The Effect of solvent extraction techniques on fatty acid composition of pistachio oil

Anna Abdolshahi, seyed ali Mortazavi, sahar naibandi
Department of Food Science and Technology
Ferdowsi university of Mashhad
Mashhad, Iran
abdoshahianna@yahoo.com
Morteza1937@yahoo.com

Anna Abdolshahi, Ali Akbar Shaebani
Biotechnology research center
Semnan University of Medical Sciences
Semnan, Iran
aashaebani@yahoo.com

***Abstract*— Pistachio oil has important nutritional and therapeutic properties because of high concentration of essential fatty acids. The extraction method used to obtain natural compounds from row matter is critical for product quality and especially for protection of their nutrition value. This study was conducted to compare fatty acid composition of pistachio oil extracted by two conventional procedures: soxhlet extraction (Sox) and maceration. Different solvents: *n*-Hexan (Hx), dichloromethane (DCM), ethyl acetate (EtAc) and ethanol (EtOH) in term of polarity index were used. The highest unsaturated fatty acid content (88.493%) was obtained by Sox with EtAc. Sox method extracted the most concentration of oleic and linolenic acids (51.99% and 0.385% respectively). Although linoleic acid had higher concentration (36.32%) in mac method.**

Keywords: ***fatty acid composition, gas chromatography, pistachio oil, solvent extraction.***

I. INTRODUCTION

Pistachio nut (Pictacia vera L.) now serve as one of the most popular tree nuts in the world (Kashani nejad et al, 2003). It is known to have high nutritional value. The pistachio kernels are good source of fat, especially low in saturated fatty acids (SFAs) and high in unsaturated fatty acids (UFAs) that are essential in the human diet. Essential fatty acids include linolenic, linoleic and oleic acids which have been shown to reduce the level of cholesterol in the blood and have cardioprotective effects (Satil et al, 2003). According to the California Pistachio Commission, the pistachio contains more magnesium and copper than milk, bread and potatoes.

Iran is the largest importer and exporter of pistachio nuts. In 2011 Iran produced more than 220,000 tons pistachio nuts (Agriculture ministry of Iran). While a part of the harvested pistachios has no or only low marketable appearance and quality, it still has nutrition value. The oil of this kind of pistachio nut has a good market and can be extracted and sold to increase the added value of pistachio cultivation (Sheibani and Ghaziaskar, 2008).

The pistachio kernel oil extraction results in an oil rich in oleic and linolenic acids so it has important therapeutic properties and it is nutritionally attractive due to the fatty acid composition. Oleic acid is mono unsaturated fatty acid (MUFA), essential in human nutrition and helps reducing triglycerids, LDL-cholesterol, total cholesterol and glycemic index (Specher, 1981). Also the increase in stability over oxidation of vegetable oil (Morrison and Boyd, 1981, IUPAC, 2007) is attributed to oleic acid. Essential fatty acids are necessary in human diet for growth maintenance and reproduction. The linoleic acids is an essential fatty acid from omega-3 group (turatti, 2000) and it is very important for development and maintenance of the nervous system and the physiological functions in humans, since it reduces total and LDL-cholesterol levels (Fagundes, 2002).

The extraction techniques used to obtain high aggregate value compounds from natural products are critical for product quality (Mezzomo et al, 2010).There are several well established conventional organic solvent extraction procedures, such as Folch, Bligh & Dyer, Soxhlet (Sox), maceration (Mac), Pont, pentane digestion, supercritical fluid extraction. Authors studied the influence of extraction time, polarity of solvent, amount of sample, flushing volume and extraction temperature in the extraction yield and fatty acid composition. Results obtained indicated that the solvent polarity was the most significant variable in these studies (Ruiz-Rodriguez et al, 2010). The Evaluation of extraction techniques on fatty acid composition in different food matrices have been studied (table1).

Table 1 . Some extraction techniques employed in the extraction of fatty acids from different food matrices.

