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Chemical composition and nutritive value of *Pinus pinea* L. seeds

Cevdet Nergiz *, İclal Dönmez

Department of Food Engineering, Faculty of Engineering, Celal bayar University, Muradiye Campus, Manisa 45140, Turkey

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Abstract

The proximate composition of stone pine *Pinus pinea* L. seeds, along with mineral and vitamin contents (ascorbic acid, thiamine and riboflavin), fatty acid and triacylglycerol compositions, were investigated. The proximate analysis of stone pine seeds showed the following composition: moisture 5%, ash 4.5%, fat 44.9%, crude protein 31.6%, total soluble sugars 5.15% and energy value 583 kcal/100 g. Oleic and linoleic acids were the major unsaturated fatty acids, while palmitic, stearic and lignoceric acids were the main saturated ones. Potassium, phosphorus and magnesium were the predominant elements present in the seeds. Zinc, iron and manganese were also detected in appreciable amounts. The contents of ascorbic acid, thiamine and riboflavin were found to be 2.50, 1.50 and 0.28 mg/100 g, respectively. The compositions of triacylglycerol of the oil were determined with ECN by HPLC. Triacylglycerols with ECNs of 44 and 48 were dominant in the oil.

Keywords: Stone pine; *Pinus pinea* L. seeds; Proximate composition; Fatty acids; Triacylglycerols; Minerals; Vitamins

1. Introduction

The species of genus *Pinus* produce seeds that are edible and highly nutritious. Edible nut-producing pines are present in Asia, Europe, the Near East and North America. Several species of *Pinus* are known and they are grouped as soft pines (haploxylon) and hard pine (diploxylon). *Pinus pinea* is present in diploxylon pine groups and its seeds are the common pine nuts present in world markets (Mirov, 1967).

Pinus pinea has been widely planted throughout the Mediterranean regions, mainly in Spain, Portugal, Italy, Greece, Albania and Turkey. Turkey has 35,000 hectares of stone pine forest. About 50% of this area is found in highlands of Bergama-Kozak in Izmir. World production of pine nut is about 20,000 tons/year. The chief producing countries are China, Spain, Italy, Portugal and Turkey. It is reported that the Turkish production of pine nut is about 1200–1300 tons/year. Nine hundred tons of this production is obtained only in the highlands of Bergama-Kozak, which is in the Aegean region of Turkey. Eighty five percent of total production is exported to several countries and the remainder is consumed within Turkey (Acun, 1982).

Pine nuts are eaten raw or roasted; they are included as ingredients in a variety of traditional dishes, such as breads, candies, sauces and cakes, as well as vegetable and meat dishes. Pine nuts are a good source of nutrients. It is reported that the seeds of *P. pinea* show a composition of 5.6% moisture, 31.1% protein, 47.4% fat, 10.7% carbohydrate and 4.3% ash. They contain vitamins, particularly B_1 and B_2 and also minerals, especially potassium and phosphorus. Apart from nutritional value, consumption of nuts aids health. Regular consumption of nuts is associated with a reduced risk of both coronary heart disease and non-fatal myocardial infarction (Savage, 2001). This effect is ascribed to fatty acid composition because of high amounts of linoleic acid.

The composition of pine nuts shows variation among species and even some subspecies depending on geographical and climatic conditions (Sagrero-Nieves, 1992; Savage, 2001). Literature data on chemical composition of the *P. pinea* seeds are very limited. There have been no reports concerning the chemical composition of *P. pinea* seeds grown in Turkey. The purpose of this work

* Corresponding author. Tel.: +90-236-241-2144; fax: +90-236-241-2143.
E-mail address: cnergiz@bayar.edu.tr (C. Nergiz).

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was to investigate the detailed composition and nutritive value of the Turkish pine nut variety.

2. Materials and methods

Pine nut samples were obtained from the Private pine nut processing factory, the Agricultural Development Cooperative and Forest Management Directorate in Bergama-Izmir. To define of some physical characteristics of the collected samples, seed length and width were determined. For the determination of pine nut fractions, seed samples were broken manually and shells, meats and skin were carefully separated and their proportions were calculated. The skinned meat samples were used for all the chemical analyses.

Moisture content was determined by the toluene distillation method according to the Turkish standard (Anon., 1974). Protein, lipids and ash were determined according to the standard AOAC method (Anon., 1990). Crude protein was calculated by using a nitrogen conversion factor of 5.30 (Greenfield & Southgate, 1992). The concentrations of sodium, potassium and calcium were determined with a flame emission photometer (Jenway PFP7 model). Lanthanum chloride was added to all samples and calibration solutions to prevent interference in the calcium analysis. The amounts of magnesium, zinc, copper, iron and manganese were also determined with a flame atomic absorption spectrophotometer (Perkin–Elmer, 2380 model). For the phosphorus content, the phosphomolybdovanadate method was used. Sugar analysis (total and invert) were conducted by the Lane and Eynon volumetric method. Sucrose content was calculated by subtracting the amount of reducing sugar from the total sugar content and multiplying the result by 0.95.

