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ORIGINAL PAPER

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DETERMINING OF THE FRUIT CHARACTERISTICS OF PISTACHIO GRAFTED ON WILD *Pistacia terebinthus* L. UNDER THE CENTRAL KELKIT BASIN (TURKEY) ECOLOGICAL CONDITIONS

Erdal Ağlar[1,✉], Ahmet Sümbül[1], Orhan Karakaya[2], Burhan Ozturk[2]

[1] Sivas Cumhuriyet University, Suşehri Timur Karabal Vocational School, Sivas, Turkey

[2] Ordu University, Faculty of Agriculture, Department of Horticulture, Ordu, Turkey

ABSTRACT

The study was carried out to determine fruit quality characteristics in 'Uzun' pistachio cultivar grafted on terebinth rootstock (*P. terebinthus* L.) in Suşehri under Central Kelkit Basin (Turkey) ecological conditions in 2017. In the study, it was determined that the ratio of the split fruit was 80%, the blank fruit ratio was 8%, nut weight was 0.93 g, kernel weight was 0.50 g and kernel ratio was 53.8%. As a result of analyses, it was assessed that the fruit consists of 19.39% protein, 3.74% moisture, 2.38% ash, 53.30% fat, 7182.45 mg kg^{-1} potassium, 4046.79 mg kg^{-1} phosphorus, 1592.96 mg kg^{-1} calcium, 974.35 mg kg^{-1} magnesium, 16.14 mg kg^{-1} iron, 4.42 mg kg^{-1} manganese, 11.38 mg kg^{-1} copper and 27.05 mg kg^{-1} zinc. It was determined that the total phenolic content was 1989.8 mg GAE kg^{-1} dw, the total flavonoid content was 363.0 mg QE kg^{-1} dw; DPPH was 30.9 mmol TE kg^{-1} dw; FRAP was 36.2 mmol TE kg^{-1} dw.

Key words: Suşehri, antioxidant activity, phenolic content, rootstock, mineral content

INTRODUCTION

Pistachio, which is defined "golden tree" or "green gold" due to its high price, was cultured by Hittite civilization settled in Southeastern Anatolia and has been consumed as a valuable fruit from that time. Turkey, where is the origin of pistachio and has unique ecological features, has led to the successful growth and widespread of this fruit species. Turkey is the most pistachio producing countries in the world [FAOSTAT 2018]. In the reproduction of the pistachio (*Pistacia vera* L.), the grafting of wild pistachio species has been used commonly. The potential of *Pistacia* species (*P. terebinthus* L., *P. khinjuk* Stocks, *P. atlantica* Desf., *P. lentiscus* etc.) that have been expanded to the different regions of Turkey enables to become widespread of pistachio cultivation [Kuru and Özsabuncuoğlu 1990].

The rootstock is one of the most effective factors for growth and development, fruit yield and quality, precocity and nutrient intake of fruit trees [Ozturk and Ozturk 2014, Yıldız et al. 2018]. The fruit yield and quality and the economic life of the tree can be varied depending on the rootstock while the rootstocks have no effect on some characteristics of the cultivar [Tarango Rivero 1993]. Gündoğdu [2019] determined that rootstocks were effective on agromorphological properties and biochemical contents of fruits. In growing of the pistachio, the using of the rootstock that are naturally placed in the flora of the region is preferred due to its adaptability to ecological conditions. *Pistacia terebinthus* L. rootstock enables to grow the pistachio in cooler regions because it is the most resistant rootstock to the cold [Ferguson et al. 2005].

✉ erdalaglar@hotmail.com

In Sivas province, where is not suitable for economically fruit growing due to its climate properties, we think that Suşehri located in the Central Kelkit Basin has a special characteristic with regards to fruit growing. Many fruit species grow easily in Suşehri. Pistachio can not be grown in cold regions like Central Anatolia due to its high temperature requirement. In Günlüce village of Suşehri, has microclimatic ecology in the region. Pistachios are grown economically by grafting on terebinth (*P. terebinthus* L.) trees. However, in the pistachio grown in the region, no study has been carried out relative to the fruit quality characteristics, which are important in terms of marketing and consumption and this is not a situation unique to Suşehri. The studies on pistachio in Turkey were generally limited to the Southeast Anatolia region [Tekin et al. 2001]. The number of studies carried out on fruit quality characteristics in pistachio obtained by grafting on wild pistachio species in many cities of Turkey is not enough. The studies carried out by Öztürk and Ataç [1982], İsfendiyaroğlu and Özeker [2001] and Satil et al. [2003] are rare studies in this sense. The aim of this study was to determine the fruit yield and quality characteristics of pistachio on grafted *P. terebinthus* L. rootstock in the Suşehri under Central Kelkit Basin (Turkey) ecological conditions.

