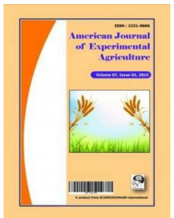

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Physical and Dietary Properties of In-shell Pine Nuts (*Pinus pinea* L.) and Kernels

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Authors' contributions

This work was carried out as a collaborative work of all authors. Authors FS and UA designed the study and wrote the protocol. Authors FS and KBO managed the field study in Kozak, collected samples and other relevant data and performed the laboratory analyses. Author FS analyzed the data and wrote the first draft of the manuscript. Author UA reviewed the draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study aims at determining physical and chemical properties of Turkish pine nut shells and kernels over a time span. Besides, changes in composition and nutritive value of the pine nut are evaluated according to the prevailing climatic conditions.

Study Design: The experiment was conducted as completely randomized design, physical analyses were performed as three, and chemical analyses as two replicates per sample prepared.

Place and Duration of Study: Kozak District Agricultural Development Cooperative and Ege University of Department of Horticulture in Izmir, Turkey, between July and August in three crop years.

Methodology: Pine nut (*Pinus pinea* L.) shell samples (*Pinus pinea*) were obtained from Kozak District Agricultural Development Cooperative. The sampling is based on sub-sampling in-shell nuts from 50 kg bags in July and August. 20 subsamples were collected from different bags and 24, 27 and 36 aggregate samples (~ 3 kg) were prepared annually.

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Results: The in-shell nut quality displayed yearly differences in terms of cracked and defected nuts cracked varying between 17 and 43% and defects ranging between 2 and 4%. The pine nut kernel composition was determined as moisture 4.64%, water activity 0.452, ash 4.19%, fat 45.8%, protein 31.98% and carbohydrates 13.16%. Potassium, phosphorus and magnesium were the predominant elements. Kernels are also rich in iron and zinc. Oleic and linoleic acids were the major unsaturated fatty acids (85.4%), while palmitic and stearic acids the as saturated fatty acids (9.8%).

Conclusion: The results confirm that pine nut kernels produced in Kozak area are a rich source of many important nutrients having positive effect on human health compared to other Mediterranean pine nuts origins.

Keywords: Pinus pinea L.; stone nut; fatty acid; physical properties; chemical composition.

1. INTRODUCTION

Some species of *Pinus* genus produce kernels that are edible and highly nutritious. Pine nuts are produced mainly in Far-eastern Asian (*Pinus chinesis* and others) and Mediterranean countries (*Pinus pinea* L.). Annual world pine kernel production ranged between 17 330 (2008) and 34 445 MT (2011) during the last five years. With the stock over, the total kernel supply is reported to vary between 24 549 and 49 621 MT during the same period. Pine nut production shows fluctuations in Turkey as in other producing countries, and the yearly kernel exports totaled to 1303, 849, 703, 1473, 1356, 2180, 2174 and 1 227 MT between 2004 and 2011 [1]. The world demand for pine nuts is very strong especially in China and Russia, and the prices display an upward trend. *Pinus chinesis* that comprise the major part of world pine nut market is produced in China, Russia, Pakistan and DPR Korea. On the other hand, Turkey, Spain, Portugal and Italy are the major producers of *Pinus pinea,* the Mediterranean pine nut [1].

In the Mediterranean basin, *Pinus pinea* is an important species in the natural flora. Additionally it is grown as plantations mainly in Turkey, Spain, Portugal, Italy, Greece and Albania due to its regional importance [2]. The Turkish kernel production ranged between 350 MT in 2012 to 1500 MT in 2008 between 2000 and 2012 [1]. Kozak plain in the western Aegean Region (İzmir province) of Turkey is historically an important pine nut production area providing more than 50% of the national supply. Pine nut production is the main income source for 55 000 families in the region. The quality of pine nuts grown in Kozak plain is also superior to the other regions with its white colour and large sizes and they are mainly destined to export markets as Italy, Spain, Switzerland and USA [1].

