JAEFS

International Journal of Agriculture, Environment and Food Sciences

e-ISSN : 2618-5946 DOI: 10.31015/jaefs.2020.1.8 www.jaefs.com

Research Article Int J Agric Environ Food Sci 4 (1): 62-69 (2020)

Determination of sugar, total phenol contents and antioxidant activity of various parts 'Uzun' pistachio cultivar (*Pistacia vera* L.)

Muhammet Ali Gündeşli[1,*]

[1]East Mediterranean Transitional Zone Agricultural Research of Institute, Kahramanmaras, Turkey

[*]Corresponding Author: maligun46@hotmail.com

Abstract

The aim of this study was conducted to determine the sugar content, total phenol and antioxidant activity of various parts of 'Uzun' pistachio trees during 2018 (high bearing, or "on"-year trees) and 2019 (low bearing, or "off"-year trees) growing season. This research was carried on 35-year old, fruitful or unfruitful (showing alternate bearing) trees that were grafted on *Pistacia vera* rootstock at the Gaziantep provinces of Turkey. Total phenolic content of the samples were determined by the Folin Ciocalteu method by Spectrophotometer. Total antioxidant activitiy of samples were evaluated using the 1, 1-diphenyl-2- picrylhydrazyl (DPPH) radical scavenging method. Radical scavenging activities and total phenolic content of the samples changed depending on the different parts of shoot, leaves, nuts (hull and hard Shell) and kernel. Sugar compounds of Pistachio were detected by using HPLC. The highest antioxidant values were observed in hull (86,02% on-year) and hard Shell (85,37 % on-year) and the lowest were in kernel samples. The highest total phenol amount was recorded hard Shell and the lowest values were in kernel (81,23% off-year) samples. Fructose content (12,10 g/100 g) was found to be higher than the contents of sucrose (3,10 g/100 g) and glucose (5,48 g/100 g) in 'off' year tree and the dominant sugar was found fructose. All tissues that the amount of sugar content in 'off' year was higher than 'on' year. The results suggest at phytochemicals in 'Uzun' pistachio variety has potent antioxidant activities that is important for human nutrition.

Keywords: Free-radicals, Antioxidant, DPPH, Pistachio, Phenol, Sugar compounds

Introduction

Turkey is a significant genetic center of pistachio (Pistacia vera L.), and growing pistachio species has a wide distribution. Pistachio is the most economically important cultivated species of Pistacia genus belonging to Anacardiaceae family, order Sapindales. Like for a lot of fruit species. Turkey is also one of the place of pistachio origin and having an important genetic sources and the center of the generation and evolution of pistachio varieties. Our country is the third largest producer of pistachios after Iran and USA. Especially, the Southeastern Anatolia region having both suitable geographical site and various climatic and soil conditions and pistachios are can be grown economically. In the production of pistachio, Southeast Anatolia Region covers 95% of the country's production area and 91.5% of the total production (Arpaci and Atli., 1996; Gundesli et al., 2018, 2019a). 'Uzun' pistachio is the most important and widely grown cultivar Gaziantep province of Southeastern Anatolia region (Ak and Fidan, 2013). Pistachio shows alternate bearing (also known as biennial bearing), a phenomenon which refers to trees with irregular crop load from year to year and is typically observed in many commercial fruit trees. In other words, a tree's yield alternates between high fruit load (on) and low fruit load (off). Due to having fluctuations in productions between "on" and "off" years, producers and consumers encounter with financial loss (Acar et al., 2006; Gundesli et al., 2019a). Recently, many researchers have made great efforts to find safe and powerful natural antioxidants from various plant species. Antioxidants have protective

