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ORIGINAL RESEARCH PAPER in HORTICULTURAL PLANTS

Effects of Altitude on the Pomological Characteristics and Chemical Properties of 'Chandler' Walnuts: A Case Study in Uşak Province

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Abstract

This research was conducted to investigate some quality parameters of nuts of walnut (*Juglans regia* L.) 'Chandler' grown at different altitudes. Studies were conducted in Eşme Town, Uşak Province, in three different villages situated at an average altitude of 650 m, 800 m, and 900 m. Studies were conducted in Karaahmetli, Takmak, and Yeşilkavak villages in the 2012–2013 production year and orchards with similar soil and topography characteristics were selected. Plant protection, plant production, and other cultivation practices in the sampled orchards were also noted and orchards with the same attributes were selected. During the harvesting period, regular measurements were taken to determine correct time for harvesting. Nuts were hand-harvested when the color of the membrane separating the kernel and outer parts turned brown. A total of 35–40 nuts were randomly selected and harvested from the three orchards and 15 of those were randomly selected for further analysis. Nut weight, internal (kernel) weight, shell thickness, kernel color, dry matter, moisture, ash, protein, oil, mineral matter, and free fatty acid composition were determined. Results showed that the site altitude significantly influences walnut's quality and it is highly important to consider the site altitude as a factor before planting new orchards. Results showed that nut height, nut width, nut length, nut weight, kernel weight, shell thickness, oil content, phosphorus content, magnesium content, oleic acid, and linolenic acid increase with an increase in altitude. On the other hand, kernel ratio, total nitrogen, and protein content were found to decrease with increasing site altitude.

Keywords

free fatty acids; protein content; minerals; palmitic acid; oleic acid; linoleic acid

1. Introduction

Walnut (*Juglans regia* L.) is an ancient nut originating in Central Asia (Fjellstrom & Parfitt, 1994). Nuts are known to be among the oldest food sources for not only human beings but also for animals (Woodroof, 1967). Walnut had the second place after almonds in the group of nuts until 2013, and then took the first place in total production. The top five largest producers in 2017, in descending order of total production, were China, the USA, Iran, Turkey, and Mexico (Food and Agriculture Organization, 2019). Walnuts have a long history of cultivation throughout the world and are easily adapted to different climates (McGranahan & Leslie, 1990). Walnuts are rich in calories (654 kcal 100 g⁻¹), proteins (15.2 g 100 g⁻¹), lipids (65.2 g 100 g⁻¹), carbohydrates (13.7 g 100 g⁻¹) (United States

Department Agriculture, 2016), and unsaturated fatty acids, i.e., oleic acid, linoleic acid, and linolenic acid (Martínez et al., 2010). Walnuts have no cholesterol and are very beneficial for human health. Jahanbani et al. (2016) reported that walnuts are high in antioxidants and exhibit possible anticancer effects by protecting against breast and colon cancer. Recent studies have also shown that walnuts are rich in health-related phytochemical compounds (Cosmulescu & Trandafir, 2015; Kafkas et al., 2017). They are also a good source of flavonoids and phenolic acids, and have high antioxidant activity (Beyhan et al., 2016; Bujdosó et al., 2014; Gharibzahedi et al., 2014; Polat et al., 2015). These phytochemicals with high antioxidant activity are known to protect human body from free radicals (Nunes et al., 2012).

