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Characterization of *Carya illinoiensis* and *Juglans regia* oils obtained by different extraction systems

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**ABSTRACT.** It is extremely important to evaluate the extraction processes of vegetable oils used in food, considering that the nuts are rich sources of triacylglycerols. Thus, the present study aimed at comparing the methods of solvent extraction and extraction by pressing of the lipid fractions of walnut (*Juglans regia*) and pecan nut (*Carya illinoensis*), in order to elucidate their influence on the content of bioactive substances. The samples were analyzed regarding fatty acid profile, tocopherols, phytosterols, carotenoids, and phenolic compounds, presenting relevant quantities of these bioactive substances. It was found that the extraction of oils by pressing minimized degradation of tocopherol when compared to solvent extraction. However, solvent extraction is more efficient to extract bioactive compounds such as phytosterols, carotenoids, and phenolic compounds.

**Keywords:** Chromatography, Soxhlet, pressing, oilseeds.

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**Introduction**

The oilseeds, in general, have a similar composition from the nutritional point of view, being characteristically foods with high fat. Epidemiological and clinical studies conducted in recent decades suggest that regular consumption of moderate amounts of nuts can bring beneficial effects to human health, particularly to the blood lipid profile and some types of cancer such as prostate, esophagus, stomach, colon, and rectum (ALASALVAR; SHAHIDI, 2008; LÓPEZ-URIARTE et al., 2010). When compared with most of the other oilseeds (olive, coconut, palm), which contain mainly monounsaturated fatty acids, the nuts are distinguished by their high content of polyunsaturated fatty acids ω-3 and ω-6, namely the essential fatty acids α-linolenic and linoleic, respectively. In addition to their favorable profile of fatty acids, walnuts also contain several other bioactive compounds such as phytosterols, tocopherols, and tocotrienols (PEREIRA et al., 2008).

Considering the nuts as rich sources of triglycerides, it is very important to evaluate the influence of extraction processes on the quality of vegetable oils used in human food.

The method of extraction of nut oil is complex and has considerable influence on its physical and chemical composition, nutritional value, and sensory properties. The main processes used for the extraction of fats and oils from oilseeds are solvent extraction and extraction by pressing (SARTORI et al., 2009).

The most serious disadvantages of Soxhlet extraction, compared to other techniques for preparing solid samples, are the time necessary for the extraction and the large amount of solvent.
needed which, besides being of high cost, also causes environmental problems. The samples are usually extracted at the boiling point of the solvent for long periods, which can result in thermal decomposition of the thermolabile target species. Moreover, a conventional Soxhlet apparatus provides no agitation, which would help to make the process more agile. Due to the large amounts of extractant used, an evaporation step is necessary after extraction. Finally, the technique is limited by the Soxhlet extractor and difficult to automate (CASTRO; PRIEGO-CAPOTE, 2010).

The extraction methods used in the past were quite simplified and the products were not always 100% pure oils. Similarly, the characteristics of oils can change, depending on the method employed, considering that their chemical properties may be totally altered depending on the conditions to which they are subjected when a certain technique is used. In the first decades of the twentieth century, presses were used for extraction of oil, and even though they extracted oil of good quality, solid waste was left. Such waste resulted in loss of oil, affecting the quality of the pie, which is also one of the waste products of the extractor (SARTORI et al., 2009). However, extraction by pressing associated with the use of a CP-Sil 88 fused-silica capillary column (60 m x 0.25 mm i.d., 0.20 µm film thickness, Chrompack, Varian Inc., Walnut Creek, CA, USA), equipped with a flame-ionization detector (GC-FID), split injector, and automatic sampler. FAMEs were separated by with the use of a CP-Sil 88 fused-silica capillary column (60 m x 0.25 mm i.d., 0.20 µm film thickness, Chrompack, Varian Inc., Walnut Creek, CA, USA). The column oven temperature was initially held at 90°C for 4 min., heated at 10°C min.¹ until 195°C, and maintained at 195°C for 20.5 min. The injector and detector temperatures were 230 and 250°C, respectively. Samples of 1.0 µL were injected, adopting a split ratio of 1:30. The carrier gas was hydrogen with a flow rate of 30 mL min.⁻¹. FAMEs were identified by comparing their retention times with those of pure FAME standards (Supelco, Bellefonte, USA) under the same operating conditions. The integration software computed the peak areas, and percentages of fatty acid methyl esters were obtained as weight percentage by direct internal normalization.