Cite this article as:
Gundesli, M.A. (2020). Determination of Sugar, Total Phenol contents-and Antioxidant Activity of various parts 'Uzun' pistachio cultivar (*Pistacia vera* L.). Int. J. Agric. Environ. Food Sci., 4(1), 62-69
DOI: https://dx.doi.org/10.31015/jaefs.2020.1.8
ORCID: [1]0000-0002-7068-8248
Received: 14 December 2019 **Accepted:** 07 February 2020 **Published Online:** 15 March 2020
Year: 2020 **Volume:** 4 **Issue:** 1 (March) **Pages:** 62-69
Available online at : http://www.jaefs.com - http://dergipark.gov.tr/jaefs

effects on food relations in different diseases such as cardiovascular disease, cancer, aging and cataracts. (Dashpande et al., 1996; Fukuda et al.,2003; Kornsteiner et al., 2006; Rohman et al., 2010; Usanmaz et al., 2018; Gul and Tekeli, 2019; Gundesli et al., 2019b). Many edible fruits as harmless antioxidant sources have been investigated in terms of antioxidant properties. One of the nutritional value of nut fruit species is pistachio. Pistachio has a unique flavor and is rich in biochemical compounds. Pistachio is known as a natural antioxidant source with its rich biochemical compounds including phenolics, vitamin and fatty acids. However, antioxidant properties of pistachio may vary depending on cultivation, planting area, climate and cultural practices (irrigation, fertilization etc.). Recently, studies on the biochemical substances of pistachio, it has been reported that pistachio is a good source of antioxidants and phenolic compounds (Tokusoglu et al., 2005; Arcan and Yemenicioglu, 2008; Tsantili et al., 2010; Taghizadeh et al., 2018). Limited studies are conducted of researches on sugar contents, total phenolic and antioxidant contents of the various parts of 'Uzun' pistachio cultivar to grown in GAP region. Thus, the objectives of this study were to determine the sugar compounds, total phenolic content and antioxidant activities of different tissues such as shoot, leaves and nuts from 'Uzun' pistachio cultivar.

Materials and Methods

Plant materials

The experiment conducted in 2018 (high bearing, or "on"-year trees) and 2019 (low bearing or "off"-year,) growing seasons at the Research and Experimental area of the Pistachio Research Institute in Gaziantep provinces in Turkey. Thirty-three years old trees belong to 'Uzun' cultivar grafted on Pistacia atlantica Desf. rootstock and planted at 10x10 m intervals were used as plant materials. In this study, shoots, leaves, and nuts (hull and hard shell) were sampled from the current year's shoots (fruiting branch) 'on' and 'off' year trees.

Characteristics of "Uzun" cultivar: The tree structure is semi-upright and strong. It is one of the middle flowering cultivars with yellowish-green flowers. Bunch intensity is medium and resistance of split is poor. Fruit splitting rate is 69.34%, and 100-nut weight is 110.69 g. Kernel fruit color is green and it is a variety with a tendency to an absolute alternate bearing. Chilling requirement is 600 days/hours and the need for total temperature is 3797 degree-days. It can be utilized in the food industry and confectionery (Atlı et al., 2003; Afshari et al., 2009; Ak and Fidan 2013).

Plant tissue sampling

In 2018 and 2019, the samplings were collected from shoot (in July) leaves (in August), nuts (harvest time: September) which is 'On' and 'Off' year trees. For total phenolic and total antioxidant capacity analysis, one-year-old branches from different directions of the canopy (north, south, east and west), per replication; young leaves (50 number), shoots (10), nuts (hull and hard Shell-50 number), and fruit kernel (50 number) were excised and immediately transferred to the on dry ice, separated into leaves, shoots, and nuts, frozen in liquid nitrogen, in laboratory. Kernels and endocarp of fruits were separated. The samples were rinsed with sterile distilled water to remove dust and soil and liyophilizied (ilShin Freeze Dryers, FD-8518, Ede, Netherlands), using a lyophilizer and then homogenized using coffee grinder and stored at +4°C.

