

## Determination of Compounds Existing in Fruits of Three Pistachio (*Pistacia vera* L.) Cultivars in Kerman Province

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### ABSTRACT

This study aimed to determine the compounds existing in three pistachio (*Pistacia vera* L.) cultivars including Ohadi, Mumtaz and Ahmad Aghaei that have been cultivated in Kerman region. The amount and type of fatty acids, amount of protein, amount and type of amino acids, amount of sugar and moisture and mineral elements (copper, zinc, iron, magnesium, calcium, potassium, and phosphorus) were measured. According to the obtained results the amount of oil in cultivars under study was 54.93% – 55.4% and the highest rate of fatty acids was related to Oleic acid (60.2% – 64.8%). Amount of protein and moisture, was determined 18.81% – 19.31% and 31.1% – 34.00%, respectively. The highest amount of sugar (6.1%) was observed in Ohadi cultivar. There was a significant difference in cultivars' amount of amino acids including Glycine, Tyrosine, Alanine,  $\alpha$  – Aminobutric acid, Valine and Isoleucine and also mineral elements including copper, zinc, magnesium, potassium and phosphorus had significant difference.

**Key Words:** Aminoacid, fatty acid, *Pistacia vera*.L, Kerman.

### INTRODUCTION

Iran is the world's largest producer of pistachio and has the highest cultivation area of this crop in the world (FAO 2008). Iran is also one of the rich resources regarding the number of pistachio cultivars and genotypes in the world. Ohadi and Ahmadaghaei cultivars are considered the most important commercial pistachio cultivars. Ohadi cultivar has the highest amount of pistachio cultivation area in Iran. Although cultivation area of Mumtaz cultivar is low, it is commercially valuable (Panahi *et al.*, 2001, Ismail-Poor 2005)

Because of variation in pistachio cultivars existing in Iran, more accurate identification of cultivars' properties and inner compounds and compare them with each other in the main areas of pistachio cultivation seem necessary. One of the most important features of pistachio is that despite the high level of oil in it (over 50 percent of its dry weight), the ratio of unsaturated fatty acids to saturated fatty acids particularly Oleic acid is high in it. Oleic acid is the most important fatty acid in pistachio and belongs to unsaturated fatty acids and has the largest rate of pistachio's fatty acids, so that it includes more than 50 percent of fatty acids in pistachio (Garcia *et al.*, 1992; Agar *et al.*, 1995; Yildiz *et al.*, 1998; Satil *et al.*, 2003; Maskan and Karatas, 1998; Kucukoner and Yurt, 2003; Chahed *et al.*, 2008). Oleic acid is effective from nutritional aspect and prevention of diseases related to atherosclerosis (Kris-Etherton 2001). Although several studies have been conducted in the field of identification and determination of amount and type of fatty acids, it should be noted that the amount of inner compounds of pistachio, including the amount of oil and ratio of fatty acids to each other change due to cultivar and climate conditions of cultivated area (Chahed *et al.*, 2008). So, every cultivar in each region should be individually identified and the compounds should be determined. Studies have been conducted based on amount of some mineral elements and amount of protein in different cultivars of pistachio (Kucukoner and Yurt, 2003) and also the amount of mineral elements and protein in pistachio Kernel influenced by the kind of rootstock (Tavallali and Rahemi, 2007). Changes in moisture levels in some pistachio cultivars in areas with different climate conditions have been reported by Chahed *et al.* (2008). Despite the studies carried out in the field of identification and determination of inner compounds of pistachio Kernel, so far identification of amino acids in pistachio has not been noticed and the current study is among the first studies conducted in this field.

### MATERIALS AND METHODS

Plant materials included 35-years-old pistachio trees, Ahmad Aghaei, Ohadi (Fandoghi), Mumtaz cultivars grafted on Badami Riz rootstock in cultivars collection kept by pistachio research station in Kerman. The test repeated three times in form of randomized complete blocks. In this study, nine pistachio trees of Ahmad Aghaei, Ohadi and Mumtaz cultivars were selected. At harvest time (23 September) four fruit clusters of each cultivar (with three replications) collected from four sides of the tree, and after mixing them, the intended traits were assessed.

