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Fatty acid compositions and nutritional value of six walnut (*Juglans regia* L.) cultivars grown in Iran

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ABSTRACT

Background and aims: Walnuts are good sources of polyunsaturated fatty acids (PUFA) and polyphenols which have beneficial effects such as proper growth, decreasing coronary heart disease, prevention of several kinds of cancer, anti-inflammatory and anti-mutagenic activities. In this study, the fatty acid content and antiradical activity of different walnut (*Juglans regia* L.) genotypes grown in Kolyaei region located in Kermanshah Province (Iran) were investigated.
Methods: In this experimental study, fatty acid compositions in different genotypes of Persian walnut were determined using a GC–FID coupled with a flame ionization detector. For antiradical activity, methanolic extracts of different genotypes affected on DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical. BHA (2-tert-butyl-4-methoxyphenol) was used as the reference compound.
Results: Total oil content of walnuts ranged from 63.3 to 78.5%. Oleic acid, linoleic acid, linolenic acid, palmitic acid and stearic acid contents ranged respectively from 17.9 to 28.6%, 46.9 to 56.8%, 10.8 to 13.9%, 5.5 to 7.2% and 2.0 to 3.9%, while trace amounts of other fatty acids (<0.1% each) were detected in the samples. The results demonstrated that fatty acid composition is genotype dependent and the highest amounts of PUFA (due to the high content of linoleic acid) were observed in B2 genotype. Among different studied genotypes, the extract of B2 had also the highest radical scavenging activity and therefore the lowest EC_{50}.
Conclusion: It was concluded that pellicle is a necessary protecting layer that can help to inhibit the oxidation of fatty acids.

Keywords: Walnut, *Juglans regia* L, Fatty acids, DPPH, Genotype.

Original article

INTRODUCTION

Omega-6 and omega-3 polyunsaturated fatty acids which have specific physiological functions are necessary for proper growth and development. They can improve blood lipids and have beneficial effects, such as helping to reduce the risk of cardiovascular disease. Both n-3 and n-6 fatty acids are essential fatty acids (EFA) because humans like all mammals cannot synthesize their parent fatty acids, linoleic acid (LA, C18:2, n-6) and α- linolenic acid (ALA, C18:3, n-3), therefore have to be provided by diet.[1, 2]

Walnuts are a good source of essential fatty acids and tocopherols.[3, 4]

The walnut fatty acid and tocopherol contents have been found to vary depending on different walnut cultivars and environmental conditions.[4]

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Several studies indicated that the frequent consumption of walnut can modify favorably the lipoprotein profile and decrease serum levels of total cholesterol.[1, 2] Walnut similar to other foods of plant origin is a natural source of antioxidants such as phenolic compounds which have positive influence on human health for example decreasing coronary heart disease, prevention of several kinds of cancer, anti-inflammatory and anti-mutagenic activities.[5]

The fatty acid composition of walnut fruits have been investigated in previous works, nevertheless there are several factors such as the cultivar, geographical origin, and agricultural practices that can affect its nutritional composition.[6, 7] In this study, six walnut genotypes (K1, G1, B1, K2, K3, B2) grown in Kolyaei regain of Kermanshah province of Iran were investigated in respect to their antioxidant potential and fatty acid compositions.

METHODS

Six walnut genotypes were harvested from Kolyaei region located in Kermanshah Province (Iran) at the end of September 2009. Three genotypes K1, K2 and K3 were picked from Kanikareh, two genotypes B1 and B2 were picked from Birgholi and the genotype G1were picked from Gerdakaneh. Green walnut fruits at full maturity stage were picked by hand. The walnut pellicle (brown thin skin) and kernels were separated, dried at room temperature and then were ground to a fine powder using mortar and pestle in order to investigate their antioxidant activity.

The extraction by ether method was used for measuring total oil content[8] with minor modifications. One gram of each sample was transferred into a test tube and 5 ml of ether was added. The tubes were placed in ultrasound for 5 min. The residual solvent was removed by flushing with nitrogen. This process was repeated for the second time. Weight difference of tubes before and after the experiment was considered as oil content.

Fatty acids were determined using a GC–FID (model 6890 N, Agilent Technologies, Wilmington, DE, USA) fitted with a J&W DB-Wax capillary column (30 m, 0.25 mm i.d., 0.25 mm film thickness), a split–split less injector with Agilent tapered liner (4mm id) and flame ionization detector according to the procedure of ISO (1978) [9] with minor modifications. Fatty acid methyl esters (FAMEs) were prepared using 2 mol/l KOH in methanol and n-heptane. The initial column temperature was maintained at 60 °C for 1 min and then raised at 20 °C/min to 180 °C and held for 10 min and then raised to 230 °C and held for 5 min. The injection port and detector were maintained at 250 and 260 °C, respectively. Nitrogen was used as carrier and make-up gas, at flow rates of 1.2 and 45 ml/min, respectively. The identification of fatty acids was based on authentic standards (Sigma, Chemical Co. St. Louis).