Fatty acid methyl esters of the lipids were prepared by saponification–esterification, according to the IUPAC Method No.: 2.301 (IUPAC, 1990). Analyses of fatty acid methyl esters were carried out with a Hewlett Packard Gas Chromatograph (Model, 439), equipped with a hydrogen flame ionisation detector and a capillary column, Supelcowax Tm, fused silica (60 m × 0.25 mm id., of 0.2 μm particle diameter). Temperatures of injector, column and detector were 260, 180 and 250 °C, respectively. Hydrogen was used as carrier gas at a flow rate of 30 ml/min. Identification and quantification of fatty acid methyl esters was accomplished by comparing the retention times of the peaks with those of standards. The analysis of triacylglycerols (TAG_s) was performed according to equivalent carbon number of triglycerides given by IUPAC Method 2324 (IUPAC, 1990). HPLC separations of TAG_s were conducted on a Hypersil ODS column (150 × 4.6 mm, id of 0.5 μm particle size). Elution was at 1.5 ml/min with acetone/acetonitrile (63.6:36.4, v/v) at ambient temperature. The instrument used was a Hewlett Packard Model 1100 Liquid Chromatograph equipped with refractive index detector. Separated triacylglycerols, as a function of equivalent carbon numbers (ECN), were identified by using reference triglycerides and their relative percentages were computed automatically.

Carbohydrate content was estimated according to Nergiz and Ötleş (1993). Energy value was expressed as kcal using the factors proposed by the Greenfield and Southgate (1992). The content of thiamine was determined according to Nergiz and Seçkin (1998). For the determination of riboflavin, the AOAC method was used (Anon., 1990). Ascorbic acid was estimated spectrophotometrically, based on the fact that it can reduce the blue dye, 2,6-dichlorophenolindophenol, to the colourless form. Descriptive statistics were calculated and results expressed as means ± SD.

3. Results and discussion

The physical characteristics of *P. pinea* seeds obtained from the Kozak district of Turkey are shown in Table 1. The moisture content was found to be 5.1%, which is below the maximum level given by the Turkish standard: TS. 1771 (Anon., 1974). The average protein content of the seeds was found to be 31.6%. Similar results were reported by Farris (1983) and Ruggeri, Cappelloni, Gambelli, Nicoli, and Carnovale (1998). Among all the *Pinus* varieties, the highest protein content (34%) was reported for *P. pinea* (Lanner, 1981). In the present study, the amount of oil was 44.9%, on average. Wolff and Bayard (1995) reported that the oil content of some varieties of pine seeds changed from 31% to 68%. Commercial pine nut samples (*P. pinea*)

Table 1
Physical and chemical characteristics of *P. pinea* L. seeds

Physical characteristics[a]	
Number of seeds/100 g	146 ± 6.83
Length (mm)	18.34 ± 2.08
Width (mm)	8.83 ± 0.9
Shell (%)	72.51 ± 1.16
Meat (%)	27.28 ± 1.16
Skin (%)	0.237 ± 0.01
Chemical characteristics[b] *(%)*	
Moisture	5.1 ± 0.01
Ash	4.5 ± 0.07
Fat	44.9 ± 0.37
Crude protein	31.6 ± 2.1
Carbohydrate	13.9 ± 2.07
Total soluble sugar	5.15 ± 0.22
Reducing sugar	0.70 ± 0.05
Sucrose	4.30 ± 0.16
Energy value (kcal)	58 ± 3.8

[a] Average value of 10 determinations ± SD.
[b] Means of three determinations ± SD.

analysed in Italy contained oil 50.3% (Ruggeri et al., 1998). In general, pine seeds are rich in oils; their contents vary due to differences in species and environmental factors.

Fatty acid compositions of the oil extracted from the seeds are given in Table 3, which shows that oleic and linoleic acids account for more than 85% of the total fatty acids. *P. pinea* seed oil is rich in unsaturated fatty acids. Linoleic and oleic acids accounted for 47.6% and 38.6% of the total fatty acids, respectively. Previous studies showed that the oils of *Pinus* varieties contained oleic and linoleic acids at relatively high levels (Sagrero-Nieves, 1992; Wolff & Marpeau, 1997). Our results are in good agreement with the reported values. In this study, saturated acids accounted for 13% of total fatty acids. Among them the main saturated acids were palmitic, stearic and lignoceric, with minute amounts of arachidic, myristic and behenic.

The distribution of triacylglycerols, with equivalent carbon number, determined by HPLC, is given in Table 4. According to the results, this oil contained six triacylglycerol species (from ECN 42 to ECN 52). The main triacylglycerols were trioctadecylglycerols (65.8%). Triacylglycerols with ECN of 44 were dominant (48.7%), followed by triacylglycerols with ECN of 48 (19.8%). It can be said that trigylcerides with ECN of 44 represent approximately one-half of the total triacylglycerols of the oil. Andrey and Long (1996) reported that the main triglycerides were trioctadecylglycerols (C-54, up to 77.2%). In our study, the four major triacylglycerols are OL_nO (23.5%), LOL (18.6%), LLL (10.8%) and OOO (10.3%). This composition is more similar to other seed oils, since the main triacylglycerols are C-54 triglycerides. It also reflects a close relationship between the fatty acids and triacylglycerol content of the oil.

