FATTY ACIDS COMPOSITION OF TOCTE (JUGLANS NEOTROPICA DIELS) WALNUT FROM ECUADOR

VILCACUNDO E\(^1\), ALVAREZ M\(^2\), SILVA M\(^2\), CARPIO C\(^2\), MORALES D\(^2\), CARRILLO W\(^1,2,2^*\)

\(^1\)Department of Research. Bolivar State University, Academic Campus Alpacha. Av Ernesto Che Guevara s/n and Av Gabriel Secaira, EC 020150, Guaranda, Ecuador. \(^2\)Laboratory of Functional Foods, Faculty of Foods Science and Engineering, Technical University of Ambato. Av Los Chasquis y Rio Payamino. Campus Huachi, CP 1801334, Ambato Ecuador. Email: wcarrillo@uta.edu.ec

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ABSTRACT

Objective: The aim of this study was to determine the fatty acids composition in a tocte seeds oil (Juglans neotropica Dieels) sample cultivated in Ecuador.

Methods: Tocte oil was obtained from tocte seeds using the cold pressing method. Fatty acids analysis was carried out using the gas chromatography method with a mass selective detector (GC/MSD) and using the database Library NIST14.L to identify the compounds.

Results: Methyl esters fatty acids were identified from tocte (J. neotropica Dieels) walnut using the GC–MS analytical method. The total lipid content of tocte walnuts seeds of plants cultivated in Ecuador was 49.01% of the total lipid content on fresh weight. Fatty acids were analyzed as methyl esters on a capillary column DB-WAX 122-7062 with a good separation of palmitic acid, stearic acid, oleic acid, linoleic acid, and linolenic acid. The structure of methyl esters fatty acids was determined using the GC–MS. Tocte walnut presents 50.55% of palmitic acid, 22.6% of stearic acid, 19.50% of oleic acid, 65.81% of linoleic acid, and 2.79% linolenic acid of the total content of fatty acids in tocte oil. Fatty acids content reported in this study were similar to the data reported for other walnuts seeds.

Conclusions: Tocte seeds are a good source of monounsaturated and polyunsaturated fatty acids. Tocte oil content oleic acid and with a good content of ω6 α-linoleic and ω3 α-linolenic. Tocte walnut can help reduce risk cardiovascular diseases in Ecuador for their good composition of fatty acids.

Keywords: Walnuts, Tocte, Juglans neotropica Dieels, Fatty acids, Lipids.

INTRODUCTION

Genus Juglans also known as walnut has about 23 species distributed in North, Central and South America, Eastern Europe, and Asia. The two most important species from the economic point of view are the Persian Walnut (Juglans regia) appreciated for the quality of its nuts and the black walnut (Juglans nigra) highly prized for the quality of its timber. In South America, there are the following species: Argentinian walnut (Juglans australis), Bolivian walnut (Juglans boliviana), and black cedar (Juglans neotropica) found in Colombia, Venezuela, Peru, and Ecuador and known as tocte [1,2].

China has a high production of walnut (J. regia) with 1655 tons in 2011, thus being the most dispersed walnut tree in the world [3,4]. Walnuts have a high nutritional value due to their high composition of oils (65%) and proteins (18–24%). They are used in the manufacturing of chocolates, and as ingredients in the production of many food and bakery products [5,6]. Composition of oils has been widely studied in other walnut species. However, there are no studies of oil composition in the J. neotropica Dieels.

The food habits play an important role in the factors related with a good health. Consumption of junk food has increased considerably, leading to a number of diseases related to important nutritional deficiencies and excess of trans fat [7,8]. At present, consumers are more interested in knowing the potential nutritional benefits, disease control or prevention of certain foods, and including healthy food as walnuts in their diets [9]. Foods and their derivate are known to play an important role in reducing health risks and improving health quality. In 2003, the Food and Drug Administration of USA has approved health claims indicating that diets rich in walnuts can reduce the risk of heart disease [10]. Nuts have become an indispensable component of healthy diets and are included in the American Heart Association dietary metrics for defining ideal cardiovascular health in their recent report on setting goals for health promotion and disease reduction for 2020 [11]. The aim of this work was to identify the fatty acids composition present in tocte (J. neotropica Dieels) walnut from Ecuador using the gas chromatography-mass spectrometer (GC–MS).

