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The Effect of Different Solvent Systems on Some Chemical Properties of Pistachio Nut Oil Contaminated with Aflatoxin

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ABSTRACT: Extraction of oil from poor quality pistachios results in increased add-value. In this study, the performance of different solvents in recovery of valuable oil and meal from the pistachio sample contaminated with aflatoxin was investigated and some chemical properties of the extracted oil were determined. The pistachio nut oil was extracted by three solvent systems including n-hexane (non- polar), ethanol 96˚ (polar) and n-hexane-ethanol 96˚ (90:10) using soxhlet method and for each solvent the mean yield was determined. Fatty acid profile, Iodine, peroxide and acid values of the extracted oil were determined. Aflatoxin contents in the produced oil and meal were measured. Extraction yield of the pistachio oil by different solvents ranged from 33.6% in the samples extracted by ethanol to 40.4% in the sample extracted by n-hexane-ethanol (90:10). The most predominant saturated and unsaturated fatty acids found in the pistachio oils were palmitic and oleic acids, respectively. The highest amount of aflatoxin was found in the oil extracted by ethanol and in the meal recovered by n- hexane.

Keywords: *Aflatoxin, Chemical Properties, Different Solvent Systems, Fatty Acids, Pistachio Nut Oil*.

Introduction

Pistachio (*Pistachia vera L.)* belongs to Anacardiacea family (Tomaino *et al.*, 2010) being one of the most important edible nuts. It grows in dry and warm regions under salinity condition (Metheney *et al.*, 1998). Pistachios are among the main agricultural products in Iran, predominantly in Kerman, Khorasan, Semnan and Pars provinces. In 2010, world pistachios export value was estimated at 320,000 t, out of which 160,000 t (50 % of the world's export value) was produced in Iran. Iran is the most important exporter of pistachio worldwide (Tavakolipour & Mokhtarian, 2012). Ohadi and Ahmadaghaei appear to be the most important pistachio varieties as the area under the cultivation of Ohadi variety is shown to be the largest as compared to other varieties in Iran (Panahi *et al.*, 2001; Ismail-Poor, 2005).

The tendency toward consumption of pistachio nuts has been increased due to their nutrient contents such as sterols, vitamins, minerals, fatty acids, phenolic compounds, protein and dietary fiber (Miraliakbari & Shahidi, 2008; Brufau *et al.*, 2006; Ryan *et al.*, 2006; Venkatachalam & Sathe, 2006; Sathe, 2006). Pistachio nuts contain about 50% oil, an oil rich in oleic

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and linoleic acids (Sheibani & Ghaziaskar, 2008) which provides important therapeutical effects (Azlan *et al.*, 2010). Oleic acid is an important monounsaturated fatty acid that helps in reducing triglycerides, low-density lipoproteins (LDL), total cholesterol and glycemic index. In addition, oleic acid is responsible for the increase of stability and reduction of oxidation in vegetable oils (Kocyigit *et al.*, 2006). Linoleic acid is an essential fatty acid (ω_6) that promotes the development and protection of nervous system and physiological functions in human (Sari *et al.*, 2010).

It should be noted that a fraction of the harvested pistachios has no or little acceptability by the consumers due to the undesirable apparent characteristics or poor quality however, they still retain the nutritional value. Extraction of the oil from these pistachio nuts might increase their add-value (Sheibani & Ghaziaskar, 2008). Among the pistachios of poor quality are pistachios contaminated with aflatoxin. Aflatoxins are secondary metabolites mostly produced by the toxigenic strains of *Aspergillus flavus* and *Aspergillus parasiticus*, hardly by *Aspergillus nomius*, which contaminate various food products especially in warm and humid areas through the world. *Aspergillus flavus* and *Aspergillus parasiticus* are the main producers of aflatoxin B1 (AFB1) and aflatoxin B2 (AFB2). Aflatoxin G1 (AFG1) and aflatoxin G2 (AFG2) are also produced by *Aspergillus parasiticus* (Santacroce *et al.*, 2008) and *Aspergillus nomius* (Wen *et al.*, 2005). The International Agency for Research on Cancer (IARC) has classified aflatoxin B1 (AFB1) as a class IA human carcinogen (IARC, 1993) because it has been identified as the most potent naturally occurring toxin which causes cancers especially liver cancer (Richard *et al.*, 1993; Verma, 2004).

Food and Agriculture Organization (FAO) has recommended that the amount of this compound in most foods except milk should not exceed 20 ppb and European Union (EU) has limited its content to 5 ppb (Rodricks, 2007). According to Iranian National Standard, maximum permissible amounts of AFB1 and total aflatoxin in pistachios are 5 ppb and 15 ppb and in meals for feed are 10 ppb and 20 ppb, respectively (ISIRI 5925, 2002).