MATERIAL AND METHODS

This study was carried out in the village of Günlüce, which has a micro-climate feature in Suşehri in 2017. A total of 100 trees was marked in population of 'Uzun' pistachio cultivar grafted on *P. terebinthus* L. trees existing in the region. At the harvest (18th October), 300 fruits on each tree were harvested to make fruit analysis and measurements. The fruits harvested were immediately transported to Food Technology laboratory of the Cumhuriyet University, Suşehri Timur Karabal Vocational School. The necessary pomological measurements (the weight and size of nuts and kernel, splitting percentage, kernel ratio) were made by removing the outer skin on fifty fresh fruits. The remaining 250 fruits were used for necessary analysis and measurements after being dried in place where is not the sunlight.

Physical Characteristics

Fruit width, fruit height and fruit length (mm). Fruit width, fruit height and fruit length were determined by measuring with a digital caliper with 0.01 mm precision.

Fruit weight (g, 100 fruits). 100 nuts and kernel weight were determined as gram by weighing with a sensitive scale of 0.001 g.

Kernel ratio (%). Kernel percentage was calculated with the following formula:

$$\text{Kernel ratio \%} = \text{kernel weight} / \text{nut weight} \times 100$$

Blank nut rate (%). The blank fruit in 100 nuts was counted and expressed as a ratio.

Splitting percentage (%). The percentage of split nuts was counted and expressed as a ratio.

Weight loss(%). The fresh and dried fruit weight of 300 nuts was weighed with a sensitive scale of 0.001 g. Weight loss was calculated with the formula:

$$\text{Weight loss (\%)} = \text{total dried weight} / \text{total fresh weight} \times 100$$

Chemical Characteristics

The following analyzes were performed on the fruit samples belong to 10 trees with superior properties as a result of pomological and physical properties.

Determination of moisture. After the harvest, the determination of moisture content in the fruits dried in shade were made according to AACC [2002]. The fruits were grinded, 3 g fruit sample was taken for each replicate. The samples were put in the oven set at 105°C for 4 h. The samples withdrew from oven after a while they were putting the desiccator. After waiting for 45 min in the room temperature, the weighing was performed and the moisture contents of the samples were determined.

Determination of ash. The ash in the kernel was determined according to ICC [1982]. For each replicate, pre-burning process was performed by putting 3 g sample milled on the crucible and by dropping 2–3 drops of ethanol on it. Then the samples milled were put 500°C ash furnace, and after the exact burning process was completed, the samples were removed from the ash furnace, they were kept in the desiccator for 45 min and the determination of the ash was made as dry weight.

Determination of protein. The amount of protein was determined using the Kjeldahl method [Bremner

1965]. In this method, which is a method of wet decomposition mainly, the nitrogen in the samples is converted to ammonia (NH_4) by burning with concentrated H_2SO_4 and nitrogen (NH_3) is determined from the amount of NH_3 obtained in titration of the ammonia (NH_3) released from the distillation made in an alkaline medium [Kacar 1972]. For each replicate 0.25 g of milled sample was taken, they were put into the tubes with the puddle paper and in pre-burning process, 1 spatula from the catalyst salt mixture was added to each Kjeldahl tube, the puddle paper was allowed to react completely with the acid by adding 5 ml salicylic-sulfuric acid on catalyst salt mixture. The samples taken to the combustion unit Kjeldatherm (Gerhardt, Germany) after the overnight pre-burning process were maintained for 1 h at temperatures of 70°C, 170°C and 270°C, and for 5 h at 370°C. After the burning process, the distillation was made in the 'Gerhardt Vapodest' device. In this process, the medium is alkalized by adding 10 ml of purified water and 20 ml of NaOH to the samples, the ammonium is converted to the ammonia and it reacts with boric acid to form ammonium borate [Kacar 1972]. After this process, which takes about 2–3 min, the % N value was determined by titration 0.37 N H_2SO_4. The protein content % was determined by multiplying the nitrogen value with the coefficient (5.30), which is obtained for the pistachios.

