Turk J Biol
33 (2009) 311-318
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doi:10.3906/biy-0808-22

Multielement determination in fruit, soaps, and gummy extract of *Pistacia terebinthus* L. by ICP OES

Mehmet SAYIM KARACAN, Fatma ÇAĞRAN
Department of Chemistry, Arts and Sciences Faculty, Gazi University, 06500 Ankara - TURKEY

Received: 22.08.2008

Abstract: Multielement analyses were carried out on whole fruit, soaps, gummy extract, and fresh infusions of *Pistacia terebinthus* using the ICP OES technique. Digestion processes were optimized in a microwave oven. Qualitatively, the elements Al, As, B, Ba, Bi, Ca, Cd, Co, Cr, Cu, Fe, Ge, Hg, In, K, La, Li, Mg, Mn, Mo, Na, Ni, P, Pb, Pd, Sb, Sc, Se, Si, Sn, Sr, Te, Ti, V, W, and Zn were found in the fresh fruit. Al, Cd, and Pb levels in the infusions of gummy extract exceeded the upper limits specified by the Turkish Food Codex and filtration was recommended before consumption of the hot beverage. Using ICP OES a determination of metals was checked with standard reference material (GBW 07605).

Key words: *Pistacia terebinthus* L., ICP OES, multielement determination, wild plants

Pistacia terebinthus L. (menengiç) meyvesi, sabunu ve sakızımsı ekstraktında ICP OES ile çoklu element tayini

Özet: *Pistacia terebinthus* meyvesi, sabunları, sakızımsı ekstraktı ve demlenmiş ekstraktının çok sayıda elementin ICP OES ile analizi yapılmıştır. Çözünürleştirme işlemi mikro dalga fırında optimize edilmiştir. Menengiç meyvesinde kalitatif olarak Al, As, B, Ba, Bi, Ca, Cd, Co, Cr, Cu, Fe, Ge, Hg, In, K, La, Li, Mg, Mn, Mo, Na, Ni, P, Pb, Pd, Sb, Sc, Se, Si, Sn, Sr, Te, Ti, V, W, Zn elementlerinin bulunduğu anlaşılmıştır. Demlenmiş ekstraktında bazı ağır metal miktarlarının Türk Gıda Kodeksi verilerinin üst sınırını aştığı gözlenmiştir ve içecek olarak tüketilirken süzülerek içilmesinin uygun olduğu tavsiye edilmektedir. Tayin yönteminin doğruluğu standart referans madde (GBW 07605) ile kontrol edilmiştir.

Anahtar sözcükler: *Pistacia terebinthus* L. (menengiç), ICP OES, çoklu element tayini, yabani bitkiler

Introduction

Pistacia terebinthus is a member of the family Anacardiaceae, native to Asia and the Mediterranean, and grows in southern Turkey (1). The plant is locally called "menengiç". It is the source of market products such as dried whole fruit, gummy extract and a special soap, known as "menengiç soap" or "bıttım soap", which contains a different proportion of terebinth oil.

Pistacia terebinthus is a broad, deciduous bushy tree that grows slowly to the height and breadth of a wide field. It produces shiny leaves, has a strong resinous smell and around March or April produces reddish-purple blossoms that grow in close compound clusters from the ends of the previous shoots. Its fruit appear like small spherical nutlets and turn brown when mature. The plant is rich in tannin and resinous substances and has been known since ancient times for its aromatic and medicinal properties. In different regions of the world, the terebinthus tree is used for various purposes. Young shoots and fruits are used for nutrition. Its fruit has been eaten as an appetizer in southern Turkey since ancient times (2-4). It is used for the treatment of sunstroke, stomachache, gastric ailments, rheumatism, and coughs, and as a stimulant and diuretic in Turkish alternative medicine (5,6). Because the plant is in current use in the form of fruit, fruit extracts (gummy extract) as well as in the production of special soaps, analysis of the inorganic constituents is useful.

Inductively coupled plasma optical emission spectrometry (ICP OES) can be applied in almost all the areas of analytical chemistry. The wide use of the technique for the analysis of different kinds of matrices may be attributed to its multi-elemental capability and to the fact that the analytical curves are generally linear over 4 to 6 orders of magnitude, which is necessary if the simultaneous use of various aspects of the technique is to be achieved (7).