Oil extraction

The oils were extracted from the nuts through the extraction method with hot petroleum ether at 40-60°C, in a Soxhlet extractor with reflux for 6 hours (AOCS, 2009). The method by cold pressing was conducted in hydraulic press, Tecnal brand, model TE-098, at room temperature, nuts pressed at 3-12 tonnes (ARANHA; JORGE, 2013).

Fatty acid profile by gas chromatography

Potassium hydroxide in methanol and n-hexane were used to transesterify the lipid fractions of samples (50 mg) to methyl esters (AOCS, 2009). Fatty acid methyl esters (FAMEs) were analyzed using a GC 3900 gas chromatograph (Varian Inc., Walnut Creek, CA, USA), equipped with a flame-ionization detector (GC-FID), split injector, and automatic sampler. FAMEs were separated by with the use of a CP-Sil 88 fused-silica capillary column (60 m x 0.25 mm i.d., 0.20 µm film thickness, Chrompack, Varian Inc., Walnut Creek, CA, USA). The column oven temperature was initially held at 90°C for 4 min., heated at 10°C min.¹ until 195°C, and maintained at 195°C for 20.5 min. The injector and detector temperatures were 230 and 250°C, respectively. Samples of 1.0 µL were injected, adopting a split ratio of 1:30. The carrier gas was hydrogen with a flow rate of 30 mL min.⁻¹. FAMEs were identified by comparing their retention times with those of pure FAME standards (Supelco, Bellefonte, USA) under the same operating conditions. The integration software computed the peak areas, and percentages of fatty acid methyl esters were obtained as weight percentage by direct internal normalization.

Tocopherols

Tocopherol composition was determined by AOCS Ce 8-86 (2009), using HPLC system (Varian Inc., Walnut Creek, CA, USA), equipped with a fluorescence detector. Forty mg of the lipid fractions of samples were diluted with n-hexane and 20 µL sample were injected. The operating conditions were: λ excitation 290 nm and λ emission 330 nm. A normal phase column (100 Si, 250 x 4.6 mm i.d., 0.5 µm particle size, Microsorb, Varian Inc., Walnut Creek, CA, USA) was used with n-hexane/isopropanol (99.5/0.5, v/v) as a mobile phase. The system was operated isocratically, at a flow rate of 1.2 mL min.⁻¹. The identification of tocopherols (α-, β-, γ- and δ-tocopherol) was conducted by comparing the HPLC retention time with those of standard compounds (Supelco,
Vitamin E is represented as the equivalent of α-tocopherol. The correction factor of 1.0 was used to calculate the α-tocopherol content, while the concentrations of β- and γ-tocopherol were multiplied by 0.25, and the correction factor 0.01 was used for the amount of δ-tocopherol. The isomers β-, γ-, and δ-tocopherols were calculated with a lower correction factor, in order to avoid the overvaluation of the α-tocopherol equivalent (KORNSTEINER et al., 2006).