Walnut has a wide genetic diversity developed through evolution under challenging environmental conditions leading to a vast differentiation in the walnut germplasm and significantly influencing the chemical composition of the fruits (Wan et al., 2017; Yang, 2005). The vast genetic variation arises mainly from seed-based plantations and from high heterozygosity (Bernard et al., 2018). This variability has led to intensive research on walnut germplasm throughout the world, as reported in Spain (Aletà & Ninot, 1997), Iran (Atefi, 1997), the Anatolia (Akça & Şen, 2001; Balci et al., 2001; Yarılguç et al., 2001), China (Gunn et al., 2010), Central Asia (Molnar et al., 2011), and Italy (Poggetti et al., 2017). Archaeological research in Anatolia indicated that the people living there for at least 3,000 years have known and benefited from walnuts (Soylu & Ertürk, 2001). Characteristics of the fruits not only vary among the different germplasm, but also differ within the similar varieties when they are grown under different conditions. The pomological characteristics and chemical composition of fruits might be influenced by the topography of the production area (Örmeci & Aşkın, 2017), growing altitudes (Eskimez et al., 2019; Usanmaz et al., 2018), production systems (Patumi, 1999), and variety (Baccouri et al., 2007; Okatan et al., 2017). Recent studies showed that the morphology of a walnut shoot is affected by its position in the canopy and that shoot morphology is directly related with the number and size of the fruits (Valdebenito et al., 2017).

Fruit production requires longer investment compared to vegetable production and it is important to know the changes in the pomological characteristics of the fruit depending on the different environmental conditions. Selection of the best suitable conditions for different walnut varieties is an important step for ensuring higher fruit quality to consumers and for improving profitability of investments. Today, the most commonly and globally produced walnut cultivar is 'Chandler' throughout the world (USDA, 2016). Kapluhan (2015) noted that the 'Chandler' walnut covers about half of the walnut production in Kırşehir, Konya, and Nevşehir cities in Turkey. Phenological, pomological, and other plant characteristics are very crucial for the selection of suitable germplasm for ecological conditions and to achieve economical cultivation. According to the authors of the present work, no specific studies exist on the differentiation of the pomological characteristics of the 'Chandler' walnut depending on altitudes. Thus, the present research was conducted to investigate some quality parameters of the 'Chandler' walnut grown at different altitudes.

2. Materials and Methods

2.1. Materials

The nuts of the 'Chandler' cultivar (*Juglans regia* L.) were considered in the present study. Nut samples were collected from three different walnut orchards, located in Uşak Province at different altitudes, during 2012–2013 growing season. The soil type and topography of the orchards were similar. The three villages where the walnut orchards were located were Karaahmetli (900 m), Takmak (800 m), and Yeşilkavak (650 m). Plant nutrition, crop protection, and other cultivation practices in the orchards were noted for the study years. Nut samples were collected at commercial maturity when the membrane turned brown and separated from the hard shell. The altitudes of the walnut orchards were determined using Google Earth and geographic information system (GIS) of the Department of Information Technologies in Uşak Province. A total of 35–40 nuts were randomly selected and

harvested from the three orchards, and 15 of those were randomly selected for further analysis. Trees affected by pests and/or diseases were avoided, and only vigorous and productive trees were chosen and represented the average orchard.

2.2. Methods

Nut samples were quickly transferred to laboratory where the inner (kernel) parts were immediately separated from their green shells and put into labeled net bags. They were allowed to dry in a cool, ventilated, and moisture-free shade area to prevent any escalation of moisture and possible darkening of the nut. The physical characteristics of the nuts were determined according to following order:

1. Nut dimensions (length, width, and height) were firstly determined with a digital caliper (sensitive to ± 0.01 mm).
2. Nut weight (whole fruit: kernel with peel) was determined with digital balance (sensitive to ± 0.01 g) after drying at room temperature according to Şen (1980).
3. Hereafter, the peel was broken, the kernel was separated from the peel and the kernel weight was determined with digital balance.
4. At that point, nut peel thickness was also determined from the center of each peel by using digital caliper.
5. Kernel yield was then calculated by using the kernel weight and total weight and expressed as % according to Şen (1980) and Beyhan (1993).
6. The peel color and kernel color (CIE L* a* b*) of the nuts were determined with Minolta CR-400 colorimeter (Minolta Corp, Ramsey, NJ, USA).
7. The kernel color of the nuts was categorized according to the standards of Dried Fruit Association (DFA) as extra light, light, light amber, and amber. Furthermore, the chemical characteristics of the kernel (dry matter, oil percentage, nitrogen content, protein, ash, mineral matter, acids, and free fatty acid composition) were also determined after crushing in a mortar. After crushing, 10 g of kernel samples were taken and dried in an oven at 105 °C for 4 to 6 hours to determine the dry matter content.
8. For the determination of ash content, the Association of Official Analytical Chemists (2000) method was followed where 1.0 g of kernel sample was taken into ash oven and the temperature was increased gradually. Samples were kept at 100 °C for 30 min, 200 °C for 30 min, 400 °C for 60 min, and 550 °C for 330 min. The final and initial weight was then used to calculate ash percentage.
9. Oil percentage of the samples was determined according to the formula of Manirakiza et al. (2001) by using Extraction Unit E-816 HE (Buchi AG, Switzerland).
10. Nitrogen content of the samples was determined according to the Kjeldahl method (Kacar & İnal, 2008). Calculation of the protein content (%) was carried out according to the formula of James (1995) by multiplying the nitrogen percentage with 6.25.
11. Furthermore, mineral analysis was carried with ICP-OES according to the method of NMKL NordVal International (2007).
12. Finally, free fatty acid concentrations were determined by following the method of Yilmazer and Seçilmiş (2006) via GC/MS.

2.3. Data Analysis

Raw data of the present study were subjected to one-way ANOVA for all parameters separately and the mean separation was performed with Tukey's honesty significance analysis at $p \leq 0.05$.

3. Results

3.1. Physical Characteristics of Nuts

The results showed that the nut length, nut width, and nut height varied from 39.73 mm to 43.70 mm, from 30.56 mm to 33.48 mm, and from 31.92 mm to 34.96 mm,

respectively, for the different altitudes (Table 1). It was concluded from the results that the nut height, nut width, and nut length increased with the increasing altitude. The nut weight and kernel weight also exhibited a significant increase as the altitude increased. The average nut weight was 8.77 g at the lowest altitude and 13.35 g at the highest. The average kernel weight was also increased as the altitude increased. However, the increase in the kernel ratio was found to be lower than the total nut weight. Therefore, in contrast to the nut weight and kernel weight results, the kernel ratio was found to decrease with the increase in the altitude. The color of the nut is an important parameter in determination of the walnut quality. All nuts analyzed in the present study were found to have an extra light color according to the DFA color scale for walnuts. However, there were differences in the color categories of the walnuts grown at the different altitudes; the lightness value (L^*) of the nuts was found to decrease as the altitude increased.

Table 1 Changes in some physical parameters of walnuts 'Chandler' according to growing altitude.

Physical characteristics	Yeşilkavak (650 m)	Takmak (800 m)	Karahmetli (900 m)
Nut length (mm)	39.73 ± 0.36 B	43.70 ± 0.40 A	42.69 ± 0.26 A
Nut width (mm)	30.56 ± 0.26 B	33.48 ± 0.12 A	32.90 ± 0.17 A
Nut height (mm)	31.92 ± 0.29 B	34.96 ± 0.14 A	34.86 ± 0.20 A
Nut weight (g)	8.77 ± 0.24 C	12.22 ± 0.16 B	13.35 ± 0.18 A
Kernel weight (g)	4.14 ± 0.17 B	5.40 ± 0.11 A	5.61 ± 0.09 A
Kernel ratio (%)	47.58 ± 5.23 A	44.40 ± 5.88 B	42.16 ± 1.90 B
Shell thickness / at top (mm)	1.55 ± 0.034 B	1.50 ± 0.027 B	1.97 ± 0.041 A
Shell thickness / side (mm)	2.50 ± 0.075 B	2.54 ± 0.053 B	3.27 ± 0.062 A
Color CIE L^* value	56.36 ± 0.52 A	50.37 ± 0.52 B	49.93 ± 0.41 B
Color CIE a^* value	13.55 ± 0.13 A	13.81 ± 0.12 A	13.61 ± 0.12 A
Color CIE b^* value	22.93 ± 0.40 A	22.80 ± 0.27 A	21.62 ± 0.20 B

Values followed by the same letter or letters within the same row are not significantly different at 5% level (Tukey's HSD multiple range test).