Sugar analyses

The samples were prepared according to the method described by Kafkas et al. (2007), with minor modifications. Briefly, sugar analysis extraction was performed using acetonitrile: ultra deionized water (50:50). 1 g of sample weighed was solved in the extraction solvent and vortexed for 30 seconds. Then the samples were treated in an ultrasonic water bath for 15 minutes. After the extract was filtered through a 0.25 µm membrane filter (Schleicher and Schuell, Dassel, Germany) prior to injection (20 µl) onto a 7.8 x 100mm HPLC column (CARBOSep COREGEL 87C) at 75 °C. The mobile phase that acetonitrile/deionized water was supplied by an isocratic pumping system (LC-10A, Shimadzu, Kyoto, Japan) at a flow rate of 0.6 ml min1. After separation, the compounds from the mixture were passed through a refractive index detector (RID-10A, Shimadzu, Kyoto, Japan) linked to a chart recorder (CRUA, Shimadzu, Kyoto, Japan). Each sample was assayed triplicated and sugars concentrations were expressed as mg/100g dry weight (D.W.). Mixed external standard solutions containing glucose, fructose, and sucrose, at different concentrations were injected into the column and peaks were used to generate calibration curves for each sugar. The levels of sugars content in different tissues was calculated using peaks and the calibration curves.

Determination of total phenolic content

The content of total phenolic compound was determined by the Folin-Ciocalteu reagent using the modified by Spanos and Wrolstad (1990). 50 µl %80 methanolic extract of dried material was diluted by water followed by adding 250 µl of Folin-Ciocalteu reagent and 750 µl sodium carbonate (20%, w/v). The extracts were centrifuged (centrifuge NF 200, Nuve, Belgium) at 5500 r.p.m. for 15 min and filtered. The solvent was incubated at room temperature in dark for 2 hours. The absorbance of all samples was measured at 760 nm using Multiskan TM GO Microplate Spectrophometer. The results were expressed as gram gallic acid equivalent per grammes of extract (g GAE/ g DW).

Determination of DPPH radicals stevenging activity

The ability of hydrogen donating or radical scavenging of sample extracts was measured bu using the stable free radical (DPPH) (1,1-dipheny 2-picrylhydrazyl) method according to the method of Brand-Williams et al. (1995), with some modifications (Duarte-Almeida et al., 2006). Abaout 1950 µl of the 1mM solution of methanolic DPPH was mixed with 50 µl extract solution in methanol. The absorbance was measured at 517 nm against the corresponding blank solution as 80% methanol and control were prepared bu taking 1950 µl od DPPH by adding 50 µl distilled water instead of sample. Percentange inhibition of radical DPPH was calculated based on control reading by the following equation.

DPPH-Percentage % of reduction power = $((Ac-As/-A_c))$ x100

DPPH % inhibition = $((A_c-(As-A_b))/A_c)$x100

A_c: absorbance of control (standart)
A_s: absorbance of sample
A_b: absorbance of blank

Quantitative and statistical analyses

All the samples were directly injected to the reverse phase chromatography column. The sugar standards, glucose, fructose, sucrose, were dissolved in water at a concentration of 30 mg ml^{-1}. All samples and standards were injected three times each and mean values were used. The data were analyzed by JMP statistical software from SAS (V7) (SAS Institute Inc. Cary, NC, USA) and all the analytical values were average of three replications. The significantdifferences were compared by the least significant differences (LSD) test executed at 5% level of probability. The means ± the standard error (SE) were calculated from three independent experiments.

Results and Discussion

Biochemical compounds and natural antioxidants have become very important in human nutrition in recent years and are increasingly consumed by consumers. For this reason, it attracts the attention of many researchers and a lot of research is being conducted on this subject. Various parts of pistachio are known to have a high content of polyphenols, vitamin and sugar etc., that are all potent antioxidants and that may have protective effects against different diseases. Pistachio shells contain greater amounts of phenolics than the skin and nuts compared to those found in previously recognized phenolic sources (Tomaino et al., 2010;Garavand et al., 2017). When the studies in previous years were examined, it was found that most of these studies were usually done on fruit. In our study, different from the literature, especially shoots and leaves were examined.