Food lipid	Extraction techniques	Refernces
Cereal, egg yolk	Sox, AHE, Folch	Toschi et al, 2003
Pistachio	Sox, PFE	Sheibani and Ghaziaskar, 2008
Peach almond	Sox,Mac, SFE, HD	Mezzomo et al, 2010
Fish	Sox, SFE	Sahena et al, 2010
Chicken	ASE, Folch	Schafer, 1998
Alga	Blight&Dyer, Folch	Kumari et al, 2011

Oleaginous seed	SFE, Sox	Suo & Cao, 2010
Different foods	Soxhlet, HD, Mac	Riverchon, 2006.,Weinhold, 2008

- Soxhlet(Sox), Automated acid hydrolysis extraction (AHE), Pressurized fluid extraction (PFE), Hydro distillation (HD), Supercritical fluid extraction (SFE).

Accurate determination of fatty acid composition in oil extracted by different extraction techniques could evaluate the efficiency of extraction techniques in obtaining high essential fatty acids. Therefore, the aim of this work was the extraction of pistachio oil by means of two conventional extraction techniques including Sox and Mac using different solvents (in term of polarity index) and the efficiency of extraction methods were evaluated through fatty acid composition.

II. METHODS AND MATERIALS

A. *Chemicals and sample*

n- Hexan, dichloromethane, ethyl acetate, ethanol, methanol, potassium hydroxide (GC grade) were supplied by Merck Co, Darmstadt, Germany. The Pistachio (pistacia vera L.) in Akbari cultivar was obtained from Pistachio Research Institute of Damghan area in Iran. All experiments were done in food quality control laboratory of Semnan University of medical sciences in Iran during January to March in 2011.

B. *Sample preparation for extraction*

The dry pistachio kernels were milled and passed through a sieve with mesh size of 16 and dried at 70 to a constant weight. They were kept within a sealed bag in a refrigerator until they were used. The pistachio kernel particle size is important to facilitate analyses mass transfer during the extraction process and to obtain reproducible extraction yield.

C. *Extraction techniques*

Soxhlet extraction (sox) was performed according to Mezzomo et al (2010). In this regard 5 g of sample (pistachio powder) were packed in a cartridge placed inside the 250 ml extractor device. The sample was submitted to Sox for 6 h at the solvent boiling point temperature. The proportion of solvent was 150 ml in a 250 ml soxhlet flask. The extraction was performed at least in duplicate, with different solvent: n-hexan (Hx) , dichloromethane (DCM), ethyl acetate (EtAc) and ethanol (EtOH) with polarities of 0 , 3.1 , 4.4 , and 5.2 respectively (Byers ,2007).

The maceration (Mac) method consists of a cold Maceration of 50 g of the pistachio kernel particles in 200 ml of each solvent Hx, DCM, EtAc and EtOH as described by Mezzomo et al (2010) with some modifications and was carried out at least in duplicate .

D. *Separation of the mixture oil/solvent*

The extract obtained by two methods were separated at reduced pressure by pressure evaporating the solvents used a rotary evaporator (Buchi ,R-210/215,Switzerland) with vacuum control and thermostatic bath (Buchi,V-700,Switzerland) .

E. *Fatty acid composition*

The fatty acid composition of the pistachio oil was determined by gas chromatography–flame ionization detector (GC-FID) according the Ce1f-96 Method of AOCS (2002). The oil samples were converted to methyl esters by vigorous shaking of a solution of oil in n-hexan (7ml) with 2 ml methanolic potassium hydroxide. The tube of solution was placed in a water bath (55) for 20 min and was shacked each 5 minutes. After decantation during final 5 min, the 1 of upper layer was injected on the GC-FID (6890N, Agilent, US) equipped with BPX70 capillary column (120m × 0.25 mm × 0.25m). The analysis followed the conditions: initial temperature of 16 and final temperature of 220 , with heating rate of 18 /min, detector temperature of 250, injector temperature of 230, nitrogen as carrier gas at pressure of 42.12 psi.

