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# Oil Content and Fatty Acid Profile of some Pine Nuts Species (*Pinus* spp.)

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A R T I C L E I N F O

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A B S T R A C T

The pine nuts, as the most expensive ones, are a source of healthy oil. Accordingly, the hypothesis was genetic differences of pine species (*Pinus* spp.) affect their nut oil content and fatty acid phytovariability. A completely randomized design experiment with three replicates was done in Isfahan Flower Garden, Iran, using four pine species of chir pine (*Pinus longifolia* Roxb.), stone pine (*P. pinea* L.), eldar pine (*P. eldarica* Medv.) and mugo pine (*P. mugo* pumilio XENARI). The mugo pine and eldar pine species contained the highest (44.10%) and the lowest (37.40%) oil percentage, respectively. The eldar pine (12.45%) and the chir pine (12.23%) species contained the highest and the stone pine species (11.18%) contained the least rate of saturated fatty acids (SFA). Palmitic (6.33%) and oleic (36.29%) acids were the highest in the stone pine specie, and stearic acid (3.86%) was the highest in the chir pine specie. For human health, high levels of unsaturated fatty acids and little amount of saturated fatty acids are recommended. In conclusion, the oil content and fatty acid composition in studied pine species were highly variable, and are considered as a potential source of polyunsaturated fatty acids. According to our results, the mugo pine species due to possess of the highest amount of oil percent and linoleic acid and the lowest amount of palmitic and stearic acids can be introduced as an elite pine specie.

## Introduction

The genus *Pinus* belongs to the Pinaceae family and also comprises about 250 species (Yang *et al*., 2010), is widely distributed in Mediterranean countries (Matthaus and Özcan, 2013). Pine tree is an important plant because of nutritional, medicinal and industrial purposes (Lutz *et al*., 2017). Moreover, pine nuts are on the list of edible nuts published by the Food and Agriculture Organization of the United States (FAO, 2015).

Nuts are good dietary sources of unsaturated fatty acids. The substantial epidemiological evidence shows that fatty acids from seeds and nuts are associated with different health effects (Li and Hu, 2002). Pine nuts are the most expensive nuts in the world and the global market for pine nut is increasing worldwide with unsatisfied increasing demand (Bracalini *et al*., 2013). Pine species (*Pinus* spp.) are ever green, monoecious and are able to grow in the soils with little fertility under different stress conditions (Mortazainezhad, 2004). The distribution of pine species in the land is highly variable and they can be found almost in all places, although their real origins are Spain, Portugal, Greece, Italy and Turkey (Destaillats *et al*. 2010; Lim 2012; Amarowicz *et al*. 2017). These plants are cultivated and found in different parts of Iran, and are used for different purposes such as medicinal, industrial and arboricultural industries (Sadeghi *et al*., 2013).

The biochemical properties of pine nuts including oil content (Ferramosca and Zara 2014; Rogachev and

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Salakhutdinov, 2015) and the composition of fatty acids, have important implications for the health of human and industrial purposes. Such natural products have medicinal usage, and are used for the production of medicine. Plant oils can positively affect the activity of cells and can prevent diseases such as cancer. Fatty acids including saturated fatty acids (SFA) and unsaturated fatty acids (UFA) are important for human metabolism and are essential for the production of compounds such as prostaglandins, leukotriene and thromboxane (Ros, 2010). Fatty acids can also affect the expression of genes, which are important for the metabolism of proteins, required for fatty acid production, by attaching to the transcription factors (Nergiz and Donmez, 2004; Vanhanen and Savage 2013; Xie *et al*. 2016).

