Determination of fatty acids, vitamins and trace elements in *Pistacia terebinthus* coffee

Harun Ciftci [1*], Ahmet Ozkaya [2] and Ergin Kariptas [3]

[1] *Department of Chemistry, Science-Art Faculty, University of Ahi Evran, 40200 Kırsehir, Turkey.* [2] *Department of Chemistry, Science-Art Faculty, University of Adiyaman, Adiyaman, Turkey.* [3] *Department of Biology, Science-Art Faculty, University of Ahi Evran,40200 Kırsehir, Turkey . *e-mail: harunciftci@yahoo.com*

Abstract

Composition of fatty acids, trace elements and fat-soluble vitamins of *Pistacia terebinthus* coffee, produced in Elazig,Turkey, was determined. Analyses were performed by using GC, ICP-OES and HPLC, respectively. The accuracy of the system was investigated by the determination of Cu, Ni, Zn, Fe and Co in standard additional method. The results of the recovery tests for samples are within the acceptable range (96% and 103%) verifying the validity of the proposed method for coffee analysis. The results were comparative with other kinds of coffee in literature. For the simultaneous determination of all vitamins, the mixture of acetonitrile/methanol (3/1, v/v) was used as the mobile phase. Detection was performed at 202 nm. It shows that this kind of coffee is a good source of trace elements and fatty acids.

Key words: Fatty acids, trace elements, vitamins, HPLC, *Pistacia terebinthus*.

Introduction

Various organs of turpentine tree are collected from different regions of the world for several intents. It is widely accepted that fresh shoots and fruits of this tree play an important role in human nutrition. In Southern Turkey the fruits of turpentine have been eaten as an appetizer for many years. Also, the fruits are used in traditional medicine for the treatment of gastralgia (internally), rheumatism and cough (externally) and as stimulant, diuretic and antitussive [1-4].

Toxic materials that cause environmental pollution with developing industry are stored by plants which take these materials through air, earth and water. Consumption of these plants directly or after some processes is an important issue in terms of human health. Therefore, it is a necessity to determine the chemical composition, and to make analyses to identify useful and harmful components of traditional foods and beverages that are major constituents of gastronomical culture of societies. In our research, the chemical composition of fruits of *Pistacia terebinthus,* known as Harput Cedene Coffee (HCC) in Elazig (East Anatolia area of Turkey), was investigated. It is sold like other coffee as roasted and squashed form in market.

Pistacia terebinthus is widely spread in the areas of Mediterranean and temperate zone climates in Turkey. The seedlings of *P. terebinthus* can grow in stony, calcarous and dry areas. They are resistant to cold and drought. For that reason, the seedlings of *P. terebinthus* growing naturally in non-agricultural areas can be grafted with pistachio cultivars and benefited from them [5].

The genus *Pistacia* belongs to the family Anacardiaceae representing 11 European species [6]. Kawashty *et al.* [7] have studied the flavonoids of four *Pistacia* species in Egypt. This chemical study showed that they were characterized mainly by the occurrence of flavonoids and flavonoid glycosides. Species *Pistacia lentiscus* has also been reported to contain phenolic compounds and triterpenoids [8].

Although chemical composition of *Pistacia terebinthus* was investigated in some areas of Turkey, the metal contents, vitamin and fatty acids of coffee plants (*Pistacia terebinthus)* grown in Elazig have not been studied and there are no reports published so far. This study is, thus, intended to assess levels of metals, vitamins and fatty acids in fruit of the coffee plants grown in Harput area. The study is expected to deliver preliminary data on chemical composition of coffee plants grown in Elazig and provide useful information for future studies which will be conducted on agronomy and physiology of the coffee plant and nutritional, medical and toxicological effects in relation to the coffee plants.

Material and Methods

Coffee material: HCC were purchased as grinded fresh from closed bazaar in Elazig in August 2008. The material was transported in polypropylene bags and held at room temperature and analyzed into two days.

Solutions and reagents: The standard metal solutions of Zn(II), Fe(III), Ni(II), Co(III) and Cu(II) (1000 mg l^{-1}, of analytical grade, Merck) were diluted to the desired concentrations with 0.2 M HNO$_3$.

