

## Determination of Vitamin E Levels in Walnuts (*Juglans regia L.*) Grown in the Lake Van Basin by HPLC

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### Abstract

*Aim of study:* The present study describes determination of vitamin E levels in Walnuts (*Juglans regia L.*) Grown in the Lake Van basin.

*Area of study:* Adilcevaz, Bahçesaray, Çatak, Edremit, Gevaş regions in the Lake Van basin and in the center of Van.

*Material and methods:* Walnuts were collected regions and stored in a deep freezer for analysis. On the day of analysis, 10 pieces of fresh and dried walnut samples belonging to each region were taken and broken, and a homogeneous mixture was obtained by pulverizing them and determined by HPLC.

*Main results:* The amounts of  $\alpha$ -Tocopherol of the samples in fresh walnuts were found to be  $2.36 \pm 0.01 \mu\text{g/g}$ ,  $5.01 \pm 0.31 \mu\text{g/g}$ ,  $6.23 \pm 0.15 \mu\text{g/g}$ ,  $7.20 \pm 0.28 \mu\text{g/g}$ ,  $3.99 \pm 0.46 \mu\text{g/g}$ ,  $5.05 \pm 0.30 \mu\text{g/g}$ , respectively, and the amounts of  $\gamma$ -Tocopherol were found to be  $23.80 \pm 0.52 \mu\text{g/g}$ ,  $16.54 \pm 1.08 \mu\text{g/g}$ ,  $14.82 \pm 0.46 \mu\text{g/g}$ ,  $17.34 \pm 0.78 \mu\text{g/g}$ ,  $11.91 \pm 0.40 \mu\text{g/g}$ ,  $15.57 \pm 0.90 \mu\text{g/g}$ , respectively, according to the regions. The amounts of  $\alpha$ -Tocopherol in dried walnuts were found to be  $5.13 \pm 0.26 \mu\text{g/g}$ ,  $6.84 \pm 0.31 \mu\text{g/g}$ ,  $7.58 \pm 0.40 \mu\text{g/g}$ ,  $10.49 \pm 0.51 \mu\text{g/g}$ ,  $8.65 \pm 0.34 \mu\text{g/g}$ ,  $5.82 \pm 0.19 \mu\text{g/g}$ , respectively, and the amounts of  $\gamma$ -Tocopherol were found to be  $33.10 \pm 1.65 \mu\text{g/g}$ ,  $21.97 \pm 0.87 \mu\text{g/g}$ ,  $20.98 \pm 0.83 \mu\text{g/g}$ ,  $29.19 \pm 0.80 \mu\text{g/g}$ ,  $21.09 \pm 0.24 \mu\text{g/g}$ ,  $18.41 \pm 0.82 \mu\text{g/g}$ , respectively, according to the regions.

*Highlights:* The results obtained in the study are considered, it is observed that the walnuts grown in Adilcevaz, Bahçesaray, Çatak, Edremit, Gevaş and Van-Center regions are of high quality.

**Keywords:** Walnut, Vitamin E, Lake Van basin, HPLC, Tocopherol

## Van Gölü Havzasında Yetiştirilen Cevizlerde (*Juglans regia L.*) Vitamin E Düzeylerinin HPLC ile Belirlenmesi

### Öz

*Çalışmanın amacı:* Bu çalışmada, Van gölü havzasında yetiştirilen cevizlerde (*Juglans regia L.*) vitamin E seviyelerinin belirlenmesi amaçlanmıştır.

*Çalışma alanı:* bulunan Adilcevaz, Bahçesaray, Çatak, Edremit, Gevaş yöreleri ile Van merkez.

*Materyal ve yöntem:* Bölgelerden ceviz örnekleri toplandıktan sonra derin dondurucuda muhafaza edilmiştir. Analiz için, her bir bölgeye ait taze ve kurutulmuş 10 adet ceviz kırılmış ve ufalama yöntemi ile homojen bir karışım haline getirildikten sonra HPLC (Yüksek Performanslı Sıvı Kromatografisi) ile tayin gerçekleştirilmiştir.