The fruits were collected and their pellicle and kernels were separated. Then they were dried at room temperature and ground to fine powder. This powder (1.5 g) was extracted with pure methanol (25 ml) in a soxhlet apparatus at 60 °C for 30 min.[10] The samples were centrifuged for 20 minutes at 4000g. The supernatant was filtered through filter paper and stored at 4°C until analysis for one week.

DPPH radical scavenging activity was determined as described by Wu et al, with slight modification.[11] Methanolic extracts (50 µl) were added to 1.5 ml 2, 2-diphenyl-1-picrylhydrazyl (DPPH) in 95% ethanol (0.15 mmol/l). The mixture was mixed vigorously and allowed to stand at room temperature in the dark for 30 min. The absorbance of the resulting solution was measured at 515 nm using a UV–visible spectrophotometer. The percentage of DPPH radical scavenging activity (RSA) was calculated using the following equation:

$$RSA\% = (A0 - A1) / A0 . 100. \quad \text{Eq. (A)}$$

Where A0 is the absorbance of control and A1 is the absorbance of the sample solutions. RSA% is the fraction of the DPPH

radicals (in percent) that eliminate in the medium after depletion of antioxidants present in the studied extracts. Also, the EC50 values defined as the concentration of an antioxidant in the reactive system necessary to decrease 50% of the initial DPPH concentration were determined and reported. BHA was used as the reference compound.

All the assays were performed in triplicate and the results presented as a mean of the three values with the standard error. The results were subjected to analysis of variance (ANOVA) and Duncan's test using SPSS 17 software at $P<0.05$.

RESULTS

The fatty acid profiles of the oils extracted from different walnut genotypes are illustrated in table 1. All the studied genotypes presented similar profile in constitution (9 fatty acids were detected) with some variations among fatty acids. In all genotypes the descending order of fatty acid contents of the extracted oils was as follows: PUFA>MUFA>SFA ranging from 57.6 to 70.2%, 17.9 to 28.7% and 7.7 to 11.1%, respectively.

Linoleic acid was the most abundant fatty acid in all genotypes ranging from 46.9% in G1 genotype to 56.8% in B2 genotype. Oleic acid was the second abundant fatty acid ranging from 17.9% (K3 genotype) to 28.6% (K1 genotype). This was followed by linolenic acid ranging from 10.8% (G1 genotype) to 13.9% (B1 genotype). In the remaining fatty acids only palmitic acid (5.5-7.2%) and stearic acids (2.0-3.9%) showed considerable amounts. The B2 genotype showed a relatively low value of total MUFA content but contained the highest amount of linoleic acid and also the highest PUFA content (70.2%).

Table 1: Fatty acid composition (percent) of oil extracted from different walnut genotypes

	Genotypes					
Fatty acids	**K3**	**K1**	**B1**	**B2**	**K2**	**G1**
C14 : 0	0.018 ± 0.001	0.014 ± 0.001	0.017 ± 0.001	0.0 ± 0.000	0.0 ± 0.000	0.055 ± 0.001
C14 : 1n5	0.007 ± 0.001	0.002 ± 0.001	0.006 ± 0.001	0.0 ± 0.000	0.0 ± 0.000	0.005 ± 0.000
C16 : 0	5.487± 0.048	6.463± 0.049	5.718± 0.095	5.559 ± 0.018	7.189 ± 0.009	5.733 ± 0.018
C16 : 1n7	0.056 ±0.001	0.047 ± 0.004	0.087 ± 0.009	0.177 ± 0.026	0.0 ± 0.000	0.113 ± 0.004
C18 : 0	2.045 ± 0.043	2.487 ± 0.007	2.485 ± 0.005	3.090 ± 0.067	3.898 ± 0.005	3.407 ± 0.004
C18 : 1n9	17.907 ± 0.562	28.618 ± 0.105	27.567 ± 0.087	19.687 ± 0.953	23.050 ± 0.039	22.027 ± 0.054
C18 : 2n6cis	53.161 ± 0.337	47.407 ± 0.138	49.679 ± 0.311	56.802 ± 0.180	52.830 ± 0.287	46.891 ± 0.079
C18 : 3n3	13.836 ± 0.057	13.634 ± 0.367	13.947 ± 0.045	13.450 ± 0.044	12.809 ± 0.029	10.764 ± 0.033
C20 : 0	0.189 ± 0.054	0.046 ± 0.013	0.068 ± 0.007	0.117 ± 0.072	0.055 ± 0.019	0.098 ± 0.053
SFA%	7.739 ± 0.146	9.01 ± 0.070	8.288 ± 0.108	8.766 ± 0.157	11.142 ± 0.033	9.293 ± 0.076
MUFA%	17.97 ± 0.564	28.667 ± 0.11	27.66 ± 0.097	19.864 ± 0.979	23.050 ± 0.039	22.145 ± 0.058
PUFA%	66.997 ± 0.394	61.041 ± 0.505	63.626 ± 0.356	70.252 ± 0.224	65.639 ± 0.316	57.655 ± 0.112
Total fat (g/100g dry weight)	63.333 ±1.258	70.867 ± 0.777	77.6 ± 1.510	78.467 ± 0.451	63.767 ± 0.750	74.7 ± 0.800

