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Determination of fatty acids, protein and amino acids in fruits of three pistachio (*Pistacia vera* L.) cultivars in Kerman Province, Iran

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Received on September 10, 2011; revised on May 12, 2012; accepted on May 13, 2012

Abstract

This study was aimed at determining the fatty and amino acids as well as protein content in three pistachio (*Pistacia vera* L.) cultivars including Ohadi, Mumtaz, and Ahmadaghaei in Kerman region, Iran. The amount and type of fatty acids, protein and the type of amino acids were measured. According to the results, the range of oil in the studied cultivars was 54.93–55.4% and the highest rate of fatty acids (60.2–64.8%) was related to Oleic acid. The amount of protein was 18.81–19.31%. There was a significant difference among cultivars for the amount of amino acids including Glycine, Tyrosine, Alanine, α -Aminobutric acid, Valine and Isoleucine.

Keywords: Amino acid, Fatty acid, *Pistacia vera*, Kerman.

Introduction

Iran is the largest producer of pistachio with the largest cultivation area of the crop in the world (Anonymous, 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 as the most important commercial pistachio cultivars. The largest pistachio cultivation area in Iran belongs to Ohadi cultivar. Although cultivation area of Mumtaz cultivar is relatively low, it is commercially valuable (Panahi *et al.*, 2001; Ismail-Poor, 2005)

Because of the variation in pistachio cultivars, more accurate characteristics are needed to identify the seed components to compare with each other in the main pistachio cultivation areas. One of the most important features of pistachio is that despite the high level of oil (over 50 percent of

its dry weight), the ratio of unsaturated to saturated fatty acids, particularly oleic acid, is high. Oleic acid forms the largest proportion of pistachio 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; Maskanand Karatas, 1998; Kucukoner and Yurt, 2003; Chahed *et al.*, 2008). Oleic acid is important from nutritional perspective and prevention of diseases (Kris-Etherton *et al.*, 2001). Several studies have been conducted on the amount and type of pistachio cultivars' fatty acids. It should be noted that the amount of oil and ratio of fatty acids are affected by cultivar and climatic conditions of cultivation area (Chahed *et al.*, 2008). Therefore, cultivars in different regions are individually important to be identified. Studies have been conducted based on the amount of some mineral elements and total protein in different cultivars of pistachio (Kucukoner and Yurt, 2003). It was shown that kernel was influenced by the type of rootstock (Tavallali and Rahemi,

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

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

Means separation was done by Duncan Test at $p \leq 0.05$.

2007). Changes in moisture levels in pistachio cultivars in areas with different climatic conditions have been reported by Chahed *et al.* (2008). The current study was carried out in order to identify and determine the kernel components of pistachio.

Materials and Methods

Plant materials included nine 35-year-old pistachio trees of Ahmad Aghaei, Ohadi and Mumtaz cultivars, all grafted on Badamiriz rootstock in Kerman Pistachio Collection, Kerman, Iran. The test was repeated three times on the basis of a Randomized Complete Block Design. At harvest time (September 23) four fruit clusters per cultivar (with three replicates) were collected from four sides of the tree, and after mixing them, the intended traits were assessed.

Measurement of oil content

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

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).

To analyze fatty acids of samples, the Agilent's gas chromatography method was used. Gas chromatography machine, the Agilent 6890N (Agilent Corporation of America) equipped with FID detector was used. Chromatography conditions were according to AOAC method No. 963/22 (28)(Anonymous, 2003), and capillary column with the length of 60 meters, diameter of 0.25 mm and thickness Polar Silica of 0.32 micrometers manufactured by G&W Co. was used.

To determine the amount of protein by Kjeldahl

method, the nitrogen rates were measured, the obtained number for each sample was multiplied by protein factor 6.25 and the amount of protein in each sample was obtained (Anonymous, 2003).

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

The conditions of HPLC was determined as follows: column: HALO C₁₈, 5cm detector: fluorescence (Wavelength Excitation: 330 nanometer Wavelength Emission: 450 nanometer) and Flow Rate: 1.1 ml min⁻¹ and Run Time: 25 min.

Statistical analysis

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

Results and Discussion

Determination of pistachio oil content

The amount of oil of the three studied cultivars was obtained as to be 54.93-55.40% and there was no significant difference between the cultivars (Table 1). Similar results have been reported by others. Kamangar and Farsam (1977) reported the amount of oil in three cultivars of Badami, Ohadi and Mumtaz as to be 58.96-60.10%, and Karaca and Nizamoghlu (1995) reported the amount of oil in Mumtaz cultivar as to be 55.4%. Okay (2002) measured the amount of oil in four cultivars of Turkish pistachios and one cultivar of Iranian pistachio as to be 55.85-59.73%. Kucukonerand Yurt (2003) found the amount of oil in the same cultivars as to be 57.85-59.60%. Similar results have been reported by Yildiz *et al.* (1998) and Shokraii (1977). In addition to cultivar, climatic conditions and management are the reasons for little difference re-