Pine nuts are utilized in the Mediterranean cuisine traditionally in bread, candies, sweets (baklava, halwa), sauces, cakes, salads and meat dishes as raw, roasted, whole or ground. It is an edible nut with a superb flavour and high protein content which make it a good nutritional source. Pine nut kernels are rich in protein, antioxidants and mono unsaturated fatty acids; are good sources of vitamin B_1 (thiamine) and minerals, especially of potassium and phosphorus [3,4]. The lipid fraction of the kernels comprises more of unsaturated fatty acids. Oleic and linoleic acids account for more than 85% of the total fatty acids [4-7].

Many nuts are identified as rich in antioxidants [8]. Although walnut, pecans and chestnut have the highest content of antioxidants among tree nuts; pine nuts also contain significant amounts of total antioxidants [9]. Since oxidative stress is the common cause in chronic degenerative diseases, it is assumed that dietary antioxidants may exert a protective effect. Several studies suggest that antioxidants in nuts are beneficial against cardiovascular and other chronic diseases [9-12]. This effect is ascribed to fatty acid composition especially because of high linoleic acid content.

Pine nut quality in-shell or kernel may show variations among species or subspecies depending upon geographical and climatic conditions [10,13,14]. Research work and data on physical and chemical properties of *P. pinea* kernels and of especially in-shell nuts are rather limited. There are no studies concerning the impact of climate or yearly conditions on the physical and chemical composition of pine nut kernels grown in Turkey.

This study aims at determining physical and chemical properties of Turkish pine nut shells and kernels over a time span. Besides, changes in composition and nutritive value of the pine nut are evaluated according to the prevailing climatic conditions.

2. MATERIALS AND METHODS

2.1 Materials

Pine nut (*Pinus pinea* L.) shell samples were obtained from Kozak District Agricultural Development Cooperative during three crop years. The sampling is based on sub-sampling in-shell nuts from 50 kg sealed jute bags one week after the removal of nuts from cones in July and August. 20 subsamples were collected from different bags and 24, 27 and 36 aggregate samples (~ 3 kg) were prepared annually. Physical analyses were performed as three, and chemical analyses as two replicates per sample prepared. The kernel samples kept at +4^{o}C under dark conditions were analyzed within a month after harvest.

2.2 Physical Analysis

From each sample, 100 g in-shell nut were examined visually and nuts were classified into 6 quality classes as no cracking, cracking only on one side the crack length being 1/3, 2/3 or 3/3 of the nut length, crack on both sides and cracked and kernel ready for germination and percentage of nuts in each group were determined as %. In-shell nuts were weighted (XB 320M, Presica Instruments Ltd., Switzerland) and number of in-shells in 100 g was calculated. These in-shell pine nuts were broken manually and shells, kernels, and skins were carefully separated. Then, each part was weighted and number of kernels in 100 g and kernel ratio (%) as the ratio of kernel weight to in-shell nut weight were calculated. The defected kernel ratio was determined by visual evaluation of kernels obtained from 100 in-shell nuts. Kernels were classified into 6 classes as intact (white), with visible mould, black, green and discoloration of the tip and the ratios were calculated. The size was also determined by measuring the dimension of the principal axes; major diameters of 20 randomly selected kernel using a digital caliper (SC-6, Mitutoyo, Japan) with a sensitivity of 0.01 mm.

2.3 Proximate Analysis

After the physical analyses, the remaining in-shell nuts were broken by special equipment and shells, kernel, and skin were carefully separated manually. Kernels (~ 600 g) were ground using a Waring blender (Blender 8011ES, USA) and used for proximate, fatty acids, and mineral analyses.

Moisture content of kernels was measured by drying samples to a constant weight, in an oven (UM400, Memmert, Germany) at 70℃ [15] and calculated based on the percentage of weight loss. Water activity was measured with a water activity meter (TH 500, Novasina, Pfaeffikon, Switzerland) at 25℃.

Total ash was determined after drying samples for 12 h at 75℃ in an oven and then transferring the crucible to a muffle furnace. The temperature was gradually raised to 550℃ and the samples were ashed for 24 h. Protein analysis in kernel samples of pine nut was determined using the Kjeldahl method [16]. The percentage of crude protein was estimated after multiplying the total nitrogen content by a factor of 5.30 [16]. Total carbohydrates were calculated by subtracting the total percentage of other components from 100. Total fat of pine nut kernel was found using the Soxhlet extraction method with petroleum ether [16].