Results of antioxidant activities and total phenolic contents of the different pistachio tissues in 'on' and 'off' year trees are presented in Table 1. It was found that the total phenolic (TPC) and antioxidant capacity (TAC) contents were significant in different tissue samples ($P < 0.05$). TPC values according to research results in the 2018 (on-year) and 2019 (off-year) years. It has been found to be change between shoot 120,034 (off-year) to 135,916 (on-year) mg gallic acid /g, leaf 88,101 (off-year) to 95,842 (on-year) mg gallic acid /g, hull 185,063 (off-year) to 272,624 (on-year) mg gallic acid /g, hard shell 268,003 (off-year) to 392,022 (on-year) mg gallic acid /g and kernel 76,379 to 95,608 mg gallic acid /g, respectively (Table 1). Figure 2 depicts individual parts expressed as a percentage of the total phenolic content and contributing over 35% of the total phenolic content of dehulled part. Total phenol contents in 'on' year were higher than in 'off'year trees. The results show that the total phenolic content of the hull and hard shell nuts was higher than that of the shoot and leaf. Goli et al., (2005) reported that shells contain antioxidant substances and can be used as an additive in foods. This variety contains more phenolic substances in their some tissues and it is advisable to consume seed with varieties because of their potential health benefits (Table 2). The data obtained from the present study are in accordance with other studies (Orhan et al., 2012; Kavak et al. 2010; Dogan et al., 2017). TPC values determined in different pistachio species and varieties 122,78 mg gallic acid /g, 120,64 mg gallic acid /g, 81.12 mg gallic acid /g and 43.81 gallic acid /g, Topcu et al. (2007), Tavakoli and Khodaparast (2013), Farhoosh et al., (2008) and Orhan et al. (2012) is lower than the study respectively, Ballistreri ve ark (2009); 184.71 to 349 mg gallic acid /g, Hatamnia et al., (2014): 189 to 330 and Polat (2016); 79.92 to 198.07 mg gallic acid /g compatible with the study, respectively, 452.95 588 mg gallic acid /g, 690.28 mg gallic acid/g, Atmani et al., (2009), Goli et al., (2005) Azadpour et al., (2015) 's were found to be higher than the study, respectively. TAC inhibition values according to research results in the 2018 and 2019 years; It has been found to be change between shoot 84,721 to 85,379 %, leaf 84,212 to 85,315 %, hull 79,839 to 86,062%, hard Shell 81,411 to 84,216% and fruit kernel 81,237 to 84,186%, respectively (Table 1 and Figure 2). The results antioksidant % radical-scavenging that to be change between shoot 69,668 to 70,373 %, leaf 67,011 to 70,063 %, shelled nuts 69,745 to 70,587%, dehulled 65,976 to 68,780% and kernel 65,870 to 68,778%, respectively (Table 1 and Figure 3). The results show that the total antioxidant activity. It was determined that antioxidant activity properties of different parts of 'Uzun' pistachio cultivar were not the same and leaves and shelled nuts had higher. Hosseinzadeh et al., (2012) found similar results in studies showing the antioxidant activity on pistachio. However, in previous studies in different pistachio species that Polat (2016), Durak and Ucak (2015), Azadpour et al., (2015) and Rezai et al., (2015) reported different results from the study that antioxidant activity in the different parts in pistachio 31,42 to 90.96%, 64.4%, 21.1% and 23.02 % respectively.

The soot, leaf and nut sugar concentrations (g/100g) and total sugar of "on" year trees in the year 2018, "off" year trees in year 2019 of "Uzun" pistachio cultivar were given in Table 2 and Figure 4, respectively. We found significant differences ($p < 0.05$) in sugars among different organs between 'on' and 'off' years in 'Uzun' pistachio cultivar. The highest sucrose content was determined of leaf (8,58 g/ 100 g) , shoot (6,54 g/100 g) and nuy (3,12 g/100 g) in 'off' year and the lowest of shoot (1,85 g /100 g) in 'on' year trees. The highest glucose content was determined of nut (5,48 g/ 100 g), leaf (5,99 g/100 g) and shoot (4, 36 g/100 g) in 'off' year and the lowest of shoot (3,20g /100 g) in 'on' year trees. The highest fructose content was determined of nut (12,10 g/ 100 g) , leaf (2,07 g/100 g) and shoot 2,47 g/100 g) in 'off' year and the lowest of leaf (1,72 g /100 g) in 'on' year trees. The results in study indicate all tissues that the amount of sugar content in 'off' year was higher than 'on' year (Table 2, Figure 4). The finding was in agreement with those of several previous researchers (Nzima et al 1997, Vemmos 1999b and Baninasab and Rahimi, 2006) reported that current different organs in 'on' years had higher amount of total sugar than 'off' year. Baninasab and Rahimi (2006) found sucrose, glucose and fructose concentration of nuts in "on" year between 19.64-121,94 mg/g, 2.58-9.23 mg/g and 14.46.3.74 mg/g, respectively. On the other hand, Karacali (1990) that suggested different plant contents of fructose, glucose, sucrose and maltose contents changed depending on species, varieties, genotypes and accessions. Kazankaya et al.