Fat analysis. Soxhelet extraction method was used to determine the total amount of fat in the fruit. For each replicate, 10 g of milled sample was placed in the cartridges and they were covered with cotton. The cartridges are placed in the SoxHellet device, and 150 ml of n-hexane was added. After extraction at 100–105°C for 6 h, fat and hexane in the balloon were evaporated under nitrogen gas, the balloon and the fat inside were weighed together and the amount of fat in the bottle was determined [Ayfer 1973, IUPAC 1987].

Mineral analysis. The amount of the mineral matter in the fruit was determined according to the method of wet burning [Kacar 1972]. For each replicate, the moisture content of 5 g of shredded fruit sample was determined by waiting in the oven, which was adjusted to 65°C, for 48 h. The samples were put into the erlenmeyer, 10 ml of nitric acid : perchloric acid was added in a ratio of 4 : 1, and erlenmeyer put on hot plates. The temperature was increased to 150–200°C slowly. The burning process continued until the intense white fumes of perchloric acid completely covered the inside of the erlenmeyer. After the samples allowed to cool, they were poured into 50 ml balloon jars using a glass funnel, and supplied with 50 ml of with distilled water. It was put into storage containers by filtering through Whatman filter paper, and the samples were kept at the refrigerator temperature for reading. A 1/10 ml dilution was made to read the mineral materials. K content was determined by the flamefotometre device (Jenway PFP 7), P content was determined by the spectrophotometer device (Shimadzu UV-160) according to vanadomolybdophosphoric method. Ca, Mg, Fe, Mn, Cu and Zn were determined by Varian 720-ES ICP (Inductively Coupled Plasma) Optical Emission Spectrometer device.

Analysis of Bioactive Compounds

Determination of total phenolics (TP) and total flavonoids (TF). TP content was measured according to the procedure described by Singleton and Rossi [1965]. Briefly, 0.2 ml sample was extracted with a buffer containing acetone, water and acetic acid (70 : 29.5 : 0.5 v/v) for 2 h at dark. Samples were replicated four times. Extracts were combined with Folin-Ciocalteu's phenol reagent and water, and incubated at room temperature for 8 min, followed by the addition of 7% sodium carbonate. After 2 h, the absorbance at 750 nm was measured in an automated UV-Vis spectrophotometer (Model T60U, PG Instruments). Gallic acid was used as the standard. The results were expressed as mg gallic acid equivalents (GAE) 100 g^{-1} on a dry weight (dw) basis.

TF content of fruit samples were determined according to the calorimetric method [Chang et al. 2002, Ozturk and Ozer 2019]. Briefly, each extract (0.1 g) was dissolved in 1 ml of the appropriate solvent. This solution (0.1 ml) was mixed with 10% $AlCl_3 \cdot 6H_2O$ and 0.1 ml of 1 M potassium acetate (CH_3COOK). The absorbance of the reaction mixture was measured at 415 nm. Quercetin was chosen as a standard. The results were expressed as mg quercetin equivalents (QE) 100 g^{-1} on a dry weight (dw) basis.

Determination of TAC as ferric reducing antioxidant power (FRAP) and radical scavenging capacity (DPPH). DPPH free radical scavenging activity: The hydrogen atom or electron donation abilities of

some pure compounds were measured by the bleaching of a purple colored methanol solution of DPPH. The free radical scavenging activities of methanol extract of fresh fruit were measured by 1.1-diphenyl-2-picryl-hydrazil (DPPH) using the method of Blois [1958] and Ozturk et al. [2019] where in the bleaching rate of a stable free radical, DPPH was monitored at a characteristic wavelength in the presence of the sample. An amount of 0.5 ml of 0.1 mm ethanolic solution of DPPH was added to 3.0 ml of all the extract samples or standard antioxidant solution (50–500 µg ml^{-1}) in water. The mixture was shaken vigorously and kept standing at room temperature for 30 min. Then the absorbance of the mixture was measured at 517 nm. The results were expressed as mmol trolox equivalents (TE) kg^{-1} on a dry weight (dw) basis.