Concentrated HNO$_3$ and HCIO$_4$ for digestion samples were of analytical grade (Merck). Water was deionized and double distilled. The vitamin standard stock solutions, 100.0 mg l^{-1}, were prepared by dissolving 10 mg of each reagent obtained from

Sigma–Aldrich (St. Louis, USA) in 100 mL of methanol using dark brown volumetric flasks. These solutions were stable for at least 1 month when stored in the dark at 4°C. Working solutions were prepared from the stock solutions by appropriate dilution with ethanol and shielded from light.

Oil extraction and preparation of fatty acid methyl esters (FAME): The coffee samples were ground using a ball mill into powder. Lipids were extracted with hexane/isopropanol (2/1 v/v). The lipid extracts were centrifuged at 10.0 g for 5 min and filtered; then the solvent was removed on a rotary evaporator at 40°C. Lipids were extracted with heptane in a straight through extractor.

Fatty acids in the lipid extracts were converted into methyl esters by means of 2% sulphuric acid (v/v) in methanol [9]. The fatty acid methyl esters were extracted with n-hexane. Then the methyl esters were separated and quantified by gas chromatography and flame ionization detection (Schimadzu GC, 17 Ver.3) coupled to a glass GC 10 software computing recorder. Chromatography was performed with a capillary column (25 m in length and 0.25 mm in diameter, Permabound 25, Machery – Nagel, Germany) using nitrogen as carrier gas (flow rate 0.8 ml/min). The temperatures of the column, detector and injector valve were 130- 220°C and 240-280°C, respectively. Identification was performed by frequent comparison with authentic standard mixtures that were analysed under the same conditions.

Determination of vitamins: The chromatographic system was equipped with a Shimadzu HPLC and photodiode array detector. Supelcosil LC 18 DB column (250 mm×4.6 mm, 5μm; Sigma, USA) were used for separation of vitamins. Extractions of fat-soluble vitamins were done according to studies of Perales et al.[10]. One g samples of HCC, 0.5 g of ascorbic acid and 10 ml of KOH–ethanol solution (prepared by dissolving 50 ml of ethanol in 15 ml of 60% (w/v) KOH) were mixed and shaken continuously overnight at room temperature. Thereafter, the samples were transferred into a separating funnel where liquid extraction with 10 mL of hexane and shaking the funnel for 5 min was carried out. This procedure was repeated two more times. The organic phases were combined and washed two times with 10 ml of water. Then, the organic phase was collected and evaporated to dryness in a vacuum rotary evaporator at 40°C and the residue was re-dissolved in 1 ml of mobile phase [10, 11]. Samples were transferred to autosampler vials of the HPLC instrument. The mixture of acetonitrile/methanol (3/1, v/v) was used as the mobile phase and the elution was performed at a flow-rate of 1 ml/min. The temperature of column was kept at 40°C. Detection was performed at 202 nm for vitamins. Identification of the individual vitamins was performed by frequent comparison with authentic external standard mixtures analyzed under the same conditions.

Determination of trace elements: Perkin-Elmer 3100 inductively coupled plasma optical emission spectrometer (ICP-OES) (Norwalk, USA) with a Gem Cone nebulizer on a cyclonic spray chamber and an auto sampler AS 91 (Perkin-Elmer) was used in the current study. Microwave-assisted acid digestions were done using a Premier microwave system.

HCC was digested microwave system. A 2.00 g portion of each sample was dried at 80°C accurately and 0.50 g directly weighed into PTFE (polytetrafluorethylen) bombs, and 4 ml of HNO_3 (65%, w/w) and 1 ml of $HClO_4$ (60%, w/w) were added. In a tightly closed system, the six-step microwave digestion program was applied [12]. PTFE bomb was left to cool for an hour and then carefully opened. Colourless solution was transferred into a beaker and evaporated to dryness with a hot plate. Afterwards final volume was diluted to 20 ml with 0.1 M HNO_3. The blank samples were digested in the same way. Sample solutions were analyzed by ICP-OES.

Results and Discussion

Metals are unique nutrients because of their important role in metabolism. They are essential part of many important enzymes and they also play roles as catalysts and antioxidants [13].

It is always stated that foods are the main source of vitamins for human and animals. Generally, the human diet does not contain appropriate amounts of vitamins necessary for the normal development of body functions. At this point, systematic unavailability of vitamins in the diet can result in deficient growing and development [14].

Unsaturated fatty acids which cannot be synthesized by human and animals are known as essential fatty acids. This group includes five fatty acids, unsaturated oleic acid (C18:1), palmitic acid (C 16:1), linoleic acid with two double bonds (C18:2), linolenic acid with three double bonds (18:3) and arachidonic acid which contains four double bonds (C 20:4) [15, 16].

