© by PSP Volume 30– No. 06A/2021 pages 6713-6727 Fresenius Environmental Bulletin

# BIOCHEMICAL AND BIOACTIVE CONTENT IN FRUITS OF WALNUT (*JUGLANS REGIA* L.) GENOTYPES FROM TURKEY

**Ummugulsum Erdogan[1,*], Sanem Argin[2], Metin Turan[3], Ramazan Cakmakci[4], Murat Olgun[5]**

[1]Bayburt University, Faculty of Engineering, Department of Food Engineering, Bayburt, Turkey
[2]Yeditepe University, School of Applied Sciences, Department of Agricultural Trade and Management, Istanbul, Turkey
[3]Yeditepe University, Faculty of Engineering, Department of Genetics and Bioengineering, Istanbul, Turkey
[4]Canakkale Onsekiz Mart University, Faculty of Agriculture, Department of Field Crop, Canakkale, Turkey
[5]Eskisehir Osmangazi University, Faculty of Agriculture, Department of Field Crop, Eskisehir, Turkey

## ABSTRACT

Walnuts (Juglans regia L.) are a good source of dietary minerals and contain significant amounts of potassium, phosphorus, magnesium and iron. In Turkey, walnut orchards are found in the Çoruh Valley and are generally composed of local varieties. Since chemical and physical properties of walnuts are very important in determining their quality, in this study, nut and kernel characteristics, fatty acid composition and phenolic composition were determined for 26 walnut genotypes collected from the valley. Total fat content was found to be between 53.75% and 71.43%, while the crude protein amount ranged from 10.21% to 20.71%. Total carbohydrates were calculated between 14.31-27.52% and ash content was found to be between 1.64% and 3.32%. The oleic acid content and linoleic acid content ranged from 18.34 to 25.58% and 37.09% to 87.51%, respectively. The linolenic acid content was determined between 5.52% and 11.03%. Our study showed that, differences in phenolic component, crude protein and fatty acid content of walnuts were caused by different growing conditions. Genotypes have a rich and stable structure in terms of fruit quality parameters, minerals, organic acids, phenolic composition, antioxidant and fatty acids amounts and amino acids. In other words, these characteristics did not show much variation in genotypes grown in the Valley. However, there was a great variation in hormone levels among genotypes. In terms of the parameters examined, GN2, GN10, GN15, GN24, GN25 and GN26 genotypes were determined as rich and stable genotypes.

**KEYWORDS**:
Amino acid, hormone, nutrient element, antioxidant and fatty acids, biplot analysis, walnut

## INTRODUCTION

Walnut (Juglans regia L.) is the most economically important species and the oldest cultivated fruit in the world. Walnut is native to a wide region in USA, European and Asian countries [1]. Total world production of walnuts is 3 million 663 thousand tons. The production of China, the world's largest walnut producer, reached 1.6 million tons in 2018. China realized 43.3 percent of the total world walnut production in 2018. Iran follows China with 16.7 percent share. The third biggest producer is the United States of America with 620 thousand tons which corresponds to 11.2 percent of the global walnut production. Turkey ranks fourth with 5.9 percent share and 215 thousand tons of production [2].

Turkey is a germplasm center of walnut since Anatolia was on the historical migration routes. Many different genotypes were brought to Anatolia throughout the history via traveling traders along the Silk Road and walnut trees are exceptionally abundant within almost all regions in Anatolia. The local walnut populations are not scattered, rather grown in indoor gardens in the Çoruh Valley. Continuous seed propagation for thousands of years in Turkey has given rise to a great number of seedling walnut trees, which stands as a valuable walnut gene resource [3]. The seeds from very ancient times showed different characteristics and in time adapted to the ecological conditions of the region, which formed a wide genetic variation. In this genetic variation, it is extremely important to ensure that the types that are highly qualified are protected by reproduction. Owing to its geological, geomorphological and climatic characteristics, Çoruh Valley has a rich biological diversity. The Valley is located within the Caucasus Ecoregion, which is among the 200 ecological regions of the world designated by the World Wildlife Fund (WWF) [4,5]. Çoruh Basin (Figure 1) has a surface area of 19 748 km$^2$ and located in the northeast of Turkey (39°40' to 42°35' longitude and 39°52' to 41°32' latitudes).

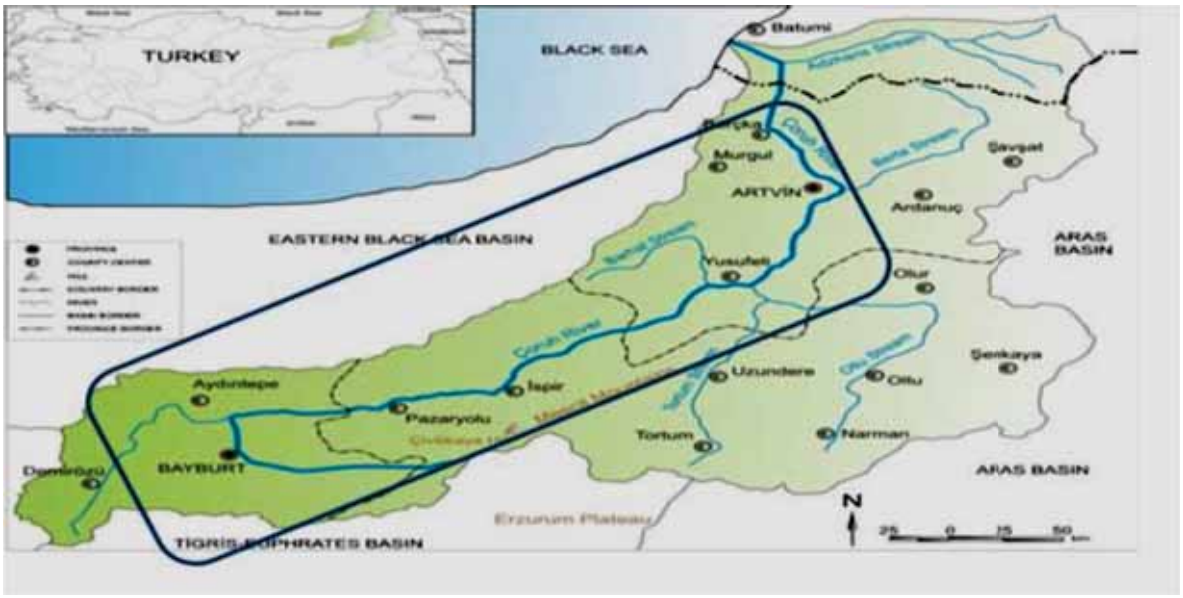


**FIGURE 1**
**Çoruh Valley**

Walnut tress have significant economic value due to the quality of the wood and nutritious nuts. The leaves and the nuts also have medicinal importance for their significant pharmacological value [6]. Walnuts are rich in vitamin E [7], ω-3 fatty acids [8] and dietary fibers [6], which provide many health benefits when consumed in sufficient amounts [9, 10, 11].

Walnut oil (up to 740 g kg-1 in some commercial varieties) is largely composed of unsaturated fatty acids, particularly α-linolenic acid (ALA; 18:3n−3) and linoleic acid (LA; 18:2n−6), and can be consumed without refining [12]. Tocopherols are the phenolic compounds found in walnuts that posses vitamin E (antioxidant) activity [13]. Walnuts are also rich in phytosterols which are important dietary components that contribute to reducing serum cholesterol levels [12].

Chemical and mineral contents of walnuts can vary by variety, genotype, ecology, agricultural practices, climate, and soil conditions. Some genotypes may have a better effect on human metabolism due to their nutrient compositions. The aim of this study was to determine walnut fruit quality parameters such as weight, crude protein, phenolic, fatty acid, amino acid, organic acid and hormone contents to elucidate the physicochemical characteristics and antioxidant capacity of twenty-six walnut genotypes from Çoruh Valley, Turkey.

## MATERIALS AND METHODS

**Plant material.** Walnut samples were collected from inside the native walnut population of Çoruh Valley, in Turkey. In the Valley, walnut has been widely cultivated since long years. Different districts were visited. The walnut fruits were harvested randomly taken from the same trees, which were all between 20 and 40 years old, in September during 2019-2020. Walnut fruits were immediately transported to the laboratory and held in an oven 3 days at 30 °C, after harvest. Physical analyses were quickly determined. The fruits were stored in the shell, closed in plastic bags, and frozen to -20 ºC, until the analyses. Chemical analyzes were performed in three, physical analyzes were performed in ten replications.

**Analysis of fatty acids.** Fatty acid composition of the air-dried seed walnut samples was determined using the modified fatty acid methyl ester (FAME) method [14]. The oil was extracted three times from 2 g samples by homogenizing with petroleum ether. For FAME, 1 ml of methylation reagent (80 ml of methanol + 0.5 g of sodium methoxide + 20 ml of isoktan) was added to 50 mg oil. The mixture was vortexed and left at room temperature for 24 hours; then 0.25 ml of isocene was added. Samples were centrifuged at 2400-x g for 5 minutes at 5 °C and the liquid was transferred to labeled Wheaton flasks and stored at 20 °C. FAME of the fatty acids (0.5 µl) were analyzed with a gas chromatograph (Perkin Elmer Auto System XL, USA) equipped with a fused silica capillary column (MN FFAP (50 m x 0.32 mm i.d.; film thickness 0.25 µm), flame ionizing detector (FID). Operated under the following conditions: oven temperature program, 120 ºC for 1 min, then raised to 240 ºC at a rate of 6 ºC/min and kept at 240 ºC for 15 min; injector and detector temperatures, 250 and 260 ºC; respectively, carrier gas, helium at a flow rate of 14 psi; split ratio, 1/20 ml/min. The contents of palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2) and linolenic (C18:3) acids were determined with a computing Integrator.

**Amino acid analysis.** 0.1 N HCl was added to one gram of fresh sample, homogenized with ultra-turraks, and incubated at 40 ºC for 12 hours. Samples were then vortexed, centrifuged at 1200 rpm for 50 minutes, and the supernatants filtered through 0.22 µm filters (Millex Millipore). Then supernatants were transferred to glass vials and sent for HPLC analysis. Amino acids were extracted from the samples and were analyzed as described by Antoine et al. [15]. The amino acid derivatives were analyzed by HPLC on a Zorbax Eclipse-AAA 4.6 x150 mm, 3.5 µm columns (Agilent 1200 HPLC). The samples were analyzed by measuring the absorbance at 254 nm, and the amino acids were identified by comparison with standards. O-phthaldialdehyde (OPA), fluorenylmethyl-chloroformate (FMOC) and 0.4 N Borate was used for derivation processes in an auto sampler. The following were used as the mobile phase in the chromatography system: mobile phase. A: 40 mM NaH2PO4 (pH 7.8) and mobile phase B: Acetonitrile/Methanol/Water (45/45/10, v/v/v) solutions. The flow rate of the mobile phase was 2 mL min−1 and the column temperature was 40 ºC. Aspartate, glutamate, asparagine, serine, glutamine, histidine, glycine, threonine, arginine, alanine, tyrosine, cysteine, valin, methionine, tryptophan, phenylalanine, isoleucine, leucine, lysine, hydroxyproline, sarcosine, proline quantities from PGPR samples were determined as pmol/µl after 26 minutes derivation process in HPLC.

