



Fatty Acid, Tocopherol and Sterol Contents of Forest Pine Seed Oil

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(Received: 18 March 2013;

Accepted: 25 October 2013)

AJC-14287

Pine seed (*Pinus pinea* L.) oil was evaluated for oil content, fatty acid composition, tocopherol and sterol contents. The main identified fatty acids were palmitic acid (4.43 %), oleic acid (18.78 %), linoleic acid (60.39 %) and linolenic acid (6.94 %). The major tocopherols of seed oil was α -tocopherol (5.38 %) and γ -tocopherol (37.46 %). The total tocopherol content had 43.88 mg/100 g. The sterol contents of pine seed oil were established as 68.80 % β -stosterol, 10.66 % campesterol and 15.02 % 5-avenasterol. The total sterol was determined as 5868.39 mg/kg.

Key Words: Pine Seed, *Pinus pinea*, Oil content, Fatty acid, Tocopherol, Sterol.

INTRODUCTION

Pine (*Pinus pinea* L.) is a member of the Pinaceae family and is widely distributed in Mediterranean countries. Pine seeds are obtained from the cones of *Pinus pinea*. It grows in Turkey and in other Mediterranean countries¹. Edible pine seeds are commercialized in France are those from *P. korainetsis* (imported from China) and *P. pinea* (imported from Spain, Portugal, Italy and Turkey). Other pine seeds are only used for forest planting². The fact that these seeds are seldom consumed may explain why little is known about their fatty acid, tocopherol and sterol contents. Limited studies have been carried out to validate the nutritional quality of pine seed^{1,2}. Large amounts of pine seeds are discharged at processing plants. A part of these is used to grow the young pine plant. Rest not only wastes a potentially valuable resource, but also aggravates already serious disposal problems.

Recently, there has been considerable emphasis on studies involving fatty acids in oil bearing seeds and kernels. But, no studies carried out tocopherol and sterol contents in pine seed oil only used for forest planting. In this study, we give the detailed oil content, fatty acid composition, tocopherol and sterol contents of seeds from forest *Pinus pinea*.

EXPERIMENTAL

Seed from *P. pinea* (voucher specimen:GM303) were collected from Mersin (Büyükeceli-Gülнар) in the south of Turkey in the summer of 2007. They are cleaned in an air

screen cleaner to remove all foreign matter such as dust, dirt and immature and broken seeds were discharged as well. Seeds were preserved until ground using a hummer mill.

Oil extraction: Pine seed oil was obtained by extraction of the meal with petroleum ether (50 °C) in a soxhlet extractor for 6 h. After extraction of the oil, the solvent was evaporated under reduced pressure. The obtained oil was kept in sealed glass bottles under deepfreezing (-18 °C) for further analysis.

Characterization of fatty acids, tocopherols and sterols

Fatty acid composition: The fatty acid composition was determined following the ISO standard ISO 5509:2000³. In brief, one drop of the oil was dissolved in 1 mL of *n*-heptane, 50 μ g of sodium methylate was added and the closed tube was agitated vigorously for 1 min at room temperature. After addition of 100 μ L of water, the tube was centrifuged at 4500 g for 10 min and the lower aqueous phase was removed. Then 50 μ L of HCl (1 mol with methyl orange) was added, the solution was shortly mixed and the lower aqueous phase was rejected. About 20 mg of sodium hydrogen sulphate (monohydrate, extra pure; Merck, Darmstadt, Germany) was added and after centrifugation at 4500 g for 10 min, the top *n*-heptane phase was transferred to a vial and injected in a Varian 5890 gas chromatograph with a capillary column, CP-Sil 88 (100 m long, 0.25 mm ID, film thickness 0.2 μ m). The temperature program was as follows: from 155 °C; heated to 220 °C (1.5 °C/min), 10 min isotherm; injector 250 °C, detector 250 °C; carrier gas 36 cm/s hydrogen; split ratio 1:50; detector gas 30

mL/min hydrogen; 300 mL/min air and 30 mL/min nitrogen; manual injection volume less than 1 μ L. The peak areas were computed by the integration software and percentages of fatty acid methyl esters (FAME) were obtained as weight per cent by direct internal normalization.

Tocopherols: For determination of tocopherols, a solution of 250 mg of oil in 25 mL of *n*-heptane was directly used for the HPLC. The HPLC analysis was conducted using a Merck-Hitachi low-pressure gradient system, fitted with a L-6000 pump, a Merck-Hitachi F-1000 fluorescence spectrophotometer (detector wavelengths for excitation 295 nm, for emission 330 nm) and a D-2500 integration system. The samples in the amount of 20 μ L were injected by a Merck 655-A40 autosampler onto a Diol phase HPLC column 25 cm \times 4.6 mm ID (Merck, Darmstadt, Germany) used with a flow rate of 1.3 mL/min. The mobile phase used was *n*-heptane/*tert*-butyl methyl ether (99 \pm 1, v/v)⁴.

Statistical analyses: The mean values were given in the tables, without the standard deviation, because this value would represent only the deviation of the method and not the variation of the appropriate sample.