The pine cones contain different products including polysaccharides, tannins, lignin's, phenolic products and terpenoids (Lutz *et al*., 2017). The seeds of pine are an important nutritional source of proteins, fats, carbohydrates, ash, vitamins (B1 and B2) and mineral nutrients such as potassium and phosphorous, which are essential for human health (Evaristo *et al*., 2010; Lutz *et al*., 2017). Pine seeds have also a high rate of unsaturated fatty acids, especially linoleic acid and oleic acid (Nergiz and Donmez, 2004; Ozguven and Vursavus 2005; Nasri *et al*. 2007; Sadeghi *et al*. 2013; Awan and Pettenella 2017). The fatty acid compositions of the seed oils from ten pine species have been reported by Wolff *et al*. (1995) and observed linoleic acid ,oleic acid or pinolenic acid were the main fatty acids. Evaristo *et al*. (2013) evaluated 27 different Portuguese populations and found that nut samples contained a predominant fraction of unsaturated fatty acids. In other study, the composition of forest pine seed oil from south of Turkey have been reported by Matthaus and Ozcan, (2013).

With respect to the wide distribution of pine species in Iran and worldwide, and because of their importance for human health, as well as for nutritional, medicinal and industrial purposes, the biochemical properties of the four most abundant pine species were investigated in this research. The hypothesis was genetic differences in pine species affect their nut oil content and fatty acid variability. The objective was to examine the oil content as well as the phytovariability of fatty acids in the seeds of the four pine species.

**Materials and Methods**

This research was conducted in 2017 to investigate the oil content and composition of fatty acids in different pine species including chir pine (*Pinus longifolia* Roxb.), stone pine (*P. pinea* L.), eldar pine (*P. eldarica* Medv.) and mugo pine (*P. mugo* pumilio XENARI) (Figs. 1 and 2). Eldar pine was originated from “Eldar” in Georgia and its seed was introduced to Iran in 1690 and it seems that this specie pine first was planted in Tehran. Seeds of chir pine were imported to Iran by Kashef-Al-Saltane about 110 years ago. Stone pine is called so because of its kernel similarity to almond (Badam in Persian) kernel and mugo pine is seemed to be planted first time in Mashhad area (Mortazainezhad, 2004).

The experiment was done in Flowers Garden, Isfahan, Iran, with the northern latitude of 32° 38′ 21″ and the eastern longitude of 51° 41′ 55″. The experimental treatment including the pine species of chir pine, stone pine, eldar pine and mugo pine in terms of oil percent and fatty acids compositions were tested using a completely randomized design with three replicates. Five trees were selected for each replicate and ten fruit samples were harvested from each replicate. The collected samples were transferred to the laboratory of Department of Horticulture, Isfahan (Khorasgan) Branch for further analyses.

Before analyzing, the collected seeds were kept in polyethylene bags at 4°C. The seeds were then peeled and the impurities removed by hand, and placed in an oven at 60°C to achieve the seed moisture of 8%; for the extraction of oil, the seeds were crushed.