*Temel sonuçlar:* Örneklerin yaş cevizdeki  $\alpha$ -Tokoferol miktarları sırasıyla  $2.36 \pm 0.01 \mu\text{g/g}$ ,  $5.01 \pm 0.31 \mu\text{g/g}$ ,  $6.23 \pm 0.15 \mu\text{g/g}$ ,  $7.20 \pm 0.28 \mu\text{g/g}$ ,  $3.99 \pm 0.46 \mu\text{g/g}$ ,  $5.05 \pm 0.30 \mu\text{g/g}$  ve  $\gamma$ -Tokoferol miktarları bölgelere göre sırasıyla  $23.80 \pm 0.52 \mu\text{g/g}$ ,  $16.54 \pm 1.08 \mu\text{g/g}$ ,  $14.82 \pm 0.46 \mu\text{g/g}$ ,  $17.34 \pm 0.78 \mu\text{g/g}$ ,  $11.91 \pm 0.40 \mu\text{g/g}$ ,  $15.57 \pm 0.90 \mu\text{g/g}$  olarak bulunmuştur. Araştırmada kuru cevizdeki  $\alpha$ -Tokoferol miktarları sırasıyla  $5.13 \pm 0.26 \mu\text{g/g}$ ,  $6.84 \pm 0.31 \mu\text{g/g}$ ,  $7.58 \pm 0.40 \mu\text{g/g}$ ,  $10.49 \pm 0.51 \mu\text{g/g}$ ,  $8.65 \pm 0.34 \mu\text{g/g}$ ,  $5.82 \pm 0.19 \mu\text{g/g}$  ve  $\gamma$ -Tokoferol miktarları bölgelere göre  $33.10 \pm 1.65 \mu\text{g/g}$ ,  $21.97 \pm 0.87 \mu\text{g/g}$ ,  $20.98 \pm 0.83 \mu\text{g/g}$ ,  $29.19 \pm 0.80 \mu\text{g/g}$ ,  $21.09 \pm 0.24 \mu\text{g/g}$ ,  $18.41 \pm 0.82 \mu\text{g/g}$  olarak tespit edilmiştir.

*Araştırma vurguları:* Elde edilen sonuçlar incelendiğinde, Adilcevaz, Bahçesaray, Çatak, Edremit, Gevaş and Van-Merkez bölgelerinde yetişen cevizlerin yüksek kalitede olduğu belirlenmiştir.

**Anahtar kelimeler:** Ceviz, Vitamin E, Van gölü havzası, HPLC, Tokoferol



## Introduction

Human beings need to take fat, protein, carbohydrate, vitamins and minerals in a sufficient and balanced way to maintain their life in a healthy manner. While different foodstuffs of vegetable and animal origin contain these components at different ratios, there are not many foodstuffs that contain all of these components at a balanced level. Most of the nuts and especially walnuts are rich in these essential nutrients. Since the composition of the walnut depends on many factors such as variety, climate and soil characteristics, harvest time, irrigation, fertilization, disease and pest control, the data determined by the researchers working in this field differ. Recently, there have been several reported studies about walnuts characteristics. The studies have focused on minor components due to their influence on walnuts quality. The most important countries in the world producing walnuts are China, USA, Iranian, and Turkey (Aydin et al., 2007) Walnuts are an important source of vitamin E due to high content of tocopherols. Total tocopherol concentration differs from 194 to 297 mg/kg in walnuts (Miller & Yang, 1985; Ferhatoğlu, 1999, 2001; Rabadán et al., 2019; Kazankaya & Güvenç, 2019). In addition to its rich oil content and qualified fatty acid composition in terms of human health, the fact that walnut oil has the highest tocopherol content after wheat embryo strengthens the importance of this valuable fruit. While the  $\alpha$ -,  $\gamma$ -, and  $\delta$ - tocopherol content of walnut oil was determined as 536, 595 and 450 mg/kg, respectively, its Vitamin E activity was evaluated as 636 mg/kg (Paunovic, 1990; Kazankaya & Güvenç, 2019). While there is information indicating that walnut was used for medical purposes in ancient periods and is especially good for wounds, it has been determined that it is also included in the composition of cosmetic products due to its moisturizing properties. The use of walnut in cosmetic products is mainly attributed to its linoleic and linolenic acid content. These acids are known to have a role in regulating the water permeability of the skin (Strila et al., 1998; Şen et al., 2006). Nowadays, there are 3 important walnut varieties, including *Juglans regia L.*, *Juglans nigra L.* and *Juglans cinerea L.* Apart from them, many other varieties are

also found. The products exported in the world are divided into five main groups according to their factor intensity, which are R&D based products that are easy and difficult to imitate, in addition to raw material, labor and capital intensive ones (Erkan et al., 2015; Kazankaya & Güvenç 2019). *Juglans regia* (Anatolian walnut, Persian walnut) variety is widely grown in our country. Lake Van basin is also among the important walnut growing regions of Anatolia (Kazankaya et al., 2003). The first thing that comes to mind when it comes to human nutrition is three main food groups. As is known, they are fats, proteins and carbohydrates. The ability of a person to have a healthy structure and to protect his/her health depends on his/her balanced nutrition in terms of basic foodstuffs. In order to determine the place of walnut in this balanced nutrition, it will be necessary to reveal its composition and its nutritional value depending on this composition. Therefore, herein, we aimed to determine vitamin E levels in Walnuts (*Juglans regia L.*) Grown in the Lake Van basin by HPLC.