(2008) identified sugar content pistachio kernels belonging to different varieties contained kernels of Siirt variety had the highest fructose content (5.04 g/100 g), followed by Siirt (4.49 g/100 g), E-1 (4.00 g/100 g), Halebi (3.59 g/100 g) and H-1 (2.67 g/100 g), respectively. Glucose content (6.26 g/100 g) of B-1 kernels was detected in the highest amount, followed by Kirmizi (4.25 g/100 g), H-1 (4.13 g/100 g), Halebi (3.96 g/100 g) and Buttum (3.94 g/100 g), respectively. Sucrose content (4.74 g/100 g) of Buttum kernels was determined at the highest level, followed by V-1 (4.17 g/100 g), H-1 (4.17 g/100 g), B-1 (3.93 g/100 g) and Halebi (3.68 g/100 g), respectively. The same researchers also studied walnut genotypes contained 0.35-2.67 g/100 g fructose, 0.13-6.26 g/100 g glucose, 1.76-4.17 g/100 g sucrose and 0.23-0.74 g/100 g maltose. Sugar components of hazelnut and almond were 0.80 and 4.00 g/100 g fructose, 1.52 and 0.86 g/100 g glucose, 2.91 and 3.23 g/100 g sucrose, respectively. In addition, in study our results had higher sugar contents than other some pistachio varieties. The finding was in agreement with those of several previous researchers (Baninasab and Rahimi, 2006; Kazankaya et al., 2008).

Table 1. Total Phenolic content and Antioxidant capacity at different tissues of 'Uzun' pistachio cultivar of 'on' and 'off' years

Tissues	Total phenolic (mg gallik asit/100 g)		Antioxidant activity % inhibition		Antioksidant activity % radical-scavenging	
	'On' year	'Off' year	'On' year	'Off' year	'On' year	'Off' year
Shoot	135,916^{c} ±3,640	120,034^{c} ±3,206	85,379^{b} ±0,515	84,721^{a} ±0,463	70,373^{a} ±0,298	69,668^{a} ±0,391
Leaf	95,842^{d} ±2,230	88,101^{d} ±2,023	85,315^{b} ±0,237	84,212^{a} ±0,447	70,063^{a} ±0,210	67,011^{b} ±0,534
Hull	272,624^{b} ±5,353	185,063^{a} ±3,748	86,062^{a} ±0,071	85,839^{d} ±0,378	70,587^{a} ±0,319	69,745^{a} ±0,802
Hard Shell	392,022^{a} ±4,163	268,003^{a} ±2,986	84,216^{c} ±0,421	81,411^{c} ±0,325	68,780^{b} ±0,418	65,976^{d} ±0,219
Kernel	95,608^{d} ±1,346	76,379^{e} ±1,207	84,186^{c} ±0,421	81,237^{b} ±0,325	68,778^{b} ±0,418	65,870^{c} ±0,219
Total	992,012	737,58				
LSD%5	6,58**	5,04**	0,62**	0,69**	0,57**	0,95**