**Organic acid analysis.** Deionized water (10 ml) was added to one gram of fresh, and homogenized with an ultra-turraks. The solution was centrifuged at 1200 rpm for 50 minutes, then the supernatants were filtered through 0.22 µm filters (Millex Millipore). The supernatants were transferred to glass vial and sent for HPLC analysis. The organic acids were analyzed by HPLC on Zorbax Eclipse-AAA 4.6 x 250 mm, 5 µm columns (Agilent 1200 HPLC) and absorbance of 220 nm in UV detector. Flow rate was 1 ml min-1 and column temperature was 25 ºC. Oxalic, propionic, tartaric, butyric, malonic, malic, lactic, citric, maleic, fumaric, and succinic acids were determined using 25 mM potassium phosphate (pH 2.5) as the mobile phase.

**Hormone analysis.** Extraction and purification processes were performed according to Battal and Tileklioglu [16]. First, methanole (%80) was cooled to -40 ºC and added to fresh samples [17]. After the mixture was homogenized with ultra-turraks for 10 minutes and then incubated for 24 hours in the dark. The samples were filtered with (Whatman No:1) and then supernatants were filtered although 0.45 µm filters [18]. Supernatants were evaporated to dryness at 35 ºC by evaporator pumps. Dried supernatants were solved using 0.1 M KH2PO4 (pH 8.0). Extracts were centrifuged at 5000 rpm for 1 hour at 4 ºC to separating fatty acids [19]. Polvinilpolipirilidon (PVPP), 1 g, was prepared and added to supernatants to separate phenolic and colored matters [20]. Supernatants with PVPP were filtered with What-man No:1 filter paper to remove PVPP [21]. For further specific separation, a Sep-Pak C-18 (Waters) cartridge was used. Hormones adsorbed by the cartridge were transferred to vials using 80% methanol. The hormones were analyzed by HPLC on a Zorbax Eclipse-AAA C-18 column (Agilent 1200 HPLC) and absorbance measured at 265 nm in a UV detector. The flow rate was set to 1.2 ml/min and at column, temperature was 25 ºC. Gibberellic acid, salicylic acid, indol-3-acetic acid (IAA), abscisic acid (ABA) was determined by using 13% acetonitrile (pH 4.98) as the mobile phase.

**Element Analysis.** The Kjeldahl method and a Vapodest 10 Rapid Kjeldahl Distillation Unit (Gerhardt, Konigswinter, Germany) were used to determine total N [22] and Ca, Mg, K, P, Fe, Cu, Mn, Zn, B, Se contents of walnut. after wet digestion of dried and ground samples using a HNO3-H2O2 acid mixture (2:3 v/v) with three steps (1st; 145 ºC, 75% RF, 5 min; 2nd; 180 ºC, 90% RF, 10 min and 3rd; 100 ºC, 40% RF, 10 min) in a microwave oven (Bergof Speedwave Microwave Digestion Equipment MWS-2) [23]. The contents Ca, Mg, K, P, Fe, Cu, Mn, Zn, B, Se of types were determined using an Inductively Couple Plasma spectrometer (Perkin-Elmer, Optima 2100 DV, ICP/OES, Shelton, CT 06484-4794, USA) [24].

**Statistical Analysis.** Biplot analysis was made to determine similarities/ dissimilarities, performances and stabilities in genotypes and parameters. Genotypes are symbolized as GN in biplot analysis.

## RESULTS

**Fruit Quality Parameters.** The fruit weight, width and length of the walnut genotypes are shown in Figure 2. The fruit weight of the genotypes varied within the wide range, from 7.33 to 13.14 g. The fruit weight was higher in genotype GN6 (13.14 g) followed by genotype GN18 (12.91g). The fruit width of the genotyes varied within the wide range, from 28.47 to 36.57 mm. The fruit width was highest in genotype GN18 (36.56 mm) followed by genotype GN19 (34.52 mm). The fruit size of the genotypes varied within the wide range, from 31.91 to 45.18 mm. The fruit size was highest in genotype GN2 (45.18 mm) followed by genotype GN18 (42.17 mm). Differences were observed among genotypes in the fruit weight, width, and size. The fruit ash of the genotypes varied within the wide range, from 1.64% to 3.32%. The fruit ash was highest in genotype GN5 (3.32%), followed by genotype GN26 (2.46%).

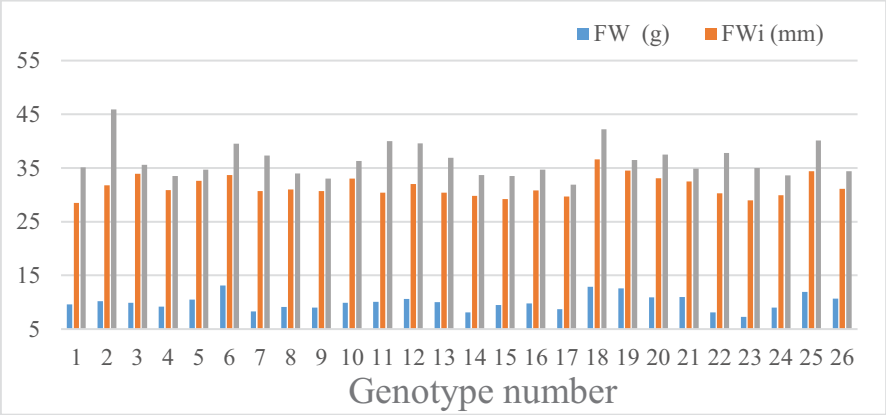


**FIGURE 2**
**Fruit Weight (FW), Fruit Width (FWi), Fruit Length (FL) of walnut genotypes**

**TABLE 1**
**Fruit nutrient element contents of walnut genotypes**

| GN | N | Cp | P | K | Ca | Mg | Na | Zn | Cu | Mn | Fe | B | Se | A |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ---- | -% | ---------- | | | | | | | | | mg/kg | | % |
| 1 | 1.9 | 12.1 | 3754 | 9215 | 3765 | 2214 | 242.2 | 29.7 | 15.6 | 24.5 | 32.6 | 22.3 | 86.6 | 1.9 |
| 2 | 2.1 | 13.0 | 3812 | 9122 | 3789 | 2245 | 230.2 | 28.1 | 16.1 | 24.6 | 31.5 | 24.1 | 83.1 | 2.1 |
| 3 | 2.2 | 13.5 | 3988 | 9134 | 3812 | 2234 | 222.2 | 29.9 | 15.4 | 25.4 | 34.5 | 23.4 | 92.5 | 2.2 |
| 4 | 2.2 | 13.9 | 3874 | 9172 | 3887 | 2213 | 228.7 | 28.7 | 16.0 | 25.4 | 32.9 | 22.1 | 88.5 | 2.2 |
| 5 | 3.3 | 20.7 | 3879 | 9233 | 3834 | 2265 | 229.8 | 27.7 | 15.2 | 26.3 | 33.1 | 21.1 | 95.4 | 3.3 |
| 6 | 2.2 | 13.6 | 3912 | 9413 | 3712 | 2287 | 227.7 | 27.0 | 16.6 | 25.5 | 34.1 | 22.3 | 97.2 | 2.2 |
| 7 | 1.7 | 10.7 | 3988 | 9230 | 3989 | 2130 | 224.5 | 28.1 | 17.0 | 24.3 | 34.2 | 21.0 | 90.9 | 1.7 |
| 8 | 1.7 | 10.6 | 3856 | 9140 | 3944 | 2209 | 227.2 | 26.7 | 17.0 | 25.7 | 35.2 | 23.5 | 92.5 | 1.7 |
| 9 | 1.6 | 10.2 | 3813 | 9187 | 3980 | 2167 | 229.8 | 25.0 | 18.1 | 25.1 | 32.9 | 22.3 | 89.7 | 1.6 |
| 10 | 2.2 | 13.5 | 3987 | 9170 | 3945 | 2200 | 228.5 | 26.8 | 18.9 | 24.5 | 35.4 | 20.3 | 96.9 | 2.2 |
| 11 | 2.3 | 14.5 | 4012 | 9121 | 4023 | 2214 | 231.1 | 26.1 | 17.3 | 25.0 | 36.1 | 21.1 | 88.7 | 2.3 |
| 12 | 1.7 | 10.6 | 4094 | 8904 | 3972 | 2175 | 224.9 | 24.5 | 19.1 | 26.1 | 35.2 | 24.6 | 80.1 | 1.7 |
| 13 | 1.7 | 10.8 | 4013 | 9234 | 3912 | 2190 | 231.5 | 25.0 | 16.7 | 25.9 | 37.1 | 21.0 | 79.5 | 1.7 |
| 14 | 2.0 | 12.5 | 4015 | 9214 | 4009 | 2234 | 230.1 | 23.2 | 16.9 | 26.4 | 37.1 | 19.9 | 89.8 | 2.0 |
| 15 | 2.1 | 13.0 | 4035 | 9087 | 4087 | 2176 | 229.8 | 26.5 | 18.1 | 26.0 | 38.2 | 22.3 | 80.7 | 2.1 |
| 16 | 1.9 | 11.7 | 4123 | 9122 | 4092 | 2114 | 224.6 | 24.3 | 19.5 | 25.4 | 35.5 | 21.9 | 86.7 | 1.9 |
| 17 | 2.0 | 12.5 | 3988 | 9100 | 4122 | 2186 | 231.9 | 20.6 | 19.2 | 26.1 | 35.1 | 19.5 | 92.6 | 2.0 |
| 18 | 2.2 | 13.5 | 4098 | 9213 | 4005 | 2209 | 226.5 | 22.6 | 18.7 | 24.3 | 38.3 | 18.8 | 94.5 | 2.2 |
| 19 | 2.0 | 12.6 | 3990 | 8977 | 4233 | 2261 | 224.9 | 23.4 | 18.1 | 25.5 | 36.1 | 17.7 | 78.6 | 2.0 |
| 20 | 2.2 | 13.5 | 3870 | 8954 | 4198 | 2289 | 218.4 | 24.5 | 19.1 | 25.0 | 35.4 | 17.1 | 88.6 | 2.2 |
| 21 | 2.0 | 12.6 | 3912 | 8844 | 4120 | 2241 | 216.9 | 20.1 | 16.6 | 27.7 | 33.1 | 18.4 | 92.7 | 2.0 |
| 22 | 1.9 | 11.6 | 4122 | 8970 | 3987 | 2277 | 227.6 | 18.8 | 18.1 | 26.1 | 36.1 | 19.2 | 88.2 | 1.9 |
| 23 | 2.2 | 13.8 | 4089 | 9032 | 4013 | 2018 | 225.1 | 22.3 | 19.2 | 25.3 | 38.8 | 15.7 | 94.6 | 2.2 |
| 24 | 2.0 | 12.7 | 4032 | 8874 | 4093 | 2097 | 220.8 | 25.5 | 19.9 | 22.3 | 34.5 | 18.8 | 75.7 | 2.0 |
| 25 | 2.2 | 13.8 | 4109 | 8942 | 4052 | 2133 | 218.9 | 21.4 | 19.0 | 24.5 | 35.4 | 20.1 | 69.4 | 2.2 |
| 26 | 2.5 | 15.4 | 4187 | 8945 | 4129 | 2172 | 221.0 | 22.3 | 18.7 | 26.8 | 39.1 | 22.3 | 67.5 | 2.5 |

