**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.\(^4\)
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 G1 were 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).

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:

\[
\text{RSA}\% = \frac{(A_0 - A_1)}{A_0} \times 100. \text{ Eq. (A)}
\]

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

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

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,
EC₅₀ 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 EC₅₀, while the highest EC₅₀ was obtained for B1 (0.20 ± 0.04 mg/ml) genotype.

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

<table>
<thead>
<tr>
<th>Genotype</th>
<th>K1</th>
<th>G1</th>
<th>B1</th>
<th>K2</th>
<th>K3</th>
<th>B2</th>
<th>BHA</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPPH Radical scavenging (%)</td>
<td>78.9 ± 0.64</td>
<td>73.77 ± 0.82</td>
<td>53.83 ± 2.83</td>
<td>86.24 ± 0.42</td>
<td>56.87 ± 1.98</td>
<td>90.38 ± 0.40</td>
<td>94.07 ± 0.10</td>
</tr>
<tr>
<td>EC₅₀ (mg/ml)</td>
<td>0.14 ± 0.01</td>
<td>0.15 ± 0.002</td>
<td>0.20 ± 0.04</td>
<td>0.12 ± 0.021</td>
<td>0.18 ± 0.04</td>
<td>0.09 ± 0.011</td>
<td>0.054 ± 0.011</td>
</tr>
</tbody>
</table>

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.15 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 EC₅₀ 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 EC₅₀ was observed for genotype B2. Low values of EC₅₀ are indicative of high antioxidant activity.

In previous investigation18 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 EC₅₀ 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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REFERENCES