Asian Journal of Chemistry Vol. 21, No. 2 (2009), 1559-1564

Studies on Physico-chemical Properties, Fatty Acid Composition of Terebinth (*Pistacia terebinthus* L.) Oil and Presence of Aflatoxins in Fruits

UMIT GECGEL* and MUHAMMET ARICI
Department of Food Engineering, Faculty of Agriculture
Namik Kemal University, Tekirdag 59030, Turkey
Fax: (90)(282)2931480 Tel: (90)(282)2931442
E-mail: ugecgel@nku.edu.tr

In present study, physico-chemical characteristics, fatty acids composition and aflatoxin contents quantities of terebinth nuts (*Pistacia terebinthus* L.) were determined. Oil contents, free fatty acid contents, peroxide values, iodine values and fatty acids composition of oils extracted from ripe fruits were determined. It is suggested that on account of high oil contents (35.26-47.52 %), low peroxide values (0.45-0.76 meqO_2/kg) and nice aroma and taste of terebinth fruits they could possibility be used for the oil industry. Palmitic acid was the main saturated fatty acid (19.91-23.58 %) while oleic acid was the main mono-unsaturated fatty acid (49.26-52.67 %); and finally total *trans* fatty acids were found (0.16-0.89 %).

Key Words: Aflatoxin, Fatty acids, Physico-chemical properties, *Pistacia terebinthus* L., Terebinth.

INTRODUCTION

Pistacia terebinthus L. is a typical representative of Mediterranean flora. The plant, a member of the family *Anacardiaceae*, is a dioecious tree which grows widely in southern and western regions of Turkey and is called menengic or melengic in Turkish. Its fruits are small globular nutlets which are dark greenish when ripe[1]. In Turkey, the turpentine tree is found growing on dry rocky slopes and hillsides or in pine forests, particularly in the Taurus mountains, from just above sea level[2] to 1600 m. In several regions of the world, different parts of the turpentine tree are exploited for various purposes. Archaeological evidence indicates that the nuts were being used as a food as early as 7000 BC. The young shoots and fruits are used for human nutrition[3]. The fruits have been regarded as an appetiser in southern Turkey for several thousand years. The fruits are also used in the baking of a speciality village bread and as a coffee substituent either before or after roasting. In folk medicine, leaf extracts are used as a stomachic and the fruits are used in the treatment of gastralgia (internally), rheumatism and cough (externally) and as a stimulant, diuretic and antitussive[2,3]. In addition, *Pistacia* species are used in eczema treatment, paralysis, diarrheic, throat infections, renal stones, jaundice, asthma and stomach-ache

and as astringent, antiinflammatory, antipyretic, antibacterial, antiviral and pectoral[4-8]. Investigation on *Pistacia* species has also revealed that crude extracts, essential oils and some triterpenoid constituents exhibit antiinflammatory, antifungal and antifeedant activities. The oil extracted from *P. terebinthus* fruits is consumed in food and also used as raw material in soap production in some regions of Turkey. The *Pistacia* resin is used in adherent production, in protecting lustre for arts of glass, porcelain, bone, wood and metal. It is also used in alcoholic and non-alcoholic refreshments, in some cosmetic mixtures and perfumery, as an ingredient of filling material in dentistry and in toothpaste production[2]. Resin is also traditionally used as chewing gum, against lip-dryness, some stomach diseases and antiseptic for respiratory system[2,9,10].

The aim of the present study was to investigate the chemical properties of ripe fruits of *P. terebinthus* from several villages of Anamur, Bozyazi and Ermenek in Turkey concerning the oil content and the composition of fatty acids, some physico-chemical properties of oil and aflatoxins of fruits.

EXPERIMENTAL

Terebinth (*Pistacia terbinthus*) fruits were collected by hand from plants growing wild in Anamur, Bozyazi and Ermenek of Turkey in August 2006. Fruits were cleaned by hand to remove unripe and broken fruits, dried in dry air oven (55-60 ºC) and than stored in polyethylene bags in refrigerator.

Oil extraction: Lipid extraction from the samples were carried out by hexane extraction under the operating conditions specified in IUPAC methods no. 1.121 and expressed as a percentage by mass of the product as received (IUPAC)[11]. Oil samples extracted from terebinth fruits were subjected to free fatty acids, peroxide value and iodine value content according to methods no 2.201, 2.501 (IUPAC)[11] and (AOCS)[12], respectively.