The oil of pistachios contaminated with aflatoxin can be recovered by those solvents with the capability of extracting the toxin along with the oil and then the toxin might be removed through refining process. Solvent extraction is one of the most widely used methods for oil recovery from oil seeds. Hexane, presently, is the most commonly used solvent worldwide because of high extraction efficiency and availability. However, hexane has been identified as a hazardous air pollutant (H.A.P.) on the list of poisonous chemicals by U.S. agency for environmental protection (Niosh, 2007). The amount of hexane in oil and meal should not exceed 5 ppm and 10 ppm, respectively (PFA act 1954). Reducing the concentration of hexane in meal to an acceptable level requires excessive energy and time. Thus, the attention of researchers has been paid to proper alternatives. Recently, new solvents have been evaluated in extraction process of which ethanol, isopropanol and hardly acetone have been recognized safe in food industries (Oliveira *et al.*, 2013). Green solvents such as ethanol does not pose toxicity problems associated with using hexane in meals for food and feed applications (Saxena *et al.*, 2011). Hexane, is also not capable of removing aflatoxins from the oil source, thus, it leaves aflatoxin in the meal. Meals containing high aflatoxin content, out of permissible limits, are not suitable for human and animal consumption, thus, they might be used as fertilizers. Therefore, the solvents which are able to remove aflatoxins along with the oil, such as ethanol, have been investigated. The aflatoxin is inactivated and removed through

alkali refining and bleaching processes of the oil, thus, it would not be found in refined vegetable oils (Hamedi, 2008). The meal resulted from the above-mentioned processes might be added to foods as a source of nutrients containing protein, dietary fiber, etc. and can also be used as a feed thereby increasing the add-value of pistachio cultivation.

Pistachios contain about 20% vegetable protein. Studies have shown that the consumption of vegetable proteins is associated with reduced risk of cardiovascular diseases. This can be attributed to low ratio of lysine to arginine in these proteins. In general, in contrast to animal proteins, vegetable proteins such as protein of nuts are rich in arginine and low in lysine contents. Consumption of foods with a high ratio of lysine to arginine is associated with higher risk of Hypercholesterolemia and atherosclerosis (Carroll & Hamilton, 1975; Kritchevsky *et al*., 1982; Sugano *et al*., 1984; Kritchevsky, 1990). The ratio of lysine to arginine in pistachios is 0·5 (Souci *et al*., 2000). The ratio is lower than that of animal proteins such as casein (1.9), whole milk (2.4) and even soya bean (0.58–1.0) (Kritchevsky *et al*., 1982).

As mentioned earlier consideration has been given to ethanol as a potential safe solvent for extraction of vegetable oils such as soya bean (Arnold & Choudhury, 1962; Rodrigues *et al*., 2010), corn (Moreau & Hicks, 2005), cottonseed (Sineiro *et al*., 1998; Abraham *et al*., 1988) and rice bran (Oliveira *et al*., 2012). Oliveira *et al*. (2013) studied the extraction of oil from passion fruit by green solvents. Oliveira *et al*. (2012) investigated the effect of extraction conditions on the yield and composition of rice bran extracted by ethanol. Saxena *et al*. (2011) compared the extraction of oil from cottonseed by n-hexane and ethanol. Nwabueze and Okocha (2008) investigated the extraction performances of polar and non-polar solvents on the physical and chemical indices of African breadfruit (*Treculia africana*) seed oil. Moreau and Hicks (2005) studied the composition of corn oil extracted by alcohol extraction. Bhowmick (2003) proposed the use of isopropanol due to higher solubility of oil and gossypol in it. Abraham *et al*. (1998) reported that 95% of the oil was extracted from cottonseed by ethanol and the resulted alcoholic micella was easily refined by caustic soda in order to produce high quality oil. Kuk *et al*. (1998) studied the extraction of cottonseed oil by the use of a new solvent system; isohexane and alcohols. Hron *et al*. (1994) investigated ethanol extraction of oil, gossypol and aflatoxin from cottonseed.

There is a little information on the effect of solvents with different polarity on the quality of oil extracted from pistachio nuts contaminated with aflatoxin, thus, in this study, the performance of n-hexane (non-polar), ethanol 96˚ (polar) and n-hexane-ethanol 96˚ (90:10) in recovery of nutritionally valuable oil and meal from pistachios contaminated with aflatoxin in respect of extraction yield, aflatoxin content in oil and meal as well as some chemical characteristics of the extracted oil consisting of fatty acid profile, acid, peroxide and Iodine values were investigated.