One of the major significant procedures in trace element determination in biological or other natural samples is the decomposition method, since samples are not homogeneous and usually contain a mixture of mineral fractions. The microwave assisted, closed-vessel digestion technique is becoming more and more popular in the digestion of various organic matrices, since this method provides rapid dissolution of the sample matrix, requires low oxidizing reagent volume and causes minimal contamination of the sample before the elemental analysis step. There are many studies aimed at the optimization of digestion procedures with natural samples (8-16). Because of this, a reliable technique is needed for the dissolution of these samples where the concentrations are of prime importance. Some metals in the edible plant species are very important from the viewpoint of human health and environment (17,18).

The literature explains that *Pistacia terebinthus* is known to be rich in tannin, essential oils, and resinous substances. There are many studies especially on the essential oil composition of various parts of the terebinthus tree (19,20). Seventy-seven constituents were characterized from the essential oil of *P. terebinthus* leaves including as major constituents a-cadinol (6.9%), phytol (5.4%), d-cadinene (5.1%), a-terpineol (5.0%), and bornyl acetate (4.4%) (21). The major fatty acid composition of terebinth fruit oil was broken down to include oleic (52.3%), palmitic (21.3%), and linoleic (19.7%) acids (22). Only one report has been found about the mineral contents of the fruit of *P. terebinthus* growing in Turkey (22).

In this study using ICP OES, a quantitative multielement analysis was carried out on the whole fruit, the soaps, gummy extract, and infusions of *P. terebinthus* growing in Turkey. In addition, a new digestion procedure for *P. terebinthus* using the microwave oven has been proposed.

Materials and methods

Materials

Terebinth fruit was collected from plants growing wild in Kilis (Gaziantep, in August 2005), and its products such as gummy extract (which is used like coffee) and bıttım soap and menengiç soap were purchased from a market in Ankara. The fruit was cleaned in the air screen cleaner to remove contaminants such as dust, dirt, pebbles, and chaff. Immature and broken fruits were discarded.

Reagents

Ultra pure water (18.3 MW.cm = 0.055 µS/cm) and analytical reagent grade chemicals were used unless otherwise specified. Stock solutions of the elements (1000 mg/L) and H_2O_2 (35%, 1.13 g/mL), HCl, HNO_3, $NaBH_4$, KI, ascorbic acid, and $KMnO_4$ were purchased from Merck.

Certified Reference Material (GBW 07605 certified tea leaf) was used for checking the accuracy of the ICP OES system.

Apparatus

A Berghoff speedwave MWS-3+ model microwave oven was used for the digestion of the samples. A Perkin–Elmer Optima 5300 DV type ICP–OES instrument was used for the qualitative and quantitative determination of metals and metalloids. C, H, N, and S determinations were performed using a CHNS-932 (LECO) Elemental Analyzer in the TÜBİTAK ATAL laboratory.

Preparation of samples

Terebinth fruit and its soaps were ground to 1-2 mm size pieces and dried to a constant weight at 120 °C in an oven. Three samples of each type (200 mg) were digested in a microwave oven according to a proposed digestion procedure. Digested samples were diluted to 50 mL with ultra pure water. It was impractical to try bringing the gummy extract used for making menengiç coffee to a constant weight; therefore, 2 procedures were performed to prepare the menengiç coffee solutions. In the first procedure, 5 g of extract was boiled in a beaker with 200 mL of water. It was then cooled and completed to 200 mL with water. From that suspension, 25 mL was taken and digested in the microwave oven and analyzed using ICP OES. In the second procedure, 25 mL of the suspension was filtered using filter paper (Schleicher and Schuell No. 589 blue ribbon) and the obtained filtrate was analyzed by ICP OES.