In our studies, FAAS and HPLC, the most common analytical techniques, have been successfully used for trace metal and vitamin analysis of plant samples [17-19]. The concentrations of elements, vitamins and fatty acids in the HCC are presented in Tables 1-3. All data are averages of three measurements of each sample with a relative standard deviation of less than 10%.

The accuracy of the method was studied by examining the recovery of metals in HCC samples fortified with known amounts of the studied metals. After microwave digestion, the recoveries of the studied metals in coffee were all between 96% and 103% (Table 1). The results of the recovery tests for samples are within the acceptable range verifying the validity of the proposed method for HCC analysis. In addition, the standard additions method was used for elimination of chemical interferences caused by the matrix. The contents of metals in HCC were: Cu 4.84, Ni 1.25, Zn 14.5, Fe 62.2 and Co 0.42 $\mu g\ g^{-1}$. The ranges of heavy metals in five different kinds of Nigerian coffees were: Cu 2.13-9.41, Ni 0.04-2.58, Zn 3.73-14.0, Fe 6.30-174 and Co 0.10-14.2 $\mu g\ g^{-1}$ [20].

The levels of vitamins ($\mu g\ g^{-1}$) were 2.50 for D_2, 9.95 for D_3, 21.00 for K_1, 15.35 for α-tocopherol, 35.45 for δ-tocopherol, 25.65 for retinol and 13.40 for α-tocopherol acetate.

For fatty acids in HCC samples the average areas % were 1.29 for C15:0, 15.45 for C16:0, 2.42 for C18:0, 35.78 for C18:1, 40.02

Table 1. The levels of metals in HCC samples.

Metal	Added ($\mu g\ g^{-1}$)	Found ($\mu g\ g^{-1}$)	Recovery (%)
Fe	-	62.2	-
	5.0	69.2	103
Zn	-	14.5	-
	2.0	16.8	102
Cu	-	4.84	-
	1.00	5.72	98
Ni	-	1.25	-
	0.20	1.39	96
Co	-	0.42	-
	0.20	0.61	98

Table 2. The levels of vitamins in HCC.

Concentration	Vitamin D_2	Vitamin D_3	Vitamin K_1	α-Tocopherol	δ-Tocopherol	Retinol	α-Tocopherol acetate
(μg g^{-1})	2.50	9.95	21.00	15.35	35.45	25.65	13.40

Table 3. Chemical composition of fatty acid from HCC by GC.

Fatty acid	C15 : 0	C16 : 0	C18 : 0	C18 : 1	C18 : 2	C18 : 3
Relative % peak area	1.29	15.45	2.42	35.78	40.02	5.04

for C18:2 and 5.04 for C18:3. The C18:1/C18:0 ratios in HCC were 14.78. Oleic and linoleic acids were the major fatty acids constituting about 76% of the total fatty acids. According to Jham et al. [21], the average area % of fatty acids in Brazil coffee was 38.2 for C16:0, 8.3 for C18:0, 8.7 for C18:1, 38.5 for C18:2, 1.6 for C18:3 and 3.6 for C20:0 . These results show that the levels of trace elements and fatty acids in HCC are in agreement with other kinds of coffee in literature.

Conclusions

There are large numbers of *Pistacia* species in the flora of Turkey. Some of these species are used as medical plants worldwide. Therefore, an investigation of the vitamin, trace element and fatty acids content in fruit of these plants is very important in terms of nutrition. The present study used some instruments (GC, HPLC and ICP-OES) to determine the vitamin D_2, D_3, K_1, α-tocopherol, δ-tocopherol, retinol, α-tocopherol acetate, Cu, Zn, Fe, Ni, Co and compositions fatty acids in HCC beverage of *Pistacia terebinthus* fruit, common in Elazig. We consider similar studies provide an important contribution to creating alternative sources of drink to nutrition quality and, thus to the coffee customer and production. The HCC coffee was found to be rich in vitamins, unsaturated fatty acids and trace elements, suggesting that they may be valuable for drink uses. The data may also be useful for the evaluation of nutritional information.

References

[1] Baytop, T. 1984. Treatment with plants in Turkey. Istanbul University Publication No. 3255. Istanbul University, Istanbul.