Materials and Methods

All the chemicals were provided by Merck, Romile, Sigma and Sapleco Chemical Companies. The pistachio nut sample, variety Ohadi, was obtained from one of the research centers of pistachios. The nut sample was ground by an electrical mill and then, passed through a sieve with the proper mesh. The chemical composition of pistachio nut, variety Ohadi, consisting of moisture, crude protein, fat, crude fibre, pectin and ash were determined according to AOAC (1995) methods in triplicate orders. Total carbohydrate was determined by the difference. The aflatoxin content of pistachio

nut was determined by reverse-phase high performance liquid chromatography method (HPLC) employing immunoaffinity column clean-up and post column derivatization (ISIRI 6872, 2013).

Pistachio nut oil was extracted by three types of solvent including n-hexane (non-polar), ethanol 96˚ (polar) and n-hexane-ethanol 96˚ (90:10). The extraction was carried out by soxhlet method according to ISO 659 (ISO 659:2009). The solvent was removed using a rotary evaporator and the extracted oil was quantified. The quality of the extracted oils was determined by identification of fatty acid composition and determination of acid and peroxide values in triplicate orders. The aflatoxin content of the oils and meals resulted from each extraction was determined by reverse-phase high performance liquid chromatography method (HPLC) and with immunoaffinity column clean-up and post column derivatization (ISIRI 6872, 2013).

The fatty acid composition of the oil samples were determined by methylation of the fatty acids according to ISO 5509:2000 followed by the identification of methyl esters by GC equipped with a flame ionization detector according to ISO 5508 (ISO 5508:1990). The acid value was determined according to ISO 729 (ISO 729:1988). The peroxide value was determined according to ISO 3960 (ISO 3960:2007). The Iodine value was calculated using the equation presented by AOSC Cd 1c - 85 directly from the fatty acid composition (Firestone, 1999).

- **Statistical analysis**

The results were statistically evaluated by one-way analysis of variance (ANOVA) in a completely randomized design using SPSS v. 18 (IBM SPSS, New York, USA). Significant differences between means were assessed at $P < 0.05$ using Duncan's Multiple Range test.

Results and Discussion

Chemical composition of the pistachio nut sample, variety Ohadi, is presented in Table 1.

Table 1. Chemical composition of the pistachio nut sample, variety Ohadi

Factor	Content
Moisture content (g/100g)	3.1
Fat content (g/100g)	40.0
Protein (g/100g)	23.4
Crude fiber (g/100g)	3.6
Pectin content (g/100g)	2.3
Ash (g/100g)	3.5
Total carbohydrate (g/100g)	30.0

The moisture content in the pistachio nut sample was 3.1%. Kamangar & Farsam (1977) determined the moisture content of 2.5- 4.1%. Drying to a proper moisture content is an important factor to ensure a good quality (Kashani Nejad *et al*., 2003).

In this study, the fat content in the pistachio nut sample was 40% as determined by soxlet method. A great difference in the fat content of pistachio nut was reported by several researchers. This factor was reported 56% by Kuecukoner and Yurt (2003) and Pala *et al.* (1994) and 40.6% - 53.5% by Koroglu (1997) (Raei & Jafari, 2011). Garcia *et al.* (1992) determined the fat content of pistachio nut, variety Ohadi, as 56.2%. Okay (2002) stated that the observed differences in the fat contents of pistachio cultivars might be due to the differences in factors like rising conditions, crop or season. Different rising regions, rootstocks or irrigation conditions had been reported to affect the fat ratio of cultivars (Kamangar & Farsam, 1977).

Total protein content was 23.4%. Kamangar & Farsam (1977) determined the protein content in Ohadi variety as 17.08 % whereas Okay (2002) reported protein content of 22.1 % for Ohadi variety. Also Kuecukoner & Yurt (2003) reported the protein content in Ohadi as 23.62%. High amount of ash in the pistachio nut sample (3.5%) indicates that the pistachio is a good

source of minerals consisting of phosphorus, potassium, magnesium, calcium and iron (Ferguson, 1995). Total carbohydrate content was 30%. U.S. Department of Agriculture (USDA) has determined the total carbohydrate content of 28% for the pistachio nut sample.

Mean oil contents of the pistachio nut sample extracted with n-hexane, ethanol 96˚ and n-hexane- ethanol 96˚ (90:10) by applying soxhlet method are shown in Figure 1.