2.4 Analysis of Fatty Acids

The fatty acid composition was determined on the lipid extracts after methylation to form fatty acid methyl esters (FAME) according to the IUPAC Method No.: 2.301 [17] using a Hewlett Packard 6890N gas chromatograph (Agilent, Polo Alto, CA), equipped with a Supelco SP2380 capillary column (60 m x 0.25 mm i.d., 0.20 µm film thickness; Supelco, Bellefonte, PA) and flame ionization detector (FID). Fatty acid standard Supelco™ 37 Component FAME mix (Supelco, Bellefonte, PA) was used for determination of fatty acid fractions. Helium was used as the carrier gas at flow rate of 1.1 ml/min; the split ratio was 1:20. An autosampler/injector HP7683 B Series was used and the injector and detector temperatures were 220℃. The oven temperature was programmed at 165℃ for 35 min; temperature was then elevated at 5℃ per min to 195℃ and held for 15 min.

2.5 Analysis of Minerals

Samples were wet-ashed with a mixture of nitric acid and perchloric acid solution, on a hot plate at 200℃. Then, the absorbances of the extract were determined using the atomic absorption spectrophotometer (Spectra AA 220 Fast Sequential, Varian, Australia), and the amounts of minerals were calculated with the standard curves. Phosphorus was analyzed spectrophotometrically in the form of vanadium phosphomolybdate [18].

2.6 Statistical Analysis

The experiment was conducted as completely randomized design. Significant differences among groups were determined using Duncan's multiple range tests at $P \leq 0.05$. Standard deviation of the mean (SD) was also calculated from the replicates. All data were subjected to analyses of variance (ANOVA) by using IBM® SPSS® Statistics 19 statistical software (IBM, NY, USA).

3. RESULTS AND DISCUSSION

3.1 Physical Properties of in-shell Nuts

The ratio of cracked in-shell nuts varied significantly ($P \leq 0.01$) according to the years and as the overall average the ratio of in-shell pine nuts having cracks was calculated as 27%. The major mode of cracking was determined as cracking of the in-shell on one side but lengthwise (3/3). The cracked ratio was the highest in the 3rd year compared to other two years. The cracking ratios were higher in lengthwise crack on one side of the in-shell, cracks on both sides and cracks where kernels are ready to germinate. In the first and second years, the ratios of cracked in-shells and the mode of cracks (except lengthwise (3/3) crack on one side) were rather similar (Table 1).

Among climatic conditions namely time and amount of precipitation and temperature may play significant role on in-shell pine nut and kernel properties. The major factors affecting higher cracked ratios in the 3rd year was found to be due to higher fluctuations in day and night temperatures during the drying period of cones in stacks. The diurnal temperatures ranged within wider limits (12.3%) during the period between April and June in the third study year compared to the other two years [19]. Despite higher cracked ratios, kernel quality was not affected from cracking. This can occur because in-shell nuts were sampled without exposing to a long-term storage. Under normal conditions, in-shell nuts can be stored for more than a year. If in-shell pine nuts are kept for longer periods under inappropriate storage conditions, cracking may advance and cracks on both sides may occur and kernels may start germination process which consequently affects the kernel quality. When cracking enhances, the kernel is more exposed to interaction with the ambient conditions, however, there is no research results on pine nuts to confirm this hypothesis.

3.2 Physical Properties of Kernels

The percentage of sound and intact kernels varied ($P \leq 0.05$) according to the yearly conditions and the mean sound and intact kernels was calculated as 96.37%. Geisler [20] report that pine nut is highly susceptible to irregular plant and climatic cycles that result in dramatic yearly fluctuations and that in USA, a good crop is obtained once in seven years. The major defect was found to be yellow kernels. The ratio of intact and sound kernels was the lowest in the first year of sampling due to higher ratio (3.46%) of yellow kernels. The other defects (mouldy, black, green or tip discoloration) were quite low (<1.0%) and similar in the three years of the study (Table 2). Changing of kernel colour to yellow or black is associated with lipid oxidation and enzymatic and non-enzymatic reactions. One of the major factors affecting the speed of these reactions is the water activity of the kernels [21]. During the first year of the study, high percentage of yellow kernels may have resulted possibly from increasing trends in water activity and water contents. The percentage of mouldy kernels was below 1.0% since many fungi species cannot grow at water activity levels below 0.60 [22].