Analysis of phytosterols

Phytosterol composition was measured by gas chromatography with saponification prior to the sample (50-80 mg). The saponification was performed according to the methodology UMA 0069 published by Duchateau et al. (2002). For the determination of the content of phytosterols, the AOCS method Ch 6-91 (2009) was used, with adaptations. The analysis was performed using a GC-2010 Plus gas chromatograph (Shimadzu, Chiyoda-ku, Tokyo, Japan), equipped with a flame-ionization detector (GC-FID), split injector and automatic sampler. Conditions of analysis: RTX 5 fused-silica capillary column (30 m x 0.25 mm i.d., 0.25 μm film thickness, Restek, Shimadzu, Chiyoda-ku, Tokyo, Japan). The programming of column temperature was started at 100°C, for 2 min., heated at 15°C min.-1 until 260°C, and maintained in isothermal condition for 35 min. The temperatures used in the injector and the detector were 280 and 320°C, respectively. Samples of 1.0 μL were injected, adopting a split ratio of 1:50. The carrier gas used was hydrogen, with linear speed of 40 mL min.-1. The phytosterols (cholesterol, campesterol, stigmasterol, β-sitosterol, and stigmastanol) were identified by comparison with the retention time of pure standards (Supelco, Bellefonte, USA), analyzed under the same conditions of the samples. The quantification of each isomer was performed by internal standardizing (5α-cholestan-3β-ol) based on the peak areas.

Total carotenoids

The verification of the content of total carotenoids was performed by scanning spectrophotometry, according to the methods described by Rodriguez-Amaya (1999): 0.5 g of oil was weighed in a 25 mL volumetric flask and the volume was completed with petroleum ether. Then, the mixture was agitated and the reading was performed in triplicate. Quantification was calculated considering the absorption at the wavelength of maximum absorption and the A value of 2592, in petroleum ether, to calculate the total amount of carotenoids (according to the equation below). Values were expressed as β-carotene, in μg g⁻¹ of the lipid fraction.

\[ C = \frac{\text{Abs} \times 25 \times 10000}{2592 \times \text{weight of sample}} \]

Total phenolic compounds

Total phenolics were quantified spectrophotometrically using the Folin-Ciocalteu reagent and gallic acid standard curve method, as described by Singleton and Rossi Jr. (1965). This method is based on the reduction of phosphomolybdic and phosphotungstic acids in alkaline solution and is mostly used for the determination of phenolic compounds in foods. The blue color produced by reduction of the Folin-Ciocalteu reagent by the phenolics was measured spectrophotometrically at 765 nm wavelength and the results were expressed in mg of gallic acid equivalent per gram of oil (GAE mg g⁻¹). The extraction of phenolic compounds was performed according to the method proposed by Parry et al. (2005). An aliquot of 1 g of sample was weighed in a tube and agitated in vortex with 3 mL of methyl alcohol. Then, it was centrifuged at 3,000 rpm, for 10 min., and the supernatant was collected. The procedure was repeated twice more. The three supernatants were combined in a 10 mL volumetric flask and the volume was completed with methyl alcohol.

Statistical analysis

All the experiments were carried out in triplicate. The results were expressed as means ± SD (standard deviation) and evaluated by analysis of variance (ANOVA) followed by Tukey’s studentized range test, carried out by ESTAT program (BANZATTO; KRONKA, 2006), version 2.0, and p < 0.05 was regarded as statistically significant.

Results and discussion

The lipids found in samples of nuts Juglans regia and Carya illinoensis were in 68.03 and 65.73% when extracted by Soxhlet, while in 59.85 and 56.44% when extracted by pressing, respectively. High levels of lipids found in seeds can be considered economically attractive for industrial extraction, especially when compared with other oilseeds, including corn and soybeans, which have lipid content from 3.1 to 5.7% and 18-20%, respectively (O’BRIEN, 2004).
Table 1 shows the fatty acid profile (%) contained in the lipid fractions of nuts. Nine major fatty acids were identified in the oils.

**Table 1.** Lipid and fatty acid profile present in lipid fractions of *Juglans regia* and *Carya illinoensis*, obtained by Soxhlet and cold pressing systems.