Concerning the extraction yield by using different polarity solvents, hexane- ethanol showed the highest yield (40.4%), however, it was not significantly different from the yield of hexane (40.1%). Hexane had higher extraction yield as compared to ethanol because oils in non- polar organic solvents are more soluble, but such oils are not much safe especially when they are not refined (Bera, *et al*., 2004). In addition, hexane has been categorized as a hazardous air pollutant (HAP) by the US Environmental Protection Agency and is included in the list of toxic chemicals (NIOSH, 2007). Lower yield of extraction by ethanol could be attributed partly to its high polarity and less solubility of oil in polar solvents. Non- polar solvents like hexane are not charged and dipole moment of them is zero, which favors oil extraction, whereas, in ethanol, as an organic polar solvent, the presence of O-H would interfere with the extraction process. It should be noted that in molecules that are composed of atoms with different electro negativities, the atoms with lower electro negativities gain partial positive charges, and the atoms with higher electro negativities gain partial negative charges. As a result, the polarization of chemical bonds occurs, which increases the dipole moment of the molecule and alters the intermolecular interactions (Oliveira, 2013).

Aflatoxin content of the pistachio nut sample and the extracted oil are presented in Figures 2 and 3 respectively.

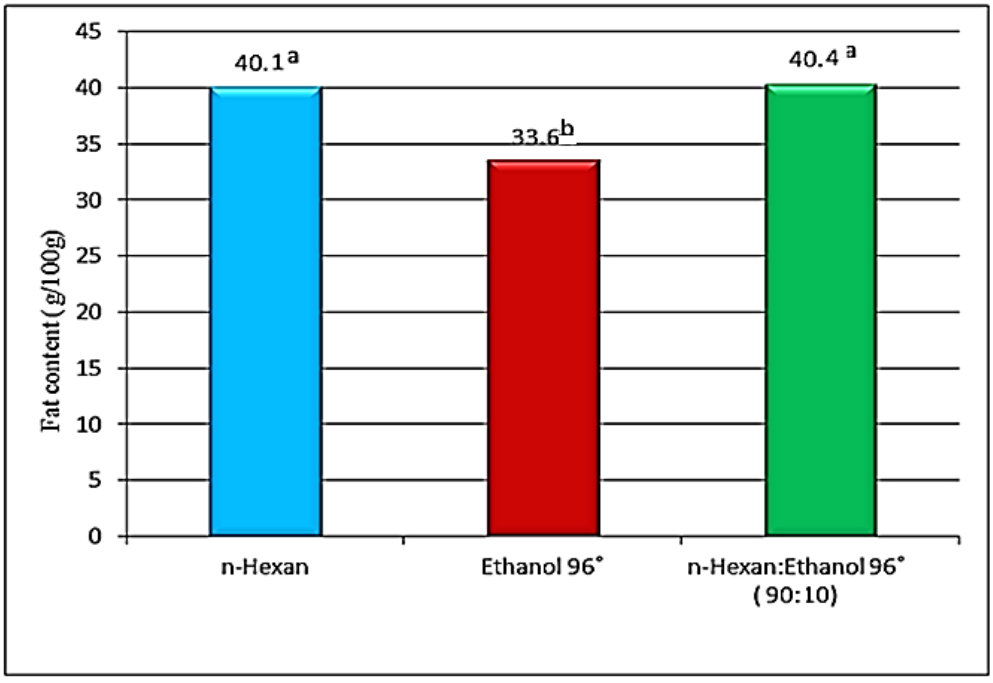

Fig. 1. Oil content of the pistachio nut

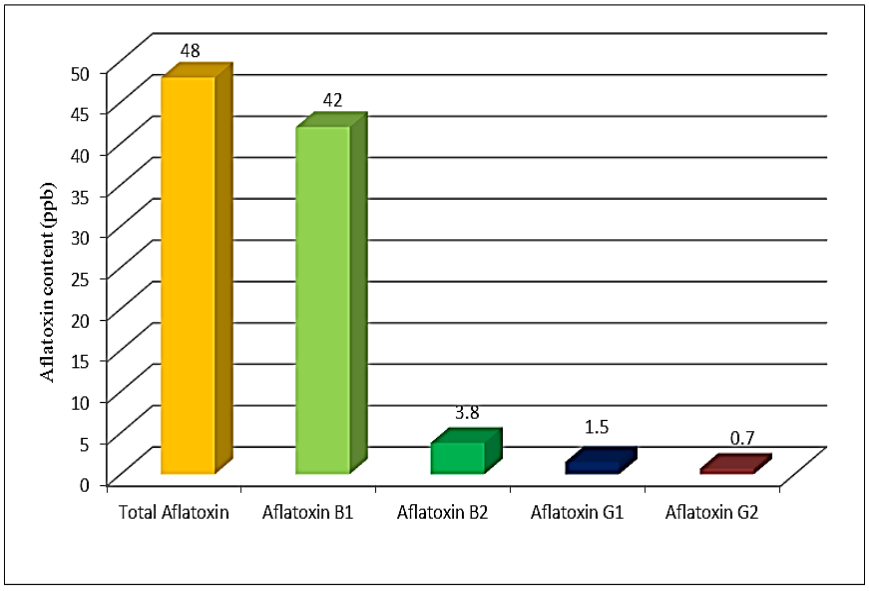

Fig. 2. Aflatoxin content of the pistachio nut