F. *Statistical analysis*

The results were statistically evaluated by a one–way analysis (ANOVA) on completely randomized design by SPSS program version 17.0.1 . The significant difference at level of 5% ($p<0.05$) were analyzed by the Dunkan test.

III. RESULTS

The chromatogram of one pistachio oil sample is shown in fig1. Obviously, in pistachio oil, oleic, linoleic and palmitic acids have higher concentration rather than other fatty acids.

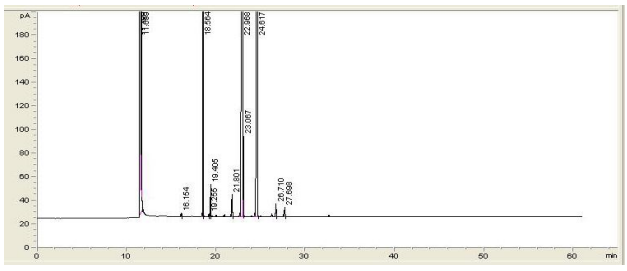

Fig.1. Chromatogram of fatty acids presented on pistachio oil obtained by GC-FID

The composition profiles for saturated and unsaturated fatty acids of pistachio oil obtained by two extraction methods (Sox and Mac) detected by GC-FID analysis, are presented in tables 1 and 2 respectively. The saturated fatty acids (SFA) represent a great variety of total fatty acid content (relative concentration) for pistachio oil extracts, ranging from 11.506 % for Sox-EtAc sample to 13.70 Mac-EtOH sample .

From table 2 it is possible to determine that palmitic and stearic acids are the most representative saturated fatty acids for pistachio oil, in relative concentration. Myristic and arachidic acids are present only in traces, for all extracts studied, with relative concentration lower than 0.9 (except for arachidic acid content of Sox-Hx).

Table 3, shows that pistachio oil contains mainly unsaturated fatty acids (UFA), with relative concentration ranging from 86.366% for oil sample obtained by Mac-EtOH, to 88.493% for Sox-EtAc, sample.

The UFA profiles indicated that oleic and linoleic acids were the major UFAS in all oil pistachio samples obtained by two methods. Comparing the extraction techniques to obtain oleic acid it was observed oleic acid content in sox method by different solvents were significantly different ($P< 0.05$). The highest and the lowest content of oleic acid were obtained in sox-EtAc sample (53.263%) and Mac-EtOH sample (43.698 %) respectively. Regarding linoleic acid the obtained range was 41.756 % in Mac-EtoH sample and 33.948 % in Sox-EtAc sample. the Palmitoleic and linolenic acids were presented in low quantities (less than 0.96 %) for all samples studied. handling, allowing the influence of solvent polarity for oil fractionation.

As it can be seen Sox and Mac methods respectively obtained the highest UFA and SFA contents.

This can be explained by the use of high temperature and reflux in Sox extraction overcoming the polarity effect for the UFAs extraction. Regarding the effect of solvent polarity index (PI) in conventional technique on fractionation of saturated and unsaturated fatty acids it is observed from the fatty acid concentration show in table 2 and 3 respectively.

Table2. Saturated fatty acid content of pistachio oil obtained by Soxhlet (Sox) and maceration (Mac) techniques