GN: Genotype number, N: Nitrogen, Cp: Crude protein, P: Phosphorus, K: Potassium, Ca: Calcium, Mg: Magnesium, Na: Sodium, Zn: Zinc, Cu: Cupper, Mn: Manganese, Fe: Iron, B: Boron, Se: Selenium, A: Ash

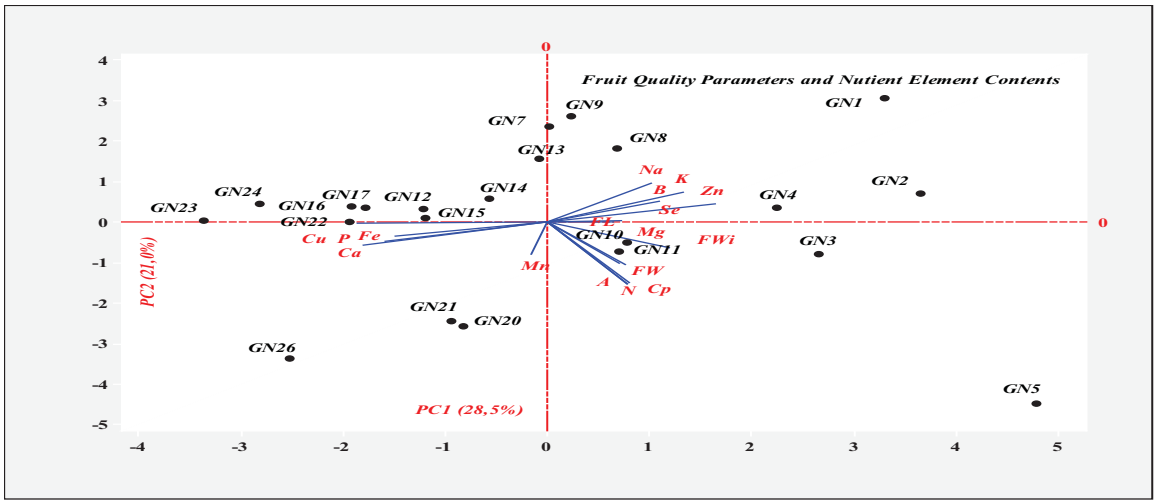


**FIGURE 3**
**Fruit quality parameters and nutrient element contents of walnut genotypes**

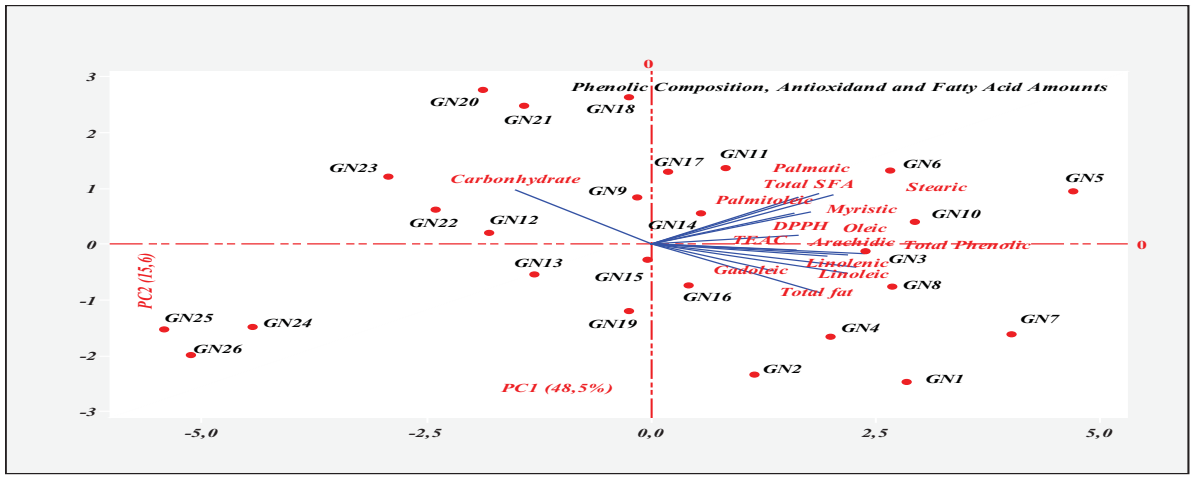


**FIGURE 4**
**Phenolic composition, antioxidant and fatty acid amounts of walnut genotypes**

Biplot analysis of fruit weight, fruit width, fruit length and minerals examined in the study are given in Figure 3. Fruit weight, fruit length, fruit width, ash amount and protein ratio were grouped under one group. In this case, it is seen that walnut genotypes do not show excessive variation in terms of these properties and exhibit similar trends. Three groups occurred for fruit quality parameters. In first group, GN26, GN21 and GN20; in second group, GN1, GN2, GN3, GN4, GN10 and GN1; and the third group had the other genotypes. Fruit weight, fruit length, fruit width, ash content and crude protein were stable and showed a high amount in terms of genotypes. As for genotypes, GN2, GN3, GN4, GN7, GN8, GN 9, GN10 and GN11 genotypes were determined as rich and stable genotypes in terms of fruit characteristics.

**Macro and Micro Nutrient Contents.** The crude protein and mineral concentrations of the walnut genotypes are shown in Table 1. The crude protein content of the genotypes varied within the wide range, from 10.21% to 20.71%. The crude protein content was highest in genotype GN5 (20.71%), followed by genotype GN26 (15.36%). The phosphorus content of the genotypes varied within the wide range, from 3754 mg/kg to 4187 mg/kg. The phosphorus content was highest in genotype GN26 (4187 mg/kg), followed by genotype GN16 (4123 mg/kg), and genotype GN22 (4122 mg/kg). The potassium content of the genotypes varied within the wide range, from 8844 mg/kg to 9413 mg/kg. The potassium content was highest in genotype GN6 (9413 mg/kg), followed by genotype GN13 (9234 mg/kg). The calcium content of the genotypes varied within the wide range, from 3712 mg/kg to 4233 mg/kg. The calcium content was highest in genotype GN19 (4233 mg/kg), followed by genotype GN20 (4198 mg/kg), and the same differences were observed among genotypes in the other nutrient elements etc. Mg, Na, Zn, Cu, Mn, Fe, B, and Se contents of walnut fruit. In biplot analysis (Figure 3), the parameters analyzed consisted of two groups. While Cu, Fe, Ca and P form one group, other minerals form another

**TABLE 2**
**Total fat, carbonhydrate, phenolic composition and antioxidant amounts of walnut genotypes**

| GN | Total fat | Carbonhydrate | Total Phenolic | TEAC | DPPH |
|---|---|---|---|---|---|
| | | % | mg GAE/100g FW | µmolTE/g FW | |
| 1 | 69.14 | 15.05 | 1698.18 | 113.212 | 83.613 |
| 2 | 66.71 | 16.49 | 1505.05 | 100.336 | 76.993 |
| 3 | 63.45 | 19.11 | 1367.88 | 111.191 | 80.050 |
| 4 | 67.86 | 14.31 | 1530.92 | 102.060 | 94.428 |
| 5 | 71.43 | 18.99 | 1825.72 | 121.714 | 86.167 |
| 6 | 63.15 | 19.41 | 1551.02 | 108.401 | 97.449 |
| 7 | 71.42 | 14.66 | 1754.09 | 116.939 | 90.449 |
| 8 | 66.29 | 19.77 | 1628.10 | 112.539 | 96.839 |
| 9 | 60.71 | 25.57 | 1491.12 | 109.407 | 88.109 |
| 10 | 64.58 | 18.18 | 1585.97 | 105.731 | 72.399 |
| 11 | 60.45 | 20.81 | 1303.20 | 99.879 | 95.562 |
| 12 | 58.74 | 27.52 | 1360.13 | 90.675 | 82.451 |
| 13 | 60.43 | 25.58 | 1484.13 | 98.941 | 86.567 |
| 14 | 63.45 | 20.50 | 1558.21 | 103.880 | 77.210 |
| 15 | 63.30 | 19.99 | 1389.79 | 118.652 | 91.237 |
| 16 | 66.87 | 18.12 | 1642.27 | 109.484 | 85.802 |
| 17 | 62.88 | 21.06 | 1544.44 | 102.962 | 81.618 |
| 18 | 57.48 | 25.30 | 1490.14 | 97.942 | 88.542 |
| 19 | 64.89 | 19.03 | 1593.76 | 106.250 | 87.344 |
| 20 | 53.75 | 29.00 | 1320.20 | 88.013 | 80.732 |
| 21 | 59.17 | 24.65 | 1453.19 | 96.879 | 81.908 |
| 22 | 60.03 | 24.12 | 1474.35 | 88.290 | 70.518 |
| 23 | 61.74 | 20.71 | 1269.33 | 80.622 | 70.843 |
| 24 | 62.02 | 21.70 | 1275.18 | 85.012 | 68.707 |
| 25 | 60.15 | 22.36 | 1236.73 | 82.448 | 70.712 |
| 26 | 60.74 | 19.98 | 1272.82 | 84.854 | 72.650 |

group. Genotypes are grouped under 3 groups. GN26, GN21 and GN20 created one group, while GN1, GN2, GN3, GN4, GN10 and GN11, created another group. The remaining genotypes formed a separate group.