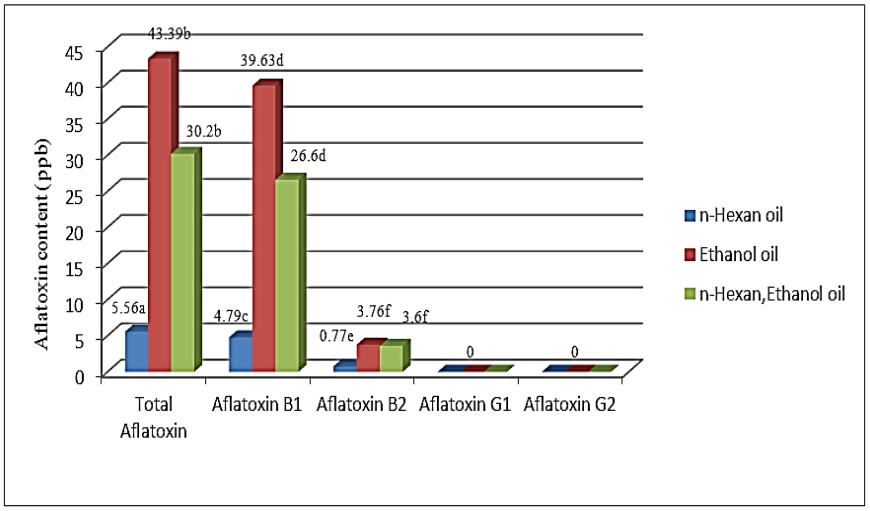

Fig. 3. Aflatoxin content of the pistachio nut oil
∗Dissimilar letters represent significant difference at $p<0.05$.
Note: The contents of AFG1 and AFG2 in all extracted oils were 0 ppb.

The highest content of aflatoxin including AFB1, AFB2 and total aflatoxin was found in the oil extracted by ethanol due to its solubility in polar solvents. As noted earlier, the solvents with the capability of extracting oil along with aflatoxins have been studied, since aflatoxin in oil is removed by alkaline refining and bleaching operations, thus they are not found in refined oils (Hamedi, 2008). Therefore, the resulted meal which contains no or little amount of aflatoxin, lower than maximum permissible level, can be added to foods as a source of nutrients such as protein, dietary fiber, etc. and might be used as animal feed. The lowest amount of AFB1, AFB2 and total aflatoxin was observed in the oil extracted by hexane.

The results of aflatoxin content in the meals resulted from each extraction process are given in Figure 4.

The highest content of aflatoxin including AFB1, AFB2 and total aflatoxin was found in the meal resulted from extraction process by hexane. The reason is the insolubility of

aflatoxin in hexane during oil extraction that allows the aflatoxin to remain in the meal. Considering the maximum permissible content of AFB1 and total aflatoxin in foods and animal feed (ISIRI 5925, 2002), the meal produced by oil extraction using hexane is not safe to be consumed.

Figures 5, 6 and 7 illustrate the fatty acid profiles of pistachio nut oil extracted using hexane, ethanol and hexane-ethanol, respectively.