Table 1. Cracking modes (%) of in-shell nuts sampled in different years

Years	No cracking	One side			Both sides	Ready for germination
		1/3	2/3	3/3		
1	78.26±5.79 a^z	0.06±0.14	0.77±0.58	14.28±4.21 b	3.89±1.92 b	2.74± 1.42 b
2	83.34±7.60 a	0.78±1.00	2.94±2.26	7.98±4.29 c	3.44 ±2.76 b	1.53±0.89 b
3	57.40±8.13 b	0.54±1.31	1.42±2.20	22.11±6.36 a	7.58±4.05 a	11.23±3.45 a
	**	NS	NS	**	*	**

^{z} Means separation within columns by Duncan's multiple range test, P≤0.05; ^{NS}, *, **, Nonsignificant, significant at P≤0.05 or P≤0.01, respectively

Table 2. Intact white (healthy) and defected pine nut kernel ratios sampled in different crop years

Years	Intact white	Defected kernels (%)				
		Yellow	Mouldy	Black	Green	Tip discoloration
1	94.08±1.08 b[z]	3.46±0.50 a	0.72±0.38	0.54±0.19	0.34±0.24	0.96±0.49
2	97.07±1.32 a	0.53±0.57 b	0.44±0.33	0.73±0.25	0.16±0.23	0.37±0.46
3	97.97±1.54 a	1.08±0.74 b	0.51±0.53	0.08±0.14	0.06±0.19	0.38±0.50
	*	**	NS	NS	NS	NS

[z] *Means separation within columns by Duncan's multiple range test, P≤0.05; [NS], [*], [**], Nonsignificant, significant at P≤0.05 or P≤0.01, respectively*

Number of in-shell nuts per 100 g was almost similar in all 3 years (Table 3). The kernels were the smallest in the third year of sampling compared to the other years. The number of kernels was 474, 481 and 541 in 100 g respectively in 2004, 2005 and 2007. In the first year, kernel ratio was the highest (28.90%) whereas the lowest (26.43%) in the third year. As the average of 3 years, number of kernels per 100 g and kernel ratio were determined as 499 and 27.73%. A previous study reported kernel ratio as 27.28% [5]. The yearly cultural practices and harvest times (generally February-April) were similar during the 3 years of study thus, kernel weight and kernel ratio were more prone to yearly changes in the climatic conditions. Excessive rainfall especially during pollination period affects fruit set and consequently yield [19]. Thus, the yearly variations in yields (e.g. very low yield in 2006) are experienced in Kozak Region, as well.

The effect of yearly conditions on kernel length was not marked, whereas created a significant ($P \leq 0.05$) effect on kernel width. The kernels were wider in the first year compared to the crop of the third year (Table 3). This result is in accordance with the kernel size and kernel ratio. Average kernel length and width were 14.25 mm and 5.48 mm respectively.

3.3 Chemical Composition of Kernels

The moisture content and water activity did not vary significantly among the three study years. The mean moisture content and water activity values are calculated as 4.64% and 0.452, respectively (Table 4). Moisture content of the kernels was lower than the maximum level (8%) allowed by the Turkish Standard (TS 1771) in all years [23]. Similarly water activity values were below the threshold levels for further aflatoxin production [24]. The measured water activity values were also at levels that will slow down the lipid oxidation process [21].

Ash content of kernels was similar in all years and averaged to 4.19% confirming the results obtained by [5]. Yearly averages in protein contents of kernels were alike and averaged to 31.98%. Among all the *Pinus* varieties, the highest protein content was reported for *P. pinea* [25]. Similar results were reported by [5,26,27]. No significant differences existed in carbohydrate content and the average is calculated as 13.36% (Table 4).