Ferric ions (Fe+3) reducing antioxidant power assay (FRAP). Portions of 120 µL were taken from the samples, 0.2 M of phosphate buffer (PO_4^{-3}) (pH 6.6) was added to obtain a volume of 1.25 ml and then 1.25 ml of 1% potassium ferricyanide ($K_3Fe(CN)_6$) solution was added. After vortexing, they were incubated at 50°C for 1 h. Afterwards, 1.25 ml of 10% TCA (trichloro acetic acid) and 0.25 ml of 0.1% $FeCl_3$ were added to the samples. The absorbances of the extract solution were read on a UV-Vis spectrometer at 700 nm. The results were expressed as mmol Trolox equivalents (TE) kg^{-1} on a dry weight (dw) basis [Benzie and Strain 1996].

RESULTS AND DISCUSSION

There is significant differences growth, procecity, yield, fruit quality, life of the tree and adaptation to the ecological conditions of the same cultivar on grafted to the different rootstocks and they have significant physiological effects on each other [Özçağıran 1974]. One of the most significant characteristics of the rootstocks is their contribution to the widespread of species due to their adaptability to different ecological conditions such as climatesand soils. Pistachio is the best example in this sense. *Pistachia terebinthus* L. provides to grow pistachios in a cold region like Central Anatolia due to its ability resistance to colds. Pistachio is economically produced by grafting on *P. terebinthus* L. rootstocks in the Suşehri where has the micro-climate property. This study was carried out to determine the fruit quality characteristics of 'Uzun' cultivar grafted on *P. terebinthus* L. in Suşehri located in the Central Kelkit Basin.

Physical characteristics

The physical properties of the nuts are given in Table 1. In the study, it had been determined that the weight of nut was 93 g (100 nuts), the weight of the kernel was 50 g (100 kernels) and kernel ratio was 53.76%. When similar studies are examined, in the study carried out in the Çanakkale-Ayvacık region, it was determined that the weight of the nut was 111 g (100 nuts) and kernel ratio was 41.88% [Satil et al. 2003]. In the study carried out in the Pervari (Siirt) between 2004 and 2005, the fruit and tree characteristics of 43 genotypes grafted on wild pistachio rootstock grown in the region have been defined. It was determined that in these genotypes, the average nut weight was between 1.21–1.93 g, kernel weight was between 0.46–0.81 g, and kernel ratio was between 31.5 to 49.0% [Öztürk 2006]. In the study conducted between 1984–1987 in Gaziantep and 'Uzun' cultivar used, it was determined that the weight of the nut was 133.82 g (100 nuts) and kernel ratio was 40.0% [Karaca and Nizamoğlu 1995]. Compared to the results of the studies, the fruit of the pistachio grown in Suşehri is relatively small, but the kernel ratio is high.

Table. 1. Physical characteristics of pistachio fruits

Characteristics	Nut	Kernel
Fruit weigth (g, 100 fruits)	93.00	50.00
Fruit width (mm)	10.65	8.54
Fruit length (mm)	20.32	17.30
Fruit heigth (mm)	8.76	6.94
Splitting percentage (%)	80.00	
Blank nut ratio (%)	8.00	
Weight loss (%)	40.74	
Kernel ratio (%)	53.76	

In the formation of the blank fruit; cultivar, rootstock, using of the pollinator, climate conditions and cultural practices have significant effects [Crane and Iwakırı 1980]. Crane [1975] reported that the for-

mation of the blank fruit in the 'Kerman' cultivar on grafted *P. vera* L. occured from parthenocarpy and seed abortion, and the ratio of the blank fruit was 26%. In a four-year study carried out in 'Siirt' cultivar, it was found that the ratio of blank fruit was 13.05% [Ak 1997]. In our study, the ratio of blank fruit was determined as 8%. Compared to other studies, this ratio will be seen to be lower. This result can be considered to be due to rootstock and climatic characteristics as well as higher fertilization as a result of a branch in each tree was grafted the pollinator when the grafting is applied to the region. Çağlar et al. [1992] reported that the formation of the blank fruit was caused by the competition between fruits, and the lack of pollination and cultural processes such as irrigation and fertilization. Goldhamer et al. [1987] suggested that the rootstocks are also effective in the formation of blank fruit.