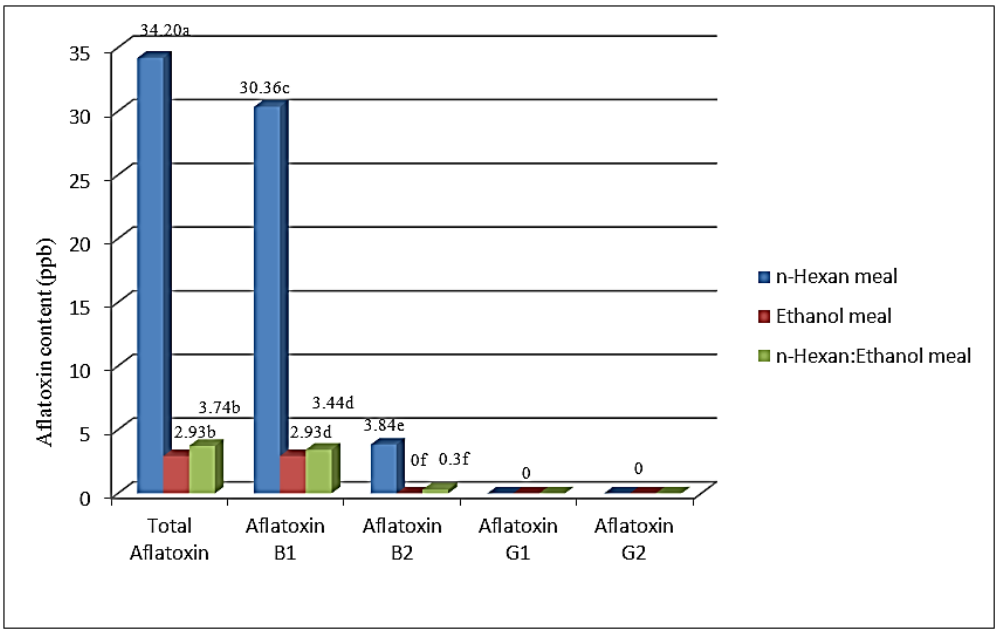

Fig. 4. Aflatoxin content of pistachio meal

∗Dissimilar letters represent significant difference at $p<0.05$.

Note: The contents of AFG1 and AFG2 in all produced meals were 0 ppb.

