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AMOUNTS OF VITAMIN A, VITAMIN E, VITAMIN C, B-CAROTENE, LYCOPENE, GHRELIN, GLUTATHIONE, AND MDA IN FRUITS OF *DIOSPYROS KAKI L.*

Zulaiha Gidado Mukhtar[1,2], Muhammad Salihu Ibrahim[1,2], Yusuf Ibrahim Ibrahim[1,2], Fikret Karatas[1*]

[1]Fırat University, Faculty of Science, Department of Chemistry, Elazig, Turkey.
[2]Kano State Polytechnic, School of Technology, Department of Science Laboratory Technology, Kano, Nigeria.

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ABSTRACT

In this study, the amounts of vitamins A, E, C, β-carotene, lycopene, ghrelin, reduced and oxidized form of glutathione (GSH, GSSG) with malondialdehyde (MDA) in ripe fruits of *Diospyros kaki L.* plant were determined by using HPLC. The amount of vitamins A, E, C, β-carotene, lycopene, ghrelin, GSH, GSSG and MDA in *Diospyros kaki L.* plant were found to be 0.40±0.06 – 0.92±0.08; 0.63±0.09 - 1.34±0.14; 47.00±0.80 - 65.61±1.56; 2.08±0.18 - 3.36±0.30; 0.69±0.08 - 1.42±0.13; 2.14±0.65 - 7.98±1.06; 398.25±34.23 - 527.90±23.36; 23.67±8.89 - 42.43±4.81 and 5.63±0.35 - 7.28±0.42 µg/g, respectively. It can be said that *Diospyros kaki L.* plant fruits are a good source of vitamin C, lycopene, ghrelin and glutathione. Findings suggest that the fruit of this plant may be a useful source of antioxidants. Differences in the amount of parameters in the *Diospyros kaki L.* fruit are thought to be due to the growth medium, climate, and environmental conditions.

Keywords: *Diospyros kaki L.,* Vitamin A, Vitamin E, Vitamin C, β-carotene, Lycopene, Ghrelin, Glutathione, MDA

TRABZON HURMASI (*DIOSPYROS KAKI L.*) MEYVELERİNDEKİ A VİTAMİNİ, E VİTAMİNİ, C VİTAMİNİ, BETA-KAROTEN, LİKOPEN, GRELİN, GLUTATYON VE MDA MİKTARLARI

ÖZ

Bu çalışmada, Trabzon hurması (*Diospyros kaki L.)*'nın olgunlaşmış meyvelerinde A, E, C vitaminleri, β-karoten, likopen, ghrelin, indirgenmiş ve yükseltgenmiş glutatyon (GSH, GSSG) ve malondialdehit (MDA) miktarları Yüksek Performanslı Sıvı Kromatografisi (HPLC) ile belirlendi. Trabzon hurması bitkisinin meyvelerindeki A vitamini, E vitamini, C vitamini, β-karoten, likopen, ghrelin, GSH, GSSG ve MDA miktarları sırasıyla 0.40±0.06 - 0.92±0.08; 0.63±0.09 - 1.34±0.14; 47.00±0.80 - 65.61±1.56; 2.08±0.18 - 3.36±0.30; 0.69±0.08 - 1.42±0.13; 2.14±0.65 - 7.98±1.06; 398.25±34.23 - 527.90±23.36; 23.67 ± 8.89 - 42.43 ± 4.81 µg/g ve 5.63 ± 0.35 - 7.28 ± 0.42 µg/g arasında olduğu gözlendi. Trabzon hurması meyvelerinin C vitamini, likopen, ghrelin ve glutatyon bakımından iyi bir kaynak olduğu söylenebilir. Bulgular bu bitkinin meyvelerinin antioksidan için yararlı bir kaynak olabileceğini düşündürmektedir. Trabzon hurmasının meyvelerindeki parametrelerin miktarındaki farklılıkların büyüme ortamı, iklim ve çevresel koşullardan kaynaklandığı düşünülmektedir.