<table>
<thead>
<tr>
<th></th>
<th><em>Juglans regia</em></th>
<th></th>
<th><em>Carya illinoensis</em></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Soxhlet</td>
<td>cold pressing</td>
<td>Soxhlet</td>
<td>cold pressing</td>
</tr>
<tr>
<td>Lipid (%)</td>
<td>68.03</td>
<td>59.85</td>
<td>65.73</td>
<td>56.44</td>
</tr>
<tr>
<td>Fatty acids (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C14:0</td>
<td>0.11 ± 0.00</td>
<td>nd</td>
<td>0.05 ± 0.01</td>
<td>nd</td>
</tr>
<tr>
<td>C16:0</td>
<td>7.78 ± 0.11</td>
<td>6.90 ± 0.05</td>
<td>7.02 ± 0.05</td>
<td>7.10 ± 0.03</td>
</tr>
<tr>
<td>C16:1</td>
<td>0.11 ± 0.00</td>
<td>0.05 ± 0.00</td>
<td>0.07 ± 0.01</td>
<td>0.07 ± 0.01</td>
</tr>
<tr>
<td>C18:0</td>
<td>2.25 ± 0.02</td>
<td>2.84 ± 0.00</td>
<td>1.95 ± 0.02</td>
<td>1.84 ± 0.02</td>
</tr>
<tr>
<td>C18:1:ω9</td>
<td>15.38 ± 0.02</td>
<td>15.66 ± 0.03</td>
<td>44.50 ± 0.02</td>
<td>45.75 ± 0.04</td>
</tr>
<tr>
<td>C18:2:n6</td>
<td>61.70 ± 0.30</td>
<td>61.52 ± 0.01</td>
<td>43.81 ± 0.30</td>
<td>42.73 ± 0.10</td>
</tr>
<tr>
<td>C18:3:n3</td>
<td>12.36 ± 0.15</td>
<td>12.71 ± 0.02</td>
<td>2.22 ± 0.02</td>
<td>2.18 ± 0.01</td>
</tr>
<tr>
<td>C20:0</td>
<td>0.09 ± 0.00</td>
<td>0.07 ± 0.00</td>
<td>0.10 ± 0.00</td>
<td>0.09 ± 0.01</td>
</tr>
<tr>
<td>C20:5:n3</td>
<td>0.08 ± 0.00</td>
<td>0.07 ± 0.00</td>
<td>0.08 ± 0.01</td>
<td>0.06 ± 0.01</td>
</tr>
<tr>
<td>Others</td>
<td>0.14 ± 0.01</td>
<td>0.18 ± 0.01</td>
<td>0.19 ± 0.01</td>
<td>0.18 ± 0.01</td>
</tr>
<tr>
<td>Saturated</td>
<td>10.29 ± 0.12</td>
<td>9.90 ± 0.04</td>
<td>9.27 ± 0.04</td>
<td>9.17 ± 0.06</td>
</tr>
<tr>
<td>Monounsaturated</td>
<td>15.52 ± 0.03</td>
<td>15.73 ± 0.03</td>
<td>44.61 ± 0.01</td>
<td>48.86 ± 0.04</td>
</tr>
<tr>
<td>Polyunsaturated</td>
<td>74.19 ± 0.15</td>
<td>74.38 ± 0.02</td>
<td>42.62 ± 0.02</td>
<td>44.96 ± 0.10</td>
</tr>
</tbody>
</table>

According to the results, the oils analyzed were proved to be highly unsaturated (90%). Oils extracted from *Juglans regia* showed fatty acid profile, especially linoleic (61%), oleic (15%) and α-linolenic (12%) acids. The major fatty acids found in *Carya illinoensis* oil were oleic, linoleic, and palmitic acids with 45, 43, and 7%, respectively. According to Amaral et al. (2003) in oil extracted from walnuts of six different cultivars, linoleic oil is in majority, with 57-62%, followed by oleic, linoleic, and palmitic acids with ranges of 14-18, 9-13, and 6-7%, respectively.

Regarding α-linolenic fatty acid (C18:3 n-3), oils extracted from *Juglans regia* and *Carya illinoensis* showed approximately 12 and 2%, respectively. Taking into account the percentage of this fatty acid in soybean (4.5 to 11%) and canola (5-13%) oils (CODEX ALIMENTARIUS COMMISSION, 2009), *Juglans regia* appeared as a similar source of α-linolenic acid.

According to Table 1, butyric fatty acid (C4:0) was detected only with Soxhlet methodology. It is possible to infer that such formation occurred due to the fact that Soxhlet is a methodology that uses mild heating, being able to cause breaks in the unsaturated bonds and formation of saturated fatty acids.