**Fig. 1.** Seeds of different pine species tested in this research.

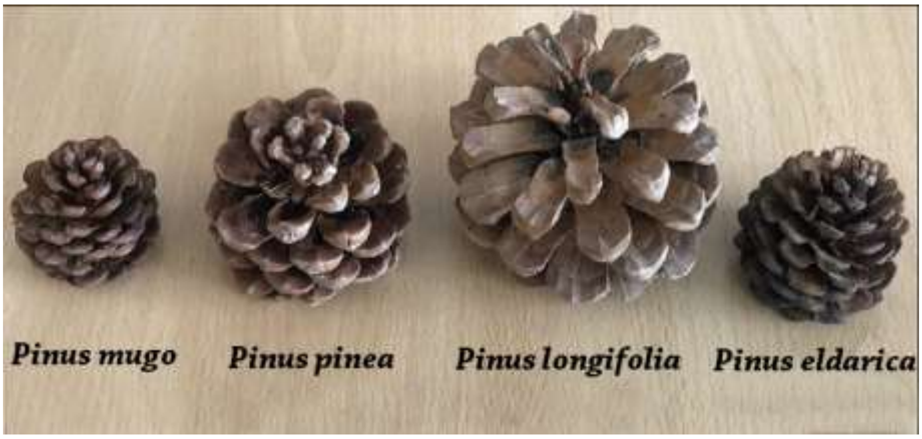


**Fig. 2.** Cones of different pine species tested in this research.

### *Oil extraction*

The oil from the seeds was extracted mainly according to the procedure of Folch *et al*. (1957). In brief, 10 g of powdered seeds was covered by a filter paper and placed in the Soxhlet extractor. The n-Hexan solvent was poured into a flask and the instrument temperature was adjusted at 67 $^oC$. The oil extraction continued for 8 h. After extraction, the solvent was evaporated under reduced pressure. The extracted oil was kept in black containers at -18 $^oC$ before further analyses (AOAC, 2000).

### *Analysis of fatty acids*

The fatty acids must become volatile (for converting to the derivatives with a less boiling point) by methyl esterification before injecting into Gas Chromatograph. The process of methyl esterification was done according to the following. First, 15 ml of oil was treated with 7 ml n-hexane and then with 2 ml of potassium hydroxide solved in methanol; during this time the solution was vortexed each 5 min. One µl of methyl esterified sample was injected into the instrument (Metcalf *et al*., 1996).

Fatty acids were isolated using GC (Germany, Column CP-SIL 88) with the column length of 100 m, internal diameter (ID) of 25 μm, carrying gas of helium and the detector of FID Flame. The initial temperature was equal to 140$^o$C, which was increased to 220$^o$C (for 2 min) at the rate of 1.5$^o$C per minute. The injection and detection temperatures were adjusted at 280 and 300$^o$C, respectively. The analysis of fatty acids by GC was conducted using the standard acid solutions and finally the composition of fatty acids was expressed as percentage of each identified fatty acid.

### *Statistical analysis*

Data were subjected to analysis of variance using SAS 9.1 software (SAS Institute Inc., Cary, NC. USA). The means were compared using Duncan's Multiple Range test at $P \leq 0.05$.

## Results

### *Oil content*

According to the analysis of variance, although oil content and sum of saturated fatty acids (SSFA) were depended on pine species, but the species effect was not significant on the sum of unsaturated fatty acids (SUFA) (Table 1). Mugo pine and eldar pine species contained the highest (44.10%) and the least (37.40%) oil percentage, respectively. However, the highest and the least rate of SSFA were obtained in eldar pine (12.45%), chir pine (12.23%) and stone pine (11.18%) species, respectively. Mugo pine (85.50%) and stone pine (85.27%) species contained the highest numerically SUFA values (Table 2).

**Table 1. Analysis of variance indicating the effects of plant species on oil percentage, sum of saturated fatty acids (SSFA) and sum of unsaturated fatty acids (SUFA).**

| | | M.S. | | |
|---|---|---|---|---|
| S.V. | d.f. | Oil (%) | SSFA | SUFA |
| Pine species | 3 | 28.570** | 0.986** | 0.722ns |
| Error | 8 | 0.048 | 0.026 | 1.278 |
| C.V. (%) | | 0.56 | 3.163 | 1.33 |

M.S.: mean of squares, S.V.: source of variation, d.f.: degree of freedom, SSFA: Sum of saturated fatty acids, SUFA: Sum of unsaturated fatty acids, C.V.: coefficient of variation, n.s.: not significant, **: Significant at P= 0.01.

**Table 2. Means of oil percentage, sum of saturated fatty acids (SSFA) and sum of unsaturated fatty acids (SUFA) affected by plant species.**

| Pine species | Oil (%) | SSFA (%) | SUFA (%) |
|---|---|---|---|
| *Pinus pinea* | 39.11b | 11.18c | 85.27a |
| *Pinus longifolia* | 37.80c | 12.23a | 84.64a |
| *Pinus eldarica* | 37.40c | 12.45a | 84.48a |
| *Pinus mugo* | 44.10a | 11.66b | 85.50a |

SSFA: Sum of saturated fatty acids, SUFA: Sum of unsaturated fatty acids

### *Fatty acid composition*

The analysis of variance indicated pine species had significant effects (P= 0.01) of on rate of palmitic, stearic, oleic, linoleic and behenic acids (Table 3). The highest rate of palmitic (6.33%) and oleic acid (36.29%) was found in stone pine species. Chir pine species contained the largest percentage of stearic acid (3.86%). The species of eldar pine (64.75%) and mugo pine (65.53%) had the highest rate of linoleic acid. The species of eldar pine (5.00%) also indicated the largest rate of behenic acid (Table 4).

