynthesis of polyphenols (Cerit et al. 2017). The place of origin has an important effect on the quality and especially the phytochemical composition of the regional product (Mo et al., 2022). The results of a four-year study (2006–2003) on eight Hungarian walnut cultivars showed that temperature has a stronger effect on phytochemical compounds compared to cultivars (Bujdoso et al., 2010). Also, differences in phytochemical compositions of different walnuts collected from different geographical regions have been reported by some researchers (Iordănescu et al. 2021; Goodarzi et al. 2023).

Percentage of protein and ash

The highest percentage of protein was related to the genotype Deliraj-2 (21.37%) and the lowest was observed in Vezg-1 and Kowkhdan-2 (13.15 and 13.33%, respectively, Table 3), which was in agreement with the results of other researchers: 14.9–19.86% (Goodarzi et al. 2023), 12.72–20.41% (Iordănescu et al. 2021), 12.25%–20.45% (Akhiani et al. 2017), 14.67%–20.38% (Golzari et al. 2013), 14.70%–20.10% (Akca et al. 2015), and 13.91%–19.04% (Jaćimović et al. 2020). The genotypes Kowkhdan-1 and Shahniz-2, the genotypes Kowkhdan-2 and Vezg-1, the genotypes of Shahniz-1, Ganjegon-1, and Tozag-2, as well as the genotypes of Stengan-2 and Ganjegon-2 did not show significant differences with each other ($P \geq 0.05$). The difference in the amount of protein can be related to the genotype and climatic conditions. Plant proteins play a significant role in human nutrition and oilseeds are valuable sources of fats and proteins (Goodarzi et al. 2023). Walnut is a rich source of high-quality protein and contains 18–24% protein based on dry weight (Sze-Tao et al., 2000). The highest percentage of walnut kernel protein has been reported to be 19% for the Pedro cultivar, and other studied cultivars, including Hartley, Pedro, 260, 230, and Jamal, respectively, contained less protein (Golzari et al.

2013). The highest protein content in 12 walnut genotypes in Markazi province, Iran was 5.14% for MS12 genotype, which is much lower than the result obtained in this study (Ghasemi et al. 2011).

The highest percentage of kernel ash was related to Deliraj-2 genotype (2.14%), and the lowest was related to Shahniz-2 genotype (1.4%, Table 3). Genotypes SiSakhet-1, SiSakhet-2, Kowkhdan-2, and Vezg-2, genotypes Deliraj-1, Stengan-1, and Ganjegon-2, genotypes Shehniz-1, Ganjegon-1, Stengan-2, and Vezg-did not show significant differences ($P \geq 0.05$). The results are consistent with the study made by Iordănescu, et al. (2021) who reported a protein content in the studied walnut genotypes from Romania between 20.41 and 12.73%, and ash between 2.49 and 1.31%. Jan et al. (2022) reported similar values to those obtained in the present work, as 12.8 to 15.8% protein and 1.82 to 1.55% ash.

## Kernel and oil color

There were significant differences ($P < 0.05$) between the color parameters of all the genotypes (Table 4, Fig. 4). The highest amount of L* was observed in the genotypes Vezg-2 and Ganjegon-2 (73.00 and 70.60, respectively), and the lowest in the genotypes Setangan-2 and Delirej-2 (2.56 and 6.56, respectively). Kwokhdan-2, Delirej-1, and Shahniz-2 genotypes, and Setangan-1, Ganjegon-1, and Ganjegon-2 genotypes did not show significant differences ($P \geq 0.05$). The highest amount of the parameter a* was observed in Setangan-2 genotype (18.18) and the lowest in Vezg-2 genotype (8.6). The genotypes Delirej-1, Delirej-2, Setangan-1, Kwokhdan-1, Shahniz-1, 2, Ganjegon-1, and Vezg-1 did not show significant differences (Table 4). The highest amount of the parameter b* was observed in the genotypes Sisakht-2 and Ganjegon-1 (41.2), while the lowest amount was observed in Ganjegon-2 genotype (33.8). Genotypes Sisakht-1, Kowkhdan-1, Kowkhdan-2, Delirej-1, Delirej-2, Shahniz-1, Shahniz-2, Vezg-1, Vezg-2, and genotypes Sisakht-2, Setangan-1, Stengan-2, and Ganjegon-1 did not show significant differences ($P \geq 0.05$).