Preparation of fatty acid methyl esters: Fatty acid methyl esters (FAME) were prepared from the alkaline hydrolysis of oils, followed by methylation in methanol with BF_3 as catalyst. The final concentration of the FAME was *ca.* 7 mg/mL in heptane (AOCS)[12].

Capillary gas-liquid chromatography (GLC): Analyses of the FAME by capillary GLC were carried out on a Hewlett-Packard 6890 chromatograph, equipped with a flame-ionization detector (FID) on a split injector (chrompack, Middelburg, The Netherlands). A fused silica capillary column was used for the FAME analysis; CP™-Sil 88, 50 m × 0.25 mm i.d., 0.2 µm film; chrompack. The column was operated isothermally at 177 ºC, injector and detector were kept at 250 ºC. The carrier gas was helium with flow rate of 1 mL/min. The peak areas were computed by the integration software and percentages of fatty acids methyl esters (FAME) were obtained as weight per cent by direct internal normalization.

Aflatoxin analyses: Samples were analyzed using the validated method of the association of official analytical chemists (AOAC)[13].

Extraction: 50 g of the sample material was added to 200 mL of methanol:water (80:20). The mixture was shaken with a horizontal shaker for 0.5 h. The extract obtained was filtered through Whatmann No. 1 filter paper and then the clear solution was extracted with 10 % NaCl solution. It was evaporated in the methanol phase rotary evaporator.

Clean-up: Ready immunoaffinity columns have been used (AflaTest P®). The sample was solved in 3 mL methanol (1 mL water + 1 mL methanol) and then passed through the column. The column has been washed with 15 mL water and with 0.5 mL methanol, respectively and than 0.75 mL methanol again. 1.75 mL water was added to eluate and used for HPLC analysis[13].

Chromatography: Aflatoxins in the samples were determined in a HPLC system (Shimadzu; Pump: LC-10AT, Detector: RF-10AXL, Column oven: CTO-10AS, Degaser: DGU-14A, Injector: Rheodyne 7725i). The analyses were conducted using a ODS-2 column (250 mm × 4.6 mm, 5 µm). The following parameters were used in the analysis:mobile phase was water:acetonitril:methanol (6:2:3, v/v/v) at a flow rate of 1 mL/min at 40 ºC, fluorescence absorbency detection was at 360 nm with excitation wavelength and 440 nm emission wavelength. To confirm the presence of AFB_1 and AFG_1, Kobra cell was disconnected and fluorescence detector was directly connected to the HPLC pump. The recoveries of aflatoxins were higher than 85 %.

RESULTS AND DISCUSSION

Oil content: The oil content of 15 samples of *P. terebinthus* fruits from different locations in Anamur, Bozyazi and Ermenek of Turkey unusual in a relatively petty range from 35.26 to 47.52 % with a mean value of 41.91 % (Table-1). This was in good agreement with results reported by Ozcan[14]. The values of physical and chemical properties of the terebinth fruit and oil samples were as follows: free fatty acid contents 0.76-2.40 %; peroxide values 0.45-0.76 meqO_2/kg and iodine values 81.9-90.8. Peroxide value of terebinth oil was lower than usually suggested for other crude vegetable oils. It is found similar peroxide value compared to Ozcan[14] result *i.e.*, the peroxide value of terebinth oil is 0.47 meqO_2/kg.

In comparison to *P. vera* (60 %)[15], the oil content of *P. terebinthus* is remarkable lower. Even so, the high oil content of *P. terebinthus* fruits is comparable to several oil seeds such as sunflower or corn. Thus, considering the oil content of *P. terebinthus* fruits could be interesting for commercial processing of the oil from an economical point view.

Fatty acid composition: The fatty acid compositions of oil from *P. terebinthus* fruits are given in Table-2. The oil included fatty acids usually present in seed oils, such as saturated fatty acids like palmitic acid, stearic acid and unsaturated fatty acids like oleic acid, linoleic acid. The fatty acid composition of terebinth oil was similar to that reported previously[14]. As seen in analyzing of the Table-2, distribution of fatty acid composition of terebinth oil samples was sorted from $C_{6:0}$ to $C_{24:1}$.