Among the saturated fatty acids, palmitic acid (C16:0) was predominant in oils, followed by stearic acid (C18:0). This result is consistent with the fact that palmitic acid is the most abundant saturated fatty acid in vegetable lipids, while stearic acid is found in smaller amounts. Although arachidic acid (C20:0) has been found in all oils analyzed, concentrations ranged from 0.07 to 0.10%. Butyric acid (C4:0) found only in solvent extracted oils, ranging from 0.05 to 0.11% for *Carya illinoensis* and *Juglans regia* oils, respectively.

When assessing the fatty acid profile of *Juglans regia* oil extracted by solvent, there was significant difference (p < 0.05) with the increase in the percentage of saturated fatty acids and the decrease of monounsaturated fatty acids, compared with the oil extracted by cold pressing. The same could not be observed regarding the polyunsaturated acids. However, in *Carya illinoensis* oil extracted by solvent it was possible to observe significant difference (p < 0.05), with increased percentage of polyunsaturated fatty acids and decreased percentage of monounsaturated fatty acids, compared with the oil extracted by cold pressing. The same cannot be observed regarding saturated fatty acids.

The quantification of bioactive compounds present in the lipid fractions of nuts, obtained by solvent and cold extraction, is presented in Table 2. While α-tocopherol presents the highest biological activity as vitamin E, γ- and δ-tocopherol present higher antioxidant activity (SHAHIDI; NACZK, 1995; SCHMIDT; POKORNÝ, 2005).

**Table 2.** Bioactive substances present in lipid fractions of *Juglans regia* and *Carya illinoensis*, obtained by Soxhlet and cold pressing systems.

<table>
<thead>
<tr>
<th>Bioactive compounds</th>
<th></th>
<th>Soxhlet</th>
<th>cold pressing</th>
<th>Soxhlet</th>
<th>cold pressing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tocopherols (mg kg⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alpha</td>
<td>4.07 ± 0.15</td>
<td>4.87 ± 0.11</td>
<td>3.70 ± 0.07</td>
<td>5.17 ± 0.16</td>
<td></td>
</tr>
<tr>
<td>Beta</td>
<td>1.20 ± 0.13</td>
<td>1.20 ± 0.00</td>
<td>0.97 ± 0.04</td>
<td>1.30 ± 0.07</td>
<td></td>
</tr>
<tr>
<td>Gamma</td>
<td>138.13 ± 0.91</td>
<td>144.97 ± 2.44</td>
<td>153.23 ± 1.38</td>
<td>165.73 ± 0.91</td>
<td></td>
</tr>
<tr>
<td>Delta</td>
<td>11.10 ± 0.47</td>
<td>14.37 ± 0.29</td>
<td>7.80 ± 0.07</td>
<td>7.90 ± 0.07</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>154.50 ± 1.47</td>
<td>165.40 ± 2.07</td>
<td>165.70 ± 1.47</td>
<td>180.10 ± 0.73</td>
<td></td>
</tr>
<tr>
<td>Vitamin E⁺</td>
<td>39.01 ± 0.25</td>
<td>41.55 ± 0.52</td>
<td>42.33 ± 0.35</td>
<td>47.01 ± 0.13</td>
<td></td>
</tr>
<tr>
<td>Phytosterols (mg 100 g⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol</td>
<td>1.91 ± 0.01</td>
<td>2.80 ± 0.01</td>
<td>1.73 ± 0.01</td>
<td>1.43 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>β-sitosterol</td>
<td>66.27 ± 0.02</td>
<td>42.61 ± 0.01</td>
<td>104.15 ± 0.02</td>
<td>83.17 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>Stigmasterol</td>
<td>2.12 ± 0.02</td>
<td>6.11 ± 0.01</td>
<td>7.55 ± 0.01</td>
<td>7.55 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>68.18 ± 0.02</td>
<td>47.53 ± 0.03</td>
<td>111.98 ± 0.01</td>
<td>92.15 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>Total carotenoids (μg g⁻¹)</td>
<td></td>
<td>6.25 ± 0.07</td>
<td>4.84 ± 0.12</td>
<td>6.86 ± 0.15</td>
<td>5.13 ± 0.15</td>
</tr>
<tr>
<td>Total phenolic compounds (mg GAE g⁻¹)</td>
<td></td>
<td>1.43 ± 0.08</td>
<td>0.88 ± 0.11</td>
<td>0.98 ± 0.03</td>
<td>0.74 ± 0.05</td>
</tr>
</tbody>
</table>