3.2. Chemical Characteristics of Nuts

The dry matter (96.74%–96.86%) content of the walnuts was found to have no significant relationship with the growing altitude. There was no significant difference in the dry matter and moisture content in the walnuts grown at the different altitudes (Table 2). The oil content in the walnuts was found to increase as the altitude increased. It was 61.73% at the 650 m altitude and increased to 65.14% and 65.91% at 800 m and 900 m, respectively. Contrary to the oil content, the nitrogen content of the walnuts was found to decrease as the growing altitude increased. Similar to the total nitrogen, as expected, the protein content in the walnuts decreased as the altitude increased. The highest protein content (17.25%) was noted in nuts from the lowest growing altitude (650 m), whereas the lowest protein content (14.81%) was determined in nuts from the highest growing altitude (900 m). The ash content of the nut samples varied from 1.60% to 1.75% and no significant relationship was observed between ash contents and the growing altitude.

Table 2 Changes in some chemical parameters of 'Chandler' walnuts according to growing altitude.

Chemical characteristics	Yeşilkavak (650 m) (%)	Takmak (800 m) (%)	Karahmetli (900 m) (%)
Dry matter	96.77 ± 0.028 A	96.74 ± 0.05 A	96.86 ± 0.042 A
Oil	61.73 ± 0.45 B	65.14 ± 0.20 A	65.91 ± 0.17 A
Total nitrogen	3.255 ± 0.40 A	2.915 ± 0.12 AB	2.795 ± 0.06 B
Protein	17.25 ± 2.11 A	15.45 ± 0.61 AB	14.81 ± 0.32 B
Ash	1.75 ± 0.00 A	1.60 ± 0.01 C	1.70 ± 0.02 B

Values followed by the same letter or letters within the same row are not significantly different at 5% level (Tukey's HSD multiple range test).

3.3. Mineral Content of Nuts

Changes in the mineral contents in the walnuts according to growing altitude are shown in Table 3. As shown by the results obtained, the nuts were found to have high amounts of phosphorus, magnesium, calcium, and potassium. Among these four most abundant minerals, phosphorus and magnesium were found to increase as the growing altitude increased. No significant correlation was obtained for calcium, whereas potassium was found to show a nonlinear increase as the altitude increased. The zinc content in the nuts decreased as the growing altitude increased, but no relationships with the altitude were detected in the case of the other minerals, i.e., manganese, iron, copper, and sodium.

Table 3 Changes in some minerals of 'Chandler' walnuts according to growing altitude.

Minerals	Yeşilkavak (650 m) (mg 100 g ⁻¹)	Takmak (800 m) (mg 100 g ⁻¹)	Karaahmetli (900 m) (mg 100 g ⁻¹)
Phosphorus (P)	417 ± 0.20 B	434 ± 0.24 AB	501 ± 0.06 A
Magnesium (Mg)	141 ± 0.07 B	141 ± 0.08 B	158 ± 0.07 A
Calcium (Ca)	148 ± 0.04 B	112 ± 0.07 C	202 ± 0.06 A
Potassium (K)	441 ± 0.0 B	509 ± 0.04 A	488 ± 0.10 A
Zinc (Zn)	1.3 ± 0.0 A	1.3 ± 0.0 A	1.1 ± 0.0 A
Manganese (Mn)	7.6 ± 0.0 A	2.5 ± 0.0 C	4.1 ± 0.0 B
Iron (Fe)	0.95 ± 0.0 B	0.85 ± 0.0 C	1.05 ± 0.0 A
Copper (Cu)	0.5 ± 0.0 A	0.4 ± 0.0 A	0.4 ± 0.0 A
Sodium (Na)	0.4 ± 0.0 A	0.3 ± 0.0 A	0.3 ± 0.0 A

Values followed by the same letter or letters within the same row are not significantly different at 5% level (Tukey's HSD multiple range test).