METHODS

Total lipid extraction

Tocte walnuts were obtained in the supermarket of Ecuador. Tocte oil sample was obtained from tocte walnuts using the cold pressed method. Oil was then stored at 4.0 ± 2°C. Oil extraction was conducted using a Soxhlet apparatus for approximately 5 h with hexane as solvent, with a solid-to-solvent ratio of 1/7 m/v. After the extraction process, the flask contents were filtered, and the liquid fraction containing the lipid extract and solvent was poured into a 250-ml flask of a rotary film evaporator to remove the solvent. The obtained oil was collected, evaporated under nitrogen, weighed, and stored in sealed amber glass vials at −20°C until analysis [12].

Methyl esters fatty acids (FAME)

FAME were prepared from 3 to 5 mg of total lipids using the two-step methylation method (1% NaOH/MeOH followed by 5% HCl/MeOH); both steps were performed at 60°C, 20 min FAME were extracted with 6 ml of hexane [13].

Analysis of FAME from tocte by GC–MS

The fatty acid composition of oil extracted from tocte walnut seeds was analyzed by injecting fatty acid methyl esters [14] into an Agilent Technologies 7980A system GC (Agilent, Santa Clara, CA) equipped with a mass selective detector 5977A GC/MSD, an auto-sampler 7693, column (60 m × 250 µm × 0.25 µm, DB-WAX Agilent 122-7062). The oven temperature was programmed as follows: From 80°C, ramp 1: To
100°C at 20°C/min during 1 min; ramp 2: At 200°C at 25°C/min during 10 min; and ramp 3: At 250°C at 2°C/min. The injector and detector temperatures were set at 250°C. Helium was used as carrier gas at a linear flow velocity of 1.4 mL/min.

Spectra were compared with the NIST14.L library and the fatty acids mass spectra archive [15]. All GC analyses of the fatty acids were carried out in triplicate, and the results were expressed as the mean value ± standard deviation.

RESULTS

The GC chromatogram of methyl esters fatty acids from tocte oil present five majoritarian peaks that were separated with a column Agilent DB-WAX 122-7062. These peaks were identified with the help of a spectrum of database NILTS14.L. The quantification of fatty acids was obtained using the peak area ratio. Fig. 1 shows five majoritarian peaks with a good separation and definition, these peaks were identified C16:0 with a retention time of 19.378 min, C18:0 with a retention time of 26.057 min, C18:1 with a retention time of 26.919 min, C18:2 with a retention time of 28.693 min, and finally C18:3 with a retention time of 31.092 min.

The concentration of FAME was measured using the peak area ratio. Table 1 summarizes C16:0: With 5.05%±0.09 of fatty acid total content, C18: With 2.26%±0.16 of fatty acid total content, C18:1 with 19.50%±0.44 of fatty acid total content, C18:2 with 65.81%±1.14 of fatty acid total content, and C18:3 with 2.79%±0.26 of fatty acid total content of tocte oil. Total lipid of tocte walnut seeds was of 49.01% of lipid in fresh weight (Table 1).

The mass spectrum of methyl esters fatty acids obtained from tocte oil is shown in Fig. 2. The identity and structures of these fatty acids were confirmed by the GC-MS method. Palmitic acid (C16:0) was identified using the mass spectrum with ions of mass/charge (m/z) between 57 and 270 m/z. In the previous range, the ions 74 and 87 m/z were the most abundant in the mass spectrum (Fig. 2a).

Stearic acid (C18:0) was identified using the mass spectrum with ions with m/z between 55-298 m/z. In the previous range, the ions 74 and 87 m/z were the most abundant in the mass spectrum (Fig. 2b).