In the present study, the amount of oil was 45.8% on average (Table 4). Wolff and Bayard [28] reported that the kernel oil content of some pine nut varieties changed from 31% to 68%. Commercial pine nut samples (*P. pinea*) analyzed in Italy contained 50.3% oil [27]. In general, pine seeds are rich in oils; their contents vary due to differences in species and environmental factors.

Fatty acid composition of the Mediterranean pine nut is reported to be identical, qualitatively. Linoleic acid is the major fatty acid followed by oleic, palmitic and stearic acids [29]. The fatty acid composition of pine nuts shows high contents of linoleic acid and especially linolenic acid have positive effects regarding the cardiovascular system [30-32]. The nutritional value of linoleic acid is due to its metabolism at tissue levels which produces the hormone-like prostaglandins [30,33]. The effect of yearly conditions on fatty acid amounts was observed to be statistically insignificant. The composition of fatty acids in pine nut kernels from Kozak region confirm that the most important fatty acid is the linoleic acid (C18:2), followed by oleic (C18:1), palmitic (C16:0), stearic (C18:0), eicosatrienoic (C20:3) and linolenic acids (C18:3) (Table 5). Previous studies also showed that the oils of *Pinus* varieties contained oleic and linoleic acids at relatively high levels [4,7,14,34,35]. In the research, other fatty acids were identified with lesser amounts, in the decreasing order as eicosenoic (C20:1), arachidic (C20:0), heptadecanoic (C17:0), palmitic (C16:1) and myristic acid (C14:0) (Table 5). Previous studies reported similar results [5,29]. In addition to the above mentioned ones, eicosadienoic (C20:2) [28] and heneicosanoic acid (C21:0) were also determined as 0.53% and 0.12%, respectively.

P. pinea kernel oil is rich in unsaturated fatty acids. The total unsaturated fatty acid contents such as oleic, linoleic and linolenic acids were at high levels (89.52%). Among the unsaturated fatty acids, 38.96% was monounsaturated fatty acids and 50.56% was polyunsaturated fatty acids. In this study, saturated fatty acids accounted for 10.47% of the total fatty acids (Table 5) whereas [5] reported saturated fatty acid percentage as 13%. [29] reported that the saturated fatty acids accounted for 11.2 to 13.1% of the total fatty acids in pine nut kernels of Turkish origin, those from Izmir possessing the lowest value which confirmed the value (10.5%) obtained in the study. Thus, the variations with the other research results are due to the origin of the pine nut samples.

Table 3. Some physical characteristics of pine nut kernels sampled in different crop years

Years	Number of in-shell nuts/100 g	Number of kernels/100 g	Kernel ratio (%)	Kernel length (mm)	Kernel width (mm)
1	137±8.7	474±21.1 b^z	28.90±1.06 a	14.31±0.74	5.58±0.15 a
2	134±5.4	481±33.9 b	27.86±1.71 ab	14.47±0.42	5.51±0.13 ab
3	143±10.3	541±42.6 a	26.43±1.47 b	13.97±0.45	5.35±0.21 b
	NS	**	*	NS	*

^{z}Means separation within columns by Duncan's multiple range test, P≤0.05; ^{NS}, *, **, Nonsignificant, significant at P ≤0.05 or P≤0.01, respectively

Table 4. Some chemical characteristics of pine nut kernels sampled in different crop years

Years	Moisture (%)	Water activity	Ash (%)	Protein (%)	Oil (%)	Carbohydrates (%)
1	4.95±0.53	0.478±0.06	4.13±0.16	31.61±1.31	45.2±0. 46	14.11±2.14
2	4.34±0.85	0.447±0.08	4.24±0.14	32.65±0.92	46.4±0. 55	12.37±2.24
3	4.62±0.77	0.431±0.05	4.21±0.13	31.66±1.12	45.9±0. 63	13.61±2.37
	NS	NS	NS	NS	NS	NS

^{NS}, Non significant

Table 5. Fatty acid composition of pine nut kernels sampled in different crop years (% of total fatty acid content)