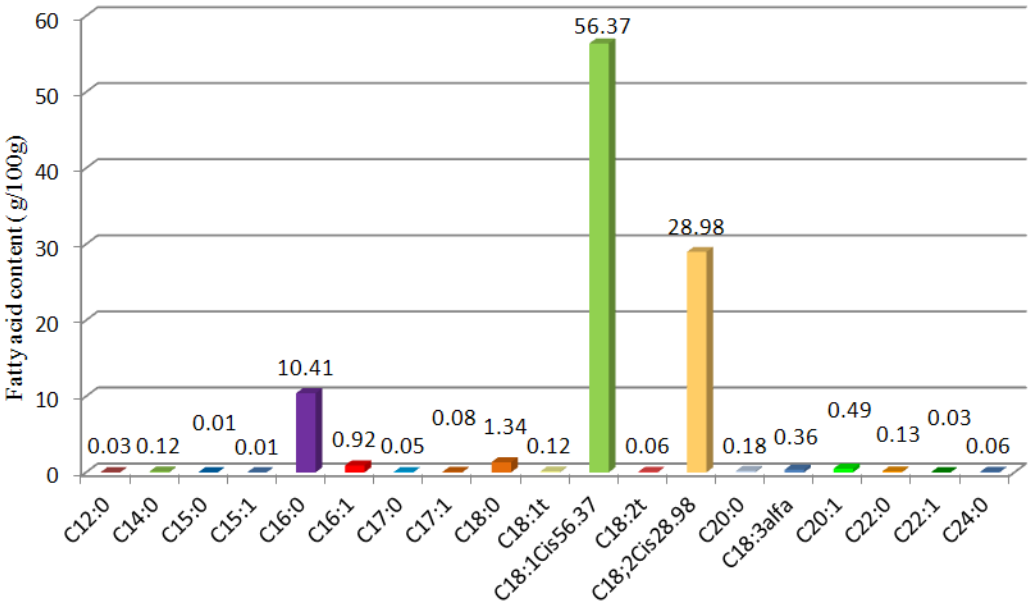

Fig. 5. Fatty acid composition of pistachio nut oil extracted by n-hexane

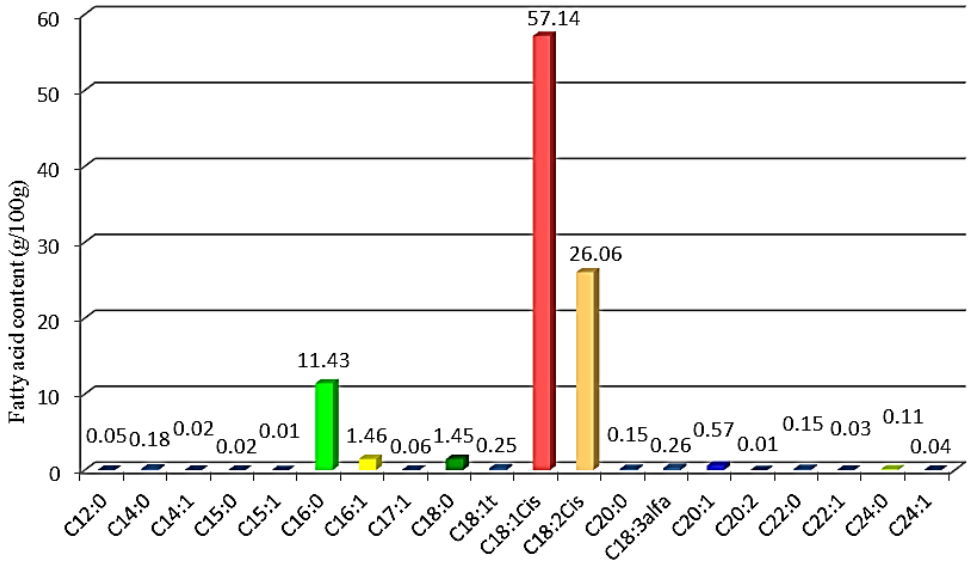

Fig.6. Fatty acid composition of pistachio nut oil extracted by ethanol

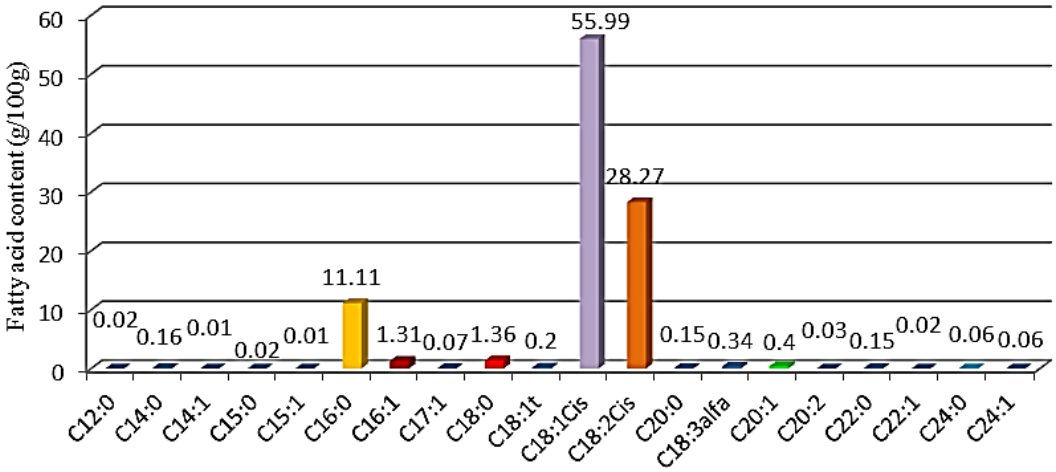

Fig.7. Fatty acid composition of pistachio nut oil extracted by hexane- ethanol (90:10)

Saturated and unsaturated fatty acids composition (%) of pistachio nut oil are presented in Tables 2 and 3, respectively.

Among the saturated fatty acids palmitic acid followed by stearic acid showed the highest concentration while among the unsaturated fatty acids, oleic acid was the predominant fatty acid followed by linoleic acid. Higher concentration of oleic acid make this oil more resistant to oxidative variations.

Pistachio nut oils extracted by hexane, ethanol and hexane- ethanol are not different in saturated and unsaturated fatty acid profiles, while their saturated fatty acids contents show significant differences. Palmitic and stearic acids in pistachio nut oils extracted by the solvents showed the highest content of saturated fatty acids. Palmitic acid content ranges from 10.41% in the oil extracted by hexane to 11.11% in the oil obtained by hexane-ethanol and stearic acid content ranges from 1.34% in the oil extracted by hexane to 1.45% in the oil obtained by ethanol. Oleic acid content ranges from 55.99% in the oil extracted by hexane-ethanol to 57.14% in the oil obtained by ethanol and linoleic acid content ranges from 26.06% in the oil extracted by ethanol to 28.98% in the oil obtained by hexane.

The effect of extraction method (Soxhlet and maceration) and polarity of solvent (n-Hexane, dichloromethane, ethyl acetate, ethanol) on the fatty acid profile of pistachio

nut oil (Akbari variety), was studied and concluded that the saturated and unsaturated fatty acid profiles of pistachio oil extracted by soxhlet and maceration method were not different but their contents were statistically different (Abdolshahi *et al.*, 2013). Okay *et al.* (2002) reported that the amounts of palmitic, stearic, oleic and linoleic acids in pistachio oil, Ohadi variety, were 10.280 ± 1.43%, 1.792 ± 0.25%, 61.570 ± 3.09%, 25.180 ± 2.02%, respectively. Mahmoodabadi *et al.* (2012) found these fatty acids in concentrations of 8.87%, 1.67%, 60.2% and 26.24%, respectively. In their study, the ratio of unsaturated to saturated fatty acids was 7.1, being approximately similar to the ratio of 7.27 determined by Okay (2002).