Optimization of digestion procedure

Several standard digestion procedures given in MWS-3 applications were used for the soaps, gummy extract, the actual beverage, fruit, and Certified Reference tea (23). The proposed digestion procedure was applied to all samples and optimum conditions were established (Table 1). In this procedure, 200 mg of the sample was weighed and put into the digestion vessel. Then 3 mL of concentrated H_2O_2 and 12 mL of concentrated HNO_3 were added dropwise. The mixture was stirred carefully with a clean Teflon bar before closing the vessels. Samples were heated in the microwave oven with the new program in Table 1. The resulting solution was diluted in a calibrated flask to 50 mL with ultra pure water. In the optimization of the digestion procedures, only 8 elements were determined.

Analysis of elements

Qualitative analysis was performed on the terebinth fruit by investigating the emission lines using ICP OES. All elements, except C and S, were also determined quantitatively by ICP OES. The instrumental parameters used in the ICP OES are outlined in Table 2. All element calibration solutions were arranged in accordance with the concentrations in the sample. Hg, As, Sb, Bi, Te, and Se were determined by hydride generation-ICP OES.

Table 1. Microwave oven digestion applications of samples.

Procedure	Species	Acid mixture	Temp (°C)	Ramp Time (min)	Time (min)
(P1)	Soaps*	5 mL HNO_3 + 2 mL HF	170	2	5
			190	5	30
			100	1	15
(P2)	Gummy extract*	10 mL HNO_3 + 2 of mL + H_2O_2	160	5	15
			210	3	15
			100	1	10
(P3)	Fruit*	2 mL of HNO_3 + 3 mL H_2O_2	145	2	5
			170	5	10
			190	2	15
			100	1	10
(P4)	CRM Tea*	10 mL HNO_3	150	5	10
			160	5	10
			190	5	20
Proposed	All samples	12 mL HNO_3 + 3 mL H_2O_2	160	5	15
			210	4	15
			130	2	10
			100	1	5

*Digestion procedures were taken from MWS applications

Table 2. Instrumental settings and apparatus parameters.

Apparatus	Perkin Elmer Optima 5300 DV	HG-ICP OES (for Hg, As, Sb, Bi, Te, Se)
RF generator	40 MHz	40 MHz
RF forward power	1300 W	1450 W
Plasma gas (Ar) flow	15 L/min	17 L/min in
Aux. gas (Ar) flow	0.2 L/min	0.2 L/min
Nebulizer gas (N_2) flow	0.80 L/min	0.45 mL/min
Pump rate	2.0 mL/min (peristaltic pump)	1.5 mL/min

Results and discussion

Digestion method for terebinth fruit

Four standard digestion procedures that are given in MWS-3 applications for soap (P1), olive oil (P2), olive leaf (P3), and tea (P4) were applied for the terebinth fruit. Determined element concentrations from 4 different digestion procedures and proposed digestion procedure are listed in Table 3. A new digestion procedure using a microwave oven was developed and optimized. In order to find an appropriate digestion procedure, the contents of 8 elements (Al, As, Cd, La, Na, Pb, Sc, and Sn) with the relative standard percent deviation were compared. As seen from Table 3, the new proposed digestion procedure has the lowest relative standard deviation values among the other digestion procedures given for MWS-3 applications.

Element analysis

Firstly, the emission line that was sensitive enough was selected for every element and is given in Table 4. Then qualitative analysis was performed for the fruit, soaps, gummy extract and infusions. As a result, a total of 36 elements (Al, As, B, Ba, Bi, Ca, Cd, Co, Cr, Cu, Fe, Ge, Hg, In, K, La, Li, Mg, Mn, Mo, Na, Ni, P, Pb, Pd, Sb, Sc, Se, Si, Sn, Sr, Te, Ti, V, W, and Zn) were determined from the terebinth fruit. The soaps and gummy extract have 32 and 30 elements, respectively. In terms of minerals, terebinth fruit, having 36 elements, is the richest. In the literature, it is reported that 26 elements were determined in the *Pistacia terebinthus* L. fruit growing wild in İçel, Turkey, in August 2000 (22). This difference could arise from both the origin of terebinth as well as the use of the digestion procedure. B, Ba, Cu, and Na were not

Table 3. Element concentrations of terebinth fruit with various digestion procedures.