Anahtar Kelimeler: Trabzon hurması, A Vitamini, E Vitamini, C Vitamini, β-karoten, Likopen, Grelin, Glutatyon ve MDA

[*]*Corresponding author* / Yazışmalardan sorumlu yazar;
✉ fkaratas@firat.edu.tr, ✆ (+90) 506 302 4088 🖷 (+90) 424 237 0000/3682

INTRODUCTION

Fruits play a crucial role in maintaining our wellbeing which is attributable to their high content of nutrients and a bioactive compound which play valuable roles in preserving homeostasis (Manach et al., 2004; Butt et al., 2015). These compounds include amino acids, sugars, enzymes, organic acids, and phytochemicals which serve as sources of key metabolites and as biochemical markers for ripeness, flavor, essence, aroma, color, and quality (Hecke et al., 2006). Free radical species generated from cellular metabolism and by external triggers are regulated by an innate cellular antioxidant mechanism which includes enzymatic and non-enzymatic antioxidants. An equilibrium between free radicals and antioxidants is crucial for normal physiological functions (Lobo et al., 2010). If left unchecked they accumulate and react with cellular components such as lipids, proteins, and DNA, modifying them, which results in oxidative stress (Finkel and Holbrook, 2000; Melov et al., 2000). Oxidative stress leads to lipid peroxidation and a consequent decrease in membrane fluidity leading to cell death. It contributes to aging and several chronic degenerative disorders such as tumors, atherosclerosis, diabetes and Alzheimer's disease (Liguori, et al., 2018). Recent findings have shown the danger of synthetic antioxidants such as butylated hydroxyl toluene and butylated hydroxyl anisole pose to human health. Thus, researches on finding natural sources of antioxidants have been intensified in a bid to protect our bodies from the ravaging effects of free radicals (Lyun et al., 2011). *Diospyros kaki L*, belonging to *Ebenaceae* family and commonly called Persimmon is one of such fruits. It is the most extensively cultivated species of the genus *Diospyros*. Though first described in 1780, it has been exploited by the Chinese for over 2000 years, with species endemic to China, Japan, Korea, Burma, Turkey and Nepal (Petersen and Martin, 2007; Celik and Ercisli, 2008). The deciduous tree has broad leaves and produces a soft, seedless, fleshy and chewy fruit that is sweet and slightly tangy; it is best taken whole when harvested and allowed to mature and soften as the unripe fruit is astringent and bitter due to high proanthocyanidin-type tannin content (Butt et al., 2015; Janick and Paull, 2008). The leaves and fruits of this species have been used throughout history as a remedy for cough, arteriosclerosis, hypertension, and apoplexy (Özen et al., 2004; George and Redpath, 2008). Persimmons are rich sources of trace mineral elements, antioxidants (lycopene, lutein, β- carotene, and zeaxanthin), phenolic acids, coumaric acids and water-soluble dietary fibre (Suzuki et al., 2005; Chenet al., 2008). Current researches on Persimmon plant have shown its biological importance as an antioxidant, antitumor, anti-hyperlipidemia, and antidiabetic agent (Lee et al., 2007; George and Redpath, 2008). The significance of antioxidants from natural dietary sources in the prevention of chronic degenerative diseases and preserving good health have been well established (Lee et al., 2012). Antioxidants are classified as enzymatic and non-enzymatic. Non-enzymatic low molecular-weight metabolites including ascorbic acid (AA), glutathione (GSH), alkaloids, phenolic compounds, non - protein amino acids, α-tocopherol, and β-carotene aid in offsetting the build-up of reactive oxygen species (ROS) prompted by various stresses (Mittler et al., 2004; Sarvajeet et al., 2011). These enzymes and metabolites not only protect plants and animals from cellular damage but also fine-tune the concentrations of ROS in order to optimize their functions in metabolism (Becana et al., 2010).