Fatty acids	Year 1	Year 2	Year 3
C14:0	0.04±0.002^{NS}	0.05±0.004	0.04±0.003
C16:0	6.30±0.16^{NS}	6.37±0.21	6.26±0.10
C16:1	0.10±0.01^{NS}	0.10±0.01	0.07±0.01
C17:0	0.12±0.01^{NS}	0.10±0.02	0.11±0.02
C18:0	3.54±0.06^{NS}	3.59±0.06	3.41±0.13
C18:1	38.44±0.53^{NS}	38.21±0.39	38.29±0.51
C18:2	46.79±0.32^{NS}	47.08±0.52	47.43±0.77
C18:3	1.20±0.04^{NS}	1.17±0.06	1.14±0.05
C20:0	0.38±0.03^{NS}	0.37±0.04	0.35±0.11
C20:1	0.52±0.04^{NS}	0.52±0.03	0.63±0.07
C20:2	0.59±0.09^{NS}	0.54±0.05	0.46±0.07
C20:3	1.85±0.18^{NS}	1.76±0.19	1.68±0.22
C21:0	0.11±0.02^{NS}	0.14±0.01	0.12±0.02
Σ Sat.	10.49±0.16^{NS}	10.61±0.19	10.30±0.17
Σ Unsat.	89.49±0.19^{NS}	89.38±0.18	89.70±0.18
Σ Punsat.	50.43±0.49^{NS}	50.55±0.42	50.71±0.37
Σ Unsat./Σ Sat.	8.53±0.34^{NS}	8.42±0.28	8.71±0.21
Σ Punsat./Σ Sat.	4.81±0.18^{NS}	4.76±0.13	4.92±0.16

^{NS}, Nonsignificant; Σ Sat., saturated fatty acid; Σ Unsat., unsaturated fatty acid; Σ Punsat., polyunsaturated fatty acid

Table 6. Mineral composition of pine nut kernels (mg/100 g) sampled in different crop years[z]

Minerals	Year 1	Year 2	Year 3
Potassium	746±64.3^{NS}	732±48.1	723±58.9
Phosphorus	628±33.7 b^{y}*	683±44.5 a	652±38.4 ab
Calcium	15.2±25.5^{NS}	16.1±16.4	16.8±11.4
Magnesium	357±11.4^{NS}	364±10.0	366±5.5
Iron	12.0±4.5^{NS}	9.5±1.8	11.0±2.1
Zinc	6.3±0.8^{NS}	6.9±1.1	6.5±0.6
Copper	3.2±0.8^{NS}	3.5±0.6	3.2±0.3
Manganese	5.4±1.0^{NS}	6.0±1.2	6.1±0.9
Sodium	4.3±1.3^{NS}	4.6±1.1	5.1±0.8

*[z] Results are the means of four replicate samples ±SD. [y] Means separation within rows by Duncan's multiple range test, P≤0.05. [NS], *, Nonsignificant or significant at P≤0.05 respectively*

The mineral compositions of the pine nut kernels were similar in all three years of study except phosphorus (P). The P content was higher in kernels sampled during the second year of the study compared to the first year. Several studies have indicated that mineral composition of tree nuts is affected by variety, geographical origin, harvest year, climate, soil characteristics, and management practices [36,37]. In the case of Kozak region, all factors except climatic conditions are negligible. Thus, the effect on P seems to occur as a consequence of yearly climatic conditions. Potassium (734 mg/100 g) was the highest mineral in pine nut kernels followed by phosphorus and magnesium. The other elements, in descending order by quantity, were Ca, Fe, Zn, Mn, Na and Cu. Compared to other tree nuts, the Mediterranean pine nut kernels were rich in phosphorus, magnesium, iron and zinc [37-39].

4. CONCLUSIONS

The Mediterranean pine nut is a specialty nut used in traditional dishes in many countries and recently differentiated from other pine nut species causing bitter mouth and thus preferred in the world markets. In the study, yearly climatic conditions exerted marked impact on quality and the major defects were found to be yellow kernels and cracked ratio. The ratio of intact and sound kernels was the lowest in the first year of sampling due to higher ratio (3.46%) of yellow kernels. The other defects (mouldy, black, green or tip discoloration) were quite low (<1.0%) and similar in the three years of the study. Especially in the third year of the study, the ratio of cracked nuts advanced to 42.6%. High number of cracked in-shells may further reduce marketable kernel ratio through promoting germination, oxidation or mould or aflatoxin formation. In this respect, drying process needs to be revisited in Kozak district and additional precautions must be implemented under unfavourable climatic conditions.