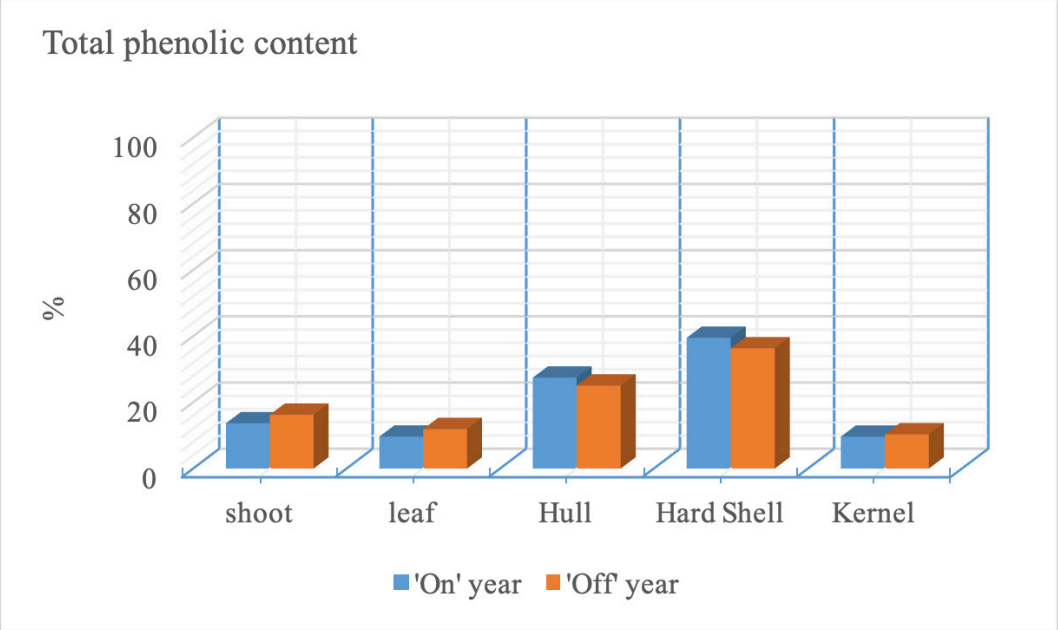

Figure 1. Total phenolic content of pistachio tissues in 'on' and 'off' years. Percentages were calculated based on the each tissue values x 100/total phenol.

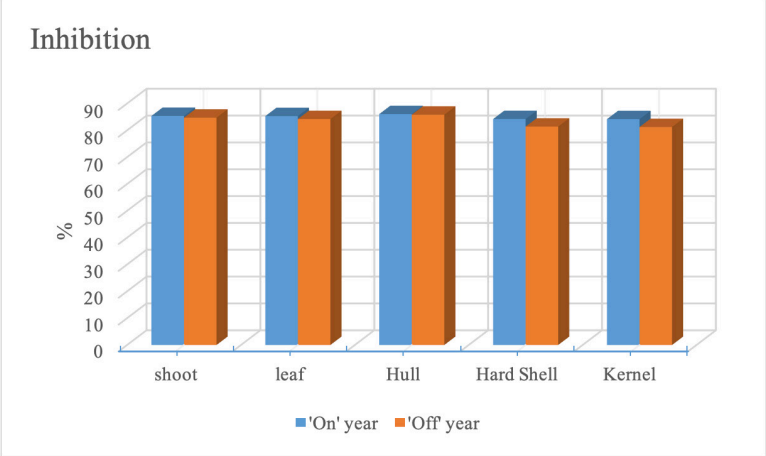

Figure 2. Antioxidant activity % inhibition in pistachio different tissue of ‘on’ and ‘off’ years

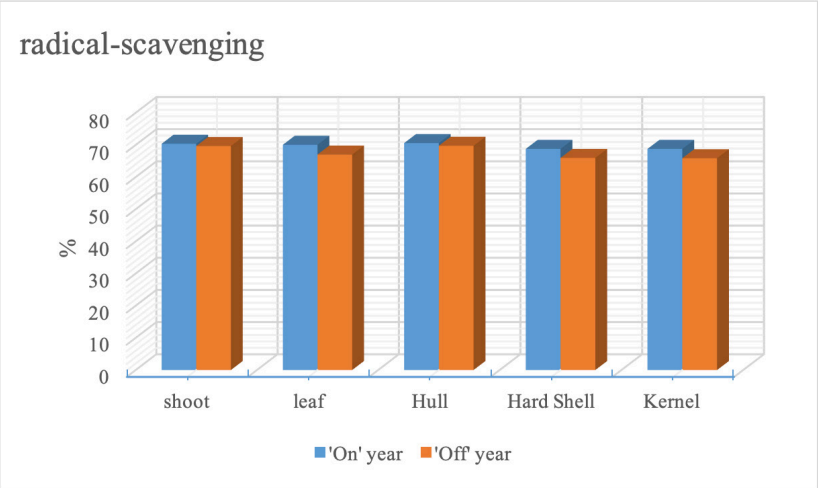

Figure 3. Antioksidant activity % radical-scavenging in pistachio different tissues of ‘on’ and ‘off’ years