Procedure	Al	RSD%	As	RSD%	Cd	RSD%	La	RSD%
(P1)	42 ± 4	9.5	0.92 ± 0.16	17.4	0.41 ± 0.16	39	3.98 ± 0.26	6.5
(P2)	41 ± 4	9.8	0.94 ± 0.15	16.0	0.42 ± 0.15	35.7	4.45 ± 0.27	6.1
(P3)	43 ± 4	9.3	0.96 ± 0.13	13.5	0.43 ± 0.14	32.6	4.45 ± 0.26	5.8
(P4)	41 ± 8	19.5	0.92 ± 0.13	14.1	0.40 ± 0.17	42.5	4.17 ± 0.25	6.0
Proposed	46 ± 2	4.3	0.99 ± 0.11	11.1	0.44 ± 0.11	25	4.61 ± 0.21	4.6
Methods	**Na**	**RSD%**	**Pb**	**RSD%**	**Sc**	**RSD%**	**Sn**	**RSD%**
(P1)	316 ± 12	3.8	1.98 ± 0.32	16.2	0.11 ± 0.02	18.2	28 ± 7	25
(P2)	343 ± 9	2.6	2.01 ± 0.31	15.4	0.13 ± 0.01	7.7	31 ± 6	19.4
(P3)	369 ± 12	3.3	2.08 ± 0.30	14.4	0.148 ± 0.008	5.4	34 ± 7	20.6
(P4)	349 ± 9	2.6	1.87 ± 0.34	18.2	0.124 ± 0.012	9.7	33 ± 8	24.2
Proposed	378 ± 8	2.1	2.12 ± 0.29	13.7	0.160 ± 0.006	3.8	36 ± 6	16.7

Values were given as mean ± standard deviation (n = 3)

Table 4. Element contents of fruit, soaps, gummy extract, and infusions of terebinth.