This research aims to assess and compare the antioxidant capacity of *Diospyros kaki L* (Persimmon) by investigating the concentration of some of its antioxidants (β-Carotene, Ascorbic acid, α-Tocopherol and Lycopene) and Glutathione (oxidised and reduced), Ghrelin and Malondialdehyde in *Diospyros kaki L*. grown in three different regions of Turkey (Adiyaman, Malatya and Antalya). The comparative assessment of its nutritive components and antioxidant effect has not been well documented (Lee et al., 2012). This, therefore, serves as a viable topic for research to further establish its nutritious and economic value, and fill in the gap in the literature, where necessary.

MATERIALS AND METHODS

Materials

In the study, ripe fruits of *Diospyros kaki L* Hachiya species, grown in Elazig, Malatya and Antalya provinces of Turkey, were used. The samples were supplied between 5-30 November 2017.

Determination of vitamin A, vitamin E, β-carotene and lycopene

3.0 g from each *Diospyros kaki L* sample was weighed and 7.0 mL ethyl alcohol was added. The suspension was shaken well in a vortex mixer and then centrifuged for three minutes at 4500 rpm. The solution was filtered (Whatman No 1). 0.5 mL n-hexane was added to the filtrate. This way, vitamin A, vitamin E, β-carotene and lycopene were extracted to the n-hexane phase. This extraction process was replicated. The n-hexane phases were collected and dried with nitrogen gas until dryness. The residue obtained was dissolved in 0.6 mL methyl alcohol and taken up in vials of HPLC which was then ready for HPLC analysis. Supelcosil LC-18 column (25.0 cm x 4.6 mm x 5.0 μm) was used in the determination of vitamin A, vitamin E, β-carotene and lycopene using a mixture of acetonitrile: methanol: water (63:33:4 v/v) as mobile phase. The mobile phase flow rate was set at 1.0 mL/min. The vitamin E 296 nm, vitamin A 326 nm, β-carotene 454 nm, and lycopene 474 nm were determined respectively (Catignani and Bieri, 1983; Miller et al., 1984; Ibrahim et al., 2017).

Determination of vitamin C, ghrelin, glutathione and MDA

3.0 g from each *Diospyros kaki L.* sample was weighed and 1.0 mL of 0.5 M perchloric acid was added to each homogenate, to precipitate the proteins. The mixture was placed in a vortex shaker. Each sample was made up to 6.0 mL with deionized water and centrifuged for 10 minutes at 4500 rpm. The supernatant was then filtered (Whatman No 1). The filtrate was divided into three equal portions. One portion was used for vitamin C while the others were used for ghrelin, glutathione and MDA determination. 1.0 mL filtrate taken up in vials of HPLC. Vitamin C, Ghrelin, GSH, GSSG, and MDA were determined according to the method of Tavazi et al. (1992), Aydin et al. (2008), Dawes and Dawes (2000) and proposed by Karatas et al. (2002), respectively. Column Exsil 100-5 ODS (25 cm, 4.6 mm ID, 5 μm) flow rate of 1.0 mL/min.

Equipment and chemicals

HPLC was performed with the SHIMADZU Prominence-İ LC- 2030C 3D Model and PDA detector. All the chemical reagents used in the analysis were analytical grade and obtained from Merck (Darmstadt, Germany). Double distilled water was used throughout the work.

Statistical Analysis

All measurements were triplicated. Mean standard deviation was determined and the results were subjected to Analysis of Variance. The SPSS 10.0 for Windows was used for variance analysis and LSD multiple comparison test was performed at $P < 0.05$ to $P < 0.005$ level.

