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# Determination of Fat, Fatty Acids and Tocopherol Content of Several Turkish Walnut Cultivars

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**Abstract**

In this study, six Turkish local walnuts (*Juglans regia* L.) cultivars grown in the Pozantı district of Adana province of Turkey were characterized in terms of fat, fatty acids, and tocopherols content. Total lipid and fatty acid content were analysed using Gas Chromatography Flame Ionization Technique (GC/FID) while, tocopherol content was analysed by High-Performance Liquid Chromatography (HPLC) technique. The total fatty acid levels varied between 62.54% (Yalova-4) to 70.20% (Yalova-2). Three saturated fatty acids were identified and quantified as stearic acid ranging from 1.96 to 3.25%, myristic acid ranging from 0.05 to 0.07% and palmitic acid ranging from 5.98 to 6.84%. As for the unsaturated fatty acids; linoleic acid which a predominant fatty acid (54.13-62.51%), followed by oleic acid (18.82-27.09%), a-linoleic acid (9.83-12.7%), and palmitoleic acid (0.04-0.09%) were detected respectively. In this study, the spectrum of tocopherols comprised of three isoforms; α, β, γ were also identified and quantified by HPLC technique. However, δ-tocopherol isoform was not detected. In the all examined walnut cultivars, the total tocopherol content varied from 67.18 (Bilecik) to 173.81 µg/g (Yalova-2). γ-tocopherol, a-tocopherol and β- tocopherol content were found between 61.1 to 164.51 µg/g, 5.43 to 11.53 µg/g and 0.60 to 5.82 µg/g, respectively. Yalova-2 was the most promising cultivar in terms of total oil content and total tocopherol content.

**Key words:** Walnut, total oil, fatty acids, tocopherols

## INTRODUCTION

Walnut (*Juglans regia* L.) is originated from Central Asia, and native to a wide range of regions from China, Iran to Turkey (Şen, 2011). Nowadays, walnut is planted in commercially scale in more than 60 countries in both hemisphere (Abdallah et al., 2015). Walnut contains relatively high level of fat, plant protein, tocopherols and antioxidants (phytosterol and polyphenols), additionally walnut is also rich in minerals such as iron, zinc, copper, magnesium and phosphorus (Ünver & Sakar, 2011). Due to the rich nutrition content walnut is widely accepted as a functional food. Walnut plays an important role in the Mediterranean diet for centuries (Ros, 2010). At present, walnut is widely used in food, pharmaceutical and cosmetic industries due to its high nutrition value (Ercisli et al., 2012). Thus, its production is constantly increasing and becomes the second-largest nut after almond in terms of production amount (FAO, 2020).

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Turkey is the fourth largest walnut producer in the world with an annual production of 215 thousand metric tons and ranked after China (1.59 million metric tons), USA (613 thousand metric tons) and Iran (394 thousand metric tons). Turkey is also the largest walnut producer in the Mediterranean region (FAO, 2020). Being one of the native regions of walnut cultivation, walnut is cultivated in many regions of Turkey. Traditionally, walnut plantation is done by seed propagation in Turkey, this leads to a great range of walnut varieties which is a valuable gene resource with many potential cultivars and genotypes with high nutrition value (Çiftçi & Gökce, 2005). In recent years, modern orchards were established by grafting the commercial local and foreign cultivars to scions. Therefore, knowing the physicochemical properties of the important local cultivars become essential to choose the proper cultivar for production also for the breeding projects.

Recent studies showed significant correlation between human diseases with their daily diet, and fatty acids was proven to be one of the most important positive health factor (Çam, 2020). In commercial cultivars, kernel represents 40-45% of the nut weight in walnut, and 59-74% of the kernel weight is fatty acids. Fatty acids (FA) in walnut are mostly unsaturated which is around 75% of the walnut oil (Crews et al., 2005). Unsaturated fatty acids have positive effect on regulating blood lipid. In contrast with other nut crops, walnut is rich in polyunsaturated fatty acids (PUFA) which is more welcomed due to its health benefits of PUFAs (Amaral et al., 2003a; Beyhan et al., 2017a). Lionelic and linolenic acids are dominant PUFAs in walnut (Liu et al., 2020). High PUFA content of walnut oil is effective in lowering the total cholesterol level and reducing the risk of cardiovascular disease (Pycia et al., 2019). FA content of the walnut is differed between cultivars so that is important to detect the FA content of the local growing cultivars in order to select the cultivars with the best nutrition value (Zwarts et al., 1999).