The split ratio in pistachio is one of the most significant quality characteristics that affect quality and marketing. The genetic variation among the rootstocks [Goldhamer et al. 1987] and the cultivar, age of the tree, nutritional, the temperature differences between day and night at ripening period have an effect in the event of the fruit split [Ak et al. 1999]. In our study, the ratio of the fruit split was determined as 80%, while this rate in similar studies was 46% [Satil 2000], 61% [Yıldız 1999] and 67.2% [Tekin and Akkök 1995]. Compared with the results of other studies, it was observed that the rate of the fruit split in pistachios in Suşehri was higher. This high fruit split ratio can be explained by the fact that the difference between day and night temperature is high at the ripening period in the region and that the used rootstock is strong. It can also be considered that the high kernel ratio of the fruit may have an effect on the rate of fruit split. As a matter of fact, Whitehouse et al. [1964] reported that the rate of the natural fruit split was higher in fruits with a high kernel ratio.

Moisture, Fat and Protein Content

Proper moisture content in dried pistachios (4–5% w/w) is an important factor for quality [Kashani Nejad et al. 2003]. Although the moisture ratio between 4 and 6% in pistachio have positive effects in some sensory properties [Kader et al. 1982], it is impossible to reduce the moisture content to exactly 5% by commercial drying. In our study, the moisture content was measured as 3.74% (Tab. 2). Compared with other studies, it has been observed that the moisture content of the fruit is low. So much so that Tsantili et al. [2010] reported that in the study carried out on the different cultivar, the moisture content of the fruit was between 4.43% ('Mumtaz') and 6.05% ('Kerman').

Table. 2. Chemical characteristics of pistachio fruits

Characteristics	Content
Protein (%)	19.39
Moisture (%)	3.74
Ash (%)	2.38
Fat (%)	53.30
Potassium (mg kg^{-1})	7182.45
Phosphorus (mg kg^{-1})	4046.79
Calcium (mg kg^{-1})	1592.96
Magnesium (mg kg^{-1})	974.35
Iron (mg kg^{-1})	16.14
Manganese (mg kg^{-1})	4.42
Copper (mg kg^{-1})	11.38
Zinc (mg kg^{-1})	27.05

The crude protein ratio in the study was found to be 19.39% (Tab. 2). When similar studies are examined, Seferoglu et al. [2006] in the study conducted with 'Uzun' cultivar in 7 different regions in the Büyük Menderes Basin, they have determined that the amount of protein is between 22.6–32.0%. Tsantili et al. [2010] in the study carried out on the different cultivars they have reported that protein content varies between 19% dw ('Joley') and 21.8% dw ('Cerasola'). Taking into consideration these results, it can be said that the protein ratio in the study is relatively low. The occurrence of differences between protein content can be explained by the effect of climate conditions. As a matter of fact, Okay [2002] and Seferoglu et al. [2006] reported that the amount of the protein may vary according to region.

Pistachio kernels are a rich source of fat and contain the essential fatty acids necessary for human nutrition [Agar et al. 1995a]. The fat percentage in pistachios may vary among the cultivars and grown ecological conditions [Agar et al. 1995b]. Environmental factors

as well as genetic factors affect the fat content [Seferoglu et al. 2006]. Seferoglu et al. [2006] have determined that the ratio of fat in the study carried out on 'Uzun' cultivar in 7 different regions in Büyük Menderes Basin is 46.8–66.5%. Kuru [1993] has reported that the fat content of pistachios in Turkey is between 54–60%. Agar et al. [1995b] whom report that the fat content in same cultivar was significantly influenced by different ecological conditions, and the ratio of fat in pistachio is between 48.5 and 58.5%. In our study, the fat ratio was determined as 53.30% in accordance with the results of this study.