**Phenolic Composition, Antioxidant and Fatty Acids Amounts.** The phenolic composition and antioxidant amount of the walnut genotypes are presented in Table 2. Considerable differences were observed among walnut genotypes in terms of phenolic composition, and antioxidant amounts.

The total oil of the genotypes varied within the wide range, from 53.8% to 71.4%. The total oil rate was highest in genotype GN5 (71.4%), followed by genotype GN7 (71.4%). The carbohydrate amounts of the genotypes varied within the wide range, from 14.3% to 29.0%. The carbohydrate amount was highest in genotype GN20 (29.0%), followed by genotype GN12 (27.5%). Similarly, the total phenolic composition of the genotypes varied within the wide range; from 1236 mg GAE/100 g FW to 1825 mg GAE/100 g FW The total phenolic composition was highest in genotype GN5 (1825 mg GAE/100 g FW), followed by genotype GN7 (1754 mg GAE/100 g FW). TEAC, DPPH, palmatic acid, myristic acid, stearic acid, arachidic acid, total SFA, palmitoleic acid, oleic acid, gadoleic acid, linoleic acid, and linolenic acid of the genotypes varied within the wide range. The highest TEAC, myristic acid, arachidic acid, total SFA, palmitoleic acid value in genotype GN5 (121 µmolTE/g FW; 1,01%; 0,176%; 7,85%, and 2,32% respectively) (Table 2, Table 3). The highest DPHH, and palmatic acid values were found in genotype GN6 (97 µmolTE/g FW, 5.40%, respectively). The highest stearic acid value was observed in genotype GN7 (1.40%). The highest oleic acid, linoleic acid, and linolenic acid values were in genotype GN3 (25.58%, 88.22%, and 11.03%, respectively). Moreover, the highest gadoleic acid was found in genotype GN2 (0.131%). Considering the biplot analysis in Figure 4, showing the phenolic composition, antioxidant and fatty acid amounts, the investigated properties formed 2 groups. While carbohydrates constitute a group alone, other features examined are grouped under one group. Genotypes are grouped under 4 groups. While GN5 created a single group, GN1, GN7 and GN8 created a second group. While GN20, GN21, GN23, GN24 and GN26 constitute the third group, other genotypes are grouped under the fourth group.

**TABLE 3**
**Fatty acid amounts of walnut genotypes**

| GN | Fatty Acid (%) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Pal-matic | Myristic | Stea-ric | Ara-chidic | SFA | Pal-mitoleic | Oleic | Gado-leic | Lino-leic | Lino-lenic |
| 1 | 4.81 | 0.60 | 1.24 | 0.169 | 6.81 | 1.93 | 22.34 | 0.129 | 87.51 | 10.94 |
| 2 | 4.62 | 0.72 | 1.02 | 0.166 | 6.53 | 2.09 | 20.18 | 0.131 | 83.00 | 10.37 |
| 3 | 5.14 | 0.71 | 1.32 | 0.117 | 7.29 | 2.11 | 25.58 | 0.126 | 88.22 | 11.03 |
| 4 | 4.92 | 0.77 | 1.03 | 0.090 | 6.81 | 2.13 | 23.12 | 0.083 | 84.71 | 10.59 |
| 5 | 5.30 | 1.01 | 1.36 | 0.176 | 7.85 | 2.32 | 19.54 | 0.028 | 81.70 | 10.21 |
| 6 | 5.40 | 0.75 | 1.39 | 0.097 | 7.64 | 2.18 | 22.15 | 0.068 | 79.78 | 9.97 |
| 7 | 5.05 | 0.69 | 1.40 | 0.172 | 7.31 | 1.71 | 23.55 | 0.125 | 83.03 | 10.38 |
| 8 | 5.14 | 0.61 | 1.32 | 0.173 | 7.24 | 1.69 | 22.45 | 0.127 | 78.89 | 9.86 |
| 9 | 4.99 | 0.60 | 1.33 | 0.091 | 7.00 | 1.64 | 19.26 | 0.029 | 73.79 | 9.22 |
| 10 | 5.38 | 0.75 | 1.38 | 0.147 | 7.66 | 2.16 | 25.34 | 0.127 | 79.07 | 9.88 |
| 11 | 4.93 | 0.80 | 1.27 | 0.127 | 7.13 | 2.32 | 23.98 | 0.099 | 57.42 | 7.18 |
| 12 | 4.45 | 0.67 | 1.04 | 0.116 | 6.28 | 1.92 | 25.10 | 0.027 | 53.84 | 8.73 |
| 13 | 4.42 | 0.60 | 1.14 | 0.163 | 6.32 | 1.73 | 18.66 | 0.126 | 54.92 | 6.86 |
| 14 | 4.99 | 0.69 | 1.28 | 0.137 | 7.10 | 2.12 | 21.50 | 0.058 | 50.87 | 8.36 |
| 15 | 4.49 | 0.72 | 1.15 | 0.064 | 6.42 | 2.08 | 25.30 | 0.028 | 58.28 | 7.28 |
| 16 | 4.82 | 0.65 | 1.24 | 0.069 | 6.77 | 1.90 | 20.66 | 0.128 | 53.42 | 6.68 |
| 17 | 5.14 | 0.69 | 1.32 | 0.093 | 7.25 | 2.00 | 22.98 | 0.031 | 44.28 | 7.53 |
| 18 | 5.25 | 0.75 | 1.25 | 0.138 | 7.39 | 2.17 | 20.13 | 0.029 | 49.73 | 6.22 |
| 19 | 4.37 | 0.70 | 1.12 | 0.092 | 6.28 | 2.01 | 21.56 | 0.123 | 51.44 | 6.43 |
| 20 | 4.92 | 0.75 | 1.27 | 0.070 | 7.01 | 2.17 | 19.66 | 0.093 | 53.86 | 6.73 |
| 21 | 5.15 | 0.70 | 1.32 | 0.074 | 7.25 | 2.02 | 18.34 | 0.022 | 39.72 | 5.97 |
| 22 | 4.90 | 0.64 | 1.13 | 0.070 | 6.74 | 1.86 | 19.20 | 0.127 | 37.09 | 5.64 |
| 23 | 5.26 | 0.58 | 1.35 | 0.075 | 7.26 | 1.21 | 17.45 | 0.025 | 44.12 | 5.52 |
| 24 | 4.21 | 0.56 | 1.08 | 0.060 | 5.90 | 1.04 | 19.23 | 0.016 | 50.27 | 6.28 |
| 25 | 3.86 | 0.60 | 0.99 | 0.055 | 5.50 | 1.21 | 18.42 | 0.014 | 42.21 | 5.28 |
| 26 | 3.75 | 0.59 | 0.96 | 0.054 | 5.35 | 1.46 | 17.12 | 0.016 | 44.05 | 5.51 |

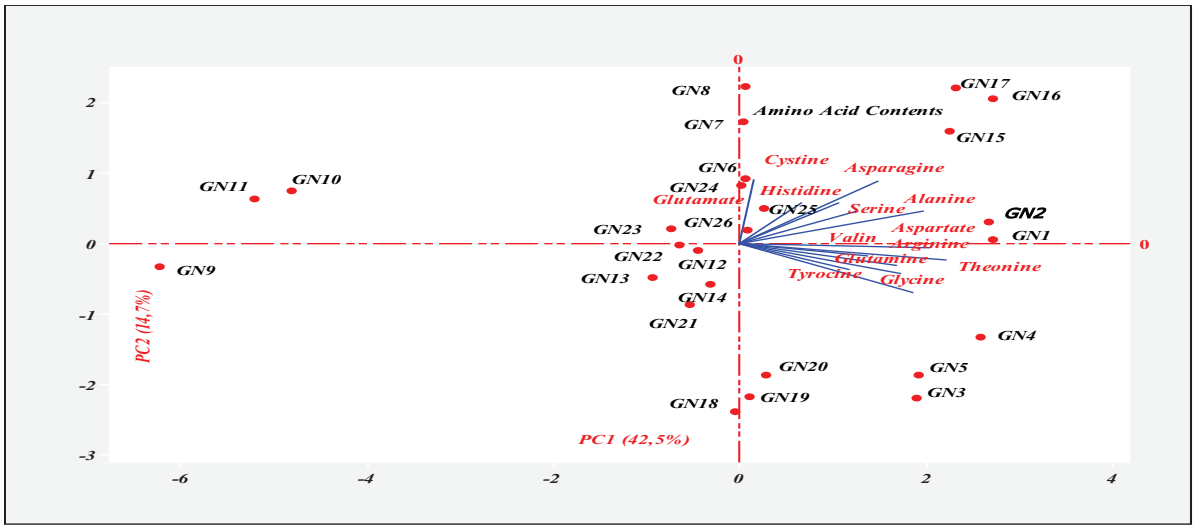


**FIGURE 5**
**Amino acid contents of walnut genotypes**

**Amino Acids Contents.** The amino acid contents of the walnut genotypes are presented in Table 4. Considerable differences were observed among walnut genotypes in terms of amino acid compositions. Overall, aspartate, glutamate, isoleucine, leucine and lycine were the most abundant amino acids, whereas all genotypes deficient in cystine, valin, and, tryptophan. In general, the highest amino acid concentrations were detected in genotype GN16. On the other hand, most of the amino acids were also high in genotype GN15, genotype GN1, genotype GN4, and genotype GN17. Cystine, valin, tryptophan, lysine, sarcosine, serine, histidine and arginine which are deficient amino acids in walnut, changed