Tocopherols has several homologs such as α, β, γ, δ-tocopherol depending on the positional and numerical difference between the methyl groups. They have natural antioxidant activity and they can protect the PUFAs in walnut oil against oxidation (Savage et al., 1999; Kodak et al., 2016). Tocopherol content of the oil is essential for its positive nutrition effects. Walnuts are rich in γ -tocopherol which is very important in reduction of the blood cholesterol levels and decreases the risk of cardiovascular diseases (Amaral et al., 2003a; Davis et al., 2007).

To our knowledge, a few Turkish local cultivars physio-chemical characteristics have been reported previously. Total fatty acid content of Yalova-1, Yalova-2, Yalova-3, Yalova-4 and Bilecik cultivars was studied, however fatty acid profile and tocopherol contents were remained unknown (Bakkalbaşı et al., 2010; Yerlikaya et al., 2012). Kaplan-86 will be studied in this study for the first time especially in the Pozantı region in Turkey. Kaplan-86 is a very important cultivar with extreme traits such as large nut (diameter 65 mm) and heavy nut (22-24g), which is widely used as paternal parent in many cultivars breeding programs (Kefayati et al., 2019). Aim of this study was to determine the phytochemical contents such as Total oil content, fatty acid profile, and different tocopherol isomer concentration of the six local Turkish walnut cultivars collected from the Pozantı district of Adana province in Turkey in order to select the cultivar with high nutrition value. This study was conducted to provide important phytochemical characteristics of some important Turkish cultivars. Which may be used in large-scale production or can be used as parents in future walnut breeding programs.

## MATERIALS AND METHODS

### Plant material

This study was conducted with the comparison of six local Turkish walnut cultivars (Yalova-1, Yalova-2, Yalova-3, Yalova-4, Kaplan-86 and Bilecik) planted in the commercial orchard in the Pozantı district located in latitude 37.4256, longitude 34.8736 and altitude 780 m. Walnut samples were collected at maturity stage in September 2019 crop year. Samples were immediately brought to the laboratory green husk removed by hand then dried in the oven at 40 $^0$C for three days.

### Oil extraction

Bligh & Dyer, (1959) method was used for walnut kernel oil extraction of the six walnut cultivars with minor modifications (Bilgin et al., 2021). At first, 20g of each kernel samples were grinned separately with mortar. Ground samples were used for the oil extraction using hexane solvent in Soxhlet equipment (Gerhardt Soxtherm). Then the samples were mixed with petroleum benzene and kept for 2h for drying at room temperature until reached a constant weight. The oil content was calculated based on the weight difference of tubes before and after the experiment. The oil samples were used for the fatty acid and tocopherol analysis.

### Fatty acid determination

Fatty acid analysis of the walnut kernel was performed by Agilent 7820A gas chromatography (GC) equipment with a flame ionization detector (Agilent Technologies, Santa Clara, CA, USA). The oil samples obtained from the Soxhlet extraction were converted to the corresponding methyl ester forms according to the method by AOCS (1993) with some modification by Ichihara et al., (1996). The column temperature program was 5 min at 140$^0$C, 4$^0$C/min to 200$^0$C, 1$^0$C/min to 220$^0$C. The injector and detector temperatures were 220 and 280$^0$C, respectively. The carrier gas was helium, flow rate was 0.3 ml/ minute. Fatty acids were identified by comparing retention time with the standard mixture of methyl esters (Sigma-Aldrich Chemicals 189-19) from butyric acid (C 4:0) to nervonic acid (C 24:1). The results were presented as % of total fatty acids.