Oleic acid (C18:1) was identified using the mass spectrum with ions m/z between 55 and 296 m/z. In the previous range, the two ions with the highest abundance were the 55-69 m/z.

Linoleic acid (C18:2) was identified using the mass spectrum with ions m/z between 55 and 294 m/z. In the previous range, the two most present ions were the 55 and 67 m/z.

When the tocte walnut fatty acid composition is compared to some common vegetable oils, it can be seen that olive oil has a high content of mono-unsaturated fatty acids, C18:1 named oleic acid with 77.6% of oleic acid. It can be seen that tocte oil from Ecuador has a good content of mono-unsaturated fatty acids with a content of 19.50% of oleic acid. Tocte oil has also a high content of polyunsaturated fatty acids with 65.81% of linoleic acid. Olive oil contains few omega-6 and omega-3 fatty acids with 9.0% and 1.0%, respectively, and macadamia oil contains 3.22% of omega 6 and 1.79% of omega 3 (Table 2).

DISCUSSION

Manos et al., 2001 [22] reported fatty acids content of J. neotropica Diels with 5.0% of palmitic acid, 3.0% of stearic acid, 39% of oleic acid, 50% of linoleic acid.

<table>
<thead>
<tr>
<th>Retention time (min)</th>
<th>Peak area ratio %</th>
<th>Carbon number: Double bound</th>
<th>FAMEs name</th>
</tr>
</thead>
<tbody>
<tr>
<td>19.378</td>
<td>5.05±0.09</td>
<td>C16:0</td>
<td>Palmitic acid</td>
</tr>
<tr>
<td>26.057</td>
<td>2.26±0.16</td>
<td>C18:0</td>
<td>Stearic acid</td>
</tr>
<tr>
<td>26.919</td>
<td>19.50±0.44</td>
<td>A9 C18:1</td>
<td>Oleic acid</td>
</tr>
<tr>
<td>28.693</td>
<td>65.81±1.14</td>
<td>A9, 12 C18:2</td>
<td>Linoleic acid</td>
</tr>
<tr>
<td>31.092</td>
<td>2.79±0.25</td>
<td>A9, 12 C18:3</td>
<td>Linolenic acid</td>
</tr>
<tr>
<td>Total lipid % fresh weight</td>
<td>49.01±0.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1: Total lipid and fatty acids composition of tocte oil sample from Ecuador by GC/MS analysis and their percentage

![Fig. 1: Analysis by gas chromatography of methyl esters fatty acid from tocte oil](image-url)

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of linoleic acid, and 3% of linolenic acid. We reported 19.50% of oleic acid and 65.81% of linoleic acid these differences can be due at system crop, quality soil, and other factors.

J. regia is the most cultivated walnut around the world with different varieties. Iqbal et al., 2016 [21] have reported fatty acids contents in two varieties (Softshell and Hardshell) from J. regia cultivated in Pakistan with 7.80% and 7.21% of palmitic acid, respectively, 2.30% and 2.20% of stearic acid, respectively, 23.10% and 23.15% of oleic acid, respectively, and 66.54% and 66.60% of linoleic acid, respectively. Pereira et al., 2008 [10] have reported the fatty acids composition of six varieties of walnut (J. regia) cultivated in Portugal. Variety Franquette with a value of 6.61% palmitic acid, 3.07% of stearic acid, 20.22% of oleic acid, and 55.51% linoelie acid. Variety Lara with a value of 6.28% of palmitic acid, 2.80% of stearic acid, 14.92% of oleic acid, and 60.30% of linoleic acid. Variety Marbot with a value of 6.35% of palmitic acid, 2.78% of stearic acid, 16.34% of oleic acid, and 59.66% of linoelie acid. Variety Mellanaise with a value of 6.31% of palmitic acid, 2.80% of stearic acid, 17.09% of oleic acid, and 58.75% of linoelie acid. Variety Parisienne with a value of 6.16% of palmitic acid, 2.90% of stearic acid, 19.50% of oleic acid, and 57.68% of linoelie acid. Data obtained on this study from walnut (J. neotropica Diels) are therefore in accordance with the content of fatty acids reported in walnut (J. regia) in previous studies. In conclusion, tocte oil has a high content of oleic acid with 19.50% and linoleic acid with 65.81%. Tocte walnuts have a similar profile of fatty acids reported as the ones reported for the traditional walnut J. regia variety. Walnuts constituents have been actively used in nutrition in slowing cancer growth by its antiproliferative and antiangiogenic mechanisms [23]. We also know that linolenic acid has different biological activities as anti-inflammatory, antioxidant, and hypolipidemic activities [24].