**TABLE 4**
**Amino acid contents of walnut genotypes**

| GN | Aspartate | Glutamate | Asparagine | Serine | Glutamine | Histidine | Glycine | Theonine | Arginine | Alanine | Tyrocine | Cystine | Valin |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | pmol/μg | | | | | | |
| 1 | 2733 | 5340 | 6137 | 3971 | 3467 | 2290 | 3181 | 2909 | 4440 | 4750 | 1261 | 556 | 940 |
| 2 | 2793 | 5180 | 5937 | 4286 | 3340 | 2123 | 3156 | 2985 | 4377 | 4769 | 1234 | 614 | 956 |
| 3 | 2690 | 5088 | 5460 | 4161 | 3429 | 1894 | 3471 | 2781 | 4321 | 4388 | 1376 | 547 | 976 |
| 4 | 2687 | 5412 | 5739 | 4094 | 3588 | 1974 | 3425 | 2864 | 4334 | 4449 | 1380 | 590 | 877 |
| 5 | 2743 | 5292 | 5499 | 4075 | 3500 | 1823 | 3336 | 2877 | 4274 | 4434 | 1332 | 577 | 912 |
| 6 | 2579 | 4916 | 5679 | 4135 | 3339 | 2123 | 3012 | 2653 | 3992 | 5058 | 1190 | 627 | 944 |
| 7 | 2639 | 5368 | 5868 | 4035 | 3244 | 2067 | 3059 | 2665 | 4061 | 5022 | 1217 | 670 | 844 |
| 8 | 2636 | 5268 | 5867 | 4211 | 3249 | 2100 | 2883 | 2741 | 3946 | 5131 | 1119 | 663 | 890 |
| 9 | 2287 | 4572 | 5083 | 3792 | 3114 | 1976 | 2641 | 2509 | 3232 | 2963 | 1097 | 551 | 807 |
| 10 | 2273 | 5360 | 5318 | 3934 | 3228 | 1870 | 2613 | 2610 | 3247 | 2939 | 1021 | 606 | 891 |
| 11 | 2204 | 5012 | 5413 | 4120 | 3266 | 1876 | 2747 | 2561 | 3185 | 2912 | 1053 | 585 | 754 |
| 12 | 2492 | 5564 | 5828 | 4064 | 3363 | 1966 | 3169 | 2794 | 3792 | 4619 | 1107 | 487 | 796 |
| 13 | 2504 | 5736 | 5558 | 3594 | 3509 | 2050 | 3109 | 2692 | 3680 | 4520 | 1182 | 534 | 821 |
| 14 | 2542 | 5492 | 5727 | 3978 | 3434 | 1894 | 3257 | 2891 | 3744 | 4665 | 1063 | 517 | 743 |
| 15 | 2507 | 5840 | 6285 | 4313 | 3514 | 2098 | 3229 | 2710 | 4224 | 5160 | 1129 | 570 | 952 |
| 16 | 2687 | 5980 | 6421 | 4465 | 3467 | 2055 | 3180 | 2748 | 4197 | 5199 | 1084 | 552 | 925 |
| 17 | 2719 | 5680 | 6227 | 4388 | 3424 | 2150 | 3100 | 2798 | 4178 | 5233 | 1058 | 606 | 925 |
| 18 | 2561 | 4876 | 5305 | 4161 | 3334 | 1929 | 3359 | 2793 | 4187 | 4294 | 1056 | 434 | 945 |
| 19 | 2617 | 4944 | 5085 | 4222 | 3393 | 2055 | 3297 | 2738 | 4256 | 4237 | 1085 | 445 | 930 |
| 20 | 2614 | 5080 | 5344 | 4075 | 3370 | 1948 | 3225 | 2890 | 4140 | 4339 | 1012 | 476 | 970 |
| 21 | 2605 | 4612 | 5729 | 4030 | 3452 | 2146 | 3036 | 2686 | 4041 | 4552 | 1134 | 459 | 817 |
| 22 | 2671 | 5032 | 5490 | 3971 | 3468 | 2203 | 2921 | 2635 | 3959 | 4597 | 1061 | 509 | 863 |
| 23 | 2662 | 4896 | 5671 | 4106 | 3362 | 2198 | 2908 | 2730 | 3987 | 4559 | 1064 | 495 | 800 |
| 24 | 2739 | 4940 | 5727 | 4167 | 3423 | 2324 | 3007 | 2618 | 4053 | 4655 | 1096 | 534 | 821 |
| 25 | 2664 | 5048 | 5426 | 4291 | 3384 | 2330 | 3075 | 2692 | 4123 | 4588 | 1109 | 551 | 866 |
| 26 | 2514 | 5372 | 5423 | 4109 | 3412 | 2309 | 3124 | 2714 | 4134 | 4500 | 1124 | 540 | 885 |

**TABLE 4 continue**
**Amino acid contents of walnut genotypes**

| GN | Methionine | Tryptophan | Phenylalanine | Isoluecine | Leucine | Lysine | Hydroxyproline | Sarcosine | Proline |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | pmol/μg | | | | |
| 1 | 2072 | 989 | 1044 | 1388 | 2850 | 2574 | 552 | 4326 | 59 |
| 2 | 1991 | 940 | 1051 | 1392 | 2662 | 2738 | 625 | 4294 | 58 |
| 3 | 1882 | 952 | 1076 | 1295 | 2573 | 2817 | 552 | 5232 | 55 |
| 4 | 2026 | 912 | 1005 | 1204 | 2453 | 2906 | 581 | 5192 | 56 |
| 5 | 1767 | 959 | 1113 | 1250 | 2665 | 2909 | 514 | 5361 | 53 |
| 6 | 1768 | 806 | 1151 | 1290 | 2750 | 2458 | 581 | 4269 | 49 |
| 7 | 1927 | 838 | 1235 | 1358 | 2629 | 2376 | 545 | 4369 | 51 |
| 8 | 1645 | 831 | 1226 | 1239 | 2563 | 2524 | 624 | 4166 | 47 |
| 9 | 1923 | 700 | 1059 | 1131 | 2606 | 2265 | 494 | 4276 | 50 |
| 10 | 1861 | 724 | 1094 | 1311 | 2752 | 2164 | 499 | 4339 | 55 |
| 11 | 1808 | 736 | 1096 | 1230 | 2698 | 2356 | 456 | 4310 | 48 |
| 12 | 1707 | 837 | 1095 | 1230 | 2263 | 2432 | 586 | 4329 | 55 |
| 13 | 1814 | 813 | 1122 | 1292 | 2312 | 2679 | 537 | 4580 | 65 |
| 14 | 1592 | 873 | 1100 | 1276 | 2489 | 2523 | 554 | 4430 | 53 |
| 15 | 1940 | 890 | 1178 | 1330 | 2535 | 2438 | 535 | 4487 | 59 |
| 16 | 1892 | 929 | 1196 | 1319 | 2447 | 2673 | 507 | 4340 | 54 |
| 17 | 1817 | 958 | 1102 | 1279 | 2434 | 2610 | 532 | 4384 | 59 |
| 18 | 2114 | 977 | 1123 | 1122 | 2517 | 2537 | 544 | 4249 | 53 |
| 19 | 2204 | 912 | 1031 | 1230 | 2588 | 2475 | 562 | 4153 | 55 |
| 20 | 1999 | 985 | 1160 | 1198 | 2610 | 2574 | 545 | 4057 | 51 |
| 21 | 1788 | 929 | 1213 | 1317 | 2247 | 2547 | 535 | 4372 | 59 |
| 22 | 1620 | 979 | 1120 | 1233 | 2070 | 2673 | 562 | 4457 | 58 |
| 23 | 1838 | 930 | 1049 | 1334 | 2220 | 2605 | 518 | 4269 | 57 |
| 24 | 1920 | 948 | 1114 | 1318 | 2262 | 2669 | 502 | 4376 | 56 |
| 25 | 1876 | 941 | 1096 | 1350 | 2403 | 2571 | 542 | 4309 | 58 |
| 26 | 1912 | 954 | 1176 | 1412 | 2322 | 2645 | 554 | 4312 | 63 |

considerably among genotypes. The range of cysteine, valin, tryptophan, lysine, sarcosine and proline contents of walnut genotypes were from 434 (GN 18) to 670 (GN 7) pmol µl-1, 743 (GN 14) to 976 (GN 3) pmol µl-1, 700 (GN 9) to 989 (GN 1) pmol µl-1, 2164 (GN 10) to 2909 (GN 5) pmol µl-1, 4057 (GN 20) to 5361 (GN 5) pmol µl-1 and 47 (GN 8) to 65 (GN 13) pmol µl-1, respectively.

In terms of biplot analysis (Figure 5), when looking at the amino acid of the studied walnut genotypes, all the amino acids examined constitute a single group, while the genotypes are grouped under 4 groups. While GN9, GN10 and GN11 form a group, GN18, GN19 and GN20 form the second group. Again, GN3, GN4 and GN5 constitute the third group, while the remaining genotypes are grouped under the fourth group.

Organic Acid Contents. As seen from the Table 5, succinic, oxalic, lactic, malic, citric, and propionic acids were the most abundant, whereas tartaric, butyric, maleic and fumaric acids were the scarcest, respectively. Organic acid content of the genotypes varied within the wide range. Generally, most of the organic acids were high in genotype GN11, GN14, GN22, GN23 and GN26, and low in genotype GN2, GN4, GN5, and GN6. The highest total organic acid contents were determined in genotype GN11 (48.80 ng µl-1). The range of oxalic acid contents of walnut genotypes were from 5.51 to 7.56 ng µl-1. The highest oxalic acid contents were determined in genotype GN8 (7.56 ng µl-1). Similarly, the range of succinic acid contents of walnut genotypes were from 5.67 to 9.64 ng µl-1. The highest succinic acid contents were determined in genotype GN26 (9.66 ng µl-1).

The biplot analysis of the organic acid amounts of the walnut genotypes showed that all organic acids examined constitute a single group, while the genotypes are grouped under 4 groups. While GN11 created a stand-alone group, GN2, GN4, GN5, GN6 and GN17 formed the second group. Again, GN1, GN3, GN7, GN8, GN9 and GN16 constitute the third group, while the remaining other genotypes are grouped under the fourth group (Figure 6).