### Tocopherol concentration

Tocopherol analysis was performed according to the method developed by Peter F. Surai et al., (1996) with some modifications by Surai & Sparks, (2000). A total of 1g oil sample from each walnut genotype was used for the tocopherol (α, β, γ, and δ-tocopherol) analysis using the HPLC system (Agilent 1100). In the analysis, Hexane: Acetic Acid: 2-propanol (1000:5:6) was used as mobile phase, samples were injected into the GL Science InterstilTM NH2 (5µm 4.6 x 250 mm) column, and the peaks were detected using the UV detector at wavelengths of 298 nm.

### Statistical analysis

All the samples were analyzed in triplicate. The statistical data were expressed as averages ± standard deviations (SD) by using Excel 2019 and a completely randomized design was used and the data were obtained by using Analysis of Variance (ANOVA) Statistical Product and Service Solutions (SPSS v23.0) software.

## RESULTS AND DISCUSSION

In this study, six important walnut cultivars (Yalova-1, Yalova-2, Yalova-3, Yalova-4, Kaplan-86 and Bilecik) cultivated in the Pozantı district of Adana province of Turkey were characterized in terms of total oil, saturated and unsaturated fatty acids, and tocopherol content.

### Total oil content

The values of the total fat (%) and the important saturated fatty acid profile of the six walnut cultivars are presented in Table 1. This study determined significant differences among the phytochemical components in six cultivars. The total oil content (TOC) was altered in the range of 62.5–70.2%. Yalova-4 had the lowest TOC, while Yalova-2 had the highest (Table 1). Bilecik (69.7%) and Yalova-2 (70.2%) had higher TOC than the average TOC (62.5 /100 g) given by the USDA Standard Reference (USDA National Nutrition Database, 2018). The TOC of commercial walnut cultivars from germplasm were varied between 62.3-66.5% in Portugal (Amaral et al., 2003a), 65.8-69.3% in Turkey (Yerlikaya et al., 2012), 68.9-72.9% in Italy (Poggetti et al., 2018), 62.2-64.2% in Spain (Li et al., 2007) and 61.7-68.7% in Poland (Pycia et al., 2019). The average TOC from six cultivars was similar to previously published results. Bakkalbaşı et al., (2010) studied the TOC of seven Turkish cultivars grown in three different locations in two consecutive years. TOC of Yalova-1, Yalova-3, Yalova-4, and Bilecik cultivars changed between 61.47 to 65.40%, 65.06-69.37%, 72.56 to 68.23%, and 65.78 to 67.80%, respectively. The significant difference of TOC between two different years in the same location showed the effect of the different climate conditions on TOC. In Bakkalbaşı et al., (2010), Yalova-4 had the highest TOC, while in our study Yalova-4 had the lowest TOC value. This is mostly due to the two very different geographical environments in which Yalova-4 cultivar was planted. The different geographical environments and different climate conditions could change fatty acid content and composition (Crews et al., 2005). This explained the differences in fatty acid content and profile among the same walnut cultivars in the different regions.

### Major fatty acid profile

A summary of fatty acid profiles of six walnut cultivars is presented in Table 1 and Table 2**.** Significant statistical differences in most of the major fatty acid profiles were determined between six walnut cultivars ($p<0.05$). In this study, fatty acid profile of six walnut cultivars showed high content of polyunsaturated fatty acids (PUFA), intermediate content of monounsaturated fatty acids (MUFA) and low content of saturated fatty acids (SFA) (Table1 and 2) which is similar to the previous studies (Amaral et al., 2003b; Beyhan et al., 2017b; Zwarts et al., 1999). SFA content of cultivars oscillated between 8.36 (Yalova-2) to 9.76% (Bilecik). Palmitic acid is the predominant saturated fatty acid in walnut oil which is followed by stearic acid and myristic acid. Content of these fatty acids was varied from 5.98 to 6.84%, 1.96 to 3.25%, and 0.5 to 0.7%, respectively (Table 1). These results of palmitic acid and stearic acid were in general agreement with previous reports (Gao et al., 2018; Ünver et al., 2016) while the result of myristic acid is statistically much higher than reported in Ünver et al., (2016) which is 0.01-0.02%. This statistical difference is most probably due to the extremely low content of the myristic acid.