The highest amount of the L* parameter was observed in Shahniz-1 and Ganjegon-2 genotypes (L* = 76) and the lowest amount in Ganjegon-1 genotype (L* = 56) (Table 4), which this value represents the oils with brighter and darker color, respectively. The genotypes Sisakht-1, Setangon 2, Shahniz-2, and Vezg-1 did not show significant differences. The highest amount of factor a* was observed in genotype Ganjegon-1 (a* = 15) and the lowest in genotypes Shahniz-1 and Ganjegon-2 (a* = 1.33). The genotypes Delirej-2 and Shahniz-2, the genotypes Setangan-1 and Vezg-2, and the genotypes Setangan-2 and Vezg-1 did not show significant differences

**Table 4** The average kernel color of 14 superior walnut genotypes

| Genotype | Kernel color | | | Oil color | | |
|---|---|---|---|---|---|---|
| | $L^*$ | $a^*$ | $b^*$ | $L^*$ | $a^*$ | $b^*$ |
| Sisakht-1 | 58.8 ± 2.6 c-e | 15.2 ± 0.56 ab | 38.2 ± 1.01ab | 66 ± 2.8 h | 8 ± 0.2 c | 48 ± 2.7 de |
| Sisakht-2 | 57.8 ± 2.1 d-e | 16 ± 0.6 ab | 41.2 ± 1.3 a | 73 ± 3.8 c | 4 ± 0.08 g | 51.33 ± 3.1 cd |
| Kowkhdan-1 | 60.2 ± 2.8 c-e | 11.2 ± 0.22 b-d | 36.4 ± 0.87 ab | 75 ± 3.92 b | 2 ± 0.06 i | 40 ± 2.2 f |
| Kowkhdan-2 | 58.4 ± 2.5 c-e | 14 ± 0.4 b-c | 37 ± 0.92 ab | 69 ± 3.3 f | 7 ± 0.18 d | 57 ± 3.4 bc |
| Delirej-1 | 58.4 ± 2.5 c-e | 14.4 ± 0.5 ab | 36.8 ± 0.91 ab | 63 ± 2.5 j | 11 ± 0.3 b | 52 ± 3.2 cd |
| Delirej-2 | 56.6 ± 1.8 e | 15.2 ± 0.56 ab | 39 ± 1.1 ab | 70 ± 3.5 e | 5 ± 0.1 f | 46 ± 2.6 d-f |
| Shahniz-1 | 57.4 ± 1.91 a-c | 11.2 ± 0.22 b-d | 38.4 ± 1.04 ab | 76 ± 4.01 a | 1.33 ± 0.05 j | 60 ± 3.52 ab |
| Shahniz-2 | 62.8 ± 3.12 b-e | 11.8 ± 0.28 b-d | 37.8 ± 1 ab | 68 ± 3.1 g | 5 ± 0.1 f | 44 ± 2.5 ef |
| Setangan-1 | 61.8 ± 3.01 b-e | 14.2 ± 0.47 ab | 40.2 ± 1.22 a | 71 ± 3.6 d | 3 ± 0.07 h | 64 ± 3.7 a |
| Setangan-2 | 56.2 ± 1.7 e | 18.8 ± 0.7 a | 40 ± 1.2 a | 66 ± 2.8 h | 6 ± 0.15 e | 52 ± 3.2 cd |
| Ganjegon-1 | 69.4 ± 3.5 ab | 12.4 ± 0.35 b-d | 41.2 ± 1.3 a | 56 ± 2.1 k | 15 ± 0.5 a | 51 ± 3 cd |
| Ganjegon-2 | 70.6 ± 3.6 ab | 9.4 ± 0.1 cd | 33.8 ± 0.8 b | 76 ± 4.01 a | 1.33 ± 0.05 j | 63 ± 3.65 ab |
| Vezg-1 | 66.8 ± 3.3 a-d | 12 ± 0.3 b-d | 37.6 ± 0.96 ab | 68 ± 3.1 g | 6 ± 0.15 e | 62 ± 3.6 ab |
| Vezg-2 | 73 ± 3.8 a | 8.6 ± 0.09 d | 36.6 ± 0.9 ab | 65 ± 2.7 i | 3 ± 0.07 h | 49 ± 2.8 de |


**Fig. 4** The kernel color of walnut genotypes

($P \geq 0.05$). The higher values of a* represent the oil color with more red tint. The highest b* value was observed in the genotypes Setangan-1, Ganjegon-2, Vezg-1, and Shahniz-1 (64, 63, 62, and 60, respectively), and the lowest amount in the genotype Kowkhdan-1 (40). The genotypes Sisakht-1 and Vezg-2, and the genotypes Sisakht-2, Delirej-1, Setangan-2, and Ganjegon-1 did not show significant differences ($P \geq 0.05$) (Fig. 5).