Element*	Conc. (mg/kg)				Conc. in infusions (mg/L)		TFC** (mg/kg)
	Fruit	Bıttım soap	Menengiç soap	Gummy extract	Non-filtrated	Filtrated	
Al (396.153)	46 ± 2	8 ± 1	16 ± 1	121 ± 17	17 ± 5	0.46 ± 0.13	2 (beer)
As (228.812)	0.99 ± 0.11	0.83 ± 0.09	3 ± 1	0.53 ± 0.07	0.095 ± 0.004	0.037 ± 0.01	0.2 (fruit juice)
B (249.772)	5 ± 1	ND	ND	4 ± 1	0.68 ± 0.02	1.77 ± 0.11	
Ba (455.403)	0.59 ± 0.13	ND	1.02 ± 0.12	1.57 ± 0.67	0.328 ± 0.007	0.33 ± 0.04	
Bi (306.766)	0.38 ± 0.06	0.40 ± 0.06	1.13 ± 0.26	ND	ND	ND	
Ca (317.933)	$(1.52 \pm 0.12) \times 10^3$	42 ± 2	399 ± 13	$(1.22 \pm 0.07) \times 10^3$	111 ± 6	145 ± 5	
Cd (228.802)	0.44 ± 0.11	0.65 ± 0.03	0.52 ± 0.04	0.45 ± 0.03	0.076 ± 0.002	0.043 ± 0.007	0.05 (fruit juice)
Co (228.616)	1.22 ± 0.03	0.220 ± 0.009	1.14 ± 0.05	1.18 ± 0.03	0.152 ± 0.007	0.142 ± 0.005	0.2 (beer)
Cr (284.325)	1.80 ± 0.13	0.76 ± 0.07	0.94 ± 0.16	1.10 ± 0.15	0.152 ± 0.001	0.089 ± 0.004	
Cu (327.393)	3.66 ± 0.42	ND	ND	ND	ND	ND	5 (fruit juice)
Fe (238.204)	30 ± 1	16 ± 3	66 ± 5	89.12 ± 2.25	13 ± 3	1.78 ± 0.04	15 (fruit juice)
Ge (209.426)	4.33 ± 0.34	6.30 ± 0.16	5.92 ± 0.47	ND	ND	ND	
Hg (253.652)	1.26 ± 0.20	1.50 ± 0.26	0.88 ± 0.09	ND	ND	ND	0.01 (fruit juice)
In (230.606)	1.58 ± 0.16	1.61 ± 0.03	1.45 ± 0.07	1.23 ± 0.07	0.133 ± 0.092	0.10 ± 0.02	
K (766.490)	$(4.71 \pm 0.17) \times 10^3$	336 ± 7	275 ± 16	$(7.91 \pm 0.19) \times 10^3$	$(1.52 \pm 0.15) \times 10^3$	$(3.33 \pm 0.15) \times 10^3$	
La (408.672)	4.61 ± 0.21	5.16 ± 0.12	4.99 ± 0.05	5.44 ± 0.61	0.30 ± 0.02	0.592 ± 0.014	
Li (610.362)	0.65 ± 0.02	0.65 ± 0.03	3.32 ± 0.04	0.57 ± 0.02	0.078 ± 0.002	0.074 ± 0.004	
Mg (285.213)	545 ± 19	26.29 ± 0.55	102 ± 2	416 ± 12	55 ± 5	137 ± 3	
Mn (259.372)	5.71 ± 0.14	0.260 ± 0.007	1.03 ± 0.07	7 ± 1	1.11 ± 0.05	2 ± 1	
Mo (202.031)	0.38 ± 0.06	0.84 ± 0.08	0.77 ± 0.12	0.74 ± 0.11	0.13 ± 0.02	0.045 ± 0.001	
Na (589.592)	378 ± 8	ND	ND	13.62 ± 0.24	4.1 ± 0.2	5 ± 3	
Ni (231.604)	1.40 ± 0.28	0.22 ± 0.06	ND	ND	ND	ND	0.1 (beer)
P (213.617)	$(1.53 \pm 0.025) \times 10^4$	985 ± 14	$(1.72 \pm 0.54) \times 10^3$	786 ± 16	45 ± 3	95 ± 7	
Pb (220.353)	2.12 ± 0.29	0.21 ± 0.01	2.27 ± 0.37	2.23 ± 0.30	0.25 ± 0.08	0.17 ± 0.01	0.05 (fruit juice)
Pd (340.458)	1.03 ± 0.12	1.45 ± 0.11	1.40 ± 0.08	1.03 ± 0.20	0.13 ± 0.02	0.15 ± 0.03	
Sb (206.836)	3.74 ± 0.24	1.32 ± 0.35	3.78 ± 0.51	0.401 ± 0.020	0.083 ± 0.030	0.038 ± 0.001	
Sc (361.383)	0.160 ± 0.006	0.22 ± 0.06	0.22 ± 0.02	0.270 ± 0.002	0.033 ± 0.004	0.031 ± 0.005	
Se (196.026)	1.85 ± 0.65	3.79 ± 0.09	1.88 ± 0.23	2.60 ± 0.06	0.50 ± 0.03	0.14 ± 0.06	
Si (251.611)	39 ± 3	16.82 ± 0.41	277 ± 16	124 ± 11	21 ± 2	7 ± 1	
Sn (189.927)	36 ± 6	109 ± 1	110 ± 3	28.77 ± 0.41	2.4 ± 0.1	4.2 ± 0.4	200 (fruit juice)
Sr (407.771)	1.43 ± 0.15	0.22 ± 0.05	6.560 ± 0.006	1.77 ± 0.11	0.24 ± 0.08	0.41 ± 0.03	
Te (214.281)	44 ± 4	59 ± 2	57 ± 3	5 ± 1	0.76 ± 0.05	0.089 ± 0.004	
Ti (334.940)	1.07 ± 0.16	0.94 ± 0.04	6.12 ± 0.25	ND	ND	ND	
V (310.230)	3.62 ± 0.54	3.87 ± 0.19	3.29 ± 0.20	3.29 ± 0.20	0.20 ± 0.02	0.42 ± 0.02	
W (224.876)	1.32 ± 0.39	11 ± 1	12 ± 1	1.154 ± 0.002	0.224 ± 0.050	0.037 ± 0.01	
Zn (213.857)	11 ± 1	1.53 ± 0.43	4.36 ± 0.23	16 ± 2	1.44 ± 0.44	2.69 ± 0.23	5 (fruit juice)