TABLE-1
SOME CHEMICAL PROPERTIES OF TEREBINTH SAMPLE AND ITS AFLATOXIN CONTENTS

Sample no.	Oil (%)	Acidity (%)	Peroksit value ($meqO_2/kg$)	Iodine value	Aflatoxin (µg/kg)		
					AFB_1	AFG_1	AF_T
1	36.03	0.76	0.69	81.9	-	-	-
2	38.05	0.91	0.46	88.1	0.09	0.13	0.23
3	39.29	1.32	0.55	85.5	-	-	-
4	35.26	0.76	0.48	86.2	-	-	-
5	37.75	0.85	0.67	88.0	-	-	-
6	39.99	1.27	0.76	87.6	-	-	-
7	45.93	1.20	0.61	85.6	-	-	-
8	46.35	2.40	0.61	90.8	-	-	-
9	47.37	2.28	0.72	89.3	-	-	-
10	43.21	2.23	0.73	82.2	-	-	-
11	37.84	2.04	0.46	88.7	-	-	-
12	47.52	1.52	0.45	90.0	0.05		0.05
13	43.75	2.12	0.61	86.8	-	-	-
14	47.17	0.93	0.63	83.7	0.08		0.08
15	43.24	1.63	0.57	89.0	-	-	-
Means	41.92	1.48	0.60	86.89			
SD	4.38	0.59	0.10	2.71			

AFB_1 = Aflatoxin B1, AFG_1 = Aflatoxin G_1, AF_T = Total aflatoxin.

$C_{16:0}$ and $C_{18:0}$ from main saturated fatty acids, $C_{18:1}$ and $C_{18:2}$ from main unsaturated fatty acids. Nutritionally unfavourable high content of saturated fatty acids, consisted of palmitic acid, which amounted between 19.91 and 23.58 % and stearic acid, which was found in a very small range between 1.51 and 2.38 %. High saturated fatty acids content possibly depending on the climatic conditions in some cases also remarkable higher values can be found (28.1 %) in seed oils of *P. vera*, when the temperature is too low[15]. However, *P. terebinthus* oil contains usually less total saturated fatty acids than palm oil. Unsaturated fatty acids, consisted of oleic acid amounted in between 49.26 and 52.67 % and linoleic acid, which was found in between 17.50 and 20.95 %. Besides linolenic acid ($C_{18:3}$) which is low (< % 1) in all samples. Oleic acid was the main monounsaturated fatty acid of all samples analyzed. High ratio of $C_{18:1}$ causes to be high ratio of total mono-unsaturated fatty acids against total polyunsaturated fatty acids. This oil consists of nearly 60 % oleic acid, which is comparable to corn and groundnut oils. Total *trans* fatty acids were found between 0.16-0.89 %. Similar results were also reported by Ozcan[14]. He has found 21.3 % palmitic, 52.3 % oleic and 19.7 % linoleic acid in terebinth oil. In addition, similar results were also reported by Agar *et al.*[16].

The present results are usually similar to the other researchers which are found by means of components. Some studies showed that various factors such as handling, ecological conditions, species and the regions where the trees were grown, affect the oil content and fatty acid composition of terebinth oil[16,17].