Mineral content

The evaluation of minerals and trace elements in foods is a significant part of nutritional and toxicological analyses. Pistachio is an excellent source of potassium, magnesium and calcium [USDA 2010]. K, the major intracellular cation in the body, is required for a normal cellular function, while Mg is the most abundant intracellular divalent cation, being an essential cofactor for more than 300 enzymatic reactions. Ca is an essential nutrient, quantitatively the most abundant of the body's minerals as well as a vital electrolyte [Segura et al. 2006]. Fabani et al. [2013] reported that the most abundant element in pistachio was potassium, followed by calcium and magnesium, and trace elements are in the following order: Na > Fe > Zn > Cu > Mn. In our study, the mineral content was determined in accordance with these results as follows: potassium: 7182.45 mg kg^{-1}, phosphorus: 4046.79 mg kg^{-1}, calcium: 1592.96 mg kg^{-1}, magnesium: 974.35 mg kg^{-1}, iron: 16.14 mg kg^{-1}, manganese: 4.42 mg kg^{-1}, copper: 11.38 mg kg^{-1}, zinc: 27.05 mg kg^{-1} (Tab. 2). Yang et al. [2009] reported that the order of mineral substances is similar in terms of content. However, mineral substance concentrations can vary depending on the region, the cultivar used and the cultural practices [Anderson and Smith 2005].

Total phenolic, total flavonoid, and total anthocyanin content

Pistachio consumption is increasing day by day due to the positive health effects of food ingredients such as phenolic compounds [Brufau et al. 2006, Ryan et al. 2006, Venkatachalam and Sathe 2006, Miraliakbari and Shahidi 2008], antioxidant and antiproliferative [Arcan and Yemenicioglu 2009, Blomhoff et al. 2006, Kornsteiner et al. 2006]. However, studies on bioactive compounds such as total phenolics, flavonoids and antioxidant activity in pistachios are limited [Grace et al. 2016]. In our study, the total phenolic content was determined as 1989.8 mg GAE kg^{-1} dw, but Fabani et al. [2013] reported that the total phenolic content in pistachio was between 360 and 463 mg GAE 100 g^{-1} dw. Also Arcan and Yemenicioglu [2009] have reported that phenolic contents in pistachio are 461 mg GAE 100 g^{-1} dw; and Yang et al. [2009] have determined as 572 mg GAE 100 g^{-1} dw. Compared with these results, it can be said that the total phenolic content in pistachio grown in Suşehri is relatively low.

Tsantili et al. [2011] have reported that the total flavonoid content in pistachio varies between 3.5 mg of CE g^{-1} dw, and 7.2 mg CE g^{-1} dw, and when it was dry, the contents of the flavonoid decrease as a ratio of 14.14%. In our study, the total content of flavonoid in dried fruit was determined to be 363.0 mg QE kg^{-1} dw. When the results are compared, it will be seen that the pistachio grown in the Suşehri is rich in terms of the flavonoid.

Antioxidant activity was determined by the DPPH radical scavenging and the ferric-reducing antioxidant power (FRAP) assays. Fabani et al. [2013] reported that DPPH and FRAP values differ between the cultivars, the highest DPPH value was 280 lg ml^{-1}; and the highest FRAP value was 20.6 mg QE 100 g^{-1} dw. Taking into consideration the results of this study, it has higher DPPH and FRAP values in pistachios grown in Suşehri. In this study, it was determined as 30.9 mmol TE kg^{-1} dw for DPPH and as 36.2 mmol TE kg^{-1} dw for FRAP (Tab. 3).

The concentrations of bioactive compounds such as total phenolics, flavonoids, and total anthocyanin may vary depending on the cultivar and the rootstock

Table. 3. Bioactive compounds content of pistachio fruits

Bioactive compounds	Content
Total phenolic (mg GAE kg^{-1} dw)	1989.8
Total flavonoid (mg QE kg^{-1} dw)	363.0
DPPH (mmol TE kg^{-1} dw)	30.9
FRAP (mmol TE kg^{-1} dw)	36.2

used, the ecological characteristics of the region, nutrition and cultural practices applied. It is foreseen that the positive differences in this sense in our work may be due to the strong of the rootstock used and the negative differences may result from the lack of cultural practices.

CONCLUSIONS

As a result; we think that the Suşehri which enables the cultivation of pistachios in a cold region like the Central Anatolia due to its mikro-climate property is a special region. The harvest in the region is being made in the middle of October, therefore the pistachio grown in the region has an economical advantage. *P. terebinthus* L., which grows naturally in the region, is a vigorous rootstock so it significantly affects fruit yield and quality. As a result of our study, we think that the results such as high kernel ratio and splitting percentage and low blank fruit percentage, which are an important criterion in terms of fruit quality, are valuable results.

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