## Material and Methods

The walnuts collected from various regions of the Lake Van basin were brought to the laboratory by marking according to the regions and stored in a deep freezer for analysis. On the day of analysis, 10 pieces of fresh and dried walnut samples belonging to each region were taken and broken, and a homogeneous mixture was obtained by pulverizing them. Then, 1 gram of this mixture was weighed on a precision scale and 6 samples were created separately for each region, both fresh and dried.

### Collection of Materials and Analysis

The extraction method specified by Miller & Yang (1985) was adapted according to our laboratory conditions and the procedures were performed to determine the vitamin E levels of the samples obtained from walnuts collected from various regions of the Lake Van basin. Then, the methanol water mixture (98:2 v/v) was prepared to be used as mobile phase in HPLC. The UV detector (DAD) was set to a wavelength of 290 nm for reading, and the peaks of these standards were taken with

the previously prepared  $\alpha$  and  $\gamma$ -tocopherol standard solutions on the HPLC device at a flow rate of 1.5 ml/min. Vitamin E amounts of walnut samples were determined by comparing the  $\alpha$  and  $\gamma$ -tocopherol peaks taken with the peak areas of the standard solutions (Zaspel & Csallany, 1983).

#### Statistical Analysis

In the study, the Kruskal-Wallis test was used to determine whether there was a difference between the regions in terms of vitamin E content in walnuts due to the low number of repetitions and the lack of normal distribution of the data. As a result of the analysis of variance, the Mann-Whitney U test was applied to determine the districts where there was a difference between the vitamin E contents of walnuts grown there (Champe & Harvey, 1997). In the study, arithmetic mean was used instead of mean rank statistics in the tables for a better understanding of interpretations.

#### Results and Discussion

The selection studies carried out in recent years have enabled the cultivation of varieties unique to Turkey (Kazankaya et al., 2003; Zaspel & Csallany, 1983). Furthermore, it was determined that the antioxidant vitamin levels in the plants growing in high altitude regions were higher compared to the plants grown in low altitude regions (Sze-Tao et al., 2000; Özkan, 1993; Aslan, 1985). The fatty acid composition, oil content and some other quality characteristics of 9 different walnut species grown in Turkey's different regions were examined, and it was determined that the ratio of the fat content to the kernel walnut weight varied between 54.68-62.28%, the main saturated fatty acid was palmitic acid, followed by stearic acid, and oleic acid was the unsaturated fatty acid containing a double bond. The most fatty acid is linoleic acid, it was determined that it constituted 53.68-60.09% of the total fatty acid in walnuts and that linolenic acid varied between 7.77-15.35% in walnuts (Ağar et al., 1995; Padley et al., 1995).

In this study, the amounts of vitamin E in walnuts taken from Adilcevaz, Bahçesaray, Çatak, Edremit, Gevaş and Van-Center regions of the Lake Van basin, which is

among the most important walnut growing regions of Turkey, were determined. The amounts of  $\alpha$ -Tocopherol of the samples in fresh walnuts were found to be  $2.36 \pm 0.01$   $\mu\text{g/g}$ ,  $5.01 \pm 0.31$   $\mu\text{g/g}$ ,  $6.23 \pm 0.15$   $\mu\text{g/g}$ ,  $7.20 \pm 0.28$   $\mu\text{g/g}$ ,  $3.99 \pm 0.46$   $\mu\text{g/g}$ ,  $5.05 \pm 0.30$   $\mu\text{g/g}$ , respectively, and the amounts of  $\gamma$ -Tocopherol were found to be  $23.80 \pm 0.52$   $\mu\text{g/g}$ ,  $16.54 \pm 1.08$   $\mu\text{g/g}$ ,  $14.82 \pm 0.46$   $\mu\text{g/g}$ ,  $17.34 \pm 0.78$   $\mu\text{g/g}$ ,  $11.91 \pm 0.40$   $\mu\text{g/g}$ ,  $15.57 \pm 0.90$   $\mu\text{g/g}$ , respectively, according to the regions (Figure 1).