.Extraction method	Solvent[A]	Saturated fatty acids (%)				
		Myristic	Palmitic	Stearic	Arachidic	Total
Sox	Hx	0.1020 ^{c} ± 0.02	10.515^{a} ± 0.1	1.535^{b} ± 0.04	1.152^{c} ± 0.04	13.304 ± 0.3
Sox	DCM	0.0801^{bc} ± 0.01	10.428 ^{a}± 0.02	1.0245^{ab} ± 0.02	0.548^{a} ± 0.05	12.080 ± 0.1
Sox	EtAc	0 ^{a}	9.9970^{a} ± 0.08	0.9730^{ab} ± 0.05	0.5360^{a} ± 0.1	11.506 ± 0.5
Sox	EtOH	0.0751^{bc} ± 0.2	10.0477^{a} ± 0.3	0.9720^{ab} ± 0.01	0.5410^{a} ± 0.01	11.636 ± 0.3
Mac	Hx	0.0730^{bc} ± 0.6	10.0030^{a} ± 0.2	0.9720^{ab} ± 0.01	0.5250^{a} ± 0.02	11.57 ± 0.5
Mac	DCM	0.0804^{bc} ± 0.1	10.373^{a} ± 0.02	0.9730^{ab} ± 0.08	0.5460 ^{a}± 0.01	12.00 ± 0.2
Mac	EtAc	0.0754 ^{bc} ± 0.1	9.989^{a} ± 0.3	0.9730^{ab} ± 0.04	0.5400^{a} ± 0.07	11.60 ± 02
Mac	EtOH	0 ^{a}	11.811^{b} ± 0.1	0.9110^{a} ± 0.1	0.9110 ^{b}± 0.01	13.70 ± 0.1

[A] Hx, hexan; DCM, dichloromethane; EtAc, ethyl acetate; EtOH, ethanol.
$^{a, b, c, d}$ Same letter indicates no significant difference at level of 5% ($p < 0.05$).

Table3. Unsaturated fatty acid content of pistachio oil obtained by Soxhlet (Sox) and maceration (Mac) techniques.

Extraction method	SolventA	Unsaturated fatty acids (%)				
		Palmitoleic	Oleic	Linoleic	Linolenic	Total
Sox	Hx	0.914a ± 0.01	49.850b ± 0.02	35.478d ± 0.2	0.379b ± 0.4	86.621 ± 0.02
Sox	DCM	0.925a ± 0.3	51.939c ± 0.03	34.670c ± 0.3	0.391b ± 0.2	87.925 ± 0.06
Sox	EtAc	0.899a ± 0.02	53.263f ± 0.2	33.948a ± 0.5	0.383b ± 0.09	88.493 ± 0.08
Sox	EtOH	0.914a ± 0.04	52.904e ± 0.06	34.0788b ± 0.01	0.392 b± 0.01	88.289 ± 0.2
Mac	Hx	0.9125a ± 0.01	52.875e ± 0.02	34.168b ± 0.3	0.398 b± 0.12	88.353 ± 0.08

Mac	DCM	0.955a ± 0.07	52.000 d± 0.06	34.591c ± 0.8	0.389b ± 0.45	87.935 ± 0.1
Mac	EtAc	0.923a ± 0.03	52.300d ± 0.15	34.738c ± 0.14	0.405b ± 0.3	88.400 ± 0.05
Mac	EtOH	0.912a ± 0.05	43.698a ± 0.1	41.756f ± 0.1	0.000a	86.366 ± 0.03

A Hx, hexan; DCM, dichloromethane; EtAc, ethyl acetate; EtOH, ethanol.
a, b, c, d Same letter indicates no significant difference at level of 5% ($p < 0.05$).

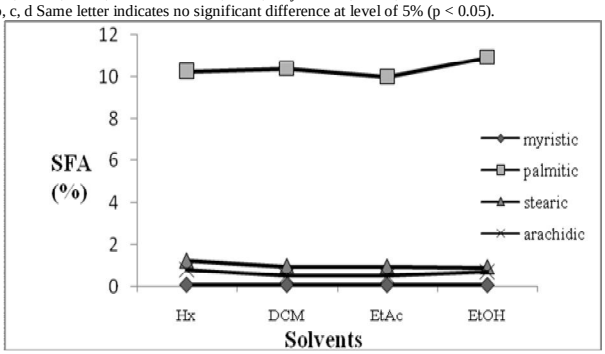

Fig. 1. Comparing solvents on fractionation of saturated fatty acids.