The least palmitic (4.29 and 4.21%), stearic (3.16 and 3.03%) and oleic (19.73 and 19.97%) acids were found in eldar pine and mugo pine species, the corresponding values for linoleic (48.98 and 53.60%) and behenic (1.41 and 2.61%) acids were related to stone pine and chir pine species (Table 4).

**Table 3. Analysis of variance indicating the effects of pine species on the seeds phytovariability of fatty acids composition**

| | | M.S. | | | | |
|---|---|---|---|---|---|---|
| S.V. | d.f. | Palmitic acid | Stearic acid | Oleic acid | Linoleic acid | Behenic acid |
| Pine species | 3 | 3.388** | 0.405** | 204.728** | 202.799** | 8.178** |
| Error | 8 | 0.024 | 0.030 | 0.345 | 0.399 | 0.040 |
| C.V. (%) | - | 2.98 | 5.10 | 2.20 | 1.09 | 5.92 |

M.S.: mean of squares, S.V.: source of variation, d.f.: degree of freedom, C.V.: coefficient of variation. **: Significant at P= 0.01.

**Table 4. Fatty acid composition in different pine species**

| Pine species | Palmitic acid (%) | Stearic acid (%) | Oleic acid (%) | Linoleic acid (%) | Behenic acid (%) |
|---|---|---|---|---|---|
| *Pinus pinea* | 6.33a | 3.44b | 36.29a | 48.98c | 1.41d |
| *Pinus longifolia* | 5.76b | 3.86a | 31.04b | 53.60b | 2.61c |
| *Pinus eldarica* | 4.29c | 3.16bc | 19.73c | 64.75a | 5.00a |
| *Pinus mugo* | 4.21c | 3.03c | 19.97c | 65.53a | 4.42b |

Means, in each column, followed by the same letter are not significantly different at P= 0.05

### *The statistical variation of oil content and fatty acids*

The oil content varied from 37.21 to 44.34% with the average of 39.60%. SSFA was in the range of 10.99-12.55%, with the average of 11.88%. The maximum, minimum and average of SUFA were equal to 83.09, 86.87 and 84.97%, respectively. The average content of palmitic acid was 5.16% and its range was 4.12 to 6.54%. Also, stearic acid was in the range of 2.87-3.88%, with the average of 3.37%. Oleic acid content was in the range of 19.45-38.64% and the average of 26.91%. Linoleic acid differed in the range of 47.97 and 65.71% and averaging 58.22%. The least and the highest behenic acid were equal to 1.23 and 44.34%, respectively, with the average of 19.15% (Table 5).

**Table 5. Statistical parameters indicating the range of fatty acids variations**

| Oil and Fatty acid | Minimum | Maximum | Average | S.D. | C.V. (%) |
|---|---|---|---|---|---|
| Oil (%) | 37.21 | 44.34 | 39.60 | 2.80 | 0.56 |
| SSFA (%) | 10.99 | 12.55 | 11.88 | 0.53 | 1.35 |
| SUFA (%) | 83.09 | 86.87 | 84.97 | 1.06 | 1.33 |
| Palmitic acid (%) | 4.12 | 6.54 | 5.16 | 0.96 | 2.98 |
| Stearic acid (%) | 2.87 | 3.88 | 3.37 | 0.36 | 5.10 |
| Oleic acid (%) | 19.45 | 38.64 | 26.91 | 7.72 | 2.20 |
| Linoleic acid (%) | 47.97 | 65.71 | 58.22 | 7.46 | 1.09 |
| Behenic acid (%) | 1.23 | 44.34 | 19.15 | 18.81 | 5.92 |

SSFA: Sum of saturated fatty acids, SUFA: Sum of unsaturated fatty acids, S.D.: Standard deviation.