Color is one of the important characteristics of oils and can be used as a guide in refining edible oils as well as the quality of frying oils (Yam and Papadakis 2004). Color is one of the most important features in determining the quality of walnuts. Although this trait is affected by environmental factors, the cultivar effect is also significant and the amount of color brightness is different in different cultivars. The parameter L* represents the brightness of color and is one of the most important features in determining the quality and the selection of superior genotypes of walnuts. In a study on the superior cultivars and genotypes of Turkey, the percentage of bright kernels varied from 15.28 to 61.41% (Bayazit and Sumbul 2012). Walnut kernel color is important in marketability so Iranian people prefer light brain color and American people prefer amber brain color (Soveili and Khadivi 2023). Most of Iran's walnut trees have been propagated through seeds, which has been achieved due to the

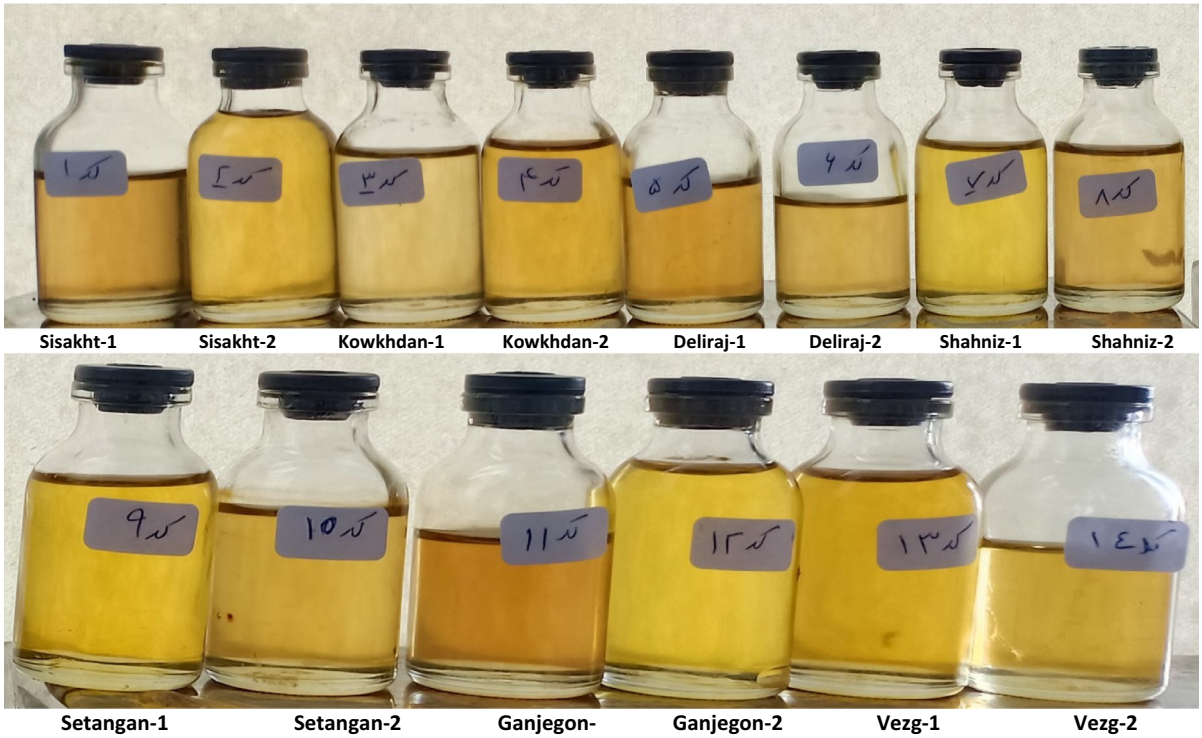



**Fig. 5** The color of walnut oil in walnut genotypes

heterozygous nature of the walnut and a wide variety among its genotypes, and there has been variation in its important horticultural characteristics such as kernel color. The kernel color is one of the desired attributes of the consumer and is decisive in the valuation of the product price. Genotypes that grow in cold and mountainous areas are mostly light in color (Jan et al. 2022). The color and thickness of the hard skin are important traits in the walnut market, and most of the evaluated genotypes have light to light to light-amber color kernels and medium skin thickness (Karamatlo et al. 2016). In another study, Kouhi et al, (2020) reported that the selected superior genotypes had light kernel color which was easily removed from the shell. Light kernel color and other nut quality characteristics are important traits to select superior genotypes (McGranahan and Leslie, 1990).