Table 4
Triacylglycerol composition of *P. pinea* L. seed oils

Triacylglycerol	ECN	RT (Min)	% of total TAG content
LLL	42	8.20	10.8
OL_nL	42	8.42	2.23
PL_nL	42	9.09	0.83
OL_nO	44	10.28	23.5
PLL	44	11.18	5.32
PL_nO	44	11.44	1.23
LOL	44	13.20	18.6
PLO	46	14.53	10.6
LPP	46	15.58	0.87
OOO	48	17.25	10.3
SLO	48	18.31	3.39
POO	48	18.70	5.38
OPP	48	19.91	0.77
SOO	50	24.05	1.87
SLS	50	25.37	0.25
POS	50	26.18	0.46
OSS	52	30.85	0.28
PSS	52	33.71	0.14

P, palmitic; S, stearic; O, oleic; L, linoleic; L_n, linolenic; RT, retention time; ECN, equivalent carbon number (carbon number – 2 × number of double bonds).

Table 2
Vitamin content and mineral distribution of *P. pinea* L. seeds[a]

Vitamins	(mg/100 g)
Ascorbic acid	2.50 ± 0.014
Thiamine (B1)	1.50 ± 0.012
Riboflavin (B2)	0.28 ± 0.015
Minerals	(mg/100 g)
Sodium	11.7 ± 0.80
Potassium	713 ± 1.14
Calcium	13.8 ± 0.58
Magnesium	325 ± 6.18
Copper	1.5 ± 0.37
Zinc	6.4 ± 0.54
Iron	10.2 ± 0.36
Manganese	6.9 ± 0.16
Phosphorus	512 ± 5.56

[a] Means of three determinations ± SD.

Table 3
Fatty acid composition of *P. pinea* L. seed oil[a]

Fatty acid	% of total FA content
Myristic (14:0)	0.05 ± 0.004
Palmitic (16:0)	6.49 ± 0.078
Palmitoleic (16:1)	0.224 ± 0.03
Margaric (17:0)	Tr
Stearic (18:0)	3.47 ± 0.1
Oleic (18:1)	38.60 ± 0.59
Linoleic (18:2)	47.6 ± 0.3
Linolenic (18:3)	0.68 ± 0.001
Arachidic (20:0)	0.54 ± 0.03
Gadoleic (20:1)	0.79 ± 0.06
Behenic (22:0)	0.13 ± 0.01
Lignoceric (24:0)	3.02 ± 0.02

Tr, trace ($\leqslant 0.05\%$).
[a] Means of three determinations ± SD.

Total sugar, invert sugar and sucrose contents of the *P. pinea* seeds are given in Table 1. The main sugar was sucrose (4.30%) and invert sugar content was found in very small amounts (0.70%). Ruggeri et al. (1998) reported total soluble sugars were 3.9% in commercial pine nut samples. They also reported that the greatest one was sucrose. Our findings are in good agreement with the reported values.

The amounts of potassium, phosphorus and magnesium were detected to be highest in *P. pinea* seeds. As seen in Table 2, potassium is the most abundant element in the seeds, followed by phosphorus and magnesium. The other elements, in descending order by quantity, were Ca, Na, Fe, Mn, Zn and Cu. Table 2 shows the vitamin contents of the *P. pinea* seeds. According to the results, the amounts of thiamine (B_1) and riboflavin (B_2) were high. The samples have only small amounts of vitamin C. Its content averaged 2.50 mg/100 g. These values are higher than the values reported by Farris

(1983) and Rosengarten (1984). However, it appears that *P. pinea* seeds are not a good source of ascorbic acid. On the other hand, pine nut is a good source of vitamin B_1. Its content was 1.50 mg/100 g of edible portion. This value corresponds to the daily requirement for adults (Anon., 1980). The seeds also provide appreciable amounts of zinc and iron from a nutritional point of view. The contents of vitamins and minerals of *P. pinea* seeds have not been previously reported.

From the results we can conclude that the seeds have high protein, fat, vitamin (B_1 and B_2), potassium and phosphorus contents. The seed lipids of the investigated samples were rich in linoleic acid, which has a beneficial effect on blood lipids, lowering blood pressure and serum cholesterol. The nutritional value of linoleic acid is due to its metabolism at tissue levels which produces the hormone-like prostaglandins (Ramadan & Mörsel, 2002; Savage, 2001). Pine nut also had a high energy value (583 kcal/100 g), since lipids are the main component. In conclusion, detailed reports on the chemical composition of the Turkish pine nut (*P. pinea*) have not been previously reported. The results in this paper confirm that the seeds are a rich source of many important nutrients that appear to have a very positive effect on human health.

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