RESULTS AND DISCUSSION

Ripe pine seed contained 15.71 % crude oil. Yazicioglu and Karaali¹ established 49 % oil in edible pine seed. Wolff and Bayard² reported between 31 to 67 % to the oil yield, of some pine seed oils. The fatty acid composition of the pine seed oil are shown in Table-1. The main fatty acid, identified by gas chromatography were stearic acid (3.10 %), palmitic acid (4.43 %), oleic acid (18.78 %), linoleic acid (60.39 %) and linolenic acid (6.94 %). According to the results in Table-1, most predominant fatty acid of pine seed oil was linoleic acid, which accounted for 67 % in oil. In addition, seed oil of pine contained higher amounts of oleic acid. The pine seed oil contained appreciable amounts of saturated fatty acids, especially palmitic acid and stearic acid. Wolf and Boyond² determined fatty acid composition of some pine seed oils. According to the same researchers, predominant fatty acids in free acids were palmitic acid (3.27 % to 5.55 %), oleic acid (14.36 % to 24.06 %), linoleic acid (44.84 % to 55.85 %) and linolenic acid (0.35 % to 21.78 %). The seeds from *P. pinea* appear to be a good source of oleic acid and linoleic acid. Wissebach⁵ reported 5.4 % palmitic acid, 0.60 % stearic acid, 48.0 % oleic acid and 46.0 % linoleic acid in pine (*P. pinea*) seed. Also, the same author determined 66 mg/kg α -tocopherol, 31 mg/kg δ -tocopherol and 4 mg/kg γ -tocopherol in pine seed oil. Yazicioglu and Karaali¹ reported that pine seed oil contained 7.5 % palmitic acid, 0.38 % palmitoleic acid, 4.31 % stearic acid, 36.31 % oleic acid, 49.38 % linoleic acid and 1.52 % linolenic acid.

The tocopherol content of pine seed oil investigated here are given in Table-2. The major tocopherol in seed oil was α -tocopherol (5.38 %) and γ -tocopherol (37.46 %). Other tocopherols are β -tocotrienol (0.59 %) and δ -tocopherol (0.45 %). The total tocopherol content had 43.88 mg/100 g. To the higher content of α -tocopherol of pine seed oil can be attributed the higher resistance to oxidation. Almond oil samples present concentrations of α -tocopherol, γ -tocopherol and δ -tocopherol in the ranges of 23.5-44.9, 2.93-6.15 and 1.27-8.06 mg/100 g of oil, respectively⁶.

TABLE-1
FATTY ACID COMPOSITIONS OF PINE SEED OILS (%)

Fatty acids	Concentration
Palmitic	4.43
Palmitoleic	0.05
16:1n-7	0.12
Stearic	3.10
Oleic	18.78
Vaccenic	0.31
Linoleic	60.39
18:4D6,9,12,15	6.94
20:1n-9	0.51
18:3D9,12,15	0.62
Total	89.26

TABLE-2
TOCOPHEROL CONTENTS OF PINE SEED OILS (mg/100 g)

Tocopherols	Concentration
α -T	5.38
α -T3	0.0
β -T	0.0
γ -T	37.46
β -T3	0.59
P8	0.0
γ -T3	0.0
δ -T	0.45
δ -T3	0.0
Total	43.88

Table-3 shows the sterols contained in the unsaponifiable matter crude pine seed oil. As can be seen, β -sitosterol was the main sterol in a relative concentration for 68.80 %, followed by 10.66 % campesterol, 0.28 % stigmasterol, 15.02 % 5-avenasterol, 0.23 % cholesterol and 0.73 % chlerosterol. Campestanol (0.06 %) and brassicasterol (0.07 %) were found in very low concentrations. Lazos and Servos⁷ determined 0.23 % cholesterol, 9.0 % campesterol, 2.77 % stigmasterol, 87.84 % β -sitosterol and 0.16 % 5-avenasterol in crude orange seed oil.

TABLE-3
STEROL CONTENTS OF PINE SEED OILS (%)

Sterols	Concentration
Cholesterol	0.23
Brassicasterol	0.07
24-methylen-cholesterol	0.19
Campesterol	10.66
Campestanol	0.06
Stigmasterol	0.28
7-Campesterol	0.62
Chlerosterol	0.73
β -Sitosterol	68.80
Sitostanol	0.32
5-Avenasterol	15.02
5,24-stigmastadienol	1.18
7-Stigmasstenol	0.17
7-Avenastanol	1.66
Sum (mg/Kg)	5868.39

Conclusion

Pinus seed seem to be an interesting source for the production of functional oil. The results indicate that the oil contains linoleic acid as the major fatty acid accompanied by

oleic acid. The content and composition of tocopherols are comparable to those of other sources such as peanut, sunflower, corn, rape seed oil and therefore the use of the pine oil in nutrition or technological applications is possible. Additional, minor compounds like vitamin E active compounds and sterols are also available in relatively small amounts in comparison to other commonly used vegetable oils.

ACKNOWLEDGEMENTS

This work was supported by DAAD (Germany). The authors are grateful to Dr. J. Fiebig (Head of Institute). Thanks are also due to E. Claudia, E. Uda and B. Bielefeld for skillful technical assistance with the GLC and HPLC.

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