Values were given as mean ± standard deviation (n = 3), ND: not detected

* Studying wavelength (nm) was given in parentheses

** Maximum levels of some contaminant (Turkish Food Codex) detected in soap 1. B, Cu, Na, and Ni were not detected in soap 2. Bi, Cu, Ge, Hg, Ni, and Ti were not detected in the gummy extract (Table 4). The absence of these elements may result from the process of production. Quantitative analysis showed that the quantity of other elements, especially Al, Ca, K, Mg, and P, diminished in the production process of soaps. However, a few elements such as Al, Ba, Fe, K, and Si in the gummy extract are rather high, compared to related values in the fruit. As can be seen in Table 4, filtrated infusions contain lower element concentrations than non-filtrated solutions. Ametals (C, H, N, and S) were also determined in terebinth fruit by elemental analysis as given in Table 5.

Metal levels in the infusions were compared with the limits specified in the Turkish Food Codex (24).

Table 5. Ametal contents of terebinth fruit (mg/100 mg).

C	H	N	S
56.84 ± 0.52	7.91 ± 0.38	2.23 ± 0.10	0.089 ± 0.005

Values were given as mean ± standard deviation (n = 3)

Table 6. Accuracy test for multielement determination.

Elements	Element Concentrations (mg/kg)		Relative Error%
	Certified	Found	
Al	0.30	0.27 ± 0.05	-10.00
As	0.28	0.26 ± 0.04	-7.14
Bi	0.063	0.059 ± 0.013	-6.35
Cd	0.057	0.059 ± 0.012	+3.50
Co	0.18	0.16 ± 0.03	-11.11
Cr	0.80	0.75 ± 0.11	-6.25
Cs	0.29	0.27 ± 0.05	-6.89
Hg	0.013	0.014 ± 0.004	+7.69
Li	0.36	0.34 ± 0.01	-5.55
Mo	0.038	0.036 ± 0.001	-5.26
Sb	0.056	0.052 ± 0.011	-7.14
Sc	0.085	0.087 ± 0.011	+2.35
Se	0.072	0.067 ± 0.012	-7.46
V	0.86	0.83 ± 0.07	-3.48
B	15.00	14.56 ± 0.52	-2.93
Cu	17.30	16.36 ± 0.16	-5.43
Ni	4.60	4.4 ± 0.3	-4.34
Pb	4.40	4.64 ± 0.27	+5.45
Sr	15.20	14.42 ± 0.35	-5.13
Ba	58.00	58.33 ± 0.24	+0.60
Na	44.00	42.4 ± 0.1	-3.64
Ti	24.00	23.10 ± 0.04	-3.75
Fe	264.00	248.58 ± 3.15	-5.84
Ca	4300.00	4189 ± 12	-2.58
Mg	1700.00	1682 ± 23	-1.06
Mn	1240.00	1198 ± 5	-3.39
K	16,600.00	15,988 ± 68	-3.69
P	2840.00	2698 ± 54	-5.00

Values were given as mean ± standard deviation (n = 3)

However, Al, As, Cd, Co, Cu, Fe, Hg, Ni, Pb, Sn, and Zn levels are found in the TFC data, in which element levels were also given as mg/kg. Al, Cd, and Pb levels in the non-filtrated infusion and Pb level in the filtrated infusion were both found to be higher than the limits specified in the TFC (density of infusions were 1 g/mL). As a result, the terebinth infusion is a beneficial hot beverage due to its mineral content, but should be used after filtration.

The accuracy of the method of determination was checked by analyzing a certified reference tea sample (GBW 07605). The tea sample was digested with the proposed digestion procedure. From the reference material of tea leaves, 28 elements were determined, and determined concentrations of elements corresponded with certified values. As can be seen from Table 6, the accuracy of the determination method is satisfactory (generally, the relative error is about 5%).

Corresponding author:
Mehmet SAYIM KARACAN
Department of Chemistry,
Science and Arts Faculty,
Gazi University,
06500 Ankara - TURKEY
E-mail : mkaracan@gazi.edu.tr

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