Fatty acid profile of the oil

The 6 genotypes were selected based on the characteristics of the walnuts that are nutritionally and commercially important. The highest percentages of palmitic acid were observed in the genotypes Shahniz-1, Delirej-1, and Kowkhdan-2 (11.93, 11.69 and 11.6%, respectively), and the lowest percentages of palmitic acid were observed in the genotypes Vezg-1, Setangan-1, and Ganjegon-2 (8.64, 8.78, and 9.5%, respectively) (Table 5). The highest percentage of stearic acid was observed in Shahniz-1 genotype (2.74%) and the lowest percentage was observed in Kowkhdan-2, Ganjegon-2, and Setangan-1 genotypes (1.11, 1.303, and 1.357%, respectively). The highest percentage of oleic acid was observed in Kowkhdan-2 genotype (35.03%), and the lowest percentage was observed in Delirej-1 and Shahniz-1 genotypes (15.63 and 17.56%, respectively). The highest percentage of linoleic acid was observed in Delirej-1 genotype (68.07%), and the lowest percentage was observed in Kowkhdan-2 genotype (50.06%). There were no significant differences between Shahniz-1 and Vezg-1 genotypes ($P \geq 0.05$). Linoleic acid content of all 6 selected genotypes was in the range of 2.20–2.75% which was not significantly different between the studied genotypes ($P \geq 0.05$).

**Table 5** Fatty acids content in six superior walnut genotypes

| Genotype | Palmitic acid (%) | Stearic acid (%) | Oleic acid (%) | Linoleic acid (%) | Linolenic acid (%) | Total unsaturated fatty acids (%) |
|---|---|---|---|---|---|---|
| Kowkhdan-2 | 11.60 ± 0.2 a | 1.11 ± 0.01 c | 35.03 ± 0.78 a | 50.06 ± 2.8 d | 2.20 ± 0.04 a | 87.29 ± 3.01 b |
| Delirej-1 | 11.69 ± 0.21 a | 1.87 ± 0.07 b | 15.63 ± 0.1 d | 68.07 ± 3.8 a | 2.74 ± 0.08 a | 86.44 ± 2.9 bc |
| Shahniz-1 | 11.93 ± 0.23 a | 2.74 ± 0.09 a | 17.56 ± 0.12 d | 65.18 ± 3.7 ab | 2.59 ± 0.06 a | 85.33 ± 2.8 c |
| Setangan-1 | 8.78 ± 0.16 b | 1.35 ± 0.04 c | 29.52 ± 0.56 b | 57.99 ± 3.2 c | 2.35 ± 0.05 a | 89.86 ± 3.5 a |
| Ganjegon-2 | 9.5 ± 0.18 b | 1.30 ± 0.04 c | 24.96 ± 0.35 c | 61.47 ± 3.5 bc | 2.75 ± 0.08 a | 89.18 ± 3.3 a |
| Vezg-1 | 8.640 ± 0.15 b | 1.63 ± 0.06 b | 23.21 ± 0.3 c | 63.78 ± 3.6 ab | 2.74 ± 0.08 a | 89.73 ± 3.47 a |

The profile of fatty acids showed that unsaturated fatty acids were dominant in the oil of walnut genotypes. Linoleic acid, oleic acid, and palmitic acids, respectively accounted for the highest percentage of fatty acids in walnut genotypes. Considering the high oil content of walnut kernels (37–64%), fatty acid compositions are important in terms of nutrition and their positive effects on human health. Therefore, to introduce superior walnut genotypes, in addition to evaluating the pomological characteristics of the fruit, phytochemical characteristics and fatty acids should also be studied.