RESULTS AND DISCUSSION

Vitamin A is essential for the normal functioning of the visual system; cellular growth and development; maintenance of epithelial cellular integrity, immune function, and reproduction (Gropper et al., 2017). The result obtained in this study showed the amount of vitamin A in *Diospyros kaki L.* fruit from Antalya, Adiyaman, and Malatya to be 0.40 ± 0.06 μg/g, 0.92 ± 0.08 μg/g and 0.46 ± 0.06 μg/g, respectively. Samples from Adiyaman had the highest ($P < 0.005$) concentration of vitamin A, followed by Antalya and Malatya (Table 1). A similar study showed the Vitamin A content of *Diospyros kaki L.* fruit to be 38 RAE μg/100g (Butt et al., 2015). Vitamin A content of fresh peach was reported as 16.3 ± 5.7 mg/kg and 18.15 ± 0.55 μg/100g for apricots (Dursta and Weaver, 2012; Kan et al., 2014) which are inconsistent with results obtained in this study.

Vitamin E is a vital compound involved in neurological and immune functions. It is an antioxidant, protecting molecules and tissues against free radicals, which contributes to the stabilization of biological membranes. In addition, it mediates in the regulation of several

enzymes (Feki et al., 2001). This study showed the vitamin E content of *Diospyros kaki L.* from Antalya, Adiyaman, and Malatya to be 0.63 ± 0.09 µg/g, 0.81 ± 0.10 µg/g and 1.34 ± 0.14 µg/g, respectively. Based on these values the vitamin E content of *Diospyros kaki L.* from Malatya is higher (P <0.005) than that of Adiyaman and Antalya (Table 1). The amount of vitamin E investigated in ripe *Ziziphus mauritiana Lam* (Ber fruit) was reported to have the concentrations of 37109.31µg/g and 29261.99 µg/g for ripe and over ripe fruit respectively (de Souza, 2008). Vitamin E concentration of irrigated cultivar of *Prunus armeniaca L*.(apricot) was found to be 37.60 ± 0.40 µg/100 g (Kan et al., 2014). The amount of vitamin E in *Prunus armeniaca* and *Ziziphus mauritiana Lam* are much higher than that of *Diospyros kaki L.* based on the result of this study.

Table 1. Some parameters in *Diospyros kaki L.* specimens from three different provinces

Parameters	1. Antalya	2. Adiyaman	3. Malatya
Vitamin A (µg/g)	0.40±0.06	0.92±0.08	0.46±0.06
Vitamin E (µg/g)	0.63±0.09	0.81±0.10	1.34±0.14
Vitamin C (µg/g)	65.61±1.56	47.00±0.80	49.20±2.93
β-Carotene(µg/g)	2.08±0.18	3.36±0.30	2.60±0.24
Lycopene (µg/g)	0.69±0.08	1.42±0.13	1.10±0.10
Ghrelin(µg/g)	2.28±0.50	2.14±0.65	7.98±1.06
Reduced glutathione (µg/g)	398.25±34.23	444.96±31.55	527.90±23.36
Oxidized glutathione (µg/g)	23.67±8.89	42.43±4.81	28.43±3.77
Reduced glutathione / Oxidized glutathione	16.83	10.49	18.57
Malondialdehyde (µg/g)	5.75±0.27	7.28±0.42	5.63±0.35

The strong reducing properties of vitamin C make it a potent antioxidant. It neutralizes superoxide and hydroxyl radicals protecting cells from oxidative damage (Aksoy, 2000). It is required for collagen formation, absorption of inorganic iron, reduction of plasma cholesterol level, inhibition of nitrosamine formation, and enhancement of the immune system. Vitamin C is also necessary for the prevention of scurvy and maintenance of healthy skin, gums, and blood vessels and is reported to reduce the risk of arteriosclerosis, cardiovascular diseases and some forms of cancer (Lee and Kader, 2000).