**Table 1.** Total fat and saturated fatty acid content (%) of the six selected walnut genotypes

| Genotypes | Total fat (%) | Saturated fatty acids (%) | | | |
|---|---|---|---|---|---|
| | | Stearic acid | Myristic acid | Palmitic acid | SFA |
| Kaplan-86 | 63.9$^{bc}$ | 3.25±0.12$^{a}$ | 0.07±0.02$^{a}$ | 6.22±0.09$^{c}$ | 9.54$^{b}$ |
| Bilecik | 69.66$^{a}$ | 3.17±0.50$^{a}$ | 0.06±0.02$^{ab}$ | 6.53±0.03$^{b}$ | 9.76$^{a}$ |
| Yalova-1 | 65.65$^{b}$ | 2.25±0.05$^{bc}$ | 0.05±0.02$^{c}$ | 6.07±0.21$^{c}$ | 8.37$^{d}$ |
| Yalova-2 | 70.20$^{a}$ | 2.33±0.14$^{bc}$ | 0.05±0.00$^{c}$ | 5.98±0.15$^{c}$ | 8.36$^{d}$ |
| Yalova-3 | 65.56$^{b}$ | 2.51±0.00$^{b}$ | 0.06±0.01$^{ab}$ | 6.21±0.35$^{c}$ | 8.38$^{d}$ |
| Yalova-4 | 62.54$^{c}$ | 1.96±0.25$^{c}$ | 0.06±0.00$^{ab}$ | 6.84±0.29$^{a}$ | 8.86$^{c}$ |

Saturated fatty acid(SFA) (Stearic acid + Myristic acid + Palmitic acid).
Values were given as mean ± SD
Different letters in a column symbolized significant differences ($p<0.05$)

Total PUFA content made up 72.77% of fatty acids in Yalova-1 and only 63.94% in Yalova-3. MUFA content changed between 18.86%(Yalova-1) to 27.09% (Yalova-4) (Table 2). In the study of Akbari et al., (2014), PUFA, MUFA, and SFA were ranged from 57.6 to 70.2%, 17.9 to 28.7%, and 7.7 to 11.1%, respectively. These results were in general agreement with our study. Yalova-1 had the highest PUFA together with the lowest MUFA. Palmitic, oleic, linoleic, and a-linolenic acid are the primary unsaturated fatty acids. Oleic acids and palmitoleic acids ranged from 18.82 (Yalova-1) to 27.09% (Yalova-4), 0 to 0.09%, respectively. Yalova-3 had the highest linoleic content (62.51%), whereas Yalova-3 had the lowest (52.13%). Linoleic is the predominant fatty acid in all walnut cultivars changing from 52.13% in Yalova-3 to 62.51% in Yalova-1. The highest a-linolenic acid content was found in Kaplan-86 (12.70%), whereas the lowest value was found in

Yalova-4 (9.83%). Similar to the result of this study, Gülsoy et al., (2019) and Wang et al., (2020) stated that linoleic, linolenic, oleic, palmitic and stearic acids were the main fatty acids compositions of walnut oils. The most dominate fatty acid linoleic fatty acid content of the cultivars in this study is 52.13 -62.51%. This result is similar to the other study from Turkey (53.2 – 64.5%) (Kafkas et al., 2017) and Argentina (52.2 – 60.9%) (Cittadini et al., 2020) while was lower than the results from Spain (59.8 – 64.7%) (Wang et al., 2020). Oil composition can be influenced by many factors such as geographical location, climate conditions, genotypes and cultural practices (Crews et al., 2005). Oleic, linolenic and palmitic acids composition were in accordance with data obtained from other previous studies (Amaral et al., 2005; Gülsoy et al., 2019; Rabrenovic et al., 2011).