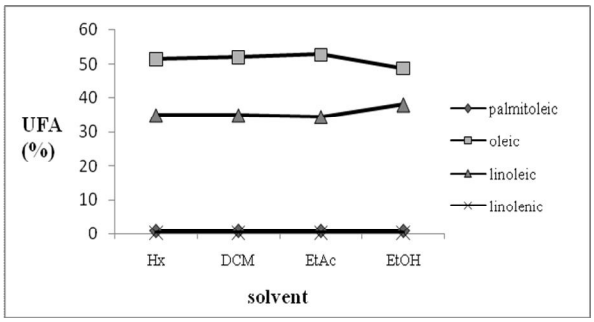

Fig. 2. Comparing solvents on fractionation of unsaturated fatty acids.

These results are statistically different in some cases but no significant correlation was found between PI and FAs in this research. Figure 1 and 2 show the comparison among different extraction techniques on fatty acid composition of pistachio oil.

IV. Discusion

Our results about differences between fatty acid concentration in different oil extraction techniques regarding method and solvent are in agreement with others researches like: Kumari et al,(2011) in extraction of red-green-brown macro alga; Schafer (1998) in oil extraction of animal and plant, texture; Suo and Cao (2010) extraction of Microula sikkimensis seed oil; Hui Tan et al,(2009) extraction of palm oil, Sheibani and Ghaziasgar (2008) in extraction of pistachio oil , Mezzomo et al,(2010) extraction of peach almond oil; Sahena et al,(2010) in oil extracted from different parts of Indian mackerel (Rastrelliger kanagurta).

In order to obtain high quality pistachio oil regarding unsaturated fatty acid especially oleic acid content, the Sox extraction method can well be applied. Sheibani and Ghaziaaskar (2008) compared methods of Sox, SFE and PFE in extraction of pistachio oil. They found the oil main fatty acid composition and the yield extracted by PFE and Sox were similar.

Pistachio nut represents an important raw material to obtain edible oil with high content of unsaturated fatty acids. The resulting oil from two conventional extraction methods showed oleic and linoleic acids as the major compounds. The Sox method provided the highest UFA, mainly due to the high operational temperature, solvent recycle and solvent /solute interactions. Mac techniques showed the lowest UFA values despite the highest concentrations of saturated fatty acids. The concentration of fatty acids and the color of oil due to use of different solvents in pistachio oil extraction were objected. So in order to obtain pistachio oil with high quality we must pay attention to the technique of oil extraction.

Conclusion

The saturated and unsaturated fatty acid profile of pistachio oil extracted by Soxhlet and maceration method was not different but its content was statistically different.

The highest unsaturated fatty acid content was obtained by Sox with EtAc.

The highest saturated fatty acid content was obtained by mac with EtOH.

Sox method could extract the highest concentration of oleic acid and linolenic acid (51.99 % and 0.385 % respectively).

Mac method could extract the highest concentration of linoleic acid (36.22 %).

Acknowledgements

The authors wish to tank Food and Drug Administration and quality control laboratory of Semnan University of Medical Sciences for helping in doing experiment.

References

[1] Agar, I.T., Sarmiento, C.,Garces, R., Kaska, N., Kafkas, et al. 1995. Compositional changes of fatty acids during the development of embryo in pistacia vera. Acta-Horticulturae 491:405-401

[2] Agar, I.T.1998. lipid characteristics of Turkish and Iranian pistachio kernels. Acta- Horticulturae 470:378-384

[3] Anonymous, http://www.foss.dk/solutions/productsdirect/soxtecsystems. aspx,2006.