3.4. Free Fatty Acids

The types of fatty acids and the percentage changes in their content in the walnuts relative to the growing altitudes are presented in Table 4. As demonstrated by the results obtained, linoleic acid was present at the highest concentration (58.185%–58.607%) and was followed by oleic acid (12.957%–14.182%) and linolenic acid (12.038%–12.911%). Among these four most abundant fatty acids, the content of linoleic acid was not correlated with the growing altitude, but the amounts of oleic acid and linolenic acid were found to increase as the altitude increases. Oleic acid is a monounsaturated fatty acid while linolenic acid, likewise linoleic acid, is a saturated fatty acid.

Table 4 Changes in free fatty acids of 'Chandler' walnuts according to growing altitude.

Free fatty acids	Yeşilkavak (650 m) (%)	Takmak (800 m) (%)	Karaahmetli (900 m) (%)
Myristic acid (C14:0)	0.061 ± 0.0 B	0.016 ± 0.0 C	0.085 ± 0.0 A
Pentadecanoic acid (C15:0)	0.020 ± 0.0 A	0.013 ± 0.0 B	0.018 ± 0.0 AB
<i>cis</i> -Pentadecanoic acid (C15:1)	0.013 ± 0.0 A	0.016 ± 0.0 A	0.023 ± 0.0 A
Palmitic acid (C16:0)	8.606 ± 0.0 B	9.399 ± 0.11 A	8.714 ± 0.02 B
Palmitoleic acid (C16:1 C7)	0.082 ± 0.04 A	0.062 ± 0.0 B	0.076 ± 0.0 AB
9-Heksenoik acid (C16:1 C9)	0.082 ± 0.0 A	0.081 ± 0.0 A	0.076 ± 0.0 A
Heptadecanoic acid (C17:0)	0.092 ± 0.0 A	0.113 ± 0.0 A	0.126 ± 0.01 A
Stearic acid (C18:0)	3.073 ± 0.05 B	3.288 ± 0.03 A	3.133 ± 0.07 AB
Oleic acid (C18:1 n9c)	12.957 ± 0.17 B	13.219 ± 0.00 B	14.182 ± 0.14 A
Conjugated linoleic acid (C18:1 t10)	1.1385 ± 0.0 A	1.1035 ± 0.04 A	1.1365 ± 0.01 A
Linoleic acid (C18:2)	58.607 ± 0.47 A	58.185 ± 0.13 A	58.343 ± 0.40 A
Linolenic acid (C18:3)	12.038 ± 0.05 B	12.262 ± 0.02 B	12.911 ± 0.17 A
Arachidonic acid (C20:0)	0.4815 ± 0.0 A	0.0330 ± 0.0 C	0.1705 ± 0.01 B

Values followed by the same letter or letters within the same row are not significantly different at 5% level (Tukey's HSD multiple range test).

4. Discussion

The results of the nut length, nut width, and nut height obtained in the present study agree with the findings reported by Oğuz et al. (2017). Our study clearly showed that these three characteristics of the nuts increased as the altitude increased. The average nut weight, kernel weight, and kernel ratio results shown in the present study are in agreement with some previous reports (Aslansoy, 2012; Beyhan, 2009; Muradoğlu & Balta, 2010; Şimşek, 2010; Ünver & Çelik, 2005). Shell thickness is an important quality parameter determining nut crackability. We documented that the shell thickness at both the top and the side increased with the increasing altitude. The nut shell thickness in the present study was found to be higher than that reported by Koyuncu and Aşkın (1995) for 39 different germplasm in Denizli Province, Turkey. However, our results are in agreement with the findings shown by Godeanu et al. (1997) in four different germplasm in Romania, and with the notes reported by Şimşek (2010) in Mardin Province, Turkey. Similarly, the study conducted by Amiri et al. (2010) showing that kernel weight is significantly correlated with the shell thickness supports our findings.