It has been reported that although high values of the ratio of unsaturated fatty acids with multiple double bonds cause the oxidation and rancidity of walnut oil, this high ratio has a direct and significant relationship with the nutritional properties of walnuts and human health (Bouabdallah et al. 2014). The presence of unsaturated fatty acids, especially omega-6 (linoleic acid) and omega-3 (linolenic acid) plays a significant role in human health and prevents many dangerous diseases such as Alzheimer's, heart attack, blood clots, and depression (Bourre 2004). Considering the variable composition of fatty acids in walnut cultivars and genotypes, these compounds can also be used as selective traits. In addition to the genetic differences related to cultivars, other factors can also affect the quality of walnut oil, such as geographical location, climatic effects, the rate of fruit ripening, and the way they are harvested and stored (Ebrahimi et al. 2010).

In the study conducted by Yazdani et al. (2017) on different walnut genotypes, the amount of oil in different walnut cultivars was between 54.4% and 72.1%. In addition, more than 90% of walnut fatty acids were unsaturated fatty acids, and the highest amount was related to linoleic acid, which was consistent with the results obtained in the present study.

Shafaei Chorush and Arzani (2018) showed that more than 88% of the fatty acid composition of walnut oil is of unsaturated type; whereas the studied walnut kernel oils consisted of linoleic acid (38–57.39%), oleic acid (20.67–41.29%), linolenic acid (7.84–12.89%), palmitic acid (6.7–33.9%) and stearic acid (1.47–4.67%), which those values were consistent with the results of the present study except for palmitic acid, which was more than linolenic acid.

In general, it was determined that the top six genotypes introduced in this research, while being superior and promising in terms of important morphological and biochemical traits of walnut, have very high amounts of mono-unsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) and can be very important in terms of nutrition and human health. Undoubtedly, paying attention to these genotypes and using them in future breeding programs will provide the possibility of introducing Iranian walnut cultivars with performance, quantity, and quality as well as high nutritional value.

Nuts are nutritionally and economically important due to their fatty acids. Among nuts, walnut oil is a rich source of omega-3 and omega-6 unsaturated fatty acids and has been considered a healthy and unique diet. Eating walnut kernels protects the heart against coronary artery diseases (Muradoglu et al. 2010). Due to the chemical composition of fatty acids, walnut kernels are nutritionally and economically important and have a positive effect on human health (Shafaei and Arzani 2017). The percentage of oil and the composition of fatty acids in walnut oil are different

among genotypes, and this feature is important in distinguishing the difference between local genotypes and identifying the genotypes that have fatty acids with the best nutritional quality (Ozcan 2009).

The content of mineral elements in kernel

The content of mineral elements in the kernel of walnut genotypes showed significant differences ($P \geq 0.05$) (Table 6). The amount of phosphorus was different from 8.94 to 26.03 mg/*100 g*, so the highest content of phosphorus was 26.03 mg/100 g in Vezg-2, and the lowest was observed in Delirej-1 genotype (8.94 mg/100 g) (Table 6). The highest amount of potassium was 614.1 mg/*100 g* (in Vezg-2 genotype) and the lowest in Delirej-1 (279.3 mg/100 g). The content of Fe in kernel was between 20.08 mg/100 g in genotype Delirej-2 and 6.58 mg/100 g in genotype Sisakht-1 with an average value of 10.36 mg/100 g. The Zn content varied between 9.01 in Delirej-2 and 2.2 mg/100 g in Delirej-1 genotypes. The highest and lowest content of Na was 107.143 (in Vezg-1) and 31.92 mg/100 g (in Delirej-2).

The quality of walnut fruit and its kernel is strongly influenced by genotype and environment and the interaction between them. Walnut kernel is known for its high nutritional and economic value, and its kernel is usually considered a rich source of minerals and essential amino acids. On the other hand, mineral elements have been identified as valuable compounds in walnut kernels. Potassium, phosphorus, magnesium, and iron are found in significant amounts in this important nut. Calcium, sodium, zinc, and copper are also present in moderate amounts (Meizhi et al. 2014). In a study, the amount of some mineral elements, including potassium (4627.6 mg/kg), phosphorus (3621.9 mg/kg), sodium (44.7 mg/kg), magnesium (1089.9 mg/kg), and manganese (46.3 mg/kg) have been reported (Ozcan 2009).