**Table 2.** Unsaturated fatty acid content (%) of the six selected walnut genotypes (mean ± SD)

| Genotypes | Unsaturated fatty (%) | | | | | |
|---|---|---|---|---|---|---|
| | Polyunsaturated fatty acids (%) | | | Monounsaturated fatty acids (%) | | |
| | Linoleic acid(%) | a-linolenic (%) | PUFA (%) | Oleic acid(%) | Palmitoleic acid (%) | MUFA (%) |
| Kaplan-86 | 58.64±2.38$^{b}$ | 12.7±0.31$^{a}$ | 71.34$^{a}$ | 19.07±2.47$^{c}$ | 0.04±0.01$^{b}$ | 19.11$^{c}$ |
| Bilecik | 56.64±0.55$^{c}$ | 11.63±1.48$^{abc}$ | 68.27$^{b}$ | 21.95±0.4$^{b}$ | 0.04±0.00$^{b}$ | 21.99$^{b}$ |
| Yalova-1 | 62.51±1.59$^{a}$ | 10.26±0.20$^{bc}$ | 72.77$^{a}$ | 18.82±1.58$^{c}$ | 0.04±0.03$^{b}$ | 18.86$^{c}$ |
| Yalova-2 | 59.92±0.36$^{b}$ | 12.63±0.22$^{a}$ | 72.55$^{a}$ | 19.08±0.13$^{c}$ | n.d | 19.08$^{c}$ |
| Yalova-3 | 52.13±5.52$^{e}$ | 11.81±0.56$^{ab}$ | 63.94 $^{c}$ | 25.51±2.79$^{a}$ | 0.09±0.00 $^{a}$ | 25.6$^{a}$ |
| Yalova-4 | 54.22±1.16$^{d}$ | 9.83±0.67$^{c}$ | 64.05 $^{c}$ | 27.09±1.87$^{a}$ | n.d | 27.09$^{a}$ |

Values were given as mean ± SD
Different letters in a column symbolized significant differences ($p<0.05$)
n.d: not detected

**Tocopherol content**

The tocopherol content and compositions in walnut oils are presented in Table 3. The studied walnut oils are rich in tocopherols and consist of three types of tocopherol isomers: a, β and γ (Table 3). The total tocopherol and tocopherol contents were showed significant statistical differences among the varieties ($p< 0.05$). The total tocopherol content changed from 67.18 (Bilecik) to 173.81 µg/g (Yalova-2). These results were higher than 19.83 – 126.00 mg/kg by Pycia et al., (2019) from Poland, while lower than 186.5 to 436.2 mg/kg by Abdallah et al., (2015) from Tunisia and 149.1 to 267.2 mg/kg by Miraliakbari & Shahidi, (2008) from Canada. The difference in the total tocopherol content could be influenced by the genotype, which is in accordance with previous publications (Abdallah et al., 2015; Kornsteiner et al., 2006; Li et al., 2007).