[4] AOAC Of fi cial Method 947.07 Fatty Acids (Wa ter-Insoluble) (WIA) in But ter (Polenske Num ber) Gravimetric MethodFirst Ac tion 1947 Fi nal Ac tion

[5] AOAC Of fi cial Method 996.01Fat (To tal, Sat u rated, Un sat u rated,and Monounsaturated) in Cereal Products Acid Hy dro ly sis Cap il lary Gas Chro mato graphic Method First Ac tion 1996

[6] Arena,E., Campisi, S., Fallico,B., Maccarone,E, 2007, Distribution of fatty acids and phytosterols as a criterion to discriminate geographic origin of pistachio seeds, Food Chemistry 104 ,403–408

[7] Arranz,S., Cert,R., Pérez-Jiménez,J., Cert,A.,Saura-Calixto,F.2008. Comparison between free radical scavenging capacity and oxidative stability of nut oils. Food Chemistry 110 , 985–990

[8] Aslan, M., Orfan, I., and Sener, B. 2002. Comparison of the seed oils of pistacia vera L.of different origins with respect to fatty acids. Int Journal of Food Science and Technology 37:333-335

[9] Aslan, M., Orhan,I.2006. Fatty acid patterns of waste parts of Turkish Pistacia Vera L. tree. Actabio CRACOVIENSIA Series Botanica 48/2: 117–120

[10] Ayfer, M. 1973. Investigation on the quantity of oil and the kind and properties of fatty acids in the fruits of some important pistacia species and the possibilities of their use in biochemical systematic. Yalova Bache Kulturleri Arastirma VE Egitim Merkezi Dergisi 6 (1-2):25-40

[11] Azlan, A., Nagendra Prasad, K., Khoo, H., Abdul-Aziz, N., Mohamad, A., Amin Ismail, Zulkhairi Amom. 2010. Comparison of fatty acids, vitamin E and physicochemical properties of Canarium odontophyllum Miq. (dabai), olive and palm oils. Journal of Food Composition and Analysis 23 , 772–776

[12] Bellomo,M.G.,Fallico,B.2007. Anthocyanins, chlorophylls and xanthophylls in pistachio nuts (Pistacia vera) of different geographic origin. Journal of Food Composition and Analysis 20 ,352–359

[13] Cao, Y., Suo, Y. 2010. Extraction of Microula sikkimensis seed oil and simultaneous analysis of saturated and unsaturated fatty acids by fluorescence detection with reversed-phase HPLC. Journal of Food Composition and Analysis 23 , 100–106

[14] Combe,N. 2002. Bioavailability of fatty acid and population reference intake. Lipids 9(2/3):135-138

[15] Costa, P.,Costa, F. A.,Lopes. P. A., Alfaia C.M,. Bessa, R.J.B.,.Roseiro L.C. 2011. Fatty acid composition, cholesterol a-tocopherolof Barrosa˜-PDO veal produced in farms located in lowlands, ridges and mountains. Journal of Food Composition and Analysis

[16] Daneshrad, A. 1987. The structure of the glycerides of pistachio kernel oil. Journal of the American oil Chemists Society 55(3):317-319

[17] Hidalgo,F.J., Zamora,R.2006. Peptides and proteins in edible oils: Stability,allergenicity, and new processing trends. Trends in Food Science & Technology 17 56–63

[18] Kamangar, T., and Farsam, H. 1977. Chemical composition of pistachio kernels of various Iranian origin. Journal of Food Science 42:1135-1138

[19] Kumari, P., Reddy .C.R.K., Jha, B. 2011. Comparative evaluation and selection of a method for lipid and fatty acid extraction from macroalgae. Analytical Biochemistry 415, 134–144

[20] Kylene Rodriguez. 2005. Nutritional Differences of Pistachio Nuts and Pistachio Butter. NTRS 519 Summer

[21] Lee a, K., Yun a, I, J., Hee Kim a, Lim a, Hamb, H., Eumc,K., Jin Ho Joo.2011. Amino acid and fatty acid compositions of Agrocybe chaxingu,an edible mushroom. Journal of Food Composition and Analysis 24 , 175–178