TABLE-2
FATTY ACID COMPOSITIONS OF TEREBINTH OIL SAMPLES

Fatty acids (%)	Samples																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	X	SD
C6:0	0.05	0.03	0.04	0.03	0.04	0.01	0.03	0.03	0.01	0.03	0.04	0.05	0.01	0.03	0.03	0.030	0.012
C8:0	0.01	0.02	0.03	0.01	0.02	0.01	0.04	0.02	0.01	0.03	0.01	0.03	0.02	0.01	0.03	0.020	0.010
C10:0	0.02	0.02	0.01	0.01	0.05	0.01	0.05	0.02	0.01	0.05	0.01	0.02	0.05	0.01	0.03	0.024	0.016
C12:0	0.02	0.01	0.02	0.02	0.07	0.02	0.04	0.02	0.01	0.03	0.02	0.01	0.03	0.04	0.02	0.025	0.015
C14:0	0.10	0.01	0.08	0.06	0.03	0.07	0.04	0.01	0.02	0.03	0.04	0.08	0.05	0.07	0.04	0.048	0.027
C14:1	0.04	0.03	0.04	0.02	0.03	0.03	0.04	0.03	0.01	0.04	0.02	0.03	0.03	0.04	0.02	0.030	0.009
C15:0	0.02	0.03	0.03	0.02	0.04	0.02	0.04	0.02	0.02	0.05	0.02	0.03	0.04	0.02	0.03	0.028	0.009
C15:1	0.01	0.05	0.01	0.03	0.03	0.01	0.05	0.01	0.02	0.03	0.01	0.04	0.05	0.01	0.02	0.025	0.015
C16:0	23.58	21.39	22.48	21.50	21.07	21.59	21.43	19.91	21.43	22.96	21.15	20.40	21.30	23.08	20.75	21.601	1.015
C16:1	3.25	4.51	3.67	3.17	3.81	3.57	3.76	6.00	4.25	3.35	3.20	3.16	4.30	3.65	4.40	3.870	0.748
C16:1 *trans*	0.04	0.05	0.05	0.05	0.06	0.06	0.10	0.07	0.04	0.07	0.06	0.04	0.04	0.05	0.06	0.056	0.015
C17:0	0.07	0.12	0.07	0.08	0.09	0.06	0.13	0.09	0.05	0.11	0.07	0.12	0.09	0.06	0.13	0.089	0.026
C17:1	0.07	0.10	0.09	0.07	0.11	0.12	0.15	0.09	0.10	0.12	0.07	0.10	0.11	0.09	0.14	0.102	0.023
C18:0	2.13	2.09	2.05	2.29	2.21	2.11	2.38	2.07	1.51	2.25	2.06	2.20	2.37	2.17	2.23	2.141	0.203
C18:1 *trans*	0.09	0.04	0.02	0.03	0.14	0.04	0.13	0.03	0.03	0.09	0.04	0.09	0.03	0.04	0.09	0.062	0.039
C18:1	51.05	49.95	49.67	50.85	49.26	51.08	50.85	51.80	51.43	51.66	52.67	51.64	51.70	49.35	51.20	50.944	0.986
C18:2	18.10	20.30	20.08	20.46	20.95	19.65	18.14	18.42	19.56	17.50	19.06	20.48	18.32	19.92	19.19	19.342	1.050
C18:2 *trans*	0.06	0.05	0.04	0.05	0.18	0.09	0.30	0.07	0.08	0.05	0.06	0.05	0.18	0.09	0.07	0.094	0.071
C18:3	0.64	0.65	0.75	0.60	0.71	0.68	0.79	0.71	0.78	0.71	0.63	0.72	0.61	0.65	0.70	0.688	0.058
C18:3 *trans*	0.06	0.03	0.04	0.02	0.18	0.02	0.28	0.06	0.04	0.10	0.03	0.05	0.04	0.02	0.06	0.068	0.071
C20:0	0.20	0.22	0.24	0.17	0.22	0.19	0.28	0.18	0.21	0.21	0.22	0.20	0.21	0.21	0.19	0.210	0.026
C20:1	0.05	0.01	0.08	0.06	0.03	0.02	0.05	0.02	0.01	0.02	0.05	0.06	0.02	0.01	0.02	0.034	0.022
C20:2	0.05	0.01	0.06	0.01	0.02	0.02	0.16	0.03	0.01	0.03	0.01	0.03	0.02	0.01	0.01	0.032	0.038
C22:0	0.06	0.05	0.06	0.07	0.08	0.07	0.03	0.06	0.09	0.08	0.05	0.07	0.06	0.07	0.04	0.062	0.015
C22:1	0.07	0.07	0.05	0.11	0.14	0.17	0.18	0.03	0.08	0.09	0.07	0.11	0.08	0.07	0.14	0.097	0.043
C22:1 *trans*	0.01	0.02	0.04	0.01	0.06	0.11	0.08	0.03	0.02	0.07	0.02	0.01	0.06	0.07	0.02	0.042	0.030
C22:2	0.09	0.09	0.11	0.10	0.15	0.12	0.17	0.09	0.09	0.11	0.15	0.09	0.11	0.09	0.12	0.108	0.025
C24:0	0.05	0.03	0.06	0.06	0.12	0.04	0.16	0.06	0.04	0.06	0.04	0.05	0.06	0.03	0.12	0.065	0.037
C24:1	0.01	0.02	0.03	0.04	0.10	0.01	0.12	0.02	0.04	0.07	0.12	0.04	0.01	0.04	0.10	0.051	0.040
Total *trans*	0.26	0.19	0.19	0.16	0.62	0.32	0.89	0.26	0.21	0.38	0.21	0.24	0.35	0.27	0.30	0.323	0.192
Total saturated	26.31	24.02	25.17	24.32	24.04	24.20	24.65	22.49	23.41	25.89	23.73	23.26	24.29	25.80	23.64	24.348	1.059
Total mono-unsaturated	54.69	54.85	53.75	54.44	53.77	55.22	55.51	58.13	56.03	55.61	56.33	55.32	56.43	53.42	56.21	55.317	1.237
Total polyunsaturated	19.00	21.13	21.08	21.24	22.19	20.58	19.84	19.38	20.56	18.50	19.94	21.42	19.28	20.78	20.15	20.338	1.019
Total unsaturated	73.69	75.98	74.83	75.68	75.96	75.80	75.35	77.51	76.59	74.11	76.27	76.74	75.71	74.20	76.36	75.652	1.059
Total unsaturated/total saturated	2.80	3.16	2.97	3.11	3.15	3.13	3.05	3.44	3.27	2.86	3.21	3.29	3.11	2.87	3.23	3.116	0.176