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#### ***Measurement of amount of pistachio oil***

To measure the amount of pistachio oil, the dried pistachio kernel became powder by an electric mill and extraction was done by Soxhlet method and solvent of ether de petrole. After removing the solvent by evaporation, the amount of oil in samples calculated and expressed as a percentage through expression of weight ratio of obtained oil sample to sample before oil extraction (Horwitz 2000).

#### ***Preparation of fatty acids methyl ester***

Preparation of fatty acids methyl ester performed by Graces and Mancha's (1993) method which is an appropriate method for plant tissues containing high oil (Graces and Mancha 1993).

#### ***Determination of fatty acid composition***

To analyze fatty acids of samples the Agilent's gas chromatography method was used. Gas Chromatography machine, the Agilent 6890N (Agilent Corporation of America) was used that was equipped with FID detector. Chromatography conditions were according to AOAC method No. 963/22 (28), and Capillary column with length of 60 meters, diameter 0.25 mm and thickness Polar Silica, 0.32 micrometers, manufactured by G and W Co. was used.

#### ***Determination of amount of protein***

To determine the amount of protein by Kjeldahl method the nitrogen rate in samples was measured, the obtained number for each sample was multiplied in protein factor 6.25 and the amount of protein in each sample was obtained (AOAC 2003).

#### ***Determination of the moisture amount of samples***

To determine the amount of moisture, the samples were put in an oven at 70°C temperature for 48 hours; samples' weight difference before and after drying was calculated and expressed as a percentage.

#### ***Determination of amount of sugar***

Considering that the constituent sugars in pistachio are glucose, sucrose and fructose, Fehling method was used for measuring pistachio's sugar. At first, sucrose was hydrolyzed to reducing sugars by hydrochloric acid, then divalent copper ion of Fehling solutions transformed into monovalent copper in an alkaline environment due to reduction by reducing sugars. The total amount of reducing sugar is calculated based on solution amount of consumed sample sugar to reduce of copper and color change of the solution (AOAC 1990).

#### ***Measurement of mineral elements***

To measure mineral elements, pistachio nutmeat was dried in an oven at 70°C temperature for 48 hours and then it became powder and after converting to ash at 550°C it was digested by hydrochloric acid and the samples were injected into atomic absorption spectrophotometer (FAO 2008).

#### ***Extraction and identification of amino acids***

To identify the amino acids after preparation of plant materials and standard solutions prepared in accordance with Bartolommeo and Maisano's (2006) method the samples were injected into HPLC.

The conditions of HPLC was determined as follows: column: HALO C<sub>18</sub>, 5cm detector: fluorescence (Wavelength Excitation: 330 nanometer Wavelength Emission: 450 nanometer) and Flow Rate: 1.1 ml/min and Run Time: 25 min.

#### ***Statistical analysis of data***

Statistical analysis of data was performed by SAS software. The mean were compared at 5% level by Duncan's test.

## RESULTS AND DISCUSSION

### *Determination of amount of pistachio oil*

The amount of oil in three cultivars under study was obtained 54.93% – 55.40% and there was no significant difference between the cultivars (Table 1). Similar results have been reported by different researchers in determining the amount of oil in different cultivars of pistachio. Kamangar *et al.* (1975) reported the amount of oil in three cultivars of Badami, Ohadi and Mumtaz 58.96% – 60.10%, and Karaca and Nizamoglu (1995) reported the amount of oil in Mumtaz cultivar 55.4%. Okay (2002) measured the amount of oil in four cultivars of Turkish pistachio and one cultivar of Iranian pistachio 55.85% – 59.73%, Kucukoner and Yurt (2003), reported the amount of oil in the same cultivars 57.85% – 59.60%. Similar results have been reported by Yildiz *et al.* (1998) and Shokraii (1977). In addition to cultivar, climate conditions of cultivation region and orchards management are the reasons for little difference reported in the amount of pistachio oil between this study and the above mentioned research. Chahed *et al.* (2008) have reported changes in the amount of oil influenced by climate conditions.