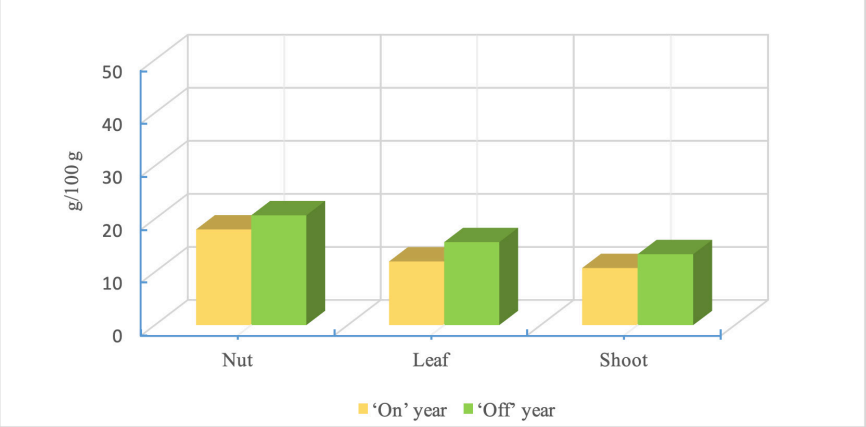

Figure 4. Total sugar concentration of different tissues of “Uzun” pistachio cultivar

Table 2. Soot, leaf and nut sugar compounds concentration (g/100g) of "Uzun" pistachio cultivar of 'on' and 'off' years

Tissues		Sucrose	Glucose	Fructose	Total sugar
Shoot	'On' year	5,71^{b}±0,30	3,20^{b}±0,21	1,85^{b}±0,08	10,76
	'Off' year	6,54^{a}±0,39	4,36^{a}±0,22	2,47^{a}±0,13	13,37
	$D\%5_{OnXoff\text{-}year}$	0,77**	0,49**	0,55**	
Leaf	'On' year	6,71^{b}±0,16	3,57^{b}±0,35	1,72^{b}±0,19	12,00
	'Off' year	8,58^{a}±0,13	4,99^{a}±0,21	2,07^{a}±0,10	15,64
	$D\%5_{OnXoff\text{-}year}$	0,33**	0,63**	0,35**	
Nut	'On' year	2,67^{a}±0,12	4,30^{b}±0,17	11,07^{b}±0,38	18,04
	'Off' year	3,12^{b}±0,15	5,48^{a}±0,25	12,10^{a}±0,43	20,70
	$D\%5_{OnXoff\text{-}year}$	0,30**	0,74**	0,72**	

Conclusion

In the fruit species (including pistachio) are of interest to researchers because of their antioxidant properties and are due to phenolic compounds and composition of sugars influences the taste and it can vary to varieties, ecological conditions, technical and cultural practice. However, this study showed that pistachio has strong radical scavengers and is a fruit that is a good source of natural antioxidants for medical and commercial uses. Thus, pistachio can be eaten as part of a diet to alleviate the symptoms of chronic and degenerative diseases that are reported to increase in the world. In Turkey, pistachio shells are used as industrial waste. However, as a result of the study, it was determined that most of sugar compositions, the total antioxidant activity and total phenolic content of 'Uzun' pistachio cultivar were found to be rich in sugars, phenolic content and antioxidant content in the shelled and dehulled nuts. Therefore we recommend the use in particular of different organs of pistachio in food and other industries (e.g. medicine, cosmetics). In addition, in respect of its antioxidant properties, it can be used as a natural antioxidant in some foods (snack, sweets, icecream etc.).

Compliance with Ethical Standards

Conflict of interest

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author contribution

The author read and approved the final manuscript. The author verifies that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Not applicable.

Funding

No financial support was received for this study.

Data availability

Not applicable.

Consent for publication

Not applicable.

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