This study showed the vitamin C content of *Diospyros kaki L.* from Antalya, Adiyaman, and Malatya to be 65.61 ± 1.56 µg/g, 47.00 ± 0.80 µg/g and 49.20 ± 2.93 µg/g, respectively, with the highest concentration (P <0.005) seen in Antalya followed by Malatya then Adiyaman (Table 1). A previous study obtained 110 mg/100g of vitamin C in fresh persimmon; 75 mg/100 g in orange, 10.6 mg/100 g in fresh tomatoes and 34 mg/100 g in mandarins (Lee and Kader, 2000). In a similar research vitamin C content in papaya fruits was found to range between 110 to 1340 µg/g in five different species of pulp puree (de Souza, 2008) while the vitamin C content in apricot was 20.75 mg/100 g) and 12 mg/100 g in fresh apple (Rasanuet al., 2005). Our findings are consistent with the literature.

β-Carotene sometimes referred to as pro-vitamin A, is a precursor of vitamin A. It is the light yellow or orange pigment found in plants which are converted to vitamin A in intestinal epithelial and is mainly stored in the liver and skin (Hamilton et al., 2014). β-carotene is a potent antioxidant known to exhibit photoprotective effects and anticancer activity (Madhunapantula and Robertson, 2012).

This study showed the β-carotene content of *Diospyros kaki L.* from Antalya, Adiyaman and Malatya to be 2.08 ± 0.18 µg/g, 3.36 ± 0.30 µg/g, and 2.60 ± 0.24 µg/g, respectively, (Table 1). The results showed the β-carotene concentration to be higher (P <0.05) in *Dipyros kaki L.* fruit from

Adiyaman, followed by Malatya then Antalya. The concentration of β-carotene content in fresh papaya pulp was shown to range from 0.19 ± 0.07 mg/100 g to 0.56 ± 0.09 mg/100 g (de Souza, 2008). The β-carotene content of three cultivars of *Prunus armeniaca L.* (apricot) was reported as 0.88 µg/g in fruits of Hacıhaliloglu, 2.20 µg/g in fruits of the cultivar. Tokaloglu and 2.62 µg/g in fruits of the cultivar. 'Kabaasi' apricots, respectively (Kan et al., 2014). The result of this study shows that the concentration of β-carotene in *Diospyros kaki L.* to be much higher than that of papaya, however, the findings are in consistent with Hacıhaliloğlu' and 'Tokaloğlu' cultivars of *Prunus armeniaca L.*

Lycopene, a non-provitamin A carotenoid, possesses antioxidant, detoxifying, signaling and chemo-preventive properties (Wang, 2012). Its antioxidant activity is enhanced by its ability to sequester singlet oxygen and its ability to trap peroxyl radicals (Basuny et al., 2009). This study showed the lycopene content of *Diospyros kaki L.* in increasing order of concentration to be, 1.42 ± 0.13 µg/g, 1.10 ± 0.10 µg/g, and 0.69 ± 0.08 µg/g, from Adiyaman, Malatya, and Antalya, respectively (Table 1). From the result, the highest lycopene concentration (P <0.05) in *Dipyros kaki L.* fruit is seen in Adiyaman, followed by Malatya then Antalya.

In similar research, the lycopene content in fresh papaya fruits ranged from 1.44 ± 0.28 mg/100 g to 3.39 ± 0.32 mg/100 g (de Souza, 2008). The lycopene content in *Solanumlycopersicum* (tomato) was reported to be 0.2 µg/g (Dewanto et al., 2002), which is inconsistent with values obtained in this study.

The gastrointestinal neuro-peptide hormone ghrelin, secreted by ghrelinergic cells in the stomach and pancreas stimulates growth hormone (GH) release from the pituitary gland. It acts on the brain to regulate food intake, body weight, adiposity, and glucose metabolism modulation of stress and anxiety. It also protects against muscle atrophy and improves cardiovascular functions such as vasodilatation and cardiac contractility (Müller et al., 2015).