Also, some researchers have reported the amount of mineral elements such as phosphorus: 280–380 mg/100 g, potassium: 230–340 mg/100 g, and magnesium: 81–99 mg/100 g in fresh walnut kernels (Çağlarırmak 2003). Previous research showed that there are 5 types of main mineral elements (potassium, phosphorus, magnesium, calcium, and manganese) in the kernels of walnut cultivars 'Franquette' and 'Hartley' from France and California, respectively, in which the content of potassium, phosphorus, and magnesium to pay attention to factors such as types of walnuts, growing conditions, and climate had significant differences (Martínez et al. 2010).

Hamidi et al. (2015) reported that the mineral content of walnut kernels in three genotypes Zh1, Zh2, and Zh3 contained sodium (443 mg/100 g), calcium (67 mg/100 g), magnesium (175 mg/100 g),

**Table 6** Content of mineral elements in kernel of 14 superior walnut genotypes

| Genotype | Content of minerals in kernel (mg/100 g) | | | | |
|---|---|---|---|---|---|
| | P | K | Fe | Zn | Na |
| Sisakht-1 | 17.14 ± 0.5 f | 574.5 ± 9.1 c | 6.58 ± 0.03 g | 3.03 ± 0.01 de | 88.94 ± 3.3 d |
| Sisakht-2 | 21.72 ± 0.82 b | 549.7 ± 7.5 f | 8.99 ± 0.05 f | 4.01 ± 0.06 c | 96.10 ± 3.6 c |
| Kowkhdan-1 | 15.787 ± 0.4 g | 515.7 ± 6.7 h | 11.02 ± 0.09 d | 6.92 ± 0.08 b | 84.24 ± 3.01 e |
| Kowkhdan-2 | 17.157 ± 0.5 f | 464.1 ± 5.5 k | 11.04 ± 0.1 d | 3.95 ± 0.05 c | 78.22 ± 2.4 g |
| Delirej-1 | 8.947 ± 0.1 j | 279.3 ± 4.4 n | 3.75 ± 0.01 h | 2.02 ± 0.006 f | 32.39 ± 0.84 m |
| Delirej-2 | 18.073 ± 0.6 de | 553.1 ± 8.1 e | 20.08 ± 0.8 a | 9.01 ± 0.1 a | 31.92 ± 0.82 m |
| Shahniz-1 | 12.19 ± 0.21 i | 409.8 ± 5.2 l l | 6.84 ± 0.04 g | 3.87 ± 0.04 c | 43.52 ± 0.91 l j |
| Shahniz-2 | 14.923 ± 0.32 h | 345.9 ± 4.8 m | 12.29 ± 0.17 bc | 2.50 ± 0.008 ef | 61.70 ± 1.8 i |
| Setangan-1 | 18.527 ± 0.62 d | 571.6 ± 8.7 d | 11.59 ± 0.12 cd | 3.23 ± 0.03 d | 82.16 ± 2.8 f |
| Setangan-2 | 11.893 ± 0.2 i | 468.5 ± 5.7 j | 9.1 ± 0.06 f | 3.11 ± 0.02 d | 98.24 ± 3.81 b |
| Ganjegon-1 | 17.587 ± 0.55 ef | 505.1 ± 6.1 i | 10.15 ± 0.07 e | 3.98 ± 0.05 c | 52.18 ± 1.01 k |
| Ganjegon-2 | 20.613 ± 0.71 c | 602.4 ± 9.7 b | 11.92 ± 0.14 c | 2.93 ± 0.01 de | 74.42 ± 2.2 h |
| Vezg-1 | 21.63 ± 0.77 b | 544.4 ± 7.1 g | 9.08 ± 0.06 f | 2.92 ± 0.01 de | 107.14 ± 3.96 a |
| Vezg-2 | 26.033 ± 0.9 a | 614.1 ± 10.1 a | 12.74 ± 0.2 b | 3.28 ± 0.03 d | 56.89 ± 1.3 j |

iron (2.63 g/mg), manganese (2.51 g/mg), zinc (2.55 g/mg), sodium (0.82 g/mg), and phosphorus (443 g/100 mg). In a study conducted in China, the content of calcium was between 155.10 and 482.03 mg/100 g with an average of 288.42 mg/100 g in *J. sigillata* species. Also, calcium content varied between 253.90 and 504.07 mg/100 g in *J. regia* with an average of 352.18 mg/100 g (Meizhi et al 2014).