[22] Mapekula, M., Chimonyo, M., Mapiye, C., Dzama, K. 2011. Fatty acid, amino acid and mineral composition of milk from Nguni and local crossbred cows in South Africa. Journal of Food Composition and Analysis 24 , 529–536

[23] Mezzomo,N., Mileo,B., Friedrich,M.T., Martínez,J., Ferreira, S.R. 2010. Supercritical fluid extraction of peach (Prunus persica) almond oil: Process yield and extract composition. Bioresource Technology 101 , 5622–5632

[24] Miraliakbari, H., Shahidi, F.2008. Antioxidant activity of minor components of tree nut oils. Food Chemistry 111 , 421–427

[25] Okay, Y.2002. The Comparison of some Pistachio Cultivars Regarding their Fat, Fatty acids and Protein Content. Gartenbauwissenschaft, 67 (3). S. 107–113

[26] Pe´rez-Jime´nez, J., Arranz, S.,Tabernero,M. 2008. Updated methodology to determine antioxidant capacity in plant foods, oils and beverages: Extraction, measurement and expression of results. Food Research International 41 , 274–285

[27] Prandini, A., Sigolo, S., Piva, G.2011. A comparative study of fatty acid composition and CLA concentration in commercial cheeses. Journal of Food Composition and Analysis 24 , 55- 61

[28] Richter, E., Shawish, K., Martin R.L. Scheeder, Paolo C. Colombani. 2009. Trans fatty acid content of selected Swiss foods: The TransSwissPilot study. Journal of Food Composition and Analysis 22, 479–484

[29] Ruiz-Rodriguez,A., Reglero,G., Ibanez, E.2010. Recent trends in the advanced analysis of bioactive fatty acids. Journal of Pharmaceutical and Biomedical Analysis 51 ,305–326

[30] Sahena, F., ,. Zaidul I.S.M S. Jinap a, A.M. Yazid a, A. Khatib a, N.A.N. Norulaini. 2010. Fatty acid compositions of fish oil extracted from different parts of Indian mackerel (Rastrelliger kanagurta) using various techniques of supercritical CO2 extraction. Food Chemistry 120 , 879–885

[31] Satil, F., Azcon, N., and baser, K. H. C. 2003. Fatty acid composition of pistachio nuts in Turkey. Chemistry of Natural Compounds 39:322-324

[32] SchaÈfer, K.1998. Accelerated solvent extraction of lipids for determining the fatty acid composition of biological material. Analytica Chimica Acta 358, 69-77

[33] Seferoglua,S.,Seferoglua,H.G.,Tekintasa,F.E.,Balta,F,2006. Biochemical composition influenced by differentlocations in Uzun pistachio cv. (Pistacia vera L.) grown in Turkey. Journal of Food Composition and Analysis 19 ,461–465

[34] Sheibani,A.,Ghaziaskar,H. 2008. Pressurized fluid extraction of pistachio oil using a modified supercritical fluid extractor and factorial design for optimization. LWT 41 ,1472–1477

[35] Tan, H., Ghazali, H.m., Kuntom, A., Tan,C., Ariffin, A .2009. Extraction and physicochemical properties of low free fatty acid crude palm oil. Food Chemistry 113, 645–650

[36] Velasco,J., Andersen,L.,Skibsted L. 2004. Evaluation of oxidative stability of vegetable oils by monitoring the tendency to radical formation. A comparison of electron spin resonance spectroscopy with the Rancimat method and differential scanning calorimetry. Food Chemistry 85 , 623–632

[37] Yang, J., Liu,R., Halim., L. 2009. Antioxidant and antiproliferative activities of common edible nut seeds. LWT - Food Science and Technology 42 ,1–8

[38] Yang, J.2009. Brazil nuts and associated health benefits: A review. LWT - Food Science and Technology 42 ,1573–1580