**Table 1.** Comparison the effect of cultivar on the amount and composition of pistachio (*Pistacia vera* L.) oil. (Data expressed as percentage).

Cultivar	Oil Content	C<14	Meristic Acid	Palmitic Acid	Palmitoleic Acid	Stearic Acid	Oleic Acid	Linoleic Acid	Linolenic Acid	Gadolic Acid
Ohadi	55.40a	1.97a	0.075a	8.87a	0.83a	1.67 a	60.2a	26.24a	0.460a	0.328a
Momtaz	54.93a	1.99a	0.078a	8.85a	0.84a	1.08 a	62.68a	22.28b	0.480a	0.350a
Ahmagaghaei	55.36a	1.05b	0.073a	8.97a	0.83 a	1.13 a	64.8a	21.95 b	0.459a	0.346a

Mean separation was done by Duncan test at  $p \leq 0.05$ .

### *Identification and determination of amount of pistachio fatty acids*

Identification and determination of amount of pistachio fatty acids showed that the highest rate of pistachio fatty acids was related to Oleic acid in all three cultivars (60.2% – 64.8%) and after Oleic acid, linoleic acid (21.95% – 26.24%) had the highest amount of fatty acids (Table 1).

Chahed *et al.* (2008) reported that the amount of Oleic acid in Mateur cultivar cultivated in four areas with different climate conditions was 54.2% – 74.8%; the lowest Oleic acid (54.2%) was obtained in the area with semi-humid climate conditions compared with the other three areas with semi-arid to arid climate.

In this study, in Ahmad Aghaei cultivar the amount of fatty acids with carbon number less than 14 (1.05%) was less than Ohadi and Mumtaz cultivars (1.97% and 1.99%, respectively) (Table 1). Also, the amount of linoleic acid in Ohadi cultivar (26.24%) compared to Mumtaz and Ahmad Aghaei cultivars (22.28% and 21.95%, respectively) was higher (Table 1). In other fatty acids in cultivars under study no significant difference was observed (Table 1). In a study conducted by Okay (2002) on fatty acids of different cultivars of Turkish pistachio there was no significant difference between the cultivars and only the amount of linoleic acid showed significant difference between cultivars (21.95% – 26.24%), which is consistent with results of this study. The other values obtained from Okay's (2002) research (cultivars of Uzun, Kirmizi, Siirt, Ohadi, Halabi) are as follows: Palmitic acid, 8.22% – 9.20%, Palmitoleic acid, 0.56% – 0.68%, Stearic acid, 0.94% – 2.01%, Oleic acid 56.6% – 74.01%, linolenic acid 0.18% – 0.30% that are relatively consistent with the results of this study. Kucukoner and Yurt (2003) also reported similar results and declared the amount of fatty acids of existing nutmeat in cultivars of Uzun, Kirmizi, Siirt, Ohadi, Halabi respectively as follows: Palmitic acid, 8.22% – 9.20%, Palmitoleic acid, 0.56% – 0.68%, Stearic acid, 0.94% – 2.01%, Oleic acid 56.66% – 74.01%, linoleic acid 14.37% – 31.00%, linolenic acid 0.19% – 0.30%. Chahed *et al.* (2008) reported the amount of fatty acids in Turkish Mateur cultivar, cultivated in four regions with different climate as follows: Palmitic acid, 11.2% – 15.7%, Palmitoleic acid, 1.5% – 2.5%, Stearic acid, 2.3% – 2.7%, Oleic acid 54.2% – 76.8%, linoleic acid 7.6% – 24.1%, linolenic acid 0.2% – 0.9%. Large changes in each of fatty acids in different cultivation areas (e.g. change of Oleic acid between 54.2% – 76.8%) reflect the impact of cultivation area on fatty acids ratio.

Also Chahed *et al.* (2008) showed that in Mateur and Ohadi cultivars cultivated in one area the amount and proportion of fatty acids is different that suggests the difference of amount and proportions of fatty acids in different cultivars.