**TABLE 5**
**Organic acid contents of walnut genotypes**

| GN | Oxalic | Propionic | Tartaric | Butyric | Malonic | Malic | Lactic | Citric | Maleic | Fumaric | Succinic |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Ng/µl | | | | | |
| 1 | 6.44 | 5.46 | 1.20 | 1.87 | 3.74 | 5.44 | 4.32 | 4.55 | 0.30 | 1.13 | 7.71 |
| 2 | 6.51 | 5.10 | 1.06 | 1.68 | 3.51 | 5.26 | 3.87 | 4.38 | 0.28 | 1.07 | 7.87 |
| 3 | 6.74 | 5.63 | 1.12 | 1.77 | 3.87 | 5.18 | 4.57 | 4.16 | 0.34 | 1.04 | 7.83 |
| 4 | 6.23 | 5.20 | 1.23 | 1.62 | 3.38 | 5.69 | 3.59 | 4.27 | 0.43 | 1.02 | 5.67 |
| 5 | 5.51 | 4.42 | 1.36 | 1.54 | 3.59 | 5.87 | 3.25 | 4.10 | 0.41 | 1.19 | 6.21 |
| 6 | 6.22 | 5.37 | 1.26 | 1.47 | 3.63 | 5.43 | 3.87 | 4.28 | 0.40 | 1.25 | 7.55 |
| 7 | 6.88 | 5.84 | 1.45 | 1.97 | 4.02 | 4.95 | 4.71 | 3.88 | 0.28 | 1.16 | 7.19 |
| 8 | 7.56 | 5.64 | 1.73 | 1.78 | 3.94 | 5.11 | 4.64 | 4.23 | 0.28 | 1.15 | 6.70 |
| 9 | 7.17 | 6.01 | 1.80 | 1.88 | 4.09 | 4.69 | 4.95 | 3.49 | 0.32 | 1.07 | 7.31 |
| 10 | 7.14 | 6.07 | 1.45 | 1.73 | 4.65 | 6.06 | 4.79 | 4.80 | 0.66 | 1.39 | 9.04 |
| 11 | 6.42 | 5.29 | 1.58 | 1.65 | 4.87 | 6.96 | 4.45 | 5.83 | 0.61 | 1.56 | 9.58 |
| 12 | 6.14 | 5.49 | 1.67 | 1.95 | 4.41 | 6.59 | 3.93 | 4.54 | 0.63 | 1.02 | 8.62 |
| 13 | 6.80 | 5.91 | 1.85 | 1.69 | 4.20 | 5.80 | 4.11 | 5.36 | 0.23 | 1.28 | 7.67 |
| 14 | 6.72 | 6.34 | 1.73 | 1.82 | 4.41 | 6.78 | 4.72 | 5.61 | 0.31 | 1.47 | 8.74 |
| 15 | 6.80 | 6.08 | 1.97 | 1.73 | 4.01 | 6.27 | 4.64 | 4.96 | 0.30 | 1.19 | 8.63 |
| 16 | 6.58 | 4.85 | 1.63 | 1.93 | 3.53 | 5.74 | 4.31 | 4.27 | 0.35 | 1.06 | 7.88 |
| 17 | 6.24 | 4.26 | 1.68 | 1.82 | 3.70 | 6.59 | 4.64 | 4.70 | 0.31 | 1.09 | 8.15 |
| 18 | 6.10 | 4.91 | 1.78 | 1.67 | 4.67 | 6.17 | 4.40 | 4.57 | 0.33 | 1.30 | 8.01 |
| 19 | 6.63 | 5.52 | 1.46 | 1.89 | 4.12 | 5.35 | 4.43 | 4.38 | 0.36 | 1.36 | 9.51 |
| 20 | 6.31 | 6.57 | 1.89 | 1.92 | 4.33 | 5.95 | 4.53 | 4.32 | 0.38 | 1.24 | 8.99 |
| 21 | 6.92 | 6.66 | 1.66 | 1.80 | 4.09 | 4.99 | 4.67 | 4.11 | 0.39 | 1.28 | 9.64 |
| 22 | 6.71 | 6.07 | 1.72 | 1.88 | 3.74 | 7.25 | 5.06 | 4.30 | 0.53 | 1.34 | 9.45 |
| 23 | 6.54 | 6.45 | 1.85 | 1.78 | 3.95 | 7.42 | 4.72 | 4.51 | 0.48 | 1.43 | 9.10 |
| 24 | 6.23 | 6.33 | 1.66 | 1.61 | 4.01 | 6.42 | 5.69 | 4.70 | 0.46 | 1.42 | 8.79 |
| 25 | 6.51 | 6.73 | 1.73 | 1.68 | 4.09 | 6.22 | 5.24 | 4.45 | 0.55 | 1.38 | 9.23 |
| 26 | 6.38 | 6.54 | 1.77 | 1.83 | 4.19 | 6.09 | 5.60 | 4.84 | 0.56 | 1.27 | 9.66 |

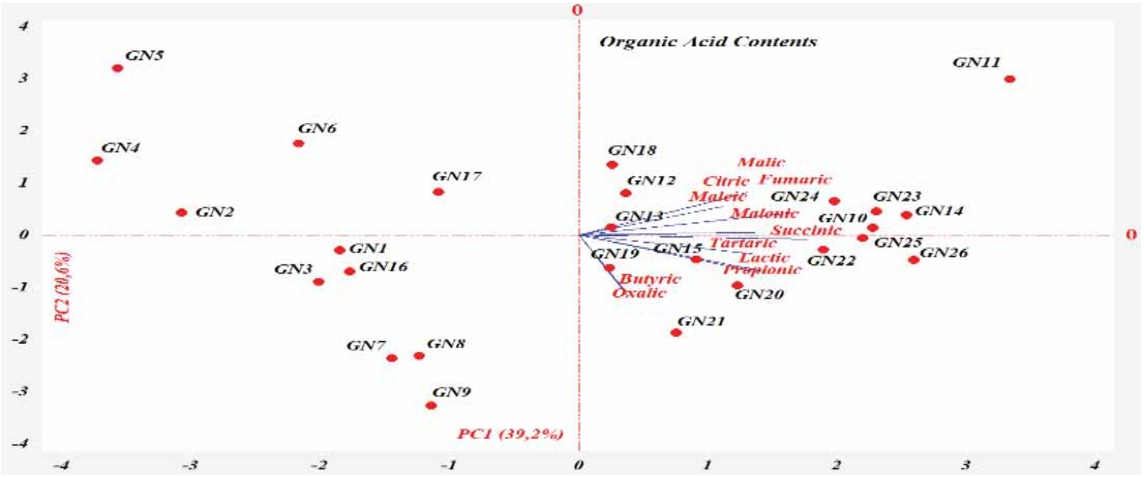


**FIGURE 6**
**Organic acid contents of common walnut genotypes**

**TABLE 6**
**Hormone contents of walnut genotypes**

| GN | Gibberellic acid | Salicylic acid | Absisic acid | Indole acetic acid |
|---|---|---|---|---|
| | | Ng/µl | | |
| 1 | 291.4 | 55.0 | 0.14 | 10.8 |
| 2 | 290.9 | 47.6 | 0.13 | 11.4 |
| 3 | 291.0 | 57.7 | 0.12 | 12.6 |
| 4 | 271.8 | 63.6 | 0.13 | 13.5 |
| 5 | 262.7 | 57.6 | 0.11 | 14.4 |
| 6 | 318.5 | 57.8 | 0.12 | 14.0 |
| 7 | 323.1 | 61.1 | 0.10 | 13.5 |
| 8 | 312.5 | 54.8 | 0.10 | 12.9 |
| 9 | 328.6 | 65.5 | 0.13 | 14.3 |
| 10 | 346.3 | 72.1 | 0.14 | 15.6 |
| 11 | 325.6 | 65.6 | 0.11 | 16.1 |
| 12 | 336.6 | 57.6 | 0.13 | 13.5 |
| 13 | 341.7 | 60.1 | 0.12 | 14.3 |
| 14 | 338.1 | 54.6 | 0.14 | 13.4 |
| 15 | 330.6 | 72.8 | 0.10 | 12.8 |
| 16 | 324.8 | 75.2 | 0.10 | 13.9 |
| 17 | 302.3 | 81.3 | 0.14 | 14.6 |
| 18 | 280.6 | 68.2 | 0.11 | 12.1 |
| 19 | 275.8 | 68.8 | 0.13 | 13.4 |
| 20 | 288.7 | 73.4 | 0.13 | 13.9 |
| 21 | 298.2 | 72.4 | 0.14 | 13.2 |
| 22 | 267.1 | 68.7 | 0.14 | 14.8 |
| 23 | 298.8 | 73.5 | 0.11 | 14.1 |
| 24 | 293.5 | 71.3 | 0.13 | 15.3 |
| 25 | 304.9 | 74.5 | 0.13 | 14.6 |
| 26 | 290.8 | 70.9 | 0.15 | 15.5 |

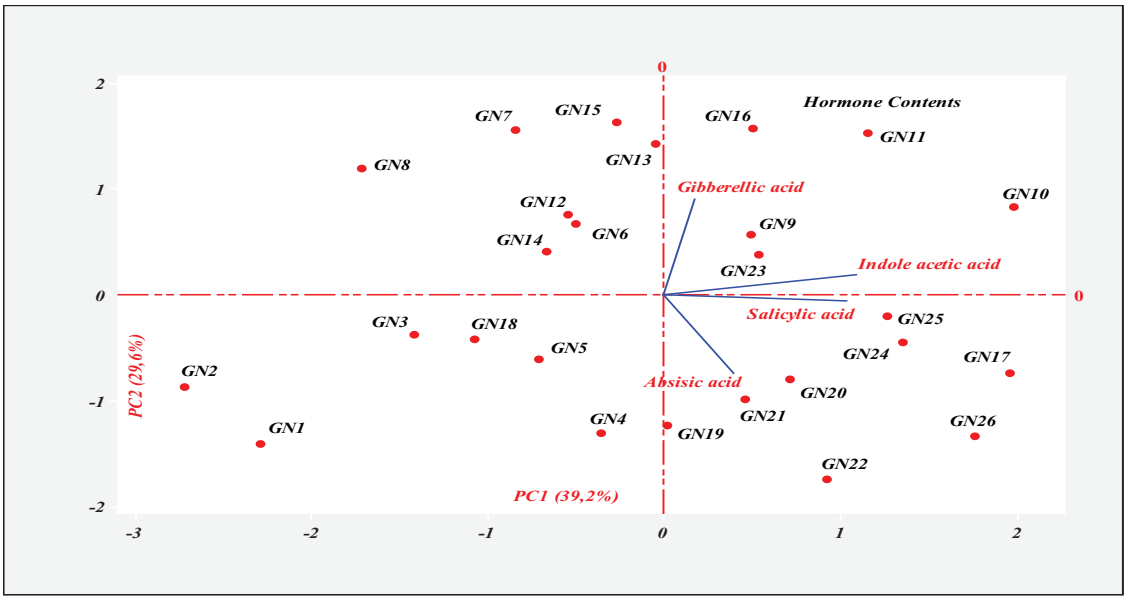


**FIGURE 7**
**Hormone contents of common walnut genotypes**

**Hormone Contents.** Walnut genotypes showed differences in terms of hormone content (Table 6). Giberallic acid content of the genotypes ranged between 262.73 ng µl-1 to 346.28 ng µl-1. The highest giberallic acid contents were determined in genotypes GN10, GN12, and GN13, whereas genotypes GN5, GN19, and GN22 had the lowest giberallic acid contents. The amount of salicylic acid, which was the second abundant hormone following giberallic acid, varied among walnut genotypes. Genotype number 17 (81.32 ng µl-1) had higher salicylic acid content than those of the other genotypes. The indole acetic acid content of the genotypes also varied within the wide range, from 10.78 ng µl-1to 16.09 ng µl-1. The amount of indole acetic acid was high in genotype GN 10, 11, 24, and 26, and low in genotype GN 1, 2, 3, 8, 15, and 18. Absisic acid content of the genotypes ranged between 0.10 ng µl-1 (genotype GN 7, 8, 15, and 16) to 0.15 ng µl-1 (genotype GN 26). If we look at the hormones in Figure 7, the parameters examined consisted of 3 groups. While gibberellic acid 1 group constituted another group of abscisic acid, indole formed a separate group in acetic and salicylic acid. In terms of genotypes, genotypes were determined to form 4 groups. While GN1, GN2, GN3, GN4, GN5 and GN18 are creating a group, GN6, GN7, GN8, GN12, GN13, GN14 and GN15 have created the second group. The third group included GN9, GN10, GN11, GN16 and GN23, while the fourth group included GN17, GN19, GN20, GN21, GN22, GN24, GN25 and GN26 (Figure 7).