Correlation between variables

The oil percentage did not show significant correlations with other traits. However, it showed negative and non-significant correlations with protein, calcium, and zinc, and positive and non-significant correlations with total flavonoid, DPPH, total phenol, and Na. Protein also had no significant correlation with the studied traits. These traits showed negative and insignificant correlations with total flavonoid, DPPH, and total phenol traits and negative and insignificant correlations with Ca and Zn. A positive and highly

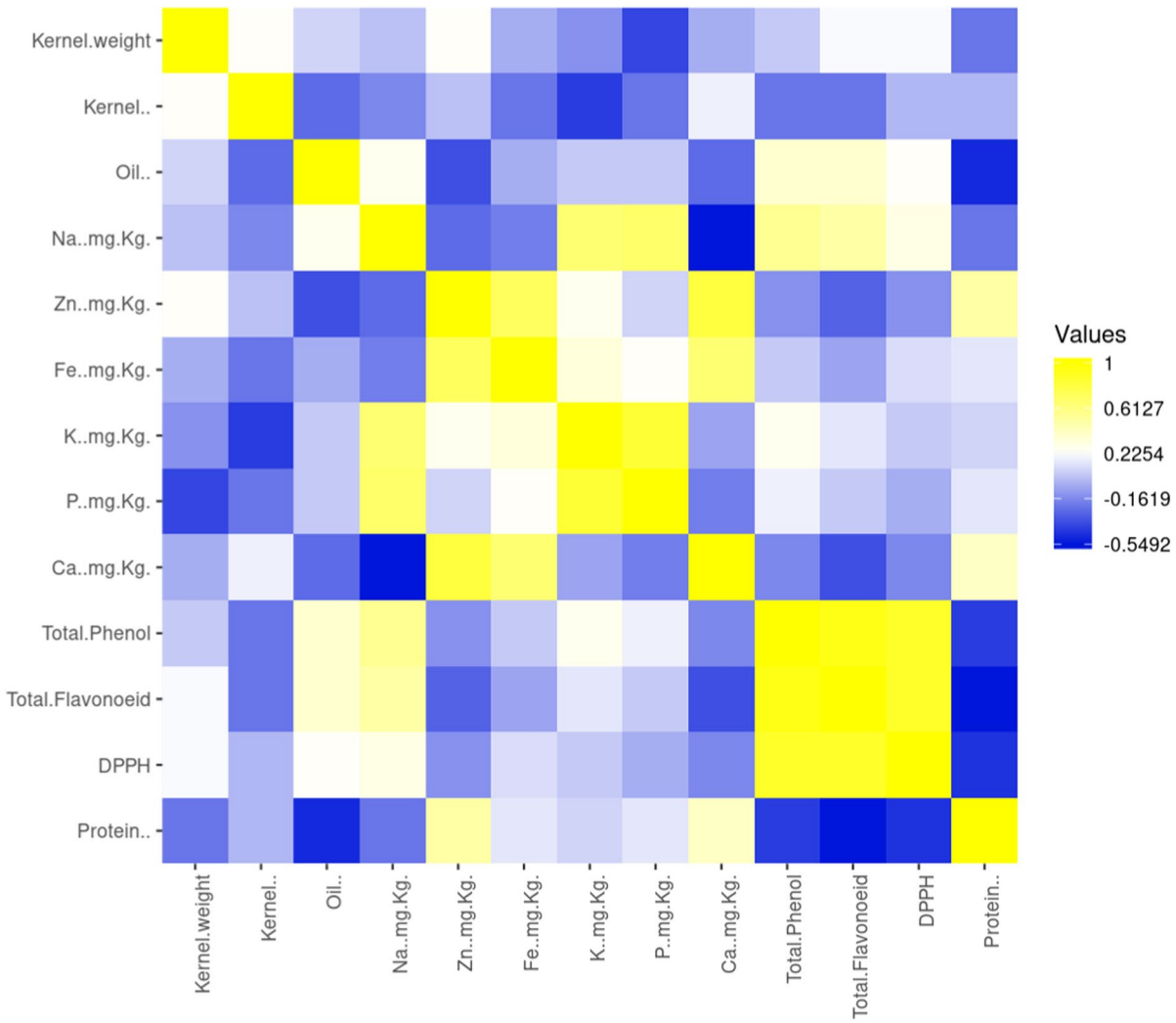



**Fig. 6** Correlations between variables measured in walnut genotypes

**Table 7** Principal component analysis (PCA) based on various traits evaluated in walnut genotypes

| Trait | PC1 | PC2 | PC3 |
|---|---|---|---|
| Oil | **0.49** | −0.26 | 0.06 |
| Na | 0.36 | −0.33 | **0.80** |
| Zn | −0.22 | **0.90** | 0.10 |
| Fe | 0.08 | **0.86** | 0.16 |
| K | 0.06 | 0.21 | **0.92** |
| P | −0.05 | 0.04 | **0.95** |
| Ca | −0.19 | **0.88** | −0.27 |
| Total phenol | **0.92** | 0.05 | 0.24 |
| Total flavonoid | **0.94** | −0.13 | 0.14 |
| DPPH | **0.92** | 0.09 | 0.00 |
| Protein | **−0.64** | 0.38 | 0.14 |
| Eigenvalue | 3.44 | 2.72 | 2.58 |
| % of variance | 31.29 | 24.70 | 23.46 |
| Cumulative % | 31.29 | 55.99 | 79.45 |

Bold values indicate the characteristic that most influence each PC

significant correlation was observed between total flavonoid trait and DPPH. These traits showed a negative and non-significant correlation with the traits Ca and Zn and a positive and significant correlation with the trait Na. The correlation between DPPH and total phenol was positive and very significant. This is even though other traits did not show significant correlations with these traits. Total phenol showed positive and significant correlation only with Na and no significant relationship was observed for this trait with other traits in this study. P showed positive and highly significant correlations with K and Na. K also had a positive and significant correlation with trait Na. Ca had a positive and highly significant correlation with Zn and a significant correlation with Fe. This is while the correlation of this attribute with Na was negative and significant. The trait Zn also showed a positive and highly significant correlation with Fe (Fig. 6).

Kernel L* showed a negative and highly significant correlation with a*. This trait had a negative but non-significant relationship with kernel b* and oil L*. The kernel L* did not show a significant relationship with two other traits, i.e. oil a* and b*. The relationship between two attributes, kernel a* and b* was positive and significant. This is the highest correlation observed between the two traits in this study. The a* had a negative but insignificant correlation with two traits of oil L* and b*, as well as