### **Determination of amount of mineral elements in pistachio kernel**

Pistachio is a rich source of phosphorus, potassium, magnesium, calcium and iron (Ferguson 1995) that these mineral elements in pistachio unlike fatty acids have been studied less. In this study, the amount of calcium (0.92% – 0.10%), iron (34.1 – 35.42 mg/kg) in three cultivars of Ohadi, Mumtaz and Ahmad Aghaei had not significant difference. While the amount of phosphorus and magnesium in Ahmad Aghaei cultivar (0.26% and 0.17%, respectively) was less than Ohadi and Mumtaz (0.31% and 0.155%; 0.32% and 0.155%, respectively), potassium levels in Ohadi cultivar (0.85%) was more than Mumtaz and Ahmad Aghaei (0.80% and 0.79%, respectively). The highest amount of iron was observed in Ahmad Aghaei cultivar (35.4 mg/kg) had not significant difference with other cultivar. The amount of copper in Ahmad Aghaei and Ohadi cultivars was 17.07 mg/kg and 16.11mg/kg, respectively (Table 2). In a study performed by Kucukoner and Yurt (2003) the amount of potassium, magnesium, sodium, copper in five cultivars of pistachio i.e. Uzun, Kirmizi, Siirt, Ohadi, Halabi was obtained 0.68% – 0.76%, 0.136% – 0.146%, 88 – 152 mg/kg, 11.90 – 15.23 mg/kg, respectively that are relatively consistent with the values obtained in this study for potassium, magnesium and copper. Tavallali and Rahemi (2007) reported that the kind of cultivar and rootstock is effective on the amount of nutrients stored in the pistachio nutmeat so that Ahmad Aghaei cultivar on Sarakhs rootstock has higher levels of mineral elements compared with Kaleghoochi and Badami cultivars on the other rootstocks.

**Table 2.** Comparison the effect of cultivar on the amount of protein and mineral elements of pistachio (*Pistacia vera* L.).

Cultivar	Protein (%)	P (%)	K (%)	Ca (%)	Mg (%)	Fe (mg/kg)	Zn (mg/kg)	Cu (mg/kg)
Ohadi	18.81a	18.81a	0.8 a	0.09 a	0.155b	34.2a	18.82b	16.11ab
Momtaz	19.31a	19.31a	0.80b	0.10a	0.155b	35.1a	21.91b	15.05b
Ahmadaghaei	19.06a	19.06a	0.79b	0.092a	0.170a	35.4a	25.42a	17.07a

Mean separation was done by Duncan test at  $p \leq 0.05$ .

### **Determination of amount of protein in pistachio kernel**

There was no significant difference in amount of protein in three cultivars under study (18.81% – 19.31%) (Table 2). In a study which conducted by Okay (2002) on cultivars of Uzun, Kirmizi, Halabi, Siirt and Ohadi, the amount of protein was measured 19.58%, 22.55%, 25.06%, 24.60% and 22.05, respectively. In a study which carried out by Kucukoner and Yurt (2003) the amount of protein in cultivars of Uzun, Kirmizi, Siirt, Ohadi and Halabi was reported 22.67%, 20.93%, 22.45%, 23/62% and 20.18, respectively. Different factors have role in determination of the total amount of protein in pistachio that among them we can refer to the kind of rootstock. Tavallali and Rahemi (2007) reported that amount of protein in pistachio nutmeat of Ahmad Aghaei cultivar on Badami rootstock and Beneh rootstock was 19.50% and 20.6%, respectively, while on Sarakhs rootstock it increased to 27.51%. Also, Kaleghoochi and Ohadi cultivars showed similar results so that the amount of protein in Kaleghoochi cultivar was 21.58% on Badami rootstock and was 19.97% on Beneh rootstock and increased to 26.63% on Sarakhs rootstock. The amount of protein in Ohadi cultivar on Badami rootstock and Beneh rootstock was 21.41% and 18.30% respectively, and on Sarakhs rootstock it reached to 28.74%. As it is observed the amount of protein measured by other researchers was reported more than the value defined in this study (18.81% – 19.31%). It is possible that low amounts of protein obtained in this study have been influenced by the kind of rootstock (Beneh rootstock).