## DISCUSSIONS

**Fruit Quality Parameters.** Turkey has an important walnut production rate due to the favorable climatic and soil characteristics. Turkey is rich in walnuts population and is one of the leading countries in the world in walnut production until the end of the year. Walnut culture in Turkey as well, each made because the seeds come from very ancient times have different characteristics from each other and adapted to the ecological conditions of the region in the form of a wide genetic variation. In this genetic variation, it is extremely important that the types which are highly qualified are protected from reproduction. Turkey's having such a rich genetic variation makes it possible to achieve success in breeding work as soon as possible. Determination of fruit quality characteristics and fatty acid composition of these Turkish walnut varieties and genotypes is very important for commercial culture and oil industry [25]. This study results showed that fruit weight and fruit quality parameters significantly varied among plants. Akca and Sen [26] showed nut length as 39.97 mm, nut diameter as 33.59 mm of the promising walnut genotype. Khattak et al. [27] determined the nut weight (1.90-12.30 g), for the walnut cultivars. Our results are higher than that shown by Ozkan and Koyuncu [28], who studied the walnut cultivars and found nut weights between 8.43-11.09 g. This could be due to the differences in the ecological and genetic properties of walnut cultivars growing in Turkey. The results of the nut properties showed that fruits of walnut cultivars have superior physical and chemical properties.

**Mineral Nutrient Contents:** Similar to the results reported in the literature [29, 30], our findings alsı showed that walnuts contain high levels of potassium (390-700 mg 100 g-1), phosphorus (310-510 mg 100 g-1) and magnesium (90-140 mg 100 g-1), and low sodium (1-15 mg 100 g-1). The calcium content in walnuts genotypes ranged from 58-91 mg

100 g-1 [31]), 640-1180 mg/kg [32]. Calcium values found in our study were considerably higher than those reported by Lavedrine et al. [31]. Magnesium and sodium contents of this study were generally higher than those reported by many studies in different walnut varieties. Copper, manganese and zinc contents were similar to values reported by other researchers [31, 32]. Content of elemental composition of walnuts can be influenced by genotype, cultivar, different ecology and different soil. While the levels of Cu, Ca, F and P are insufficient on the basis of genotypes, genotypes have a higher amount of minerals in terms of the minerals that make up the third group. Although all minerals except Mn have a stable course in genotypes, genotypes have a richer structure in terms of Na, K, B, Zn, Se, Mg and N. This shows that genotypes have given a higher quality product in terms of these minerals throughout the Valley. Genotypes of GN2, GN3, GN4, GN10 and GN11 are both stable and richer genotypes in terms of mineral substance.

**Phenolic Composition and Antioxidant Amounts.** Our results concerning phenolic composition and fatty acid were in agreement with the results obtained from walnut cultivars [33] but higher than those of Ozcan et al. [35]. Linoleic acid and linolenic acid contents were similar to the results of Zwartz et al. [33] but higher than the results of Çaglarırmak [36]. There were some differences in the fatty acid composition between our findings and the literature. It is thought that this difference may come from many factors such as the region where it is cultivated, the harvesting time, the year of harvest, and the cultural processes applied, such as irrigation [7]. Although they contain very different nutrients, the most important nutrient provided by walnuts is the dietary fat. The walnut oil is rich in essential fatty acids and the fatty acid composition is mainly composed of oleic, linoleic and linolenic acids. The walnut genotypes with high fatty acid, especially linoleic and linolenic acids, have beneficial effects on human health especially for cardio vascular system [37]. The ratios of oleic, linoleic and linolenic acids to each other are important to the economic and nutritional value of the nuts [25]. Therefore, fatty acid compositions are an important criterion for identification of walnut genotypes in different regions. The consumption of walnut is increasing as consumers are showing an interest in their valuable fatty acid content. It is reported that polyunsaturated fatty acids have a very important role in human nutrition. Polyunsaturated fatty acids found in walnut play an important role in the prevention of cardiovascular diseases and prevention of vascular occlusion [38]. Pereira et al. [37] determined fats (68.83%-72.14%), carbohydrates (3.75-7.16%), and ash (3.31-4.26%) for the six walnut cultivars grown in Portugal. In Italy, internal fat content (54.2-72.2%) linoleic (46.9-68.6%), oleic (10.0-25.1%), linolenic (6.9-17.6%), palmitic (3.9-11.4%) and stearic fatty acids (1.1-5.2%) were determined for 190 wild walnut types [38]. The results of this study are in good agreement with this notion except for the percent carbohydrates and ash contents. Al-Bachir [39] determined the protein (22.85%), fat (67.35%) and ash (1.26%) content. The results of the fat content and ash content are in agreement with the results of this study but there is variation in term of protein content. These differences and variations can be attributed to environmental conditions, horticulture procedures and composition of walnut fruits. The fact that other studied properties except carbohydrates show similar stability in genotypes reveals that the genotypes sampled throughout the Valley do not show much variation in terms of these substances. Therefore, these substances show almost similar variations on the basis of genotypes. Genotypes showed a rich and stable structure in terms of all phenolic and antioxidant compounds except carbohydrates. Regarding the genotypes, GN1, GN2, GN3, GN4, GN8, GN10, GN11, GN14 and GN16 have been determined as rich and stable varieties in terms of phenolic and antioxidant compounds.

**Amino Acid Contents.** Previous studies showed that amino acids may contribute to yield, growth and nutrient element uptake from soil in different plant species under stress conditions for different crops. Proline, alanine, serine, and asparagine also delayed wilting of maize under stress conditions, proline, glycine, alanine, leucine, threonine, lysine, arginine, tryptophan and phenylalanine inhibited stomatal opening while histidine, methionine, aspartic acid, glutamic acid, asparagine and glutamine promoted stomatal opening of Vicia faba, histidine, proline, glutamine, methionine and glycine promoted calcium uptake in phaseolus seedlings, proline relieved salt toxicity in barley plant lets by changing salt transport from root to shoot and increasing proline content increased K+ content and alleviated salt stress effects on growth of Vigna radiate cultures [40]. In the literature, the walnut plant was reported to have a very rich amino acids content. We also found that although the amounts in the plant varied, almost all the genotypes examined throughout the Valley showed quite stable amino acid levels and no major variations were observed in this change (Figure 3). In terms of amino acid levels and stability, GN1, GN2, GN15, GN24 and GN25 genotypes were determined to have a rich and stable structure in terms of amino acids.

**Organic Acid Contents.** Organic acids make osmotic adjustment and provide a greater cation balance in the plant. Acetic, abscisic, glycolic, malonic, oxalic, and formic acid play a very important role in nutrient uptake (P, Fe and Mn) of plants grown in low nutrient soils. These acids differ from plant species in response to nutrients starvation [41]. Similarly,

these acid anions form complexes with Ca, Al and Fe present in soil as in-soluble phosphates of calcium, iron and aluminium, and liberate P for uptake by roots [42]. Additionally, these acids can desorb P from sesquioxide surfaces by anion exchange [43], and also maintain sulphate mobility in rhizosphere soil through competitive displacement from adsorption sites. The concentrations of fumaric, malic and citric acids can also chelate Fe and Mn in iron and manganese oxides (i.e. Fe2O3 and MnO2), thus making them available for uptake by the plant [42]. Organic acids are the main inputs for many biochemical events in walnuts, as in all plants. Organic acids in genotypes examined in Çoruh Valley were found to be similar in terms of both quantity and change. In this case, the effect of both environmental and genotypic characteristics in the whole genotypes throughout the Valley is almost similar and the organic acid levels in walnuts showed very close changes. All the organic acids examined were determined in high amounts and stable on the basis of genotypes, while the fruit characteristics, protein ratio and ash were found to be not stable. Briefly, GN10, GN11, GN12, GN13, GN14, GN15, GN20, GN22, GN23, GN24, GN25 and GN26 were determined as high and stable varieties in terms of organic acid (Figure 3).

**Hormone Contents.** The effects of indol-3-acetic acid (IAA), a main auxin in plants, on plants are as follows: to promote root formation of steels, to provide parthenocarpic fruit, to provide adventitious root formation, to prevent fruit and leaf breakdown, to increase fruit attitude, to provide early flowering of buds and to prevent weed growth. In plant cells, The IAA is produced from tryptophan (TRP) in a variety of semi-independent and tryptophan-independent ways. Synthesis of auxin is controlled developmentally and environmentally. Indole-3-acetic acids are known for their effects such as cell division, root development, expansion of root surface area, helping the plant at the entrance of nutrients [44]. Cytokinins are the hormones that initiate the cell division. It is especially synthesized in the root meristems and then transported to the green parts of the plant through xylem. Cytokinins stimulate plant cell division, control root meristem differentiation, inhibits primary root elongation and lateral root formation, but can promote root hair development. The most obvious effect of gibberellins is to increase the prolongation of cells. Gibberellins enhance particularly stem tissue, promote root elongation and lateral root extension. In biplot analysis, although genotypes differed in terms of hormones, IAA and salicylic acid levels in genotypes were determined to be more stable and higher. In other words, the samples examined in the Valley in terms of these two hormones did not change much and they were rich in these two hormones. Salicylic and indole acetic acid have been determined as hormones that are stable on the basis of genotypes and contain high amounts of genotypes. Again, GN9, GN10, GN17, GN20, GN23, GN24 and GN25 have been determined as stable varieties with high hormone content. In addition, it has been demonstrated that genotypes have different hormone levels throughout the Valley in terms of hormone levels and were not stable in this distribution (Figure 6).