The results of determination of Iodine, peroxide and acid values of pistachio nut oil extracted by the solvents are presented in Table 4.

Higher Iodine value in the oil extracted by hexane (101 gI_2/100g) is attributed to its relatively higher content of $C_{18}:2cis$ and $C_{18}:3alfa$. Kamangar et al. (1975) reported that Iodine value of Iranian pistachio oil was 98.1-100.5 (gI_2/100g). Iodine value represents the degree of unsaturation in the oils and here indicates the presence of unsaturated fatty acids particularly the monounsaturated fatty acids (MUFA).

Table 2. Saturated fatty acids content (%) in pistachio nut oil

Extraction solvent	Saturated fatty acid (%)					
	Myristic $C_{14}:0$	Palmitic $C_{16}:0$	Stearic $C_{18}:0$	Arachidic $C_{20}:0$	Behinic $C_{22}:0$	Lignoceric $C_{24}:0$
n-Hexane	0.12 ^{a}	10.41 ^{b}	1.34 ^{e}	0.18 ^{g}	0.13 ^{h}	0.06 ^{i}
Ethanol 96˚	0.18 ^{a}	11.43 ^{c}	1.45 ^{f}	0.15 ^{g}	0.15 ^{h}	0.11 ^{j}
n-hexane-ethanol 96˚(90:10)	0.16 ^{a}	11.11 ^{d}	1.36 ^{e}	0.15 ^{g}	0.15 ^{h}	0.06 ^{i}

∗Dissimilar letters represent significant difference at p<0.05.

Table 3. Unsaturated fatty acids content (%) in pistachio nut oils

Extraction solvent	Unsaturated fatty acid (%)			
	Linolenic $C_{18}:3alfa$	Linoleic $C_{18}:2cis$	Oleic $C_{18}:1cis$	Palmitoleic $C_{16}:1$
n-Hexane	0.36 ^{j}	28.98 ^{g}	56.37 ^{d}	0.92 ^{a}
Ethanol 96˚	0.26 ^{j}	26.06 ^{h}	57.14 ^{e}	1.46 ^{b}
n-hexane-ethanol 96˚(90:10)	0.34 ^{j}	28.27 ^{i}	55.99 ^{f}	1.31 ^{c}

∗Dissimilar letters represent significant difference at p<0.05.

Table 4. Results of determination of iodine, peroxide and acid values of pistachio nut oil extracted by different solvents

Extraction solvent	Iodine Value (gI_2/100g)	Peroxide Value (meq/Kg)	Acid value (mg KOH/g)
n-Hexane	101 ^{f}	2 ^{d}	2 ^{a}
Ethanol 96˚	97 ^{g}	10 ^{e}	4.3 ^{b}
n-hexane-ethanol 96˚(90:10)	99.5 ^{h}	10 ^{e}	3.1 ^{c}

∗Dissimilar letters represent significant difference at p<0.05.

Higher acid value in the oil extracted by ethanol (4.3 mg KOH/g) might be the result of hydrolysis of a part of triglycerides during oil extraction by ethanol due to the presence of water in this solvent and its high efficiency for extraction of most compounds with the ability to interact with alkali in acidity test as compared to hexane. High amount of free fatty acids reduce the smoke point of oil thereby increasing the possibility of autoxidation occurrence.

Peroxide value of pistachio nut oil varies depending on the type of solvent. This value is an indicator of oil rancidity. Higher peroxide value of the oil extracted by ethanol and ethanol- hexane might be the result of oxidation during extraction by ethanol and also ethanol-hexane (because ethanol requires higher temperature for extraction due to its higher boiling point) resulting in formation of more hydroperoxide and, thus, higher peroxide value.

Conclusion

In this study, the effects of polar and non-polar solvents and their mixture on chemical characteristics of pistachio nut oil containing aflatoxin were investigated. Some results and findings indicated significant differences. The oil extracted from pistachio nuts is rich in oleic and linoleic acids and provides important therapeutical properties. Pistachio nut oil contaminated with aflatoxin can be recovered by the use of safe polar solvents such as ethanol. Aflatoxin contained in the oil is removed by solvents with high polarity, thus, the content of aflatoxin in the produced meal is reduced to a permissible level. Considering the results of previous studies, aflatoxin contained in the oil might be removed through refining operations, thus, the valuable aflatoxin- free pistachio oil might be consumed by human. The safe meal resulted from oil extraction might be added to human food as a rich protein source or used as animal feed.

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