### **Determination of amount of sugar and moisture in pistachio kernel**

The amount of sugar in cultivars under study was obtained 5.3% – 6.1%. The highest amount of sugar was determined in Ohadi cultivar and Mumtaz and Ahmad Aghaei cultivars had no significant difference with each other(5.5%-5.3% respectively) (Table 3). The amount of moisture in cultivar was 31.1%-34% that had no significant difference with each other. In a study conducted by Chahed *et al.* (2008) the amount of moisture in pistachio kernel of Mateur cultivar collected from four regions with different semi-humid to semi-arid and arid climate conditions was different and varied between 30% – 38%. Also, a significant difference was observed in moisture between the two cultivars of Mateur and Ohadi cultivated in one area (25% and 30%, respectively). So it can be said that climate conditions and in some cases the kind of cultivar can change the amount of moisture of kernel.

**Table 3.** Comparison the effect of cultivar on the amount of moisture and sugar pistachio (*Pistacia vera* L.).

Cultivar	Moisture (%)	Sugar (%)
Ohadi	31.7a	6.1a
Momtaz	34.00a	5.5b
Ahmadaghaei	31.10a	5.3b

Mean separation was done by Duncan test at  $p \leq 0.05$ .

### Identification and determination of type of amino acids in pistachio kernel

In the field of identifying and determining the type of amino acids in pistachio no report has been published yet and the current study is one of the first reports in this regard. Mumtaz cultivar had higher levels of amino acids of Tyrosine, Alanine,  $\alpha$ -Aminobutyric acid, Valine and leucine (Tables 4 and 5) and Ohadi and Ahmad Aghaei cultivars had no significant difference with each other in this regard. Amounts of other amino acids in cultivars under study had no significant difference (Tables 4 and 5).

**Table 4.** Comparison the effect of cultivar on the amount of aminoacids of pistachio (*Pistacia vera* L.). (Data expressed as mg/kg).

Cultivar	Aspartic acid	Glutamic acid	Asparagine	Serine	Glutamine	Histidine	Glycine	Tyrosine	Citroline	Arginine
Ohadi	1.01a	1.33a	1.14a	0.8a	0.26a	0.11a	0.18a	0.23ab	0.05a	0.05a
Momtaz	1.05a	1.40a	1.28a	0.85a	0.25a	0.11a	0.19a	0.27a	0.05a	0.05a
Ahmad-aghaei	0.97a	1.35a	1.15a	0.77a	0.25 a	0.11a	0.18a	0.21b	0.05a	0.05a

**Table 5.** Comparison the effect of cultivar on the amount of aminoacids of pistachio (*Pistacia vera* L.). (Data expressed as mg/kg).

Cultivar	Pro-line	Ala-nine	Threo-nine	$\alpha$ -Amino-butyric acid	Trypto-phan	Methio-nine	Valine	Phenyl-alanine	Leusine	iso-leusine
Ohadi	0.1a	0.95b	0.8a	0.07b	0.13a	0.03a	0.28b	0.17a	0.16b	0.13a
Momtaz	0.08a	1.27a	0.09a	0.10a	0.13a	0.04a	0.31a	0.17a	0.18a	0.14a
Ahmad-aghaei	0.1a	1.03b	0.09a	0.08b	0.1a	0.03a	0.28b	0.17a	0.16b	0.14a

Mean separation was done by Duncan test at  $p \leq 0.05$ .

## CONCLUSIONS

Result of this research show that the composition of pistachio kernel variable by the effect of cultivar. Other researcher such as Kukukoner and yourt (2003), Chahed *et al.*, (2008), Okay (2001) Tavallali and Rahemi (2007) confirm the effect of cultivar on the internal composition of pistachio. Regarding genetic diversity of pistachio cultivars and the impact of different factors such as climate conditions of cultivation area and orchards management on inner compounds of pistachio nutmeat, it is recommended that in main areas of pistachio cultivation the compounds of pistachio kernel and other compounds not reviewed in this study such as vitamins etc be identified and determined.

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