In terms of the average dry matter and moisture contents, our results are in agreement with the studies carried out by Başer et al. (2016). The oil content obtained in the present study is higher than that reported by Muradoğlu and Balta (2010) but similar to the values documented by Savage (2001), Doğan and Akgül (2005), Çelik et al. (2011), Gharibzahedi et al. (2014), Keles et al. (2014), and Yarılgaç and Yılmaz (2016). However, these previous studies only reported the oil content in walnuts but did not study the influence of growing conditions on the oil content. To our knowledge, no previous studies aimed to determine the influence of the growing altitude on the protein content in walnuts. However, in several previous reports (e.g., Çelik et al., 2011; Koyuncu et al., 2005; Yarılgaç et al., 2003; Yarılgaç & Yılmaz, 2016), the protein levels in walnut differed depending on the growing environments. The results of the ash content (from 1.60% to 1.75%) obtained in the present study supported the findings reported by Yarılgaç et al. (2003) but were lower than that documented by Gharibzahedi et al. (2014).

The nuts analyzed in this study were found to have a high amount of phosphorus, magnesium, calcium, and potassium. The results of the average potassium and magnesium concentrations are in agreement with the research conducted by Yerlikaya et al. (2012). In turn, the iron concentration was lower than that determined by Tapia et al. (2013). Concurrently, the manganese concentration was higher compared to that reported by Tapia et al. (2013). To the best of our knowledge, there are no results showing a relationship between the walnut quality parameters and the growing altitude. However, the results of our analyses and other reports showing the environmental impact on walnut components suggest that the altitude has a significant influence on the quality parameters of walnuts, including minerals.

The concentration of linoleic acid in the walnuts analyzed in the current study confirms the findings reported by Beyazit and Sümbül (2012), but the contents of palmitic acid and linolenic acid are higher. In turn, the amounts of oleic acid and stearic acid are lower compared to those presented by Beyazit and Sümbül (2012). The oleic acid concentration obtained in the present study is also lower than that documented by Yerlikaya et al. (2012) and Şimşek (2016). The linoleic acid concentration in the walnuts determined in our study is in agreement with the results reported by Yerlikaya et al. (2012) and Şimşek (2016). Our results also agree with the study conducted by Beck et al. (2014), who reported that walnuts are rich in linolenic acid. Overall, the concentrations and diversity in the fatty acid composition are in agreement with the results presented by Bouabdallah et al. (2014) and Gao et al. (2019).

It is possible to conclude that some of the walnut fruit characteristics are significantly affected by the altitude, whereas some others are not. It was also found that 'Chandler' nuts may exhibit different response to the growing altitude, compared to other walnut cultivars.

5. Conclusion

Results of the present study suggested that site altitude has significant influence both on the pomological characteristics and on the fatty acid composition of walnuts. Results showed that nut height, nut width, nut length, nut weight, kernel weight, shell thickness, oil content, phosphorus content, magnesium content, oleic acid, and linolenic acid increased with growing altitude. On the other hand, some other characteristics, i.e., kernel ratio, total nitrogen, and protein content showed a decreasing trend while the site altitude increased. Site altitudes of the present study were found to have slight influence on the lightness of kernel color but did not affect the nut color. Walnuts at higher altitudes were found to have slightly darker kernel than those at lower altitudes. Some other parameters such as dry matter, moisture content, ash content, calcium content, potassium content, and linoleic acid were also noted to have no significant relationship with the site altitude. In conclusion, results showed that the site altitude significantly influenced walnuts quality and it is highly important to consider the site altitude as a factor before planting new orchards.

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