This study shows the ghrelin content of *Diospyros kaki L.* from Antalya, Adiyaman, and Malatya to be 2.28 ± 0.50 µg/g, 2.14 ± 0.65 µg/g and 7.98 ± 1.06 µg/g, respectively. The ghrelin content of fruits from Malatya was the highest (P <0.005) followed by that of Antalya then Adiyaman (Table 1). The ghrelin content of *C. laevigata* from Elazig, Adiyaman, and Malatya to be 67.67 ± 6.16 µg/g, 18.96 ± 6.73 µg/g and 79.96 ± 12.14 µg/g, respectively. The ghrelin content of fruit from Malatya was higher followed by that of Elazig, then Adiyaman (Ibrahim et al., 2017). Our findings are much lower than those of *C. laevigata.*

Glutathione is required for a lot of cellular activities including the protection of cells of the immune system, brain, kidneys, eyes, liver, heart, lungs and skin tissues against oxidative damage. The reduced form of glutathione (GSH), is the most important intracellular antioxidant molecule. GSH has many functions such as the detoxification of xenobiotics, the transport of amino acids, and the reduction of sulfhydryl groups in proteins. In particular, glutathione is the most important metabolite against oxidative stresses in plants and it is found in almost all cells of plants (Esterbauer et al., 1992).

This study revealed the content of GSH in *Diospyros kaki L.*to be 398.25 ± 34.23 µg/g, 444.96 ± 31.55 µg/g and 527.90 ± 23.36 µg/g while the GSSG content was found to be 23.67 ± 8.89 µg/g, 42.43 ± 4.81 µg/g and 28.43 ± 3.77 µg/g from Antalya, Adiyaman and Malatya, respectively. The results showed the GSH concentration to be higher (P <0.05) in *Dipyros kaki L.* fruit from Malatya, followed by Adiyaman then Antalya. However, the GSH/GSSG ratio was found to be 16.83, 10.49 and 18.57. The GSH content of *Diospyros kaki L.* from Malatya had the highest (P <0.05) concentration followed by Adiyaman then that of Antalya. On the other hand, the GSSG content of Adiyaman is higher followed by that of Malatya, then Antalya (Table 1).

The level of glutathione in *Musa paradisiaca L.* (plantain) was reported to be 54.10 ± 0.60 µg/g (Agoreyo et al., 2017). The amount of reduced

glutathione in mango has been found to be 159.1 μmol/g (AbdAllatif et al., 2015). The amount of total glutathione content in ripe ber (*Ziziphus mauritiana Lam*) fruit was also found to be 6598.2 μmol/g (Kumar et al., 2011). Our findings are inconsistent with the literature.

Free radicals affect lipid peroxidation by acting on unsaturated fatty acids in the membranes. The resulting lipid peroxides break down rapidly to form reactive carbon compounds one of which is MDA (Gawel et al., 2004).In this study, the MDA content of *Diospyros kaki L.* from Antalya, Adiyaman, and Malatya was found to be 5.75 ± 0.27 μg/g, 7.28 ± 0.42 μg/g and 5.63 ± 0.35 μg/g respectively. The MDA content of *Diospyros kaki L.* from Adiyaman was higher (P <0.05) followed by that of Antalya then Malatya. The amount of MDA in ripe ber (*Ziziphus mauritiana Lam*) fruit was found to be 4.498 nmol/g (Kumar et al., 2011). The amount of MDA in red ripe tomato fruit was found to be 2.3 nmol/g (Mondal et al., 2004).

CONCLUSION

Based on the results obtained from this research, the variation in values obtained from one region to another may be due to differences in environmental factors like geographical location, soil, temperature, altitude, and pre/post-harvest treatments. These dynamics affect the biochemistry of the plants and hence its concentration of bioactive compounds (Liu et al., 2015). The outcomes of this research on the levels of these biomolecules show it to be a rich source of vitamins C, β-carotene; glutathione, lycopene, and ghrelin. We can say that *Diospyros kaki L.* fruit can be a very good source of some antioxidants that are very important in protecting the body against the dangerous effect of free radicals that lead to aging and degenerative diseases such as diabetes and cancer. Its nutritional value can be of optimal importance to the food industry where its antioxidant value can be used and optimized in order to satisfy a wide variety of consumer preference.

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