## Discussion

The biochemical properties of pine nuts can greatly determine their medicinal as well as their economical values (Matthaus and Ozcan, 2013). However, such properties are affected by different parameters, especially the pine species (genetic combination). Fatty acid composition is a strong chemotaxonomic property, which can be used for differentiation among different plant families, genera and species (Matthäus *et al*. 2018). According to the results, there was high variability among the four tested pine species, in terms of oil content and the variability of fatty acids (SSFA and SUFA). Similar to the results by Matthäus *et al*. (2018) linoleic acid and oleic acid were the predominant fatty acids. These observations suggest that little amount of SFA including palmitic acid and stearic acid, is the characteristics of pine nuts. Such results can be used for different purposes including differentiation of different pine species according to their oil content and fatty acid composition, determination of the nutritional values of each pine species, breeding, and their use for medicinal purposes.

Similar to our results, Matthäus *et al*. (2018) found that the predominant fatty acids in the seed oils of *P. armandii* were linoleic acid (35.2-58.2 g/100 g) and oleic acid (14.6-48.5 g/100 g). They found that there were no differences between the biochemical properties of *P. armandii* and other pine species. Kadri *et al*. (2015) also found that the dominant seed oils (unsaturated) in the Algerian pine species were linoleic acid (30-59%) and oleic acid (17.4-34.6%). Similar results were also found by Kornsteiner-Krenn *et al*. (2013), for pine nuts for oil content (SFA and UFA) and fatty acid composition. The importance of polyunsaturated fatty acids as essential fatty acids, in terms of nutritional value for human health has been emphasized in recent studies (Abedi and Sahari, 2014). The absence of appropriate enzymes in human body causes the inability of synthesizing polyunsaturated fatty acids. Therefore, these acids should be obtained through proper diet (Andrew *et al*., 2006).

The two species of stone pine and chir pines mostly grow in the northern part of Iran, with a humid climate; however, the species of eldar pine and mugo pine are grown in dry and semi-arid region of Iran. Although genetic is the most important factor affecting the biochemical properties of pine species, climate and geographical location can also affect the oil content and fatty acid composition of pine nuts (Derewiaka *et al*. 2014; Lutz *et al*., 2017).

The chemotaxonomic differences including oil content and fatty acid composition may also be used for other implications. For example, Matthäus *et al*. (2018) proposed that it is possible to determine the species of pine, which are susceptible to pine nut syndrome by determining different biochemical properties such as the ones investigated in this research work. They determined the oil content of different pine species and found some results, which are similar to our results, for example, in *P. pinea* it was in the range of 46.5 and 55 (g/100 g), in *P. eldarica* equal to 47.6 (g/100 g), and in *P. mugo* in the range of 30.9 and 34.1 (g/100 g).

## Conclusions

Biochemical properties of nuts are important chemotaxonomic characteristics, and are of ecological significance determining the botanical origins of pine species. The oil content and fatty acid composition of pine nuts for four different pine species were determined in this research. The results indicated a high rate of variability among the four species of pine. The four pine species, investigated in this research, as some of most dominant ones in Iran and different parts of the world can be used as healthy sources of oil, which is of medicinal and nutritional values. In terms of nutritional value, for human health high levels of unsaturated fatty acids and little amount of saturated fatty acids are recommended. Therefore, in studied pine nuts species, mugo pine species due to possess of these characteristics such as little amount of palmitic, stearic acid and high levels of linoleic acid and also the highest oil percent can be introduced as best of studied species.

## Conflict of interest

The authors declare that they do not have any conflict of interest.

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