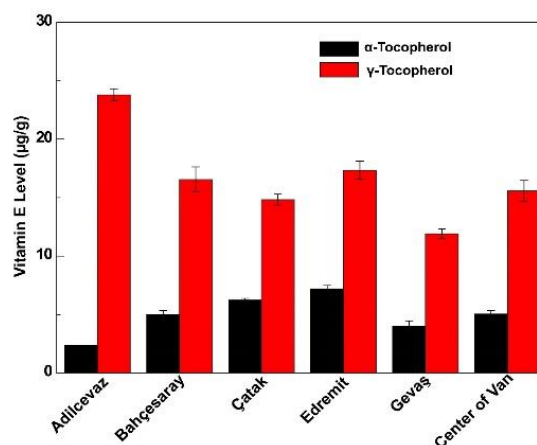


Figure 1. Statistical results of Vitamin E levels in fresh walnuts of the regions (sample number: 6, the difference between the different lettered area means is statistically significant ( $p < 0.05$ ))

In the study, the amounts of  $\alpha$ -Tocopherol in dried walnuts were found to be  $5.13 \pm 0.26$   $\mu\text{g/g}$ ,  $6.84 \pm 0.31$   $\mu\text{g/g}$ ,  $7.58 \pm 0.40$   $\mu\text{g/g}$ ,  $10.49 \pm 0.51$   $\mu\text{g/g}$ ,  $8.65 \pm 0.34$   $\mu\text{g/g}$ ,  $5.82 \pm 0.19$   $\mu\text{g/g}$ , respectively, and the amounts of  $\gamma$ -Tocopherol were found to be  $33.10 \pm 1.65$   $\mu\text{g/g}$ ,  $21.97 \pm 0.87$   $\mu\text{g/g}$ ,  $20.98 \pm 0.83$   $\mu\text{g/g}$ ,  $29.19 \pm 0.80$   $\mu\text{g/g}$ ,  $21.09 \pm 0.24$   $\mu\text{g/g}$ ,  $18.41 \pm 0.82$   $\mu\text{g/g}$ , respectively, according to the regions (Figure 2).

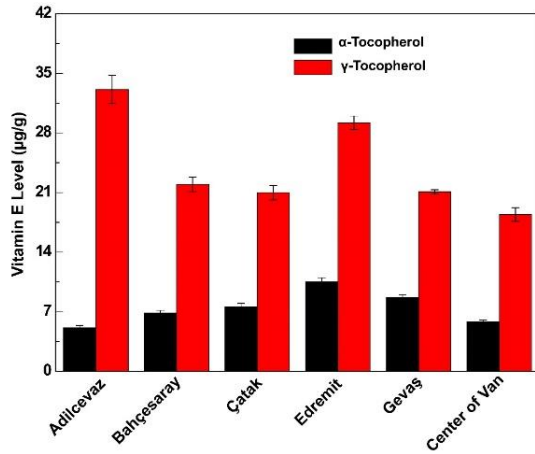


Figure 2. Statistical results of Vitamin E levels in dried walnuts of the regions (sample number: 6, the difference between the different lettered area means is statistically significant ( $p < 0.05$ )).

### Conclusion

HPLC method is one of the techniques successfully applied in the analysis of tocopherol in oil. The separation of the mixtures, recognition of components and their quantities can be performed precisely and in a short time. The method provides better results than colorimetric and other electrochemical methods. In this study, the amounts of  $\gamma$ -tocopherol in dried and fresh walnuts in Adilcevaz region were found to be higher compared to other regions. Our results are in parallel with a previous study (Küçük et al., 2003). The vitamin content of a plant variety depends on many factors. Climate conditions and ecological conditions can be listed as the main factors. The amount of antioxidant vitamins produced by the plant also changes due to reasons such as climate type, high or low altitude, soil type and structure, and exposure to radioactive. We considered that altitude and climatic conditions were among the factors that played a role in this difference in the Lake Van basin. We do not have enough information on whether taking vitamins required for sports performance in large quantities has a positive effect on performance. It is known that the lack of minerals and vitamins negatively affects the performance and the intake of minerals and vitamins from outside affects it positively. Vitamins that play a role in biochemical

events are used in required amounts in our body to sustain these events.

When the results obtained in the study are considered, it is observed that the walnuts grown in Adilcevaz, Bahçesaray, Çatak, Edremit, Gevaş and Van-Center regions are of high quality. In this context, it has been concluded that focusing on the selection of walnuts in these regions and concentrating on the production of standard varieties that are compatible with the conditions of our country may significantly contribute to the national economy and science.

### Ethics Committee Approval

N/A

### Peer-review

Externally peer-reviewed.

### Author Contributions

Conceptualization: F.D., M.B., I.H.Y.; Investigation: F.D., M.B.; Material and Methodology: F.D., M.B.; Supervision: F.D., I.H.Y.; Visualization: F.D., M.B.; Writing-Original Draft: F.D., M.B.; Writing-review&Editing: F.D. All authors have read and agreed to the published version of manuscript.

### Conflict of Interest

The authors have no conflicts of interest to declare.

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