## CONCLUSIONS

The present study showed that cultivars affected walnut fruit quality parameters, weight, nutrient contents, crude protein, phenolic components, fatty acid, amino acid, organic acid and hormone contents of walnut in Çoruh Valley, Turkey. The selection of cultivars can improve the economic yield and directly influence the yield components. It can reasonably be concluded that the results of this study showed that cultivars of walnut had a significant effect on fruit quality parameters, weight, nutrient contents, crude protein, phenolic components, fatty acid, amino acid, organic acid and hormone contents of walnut. Different cultivars conditions were caused by a variation in phenolic component, crude protein, fatty acid and nutrient contents. The nut weight of walnut was affected by other components of yield parameters. However, probability in the variations of nut weight and yield parameters was affected by amino acid, organic acid and hormone contents of walnut. Genotypes have a rich and stable structure in terms of other fruit quality parameters, minerals, organic acids, phenolic composition, antioxidant and fatty acids amounts and amino acids. In other words, these characteristics did not show much variation in genotypes grown in the Valley. However, there was a great variation in hormone levels among genotypes. This means that although there may be variations in hormone content in walnut cultivation along the Çoruh Valley, walnut genotypes are expected to display a rich and stable feature across the Valley in terms of other parameters. In conclusion, GN2, GN10, GN15, GN24, GN25 and GN26 genotypes were determined as rich and stable genotypes.

## ACKNOWLEDGEMENTS

The authors certify that there is no conflict of interest regarding the material discussed in the manuscript.

## REFERENCES

[1] Sen, S.M. (2011) Walnut, cultivation, nutritional value, folklore (4th Ed.). ICC Publication: Ankara, Turkey.(in Turkish)

[2] FAOSTAT (2020). http://www.fao.org/faostat/en/#data/QC-2018 [date of access: 04.10.2020].
[3] Bayazit, S., Kazan, K., Gülbitti, S., Çevik, V., Ayanoglu, H., Ergül, A. (2007) AFLP analysis of genetic diversity in low chill requiring walnut (Juglans regia L.) genotypes from Hatay, Turkey. Sci. Hort. 111, 394-398.
[4] Aslantas, R. (2006) Identification of superior walnut (Juglans regia L.) genotypes in north eastern Anatolia, Turkey. New Zeal. J. Crop Hort. 34, 231-237.
[5] WWF (2018) World Wide Fund for Nature Caucasus ecological corridor Available: http://www.wwf.org.tr/ne_yapiyoruz/doga_koruma/doal_alanlar/kafkasyaekolojikkoridoru/ [date of access: 04.09.2020] (in Turkish)
[6] Sen, S.M. (2013) Eat Walnuts, Live Healthy. Venedik Publishing: Ankara, Turkey. p. 215 (in Türkish)
[7] Reiter, R.J., Manchester, L.C., Tan, D.X. (2005) Melatonin in walnuts: Influence on levels of melatonin and total antioxidant capacity of blood. Nutrition. 21, 920-924.
[8] Amaral, J.S., Casal, S., Pereira, J., Seabra, R., Oliveira, B. (2003) Determination of sterol and fatty acid compositions, oxidative stability, and nutritional value of six walnuts (Juglans regia L.) cultivars grown in Portugal. Agric. Food Chem. 51, 7698-7702.
[9] Savage, G.P. (2001) Chemical composition of walnuts (*Juglans regia* L.) grown in New Zealand. Plant Food Hum. Nutr. 56, 75-82.
[10] Grosso, G., Estruch, R. (2016) Nut consumption and age-related disease. Maturitas. 84, 11-16.
[11] Miraliakbari, H., Shahidi, F. (2008) Antioxidant activity of minor components of tree nut oils. Food Chem. 111, 421-427.
[12] Martínez, M.L., Labuckas, D.O., Lamarque, A.L., Maestr, D.M. (2010) Walnut (Juglans regia L.): genetic resources, chemistry, by-products. J. Sci. Food Agric. 90, 1959-1967.
[13] Regueiro, J., Sánchez-González, C., Vallverdú-Queralt, A., Simal-Gándara, J., Lamuela Raventós, R., Izquierdo-Pulido, M. (2014) Comprehensive identification of walnut polyphenols by liquid chromatography coupled to linear ion trap-Orbitrap mass. Food Chem. 152, 340-348.
[14] Baydar, H., Marquard, R., Turgut, I. (1999) Pure line selection for improved yield, oil content and different fatty acid composition of sesame, Sesamum indicum. Plant Breed. 118, 462-464.
[15] Antoine, F.R., Wei, C.I., Littell, R.C., Marshall, M.R. (1999) HPLC method for analysis of free amino acids in fish using ophthaldialdehyde precolumn derivatization. J. Agric. Food Chem. 47, 5100-5107
[16] Battal, P., Tilekliоğlu, B. (2001) The effects of different mineral nutrients on the levels of cytokinins in maize (Zea mays L.). Turk J. Bot. 25, 123-130.
[17] Davies, P.J. (1995) Plant hormones-physiology, biochemistry and molecular biology. In Biochemistry and Molecular Biology. Kluwer Academic Publishers, Dordrecht, The Netherlands: Norwell, MA, USA.
[18] Cutting, J.G.M. (1991) Determination of the cytokinin complement in healthy and witches broom malformed protease. J. Plant Growth Regul. 10, 85-89.
[19] Palni, L.M.S., Summons, R.E., Letham, D.S. (1983) Mass spectrometric analysis of cytokinins in plant tissues, V, Identification of the cytokinin complex of Datura innoxia crown all tissue. Plant Physiol. 72, 858-863
[20] Qamaruddin, M. (1996) Apperance of the zeatin riboside type of cytokinin in Pinus sylvestris seeds after red light treatment. Scand. J. Forest Res. 6, 41-46.
[21] Cheikh, N., Jonmes, R.J. (1994) Distruption of maize kernel growth and development by heat stress. Plant Physiol. 106, 45-51.
[22] Bremner, J.M. (1996) Nitrogen-Total. In: Methods of Soil Analysis: Part 3 Chemical Methods Sparks, D.L., Page, A.L., Helmke, P.A., Loeppert, R.H., Soltanpour, P.N., Tabatabai, M.A., Johnston, C.T., Sumner, M.E. (eds). SSSA Book Series 5. 1085-1121.
[23] Mertens, D. (2005a) Plants Preparation of Laboratory Sample. In: Official Methods of Analysis 18th Chapter 3, Horwitz, W., Latimer, G.W. (eds). Gaitherburg, Maryland USA. 1-2.
[24] Mertens, D. (2005b) Metal in Plants and Pet Foods. In: Official Methods of Analysis 18th Chapter 3, Horwitz, W., Latimer, G.W. (eds). Gaitherburg, Maryland USA. 3-4.
[25] Bayazit, S., Sumbul, A. (2012) Determination of fruit quality and fatty acid composition of Turkish walnut (Juglans regia) cultivars and genotypes grown in subtropical climate of eastern Mediterranean region. Int. J. Agric. Biol. 14, 419-424.
[26] Akca, Y., Sen, S.M. (1995) Relationship between and yield nut characteristic in Juglans regia. Acta Hort. 442, 81-82.
[27] Khattak, M.S., Ali, S., Wahab, F., Shah, M. (2000) Genetic bio-diversity in the segregating population of walnut (Juglans regia L.) at Kurrum agency, Parachnar. Pak. J. Biol. Sci. 3, 965-966.
[28] Ozkan, G., Koyuncu, M.A. (2005) Physical and chemical composition of some walnut (Juglans regia L) genotypes grown in Turkey. Grasas Aceites. 56, 141-146.

[29] Klepping, J., Guilland, J.C., Fuchs, F., Marcer, I., Houard-Malval, M. (1989) Data collection on food composition, CEIV, Roche, Neuilly Sur Seine.(in France)
[30] Ravai, M. (1992) Quality characteristics of Califonia walnuts. Cereal Foods World. 37, 362-366.
[31] Lavedrine, F., Ravel, A., Villet, A., Ducros, V., Alary, J. (2000) Mineral composition of two walnut cultivars originating in France and California. Food Chem. 68, 347-351.
[32] Koyuncu, F., Koyuncu, M.A., Erdal, I., Yavic, A. (2002) Chemical Composition of fruits of some walnut (J. regia L) selections. Gıda. 27, 247-251.
[33] Zwarts, L., Savage, G.P., Mcneil, D.L. (1999) Fatty acid content of New Zealand-grown walnuts (Juglans regia L.). Int. J. Food Sci. Nutr. 50, 189-194.
[34] Ozcan, M.M., Iman, C., Arslan, D. (2010) Physico-chemical properties, fatty acid and mineral content of some walnuts (Juglans regia L.) types. Agric. Sci. 1, 62-67.
[35] Cağlarirmak, N. (2003) Biochemical and physical properties of some walnut genotypes (Juglans regia L.). Food/Nahrung. 47, 28-32.
[36] Pereira, J.A., Oliveira, I., Sousa, A., Ferreira, C.F.R., Bento, A., Estevinho, L. (2008) Bioactive properties and chemical composition of six walnuts (Juglans regia L.) cultivars. Food and Chem. Toxicol. 46, 2103-2111.
[37] Abbey, M., Noaks, M., Belling, G.B., Nestel, P.J. (1994) Partial replacement of saturated fatty acids with almonds or walnuts lowers total plasma cholesterol and low-density lipoprotein cholesterol. Am. J. Clin. Nutr. 59, 995-999.
[38] Poggetti, L., Ferfuia, C., Chiabà, C., Testolin, R., Baldini, M. (2018) Kernel oil content and oil composition in walnut (Juglans regia L.) accessions from north-eastern Italy. J. Sci. Food Agric. 98, 955-962.
[39] Al-Bachir, M. (2004) Effect of gamma irradiation on fungal load, chemical and sensory characteristics of walnuts (Juglans regia L.). J. Stored Products Res. 40, 355-362.
[40] Rana, U., Rai, V.K. (1996) Modulation of calcium uptake by exogenous amino acids in Phaseolus vulgaris seedlings. Acta Physiol. Plant. 18, 117-120.
[41] Ae, N., Arihara, J., Okada, K., Yoshihara, T., Johansen, C. (1990) Phosphorus uptake by pigeon pea and its role in cropping systems of the Indian subcontinent. Science. 248, 477-480.
[42] Marschner, H. (1995) Mineral Nutrition of Higher Plants. 2nd Edn. Academic Press: London.
[43] Jones, D.L. (1998) Organic acids in the rhizosphere - a critical review. Plant and Soil. 205, 25-44.
[44] Zaidi, A., Khan, M.S., Ahemad, M., Oves, M. (2009) Plant growth promotion by phosphate solubilizing bacteria. Acta Microbiol. Immunol. Hung. 56, 263-284.

**Received:** **09.01.2021**
**Accepted:** **17.02.2021**

**CORRESPONDING AUTHOR**

**Ummugulsum Erdogan**
Faculty of Engineering,
Department of Food Engineering,
Bayburt University,
Bayburt – Turkey

e-mail: gulsum25@gmail.com