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Differences in omega-6/omega-3 ratio and polyunsaturated fatty acid content in five Iranian walnuts (*Juglans regia*) cultivars

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Race and environmental factors like nutrition and life style in each location are known that affect the incidence of myocardial infarction (Ilali and Taraghi 2010), thus research has been dedicated into determining the food stuffs that may play roles in the induction or prevention of coronary heart disease (CHD). While numerous studies have indicated the adverse effects of consuming saturated fatty acid (SFAs) on the development of CHD, some types of unsaturated fatty acids (USFAs), particularly, the polyunsaturated fatty acids (PUFAs), such as alpha linolenic acid C18:9,12, ω_3, have been found to possess protective effects against this disease (Simon *et al.* 1995). Meanwhile, PUFAs in the diet that consist mainly of ω_3 and ω_6 fatty acids, have diverse effect on CHD; contrary to ω6 fatty acids, ω3 fatty acids decrease the risk of CHD(Zavar-Reza *et al.* 2006).

Walnut (*Juglans regia* L.), rich in both monounsaturated fatty acids (MUFAs) such as oleic acid, and PUFAs including both ω_3 (α-linolenic acid) and ω6 (linoleic acid) fatty acids, but a low ratio of ω_6/ω_3 has a unique nutritional value (Muñoz *et al.* 2001). Studies show daily intake of 40g walnut in breakfast has beneficial effect on HDLc, tChol, LDLc and TG of obese females (Mushtaq *et al.* 2009).

Fatty acid composition seems to vary significantly among different walnut varieties or cultivars and also is related to location of farming (Li *et al.* 2007). Ali *et al.* (2010), studied 6 cultivars of walnut that grow in Pakistan and reported that range of total fat of these walnut kernels were from 63.54–69.92%. Although the main source of walnut in literatures is Persia (Nasir *et al.* 2001) which includes Iran as well, and Iran is the third producer of walnut in the world (Balta *et al.* 2007). A few studies have been conducted so far on the characterization of the Iran's walnut fatty acid composition. The diversity of fatty acid composition is due to both genetic and environmental factors and because walnut production is under relatively high genetic control (Dogan *et al.* 2005) the results of this study will produce useful information about the nutritional properties of the local walnut cultivars and may help in designing strategies that maximize desirable quality and utility of walnut germplasm. The present study was an attempt to determine the fatty acid composition of just five of the many Iranian walnut cultivars.

We evaluated five fatty acids in five cultivars of Iranian walnut, called Soozani, Kaghazi, Sangi, Kalaghi and Makuei, collected during the 2006 crop from five different cultivars grown in Hamadan, west of Iran. The measured fatty acids were palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1)(ω_9), linoleic acid (18:2)(ω_6) and linolenic acid (18:3) (ω_3). Air-dried walnut seeds (100 g) were ground in a coffee blender and extracted with 500 ml petroleum ether in Soxhlet apparatus for 48 hr. After removing the solvent and filtering, the weight and volume of remained yellow oil was measured and stored in the dark. For preparing fatty acid methyl esters (FAMEs), 0.5g oil was added to 1.5 ml $NaOCH_3$/methanol (1%) and sonicated for 5 min and heated at 40°C for 1 hr. After adding 100 µl HCl (0.1N), extraction with 3ml n-Hexane was performed twice. The hexane (FAMEs) layer was removed and dried under nitrogen flow. FAMEs were analyzed using a Gas Chromatography (GC) with a 50 m× 0.25 mm Cp-Sil 88 fused capillary column (Chrompack). The FAMEs were identified by comparison with authentic standards as well as an internal standard (butyl linolenate); the peak areas were integrated as relative weights with the use of microprocessor.

Statistical analysis was performed using ANOVA, followed by Scheffe test for non-significant homogeneity of variances and Dunnett T_3 for significant homogeneity (SPSS 11.5).

The total oil content of the five cultivars of walnut

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Table 1 Fatty acid composition of oils extracted from five Iranian walnut cultivars

Fatty acid	Walnut cultivars (%)						Iranian cultivar
	Soozani	Kaghazi	Sangi	Kalaghi	Makuei	P value	average [%]
Fatty acids			*SFA*				
Palmitic acid(C_{16}:0)	9.10	7.95↓↓↓	8.59	**9.22**↑↑↑	8.65	<0.001	8.78
Stearic acid (C_{18}:0)	4.53	4.59	4.66	4.69↑↑↑	4.23???	<0.02	4.54
∑ SFA	13.63	12.54↓↓↓	13.25	**13.91**↑↑↑	12.88	<0.001	13.24
			MUFA				
Oleic acid(C_{18}:1;9,ω_9)	23.3???	24.33	26.68	**27.17**↑↑↑	23.43	<0.03	24.98
∑MUFA	23.3???	24.33	26.68	**27.17**↑↑↑	23.43	<0.03	24.98
			PUFA				
Linoleic acid (C_{18}:2;9,12,ω_6)	37.99	37.00	36.06↓↓↓	37.95	**38.08**↑↑↑	<0.01	37.42
Linolenic acid (C_{18}:3;9,12,15,ω_3)	25.03	**26.00**↑↑↑	24.00	20.90↓↓↓	25.59	<0.05	24.31
∑ PUFA	63.33	63.11	60.06	58.90↓↓↓	**63.68**↑↑↑	<0.001	61.82
			USFA(MUFA+PUFA)				
∑ USFA	79.79↓↓↓	**87.45**↑↑↑	86.74	86.08	87.11	<0.001	85.43
			Ratio				
USFA/SFA ratio	6.38	6.94↑↑↑	6.57	6.20↓↓↓	6.80	<0.05	6.58
PUFA/MUFA ratio	2.77↑↑↑	2.58	2.26	2.17↓↓↓	2.73	<0.03	2.50
ω_6/ω_3 ratio	1.52	1.44↓↓↓	1.47	1.81↑↑↑	1.50	<0.03	1.55