a positive and non-significant correlation with the trait kernel b*. The oil L* showed a negative and highly significant correlation with a*. This attribute had a negative and insignificant correlation with other attributes except oil L*. This is while the correlation of the oil a* trait with other traits except oil L* was positive but non-significant. The oil b* trait did not show any significant relationship with other traits in this study (Fig. 6).

### PCA

Based on PCA, the first three components explained 79.45% of the total variance (Table 7). The first component (PC1) included kernel oil, total phenol, total flavonoid, DPPH, and protein, with explaining 31.29% of the total variance. The second component (PC2) justified 24.70% of the total variance and included Zn, Fe, and Ca. The third component (PC3) explained 23.46% of the total variance and included Na, K, and P. According to the results PCA, kernel oil, total phenol, total flavonoid, DPPH, and protein had the largest share of the variance of the traits, and therefore have a more favorable variety for selection and to achieve the desired oil.

## Conclusions

The quality of walnut fruit and its kernel is strongly influenced by the species, the environment, and the interaction between them. Walnut is known for its high nutritional and economic value, and its kernel is usually considered a rich source of minerals and essential amino acids. On the other hand, mineral elements have been identified as valuable compounds in walnut kernels. Walnuts are a rich source of fatty acids composition (high linoleic acid and oleic acid), phenol content, antioxidant activity, and protein in the diet. The present results indicated that Setangan-1, Kowkhdan-2, Delirej-1, Delirej-2, and Vezg-1 genotypes are rich in minerals, fatty acids, and bioactive compounds. Therefore, these genotypes with high nutritional value and health-important traits can be used as genetic material in walnut breeding programs and to develop new walnut cultivars.

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**Data Availability** The data that support the findings of this study are available from the corresponding author upon reasonable request.

**Declarations**

**Competing interests** The authors declare no competing interests.

**Consent for publication** Not applicable.

**Informed consent** Not applicable.

**Research involving Human Participants and/or Animals** Not applicable.

**Statement on experimental research and field trials on plants** The either cultivated or wild-growing plants sampled comply with relevant institutional, national, and international guidelines and domestic legislation of Iran.

**Statement specifying permissions** For this study, we acquired permission to study walnut issued by the Agricultural and Natural Resources Ministry of Iran.

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