According to the aflatoxin analyses results, it was determined that 3 of the samples had been contaminated with 0.05-0.09 µg/kg aflatoxin B_1 and 1 sample with 0.13 µg/kg aflatoxin G_1. The sample determined to have relatively high levels of aflatoxin B_1 and aflatoxin G_1 was the one harvested in 2006 and stored under room-temperature conditions. The total aflatoxin levels of the samples were ranged between 0.05 and 0.23 µg/kg. According to the results, none of the samples was determined to exceed the maximum limits stated by Turkish Food Regulations.

Due to high oil content, high oleic acid content, relatively low saturated fatty acids and *trans* fatty acids contents and low peroxide value of *P. terebinthus* seem to be an interesting source for the production of vegetable oil.

REFERENCES

1. P.H. Davis, Flora of Turkey and the East Aegean Islands, Edinburgh University Press, Edinburgh, Vol. 2 (1967).
2. T. Baytop, Therapy with Medicinal Plants in Turkey, Nobel Tip Kitap Evleri Press, Istanbul (1999).
3. L. Walheim, Western Fruit and Nuts, HP Books, p. 166 (1981).
4. E.M. Giner-Larza, S. Manez, R.M. Giner, M.C. Recio, J.M. Prieto, M.M. Cerda-Nicolas and J.L. Rios, *Planta Med.*, **68**, 311 (2002).
5. P. Marone, L. Bono, E. Leone, S. Bona, E. Carretto and L. Perversi, *J. Chemother.*, **13**, 611 (2001).
6. S.G. Wyllie, J.J. Brophy, U. Sarafis and M. Hobbs, *J. Food Sci.*, **55**, 1325 (1990).
7. L. Bonsignore, F. Cottiglia and G. Loy, *Fitoterapia*, **69**, 537 (1998).
8. F. Mouhajir, J.B. Hudson, M. Rejdali and G.H.N. Towers, *Pharm. Biol.*, **39**, 364 (2001).
9. H.L. De Pooter, N.M. Schamp, E.A. Aboutabl, S.F. Eltohamy and S.L. Doss, *Flav. Fragr. J.*, **6**, 229 (1991).
10. E. Tuzlaci and P.E. Aymaz, *Fitoterapia*, **72**, 323 (2001).
11. IUPAC, Standard Methods for the Analysis of Oils, Fats and Derivatives, International Union Pure and Applied Chemistry Division Commission on Oils, Fats and Derivatives, Blackwell Jevent Publishers, Oxford, edn. 7 (1987).
12. AOCS, Official Methods and Recommended Practices of the American Oil Chemists' Society, American Oil Chemists' Society, Champaign, Method Ce, edn. 4, pp. 2-66 (1992).
13. J. Stroka, E. Anklam, U. Jörissen and J. Gilbert, *J. AOAC Int.*, **2**, 320 (2000).
14. M. Ozcan, *J. Sci. Food Agric.*, **84**, 517 (2004).
15. F. Satil, N. Azcan and K.H.C. Baser, *Chem. Nat. Comp.*, **39**, 322 (2003).
16. I.T. Agar, N. Kaska and N. Kafkas, *Acta Horticult.*, **419**, 417 (1995).
17. M. Couladis, M. Ozcan, O. Tzakou and A. Akgul, *J. Sci. Food Agric.*, **83**, 136 (1998).

(*Received*: 15 April 2008; *Accepted*: 15 October 2008) AJC-6946