Table 2. Comparison of the effect of cultivar on the amount of protein and amino acids of pistachio (*Pistaciavera* L.) oil. (Data expressed as mg kg⁻¹)

Cultivar	Protein	Aspartic acid	Glutamic acid	Asparagine	Serine	Glutamine	Histidine	Glycine	Tyrosine	Citroline	Arginine
Ohadi	18.81 a	1.01 a	1.33 a	1.14 a	0.8 a	0.26 a	0.11 a	0.18 b	0.23 ab	0.05 a	1.85 a
Mumtaz	19.31 a	1.05 a	1.40 a	1.28 a	0.85 a	0.25 a	0.11 a	0.19 a	0.27 a	0.05 a	1.84 a
Ahmad Aghaei	19.06 a	0.97 a	1.35 a	1.15 a	0.77 a	0.25 a	0.11 a	0.18 b	0.21 b	0.05 a	1.90 a

Means separation was done by Duncan Test at $p \leq 0.05$.

ported in the amount of pistachio oil between this study and other researches. Chahed *et al.* (2008) reported that climatic conditions changed the amount of oil in different cultivars.

Identification and determination of the content of fatty acids

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

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

In the current study, in Ahmad Aghaei cultivar the sum of the fatty acids with ¹⁴C (1.05%) was lower than that in Ohadi and Mumtaz cultivars (1.97% and 1.99%, respectively) (Table 1). Also, linoleic acid in Ohadi (26.24%) compared with Mumtaz and Ahmad Aghaei cultivars (22.28% and 21.95%, respectively), showed the highest value (Table 1). No significant difference was observed in other fatty acids of the cultivars (Table 1). Okay (2002) reported no significant differences between the fatty acids of Turkish pistachio cultivars except linoleic acid (21.95% – 26.24%), which is consistent with results of this study. The other values obtained by Okay's (2002) who studied the cultivars 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%, and 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 kernel in cultivars 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, 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%, and linolenic acid 0.2% – 0.9%. Large changes in each of fatty acids in different 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 protein content

There was no significant difference in the amount of protein in three cultivars under study (18.81% – 19.31%), (Table 2). In a study conducted by Okay (2002) on cultivars Uzun, Kirmizi, Halabi, Siirt and Ohadi, protein contents were 19.58%, 22.55%, 25.06%, 24.60% and 22.05, respectively. In another study carried out by Kucukoner and Yurt (2003), the amount of protein in cultivars Uzun, Kirmizi, Siirt, Ohadi and Halabi was reported to be 22.67%, 20.93%, 22.45%, 23/62% and 20.18, respectively. Different factors such as rootstock have key role in determination of the total protein in pistachios. Tavallali and Rahemi, (2007) found the higher amount of protein in Ahmad Aghaei on Badami and Beneh rootstocks with 19.50% and 20.6%, respectively, while on Sarakhs it increased to 27.51%. Also, Kaleghoochi and Ohadi cultivars showed similar results so that the amount of protein in Kaleghoochi was 21.58% on Badami and 19.97% on Beneh and increased to 26.63% on Sarakhs rootstock. The amount of protein in Ohadi cultivar on Badami and Beneh was 21.41% and 18.30% respectively, whereas on Sarakhs increased to 28.74%. It is observed that the amount of protein

Table 3. Comparison of the effect of cultivar on the amount of amino acids of pistachio (*Pistaciavera* L.) oil. (Data expressed as mg kg⁻¹)

Cultivar	Protein	Alanine	Threonine	α -aminobutyric acid	Tryptophan	Methionine	Valine	Phenylalanine	Leucine	Isoleucine
Ohadi	0.1 a	0.95 b	0.8 a	0.07 b	0.13 a	0.03 a	0.28 b	0.17 a	0.16 b	0.13 a
Mumtaz	0.08 a	1.27 a	0.09 a	0.10 a	0.13 a	0.04 a	0.31 a	0.17 a	0.18 a	0.14 a
Ahmad Aghaei	0.1 a	1.03 b	0.09 a	0.08 b	0.1 a	0.03 a	0.28 b	0.17 a	0.16 b	0.14 a

Means separation was done by Duncan Test at $p \leq 0.05$.

reported by other researchers was higher than the value found in the current study (18.81% – 19.31%). Low protein in cultivars such as Beneh highlighted the influence of rootstock on kernel components.

Identification and determination of type of amino acids

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 Glycine, Tyrosine, Alanine, α -Aminobutyric acid, Valine and Isoleucine (Tables 2 and 3) and Ohadi and Ahmad Aghaei cultivars had no significant differences with each other in this regard. Amounts of other amino acids in cultivars under study had no significant difference (Tables 2 and 3).

Conclusions

Results of this research show that the composition of fatty acids and amino acids in pistachio kernel varied by the effect of rootstock and cultivar. Other researchers such as Kukukoner and Yourt (2003), Chahed et al. (2008), Okay (2001) Tavallali and Rahemi (2007) confirm the effect of cultivar as well as other factors such as climatic conditions and orchard management on the composition of the pistachio kernel, resulting in diversity of pistachio cultivars.

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