[2] Matthaus, B. and Özcan, M. 2006. Quantification of fatty acids, sterols and tocopherols turpentine (*Pistacia terebinthus* Chia) wild growing in Turkey. Journal of Agriculture and Food Chemistry **54**:7667–7671.

[3] Özcan, M. 2004. Characteristics of fruit and oil of terebinth (*Pistacia terebinthus* L.) growing wild in Turkey. Journal of the Science of Food and Agriculture **84**:517–520.

[4] Walheim, L. 1981. Western Fruit and Nuts. HP Books, 166 p.

[5] Tekin, H., Arpaci, S., Atli, H.S., Karaca, R., Mart, C. and Turan, K. 1995. Pistachio Nut Growing Techniques. Pistachio Research Institute, Pub. No.4, Gaziantep.

[6] Tutin, T. G., Heywood, V. H., Burgess, N. A., Moore, D. M., Valentine, D. H., Walters, S. M. and Webb, D. A. 1968. Flora Europaea. Cambridge University Press, Cambridge, UK, 237 p.

[7] Kawashty, A., Mosharrata, S. A. M., El-Gibali, M. and Saleh, N. A. M. 2000. The flavonoids of four *Pistacia* species in Egypt. Biochem. Syst. Ecol. **28**:915-917.

[8] Marner, F. J., Freyer, A. and Lex, J. 1991, Triterpenoids from gum mastic, the resin of *Pistacia lentiscus*. Phytochemistry **30**:3709-3712.

[9] Christie, W. W. 1990. Gas Chromatography and Lipids: A Practical Guide. The Oily Press, Ayr, 307 p.

[10] Perales, S., Delgado, M. M., Alegria, A., Barbera, R. and Farre, R. 2005. Liquid chromatographic determination of vitamin D_3 in infant formulas and fortified milk. Anal. Chim. Acta **543**:58-64.

[11] Kienen, V., Costa, V. F., Visentainer, J. V., Souza, N. E. and Oliveira, C.C. 2000. Development of a green chromatographic method for determination of fat-soluble vitamins in food and pharmaceutical supplement. Talanta **75**:141–146.

[12] Ciftci, H. 2008. Determination of cobalt in some plants by reversed-phase high-performance liquid chromatography. Asian Journal of Chemistry **20**(4):3025-3032.

[13] Ciftci, H. and Bakal, U. 2009. The effect of lipoic acid on macro and trace metal levels in living tissues exposed to oxidative stress. Anti Cancer Agents in Medicinal Chemistry **9**(5):560-568.

[14] Sebrel, W. H. J. and Harris, R. S. 1971. The Vitamins: Chemistry, Physiology, Pathology, Methods. 2nd edn. Academic Press.

[15] Kralovánsyki, U. P. and Martné Schill, J. 1994. Adatok a kendermag beltartalmi és használati értékeléséhez. Növénytermelés **43**:436-446.

[16] Then, M., Simándi, B., Illés, V., Preneczki, S., Daood, H. and Szentmihályi, K. 1996. Különböző kinyerésű zsíros olajok biológiailag aktív anyagainak összehasonlító vizsgálata. Olaj, Szappan, Kozmetika **45**:75-82.

[17] Karatas, F., Cansiz, A., Cifci, H. and Serbetci, Z. 2007. The determination of folic acid level in some foodstuffs. Chemistry of Natural Compounds **3**:310-311.

[18] Bakoglu, A., Bagci, E. and Ciftci, H. 2009. Fatty acids, protein contents and metal composition of some feed crops from Turkey. Journal of Food, Agriculture & Environment **7**(2):343-346.

[19] Ciftci, H., Olcucu, A., Ozkaya, A. ad Ciftci, T. 2009. The optimization of analytical parameters for determination of iron, nickel and cobalt in plants with RP-HPLC. Asian Journal of Chemistry **21**(5):2643-2652.

[20] Onianwa, P. C., Adetola, I. G., Iwegbue, C. M. A., Ojo, M. F. and Tela, O. O. 1999. Trace heavy metals composition of some Nigerian beverages and food drinks. Food Chemistry **66**:275-279.

[21] Jham, G. N., Berhow, M. A., Manthey, L. K., Palmquist, D. A. and Vaughn, S. F. 2008. The use of fatty acid profile as a potential marker for Brazilian coffee (*Coffea arabica* L.) for corn adulteration. J. Braz. Chem. Soc. **19**(8):1462-1467.