↑↑↑ the most percent, ↓↓↓ the lowest percent, Monounsaturated fatty acid = MUFA, Omega-6 = ω_6, Omega-3 = ω_3,Polyunsaturated fatty acids = PUFA, Saturated fatty acid = SFA, Unsaturated fatty acids = USFA, ∑ = summation

available in Iran ranged from 59% (Soozani) to 70% (Sangi). The fatty acid composition varied among the cultivars; qualitatively, the five predominant fatty acids found were linoleic, linolenic, oleic, palmitic, and stearic acids and the quantity of these fatty acids in the five cultivars are summarized in Table 1.

Differences between the fatty acids content of the analyzed cultivars of the Iranian walnut were small but statistically significant in most of the cases. Also according to Table 1, USFA/SFA ratio was more than 6 in all of the analyzed walnut cultivars. In all of the analyzed walnut cultivars the PUFA content was more than twice greater than MUFA content. Comparison of PUFA/MUFA ratios between the cultivars showed the average 2.50. Overall, the average percentages of different fatty acids in studied Iranian cultivars were as follows: Palmitic acid (C_{16}:0) = 8.78%, stearic acid (C_{18}:0) = 4.54%, MUFA or oleic acid (C_{18}:1,ω_9) = 24.98%, linoleic acid (C_{18}:2,ω_6) = 37.42%, linolenic acid (C_{18}:3,ω_3) = 24.31%. Also the averages of nutritionaly important ratios were: USFA/SFA 6.58, PUFA/MUFA 2.50 and ω_6/ω_3 1.55.

The CHD preventing effect of the walnut diet can be explained in part by its fat content. USFA or PUFA could help to reduce cholesterol (especially LDL-C) levels if they replaced for SFA (Zambón *et al.* 2000, Li *et al.* 2007).

In this study, the average SFA in Iranian walnut cultivars was approximately 13.21% which is lower than what reported by Zlatonov (1999) and Tsamouris (2002), and yet others have reported even lower levels of SFA in the Persian walnut, results ranging from as low as 4.37% (Feldman 2002) to 9.11% (Li *et al.* 2007).

The USFA/SFA ratio which is an important index of oily nutrient was approximately 6.57. Zlatonov (1999) and Tsamouris (2002) reported lower approximations and others have reported higher ratios (Zwarts *et al.* 1999. Amaral *et al.* 2003, Li *et al.* 2007). These differences among USFA/SFA ratios reflect the different contents of USFA and SFA in different cultivars. A high ω_6/ω_3 ratio is a risk factor for CHD. The dietary recommendation to prevent CHD is to reduce this ratio to less than 4, either by increasing the ω_3 fatty acids and/or reducing ω_6 fatty acids in the diet (Li *et al.* 2007). In the present study ω_6/ω_3 ratio ranged from 1.44 to 1.81 with an average of 1.53, and to our knowledge this is the first time such a low ω_6/ω_3 ratio has been reported. The oleic acid (MUFA) content (approximately 25%) in this study was higher than all the other reports that we could find with results ranging from trace (Tsamouris *et al.* 2002) to 19.9% (Feldman 2002) except that reported by Zwarts (1999) , a range of 14.3% to 26.1% . We found higher average percentage of linolenic acid (ω_3) (approximately 24.4%) in Persian walnut compared to other studies that have reported a value of 1.9% (Zlatonov *et al.* 1999) and 15.8% (Tsamouris *et al.* 2002).

Our investigations indicate that Iranian walnut ω_6/ω_3 ratio is closer to the natural ratio and we concluded that in relation to CHD prevention, the best of the five cultivars is Kaghazi (which is high in ω_3, USFA, USFA/SFA ratio and low in SFA and ω_6/ω_3 ratio) also the least useful cultivar is Kalaghi (high in: SFA, ω_6/ω_3 ratio and low in: ω_3, PUFA, USFA/SFA ratio, PUFA/MUFA ratio). Therefore assessment

of fatty acid composition in studied samples indicated that Kaghazi is superior walnut cultivar compared to others in CHD prevention and can be recommended for further cultivation.

SUMMARY

Results obtained in the present study show the differences in fatty acid composition among the different cultivars of Iranian walnut. The ω_6/ω_3 ratios were found to be close to the natural ratio, and therefore, consumption of Iranian walnut may be more helpful for patients with coronary heart disease. In our knowledge this is the first report of the fatty acid composition of walnut in this region of Iran. According to the found fatty acid composition of the studied walnuts we can suggest Kaghazi cultivar of walnut may be more effective in CHD prevention compared to other cultivars.

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