CONCLUSION

Tocte walnut seeds possess a good proportion of monounsaturated and polyunsaturated acids. The content of oleic acid presents in tocte walnut seeds was high. J. neotropica Diels can be used to reduce the risk of cardiovascular diseases. Tocte oil can also be used as a good source of fatty acids with possible biological activities.

ACKNOWLEDGMENTS

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Table 2: Fatty acid composition (%) of 10 vegetable oils

<table>
<thead>
<tr>
<th>References</th>
<th>Vegetal oil</th>
<th>C16:0</th>
<th>C16:1</th>
<th>C18:0</th>
<th>C18:1</th>
<th>C18:2</th>
<th>C18:3</th>
</tr>
</thead>
<tbody>
<tr>
<td>[16]</td>
<td>Olive oil</td>
<td>13.8</td>
<td>1.4</td>
<td>2.8</td>
<td>71.6</td>
<td>9.00</td>
<td>1.0</td>
</tr>
<tr>
<td>[16]</td>
<td>Sunflower oil</td>
<td>5.2</td>
<td>0.1</td>
<td>3.7</td>
<td>33.7</td>
<td>56.5</td>
<td>0.0</td>
</tr>
<tr>
<td>[16]</td>
<td>Palm oil</td>
<td>44.8</td>
<td>0.0</td>
<td>4.6</td>
<td>38.9</td>
<td>9.5</td>
<td>0.4</td>
</tr>
<tr>
<td>[16]</td>
<td>Soybean oil</td>
<td>10.1</td>
<td>0.0</td>
<td>4.3</td>
<td>22.3</td>
<td>53.7</td>
<td>8.1</td>
</tr>
<tr>
<td>[16]</td>
<td>Cori oil</td>
<td>11.6</td>
<td>0.0</td>
<td>2.5</td>
<td>38.7</td>
<td>44.7</td>
<td>1.4</td>
</tr>
<tr>
<td>[17]</td>
<td>Sacha inchi oil</td>
<td>3.98</td>
<td>0.0</td>
<td>3.12</td>
<td>8.58</td>
<td>34.98</td>
<td>47.04</td>
</tr>
<tr>
<td>[18]</td>
<td>Sambo oil</td>
<td>9.33</td>
<td>0.0</td>
<td>6.84</td>
<td>41.36</td>
<td>33.98</td>
<td>0.0</td>
</tr>
<tr>
<td>[20]</td>
<td>Kahai oil</td>
<td>19.32</td>
<td>0.0</td>
<td>3.47</td>
<td>18.59</td>
<td>68.04</td>
<td>0.0</td>
</tr>
<tr>
<td>[21]</td>
<td>Walnut Juglans rega</td>
<td>7.80</td>
<td>N.D</td>
<td>2.30</td>
<td>23.10</td>
<td>66.54</td>
<td>N.D</td>
</tr>
<tr>
<td></td>
<td>Tocte oil</td>
<td>5.05</td>
<td>0.0</td>
<td>2.26</td>
<td>19.50</td>
<td>65.81</td>
<td>2.79</td>
</tr>
</tbody>
</table>
AUTHOR CONTRIBUTIONS
Vilcacundo E, Carpio C, Morales D, and Carrillo W conceived and designed the experiments. Silva M and Alvarez M performed the gas chromatography analyses. Carrillo W wrote the paper.

CONFLICT OF INTERESTS
The authors declare no conflict of interest.

REFERENCES