Data in table were expressed by Mean±SE in each column

In the present study, the antioxidant potential of walnut samples was measured by scavenging activity on DPPH radicals (table 2). All the assessed extracts revealed a remarkable antioxidant activity. Significant differences ($P<0.05$) were observed among tested genotypes. In comparison with BHA, B2 genotype had higher scavenging capacity than those obtained from the other genotypes. The order of radical scavenging activity was as follow: B2>K2>K1>G1>K3>B1. On DPPH assay,

EC_{50} values were obtained for samples and BHA. As shown in Table 2, among different genotypes used in this study B2 (0.09 ± 0.011 mg/ml) had the lowest EC_{50}, while the highest EC_{50} was obtained for B1 (0.20 ± 0.04 mg/ml) genotype.

Table 2: DPPH radical scavenging (%) in six genotypes of *Juglans regia* L.pellicles

	Genotype						
	K1	**G1**	**B1**	**K2**	**K3**	**B2**	**BHA**
DPPH Radical scavenging (%)	78.9 ± 0.64	73.77 ± 0.82	53.83 ± 2.83	86.24 ± 0.42	56.87 ± 1.98	90.38 ± 0.40	94.07 ± 0.10
EC_{50} (mg/ml)	0.14 ± 0.01	0.15 ± 0.002	0.20 ± 0.04	0.12 ± 0.021	0.18 ± 0.04	0.09 ± 0.011	0.054 ± 0.011

Each value is expressed as Mean±SE

DISCUSSION

Epidemiological and clinical studies indicated that PUFA may have a significant role in the secondary prevention of cardiovascular disease.[12] The result of this study showed that PUFA were the main group of fatty acids in walnut oil in all studied genotypes followed by monounsaturated fatty acids (MUFA) and SFA, respectively. The major fatty acids identified in the oil extracted from all walnut genotypes were linoleic, oleic and linolenic acids. Substituting walnuts for part of the monounsaturated fatty acids have been associated with beneficial effects on serum lipids.[2] Also, it has been shown that walnut – enriched diets significantly decrease total LDL cholesterol.[13] In general terms, the obtained results in this study were in accordance with the results reported by Amaral et al (2003)[3] and Li et al (2007) [14] . However, comparing between the main fatty acids obtained from this study with fatty acids reported in walnuts grown in Portugal, some differences were observed. [3, 12, 15] These seem to be associated with environmental conditions, as well as genotypes of different cultivars.

The DPPH radical is one of the few stable organic radicals and the test is simple and rapid which has widespread use in antioxidant screening.[16] In this method, the purple chromogen radical DPPH is reduced by antioxidant/reducing compounds to the corresponding pale yellow hydrazine, [17] and the loss of DPPH color after reaction with test compounds was monitored at 517 nm. The results were expressed as EC_{50} values, which is the amount of antioxidant necessary to decrease by 50% the initial DPPH concentration. In this study, B2 genotype showed higher scavenging activity than those obtained from the other genotypes. The obtained results are comparable with that achieved for BHA (96.0% at 3.6 mg/mL) standard. Also, the lowest EC_{50} was observed for genotype B2. Low values of EC_{50} are indicative of high antioxidant activity.

In previous investigation[18] proved that the descending order of DPPH radical scavenging activity of the methanolic extracts of each genotype was as follows: pellicle>hull>shell>kernel. It seems that pellicle as a protecting layer, can help to protect fatty acids particularly PUFA from oxidation by radicals.

It is concluded in literature that the antioxidant activities of the nut oils are attributed to the phenolic and non-phenolic compounds present in the samples.[19, 20, 21] Miraliakbari and Shahidi (2008a), [21] also reported that a strong antioxidant activity of WOs may be due to its high content of tocopherols especially γ-tocopherol.

CONCLUSIONS

In general, variation in fatty acid profiles and antioxidant activity was detected among the genotypes grown in the Kolyaei region. The highest content of

PUFA was observed in B2 genotype. In comparison with BHA, the extract from B2 genotype had also higher radical scavenging activity as well as lower EC_{50} than those obtained from the other genotypes.

The differences can be emanated from ecological, nutrition and genetically factors. The variation in the fatty acid composition of the nuts from different genotypes may affect the final use of the product. So, the nuts containing high levels of PUFA and MUFA should be preferred if the nuts were destined for use in a cholesterol-lowering diet. In addition we conclude that pellicle of fruit is a necessary protecting layer contain high quality antiradicals that shouldn't separate for nutritional value.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interests.

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