This research clearly showed that γ-tocopherol is the major tocopherol form in walnut oil. The highest content of γ-tocopherol was found in Yalova-2 (164.51 µg/g), while the lowest content was found in the Bilecik (61.1 µg/g). a-Tocopherol is the second predominant tocopherol composition ranging from 5.43 (Bilecik) to 11.53 µg/g (Kaplan-86). The β-tocopherol is a minor tocopherol isomer and ranging from 0.60 to 5.82 µg/g. In this study, γ-tocopherol is the major tocopherol form in walnut oil. This result is consistent with the previous authors (Abdallah et al., 2015; Kornsteiner et al., 2006; Li et al., 2007). According to them, γ-tocopherol also is the major tocopherol fragment in walnut oil. While Cittadini et al., (2020) suggested that the β- and δ-tocopherol is the main tocopherol from. On the other hand, some authors (Abdallah et al., 2015; Kornsteiner et al., 2006; Li et al., 2007) stated that the smallest tocopherol form is a-tocopherol, which is not congruent with the current study in which β-tocopherol is the minor tocopherol form. Besides, Kornsteiner et al., (2006) determined that a-tocopherol is not detected in walnut taken from Austria, whereas β- and γ-tocopherol are the main tocopherols fragments. In addition, γ-tocopherol is determined as predominate tocopherol in the Canadian walnut cultivars, but β- tocopherol is not found. The main reasons for variability of the tocopherols composition in the different experiments could be caused by the difference of genotypes (Kodad et al., 2016), the effect of cultivated environment (Amaral et al., 2005) and interaction between genotype and environment (Cittadini et al., 2020), abiotic stress factors (Munné-Bosch & Alegre, 2002), climatic conditions during cultivated years as well as phenomena of drought during fruit development (Munné-Bosch & Alegre, 2002). Tocopherols are a group of bioactive components that have potent antioxidant properties. They are used as precursors compound for producing vitamin E. Besides, they have active roles in inhibiting the lipid oxidation processing in oil seeds and vegetable oils. Some researchers stated that γ-tocopherol is more potent than a-tocopherol in some processes, such as reducing platelet aggregation, LDL oxidation, and put-off intra-arterial thrombus formation (Abdallah et al., 2015; Pycia et al., 2019). Considering all examined walnut genotypes, Yalova-2 is a superior cultivar in terms of total tocopherol and total lipid content.

**Table 3.** Total tocopherol contents ( µg/g oil) and composition (%) in six walnuts cultivars.

| Genotypes | α | | β | | γ | | Total (µg/g ) |
|---|---|---|---|---|---|---|---|
| | µg/g | % | µg/g | % | µg/g | % | |
| Kaplan-86 | 11.53±0.53[a] | 8.24 | 1.33±0.26[c] | 0.95 | 126.96±1.83[d] | 90.80 | 139.82[d] |
| Bilecik | 5.43±0.24[c] | 8.08 | 0.65±0.02[d] | 0.97 | 61.1±0.66[f] | 90.95 | 67.18[f] |
| Yalova-1 | 8.78±0.33[b] | 6.07 | 2.36±0.06[b] | 1.63 | 133.51±2.18[c] | 92.30 | 144.65[c] |
| Yalova-2 | 8.66±0.19[b] | 4.98 | 0.64±0.08[d] | 0.37 | 164.51±2.12[a] | 94.65 | 173.81[a] |
| Yalova-3 | 8.67±0.27[b] | 5.69 | 5.82±0.07[a] | 3.82 | 137.99±1.45[b] | 94.50 | 152.48[b] |
| Yalova-4 | 9.01±0.54[b] | 8.37 | 0.6±0.05[d] | 0.56 | 98.01±2.95[e] | 91.07 | 107.62[e] |

Values were given as mean ± SD
Different letters in a column symbolized significant differences ($p<0.05$)

## CONCLUSION

The current study revealed the phytochemical quality of the critical six Turkish local walnut genotypes cultivated in the Pozantı district of Adana, Turkey. The results obtained by this study showed that the walnut genotypes differ in their total oil content, fatty acid components, and tocopherol contents basing on walnut genotypes. Walnut is a rich source of unsaturated fatty acids and tocopherols. Linoleic acid is predominant in unsaturated acids, and γ-tocopherol is the major tocopherol form. Unsaturated fatty acids and tocopherols are very important in human health. Thus in recent years, these parameters have become one of the main objectives of farmers and breeding projects. This study presented essential information to farmers and breeders who aimed to develop walnut cultivars with high nutrition value. Besides, it adds a new report on phytochemical properties of six important walnut genotypes from Turkey.

## AUTHOR CONTRIBUTIONS

MA, SH, and OB prepared the plant materials. SH, EK, AP, and MA performed the analysis. AP, NT and SK wrote the manuscript. All the authors contributed to the article and approved the submitted version.

## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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