The results confirm that the pine nut kernels are a rich source of many important nutrients that appear to have positive effects on human health. Kozak pine nuts are rich in protein, potassium, phosphorus, and magnesium contents contributing also to daily zinc and iron intake. The fatty acid composition of pine nut with high linoleic acid content displays importance in terms of human nutrition and health. The saturated fatty acid ratios were determined between 10.3 to 10.6% in Kozak (İzmir) pine nuts comparatively lower than the other reported ratios [5,29]. These results confirm that the pine nut kernels produced in Kozak area are a rich source of many important nutrients that appear to have a very positive effect on human health and possess additional quality attributes compared to Mediterranean pine nuts from other origins.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Anonymous. International Nut and Dried Fruit Council (INC). Available:http://www.nutfruit.org/what-we-do/industry/statistics (Accessed May 2013)
2. Ozguven F, Vursavus K. Some physical, mechanical and aerodynamic properties of pine (*Pinus pinea*) nuts. J. Food Eng. 2005;68:191-196.
3. Gomez-Ariza JL, Arias-Borrego A, Garcia-Barrera T. Multielemental fractionation in pine nuts (*Pinus pinea*) from different geographic origins by size-exclusion chromatography with UV and inductively coupled plasma mass spectrometry detection. J. Chromatogr A. 2006; 1121:191-199.
4. Derewiaka D, Szwed E, Wolosiak R. Physicochemical properties and composition of lipid fraction of selected edible nuts. Pak. J. Bot. 2014;46(1):337-343.
5. Nergiz C, Donmez I. Chemical composition and nutritive value of *Pinus pinea* L. seeds. Food Chem. 2004;86:365-368.
6. Nasri N, Triki S. Analyse des lipids des garnies de pind de Tunisie: *Pinus halepensis* Mill. Et *Pinus pinea* L. La Rivista Italiana Delle Sostanze Grasse, Luglio/Agosto. French. 2004;81:244-247.
7. Gölge E, Ova G. The effects of food irradiation on quality of pine nut kernels. Rad. Phys. and Chem. 2008;77:365-369.
8. Halvorsen BL, Holte K, Myhrstad MC. A systematic screening of total antioxidants in dietary plants. J. Nutr. 2002;132:461-471.
9. Blomhoff R, Carlsen MH, Andersen LF, Jacobs DR. Health benefits of nuts: potential role of antioxidants. Brit. J. Nut. 2006;96:52-60.
10. Savage GP. Chemical composition of wallnuts (*Juglans regia* L.) grown in New Zealand. Plants Foods for Human Nutr. 2001;56:75-82.
11. Ellsworth JI, Kushi LH, Folsom AR. Frequent nut intake and risk of death from coronary heart disease and all causes in postmenopausal women: The Iowa Women's Health Study. Nutr. Metab. Cardiovasc Dis. 2001;11:372-377.
12. Mukuddem-Peterson J, Oosthuizen W, Jerling JC. A systematic review of the effects of nuts on blood lipid profiles in humans. J. Nutr. 2005;135:2082-2089.
13. Rosengarten F. The Book of edible nuts. New York: Walker and Company; 1984.
14. Sagrero-Nieves L. Fatty acid composition of Mexican pine nut (*Pinus cembroides*) oil from three seed coat phenotypes. J. Sci. Food Agric. 1992;59:413-414.
15. AOAC. Official methods of analysis of AOAC International. 15th ed, USA: Association of Official Analytical Chemists; 1990.
16. AOAC. Official methods of analysis of AOAC International. In: Horwitz, W, editor. Association of Official Analytical Chemists International. 17th ed. Gaithersburg, MD, USA; 2000.
17. IUPAC. Standard methods for the analysis of oils, fats and derivatives. Pergamon Press, Oxford: International Union of Pure and Applied Chemistry (IUPAC); 1990.
18. James GS. Analytical chemistry of foods. London: Blackie Academic and Professional; 1995.
19. Anonymous. Turkish State Meteorological Service. Available:http://www.mgm.gov.tr (Accessed April 2008)
20. Geisler M. Pine nuts profile. AgMRC, Lowa State University. Available:http://www.agmrc.org/commodities_products/nuts/pine-nuts-profile (Accessed October 2015)
21. Labuza TP. Kinetics of lipid oxidation in foods. Crit. Rev. Food Technol. 1971;2:355-405.
22. Jay JM. Modern food microbiology. Maryland: Apsen Publisher Inc.; 2000.
23. Anonymous. Pine Nut Standard TS 1771. Turkish Standards Institute (Ankara), ICS 67.220.10; 2003.
24. Northolt MD, Van Egmond HP, Paulsch WE. Differences between *Aspergillus flavus* strains in growth and aflatoxin B1 production in relation water activity and temperature. J. Food Prod. 1977;40:778-781.
25. Lanner RM. The piñon pine: A natural and cultural history. Nevada: University of Nevada Press; 1981.
26. Farris GJ. California pignolia: Seeds of *Pinus sabiniana*. Econ. Bot. 1983;37:201-206.
27. Ruggeri S, Cappelloni M, Gambelli L, Nicoli S, Carnovale E. Chemical composition and nutritive value of nuts grown in Italy. It. J. Food Sci. 1998;10:243-252.

28. Wolff RL, Bayard CC. Fatty acid composition of some pine seed oils. J. Am. Oil Chem. Soc. 1995;72:1043-1046.
29. Nasri N, Khaldi A, Fady B, Triki S. Fatty acids from seeds of *Pinus pinea* L.: Composition and population profiling. Phytochemistry. 2005;66:1729-1735.
30. Sabate EJ, Fraser GE, Burke K, Knutsen S, Bennett HK, Lindsted D. Effects of walnuts on serum lipids levels and blood pressure in normal men. New England J. Med. 1993;328:603-607.
31. Abbey M, Noakes M, Belling GB, Nestel PJ. Partial replacement of saturated fatty acids with almonds or walnuts lowers total plasma cholesterol and low-density-lipoprotein cholesterol. Am. J. Clin. Nut. 1994;59:995-999.
32. Cunnane SC, Ganguli S, Menard C, Liede AC, Hamadeh MJ, Chen ZY. High alpha-linolenic acid flaxseed (*Linum usitatissimum*): Some nutritional properties in humans. Brit. J. Nutr. 1993;69:443-453.
33. Ramadan MF, Morsel JF. Neutral lipids classes of black cumin (*Nigella sativa* L.) seed oils. Eur. Food Res. Technol. 2002;214:202-206.
34. Wolff RL, Marpeau AM. Δ5-olefinic acids in the edible seeds of nut pines (*Pinus cembroides edulis*) from the United States. J. Am. Oil Chem. Soc. 1997;74: 613-614.
35. Ryan E, Galvin K, O'Connor TP, Maguire AR, O'Brien NM. Fatty acid profile, tocopherol, squalene and phytosterol content of Brazil, pecan, pine, pistachio and cashew nuts. Int. J. Food Sci. Nutr. 2006;57:219-228.
36. Bonvehi JS, Coll FV. Evaluation of the bitterness and astringency of polyphenolic compounds in cocoa powder. Food Chem. 1997;60:365-370.
37. Köksal Aİ, Artik N, Şimşek A, Güneş N. Nutrient composition of hazelnut (*Corylus avellana* L.) varieties cultivated in Turkey. Food Chem. 2006;99:509-515.
38. Bostan SZ. Important chemical and physical traits and variation in these traits in 'tombul' hazelnut cultivar at different elevations. Grasas Aceites. 2003;54:234-239.
39. Wuilloud RG, Kannamkumarath SS, Caruso JA. Speciation of nickel, copper, zinc and manganese in different edible nuts: A comparative study of molecular size distribution by SEC–UV–ICP–MS. Anal. Bioanal. Chem. 2004;379:495-503.

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