The mean values ± standard deviation of three determinations followed by the same letters in the lines do not differ by Tukey test (p < 0.05). α-Tocopherol presents the highest biological activity as vitamin E, γ- and δ-tocopherol present higher antioxidant activity. The activity of vitamin E expressed as the equivalent of α-tocopherol. Milligram of gallic acid equivalents per gram of sample. nd: not detected, defined as ≤ 0.1 mg GAE g⁻¹.
It is noteworthy that the oils analyzed showed similar profiles of tocopherols. In these oils, \( \gamma \)-tocopherol represented between 87.65 and 92.47% of total tocopherols and \( \delta \)-tocopherol, between 4.38 and 8.69%. The oils which have \( \alpha \)-tocopherol as predominant are sunflower (403-935 mg kg\(^{-1}\)), palm (30-280 mg kg\(^{-1}\)), and grape (16-38 mg kg\(^{-1}\)) oils. In contrast, corn (268-2468 mg kg\(^{-1}\)), soybean (89-2307 mg kg\(^{-1}\)), and sesame (521-983 mg kg\(^{-1}\)) oils have \( \gamma \)-tocopherol as predominant (CODEX ALIMENTARIUS COMMISSION, 2009).

Although \( \delta \)-tocopherol is found naturally in small quantities in lipids, concentrations similar to those found, for example, in peanut (13.4 mg kg\(^{-1}\)), sunflower (9.2 mg kg\(^{-1}\)), and canola (6.1 mg kg\(^{-1}\)) oils (TUBEROSO et al., 2007) were quantified in the oils analyzed (from 7.8 to 14.4 mg kg\(^{-1}\)).

The oils extracted from the nuts by cold pressing presented quantity of total tocopherols significantly superior to solvent extracted oils, but all oils analyzed are good source of tocopherols.

According to a study by Miralakbari and Shahidi (2008), the content of total tocopherols in the oil extracted from Brazil nut was 106.80 mg kg\(^{-1}\). On the other hand, Yang (2009), reviewing the benefits of chestnuts related to health, found 122 mg kg\(^{-1}\) of total tocopherols for macadamia (Macadamia integrifolia) and 291 mg kg\(^{-1}\) for pistachio (Pistacia vera L.) nuts.

It was found that the activity of vitamin \( E \), expressed as \( \alpha \)-tocopherol equivalents, ranged from 39 to 47 in the oils analyzed. In a study of chestnuts and walnuts regarding the activity of vitamin \( E \), it was possible to determine levels ranging from not detected, in macadamia oil, to 33.1 of \( \alpha \)-tocopherol equivalent, in hazelnut oil. In relation to health, low levels of vitamin \( E \) may be associated with increased risk of development of degenerative diseases such as atherosclerosis. Adequate intake of vitamin \( E \) exerts cardioprotective effect by inhibiting the oxidation of the cholesterol fraction of low density lipoproteins (LDL-c), which is a key role in the atherogenic process. In addition, \( \alpha \)-tocopherol is considered the most active form in living beings. However, \( \gamma \)-tocopherol is the most prevalent in plant seeds, especially in oilseeds (KORNSTEINER et al., 2006).

Phytosterols are constituents that are present in smaller amounts in the unsaponifiable fraction of vegetable matter. Most vegetable oils contain from 100 to 1500 mg 100 g\(^{-1}\) of oil, and the \( \beta \)-sitosterol is present in higher amount. Table 2 shows the results of means tested with Tukey. While evaluating the sterol composition for nuts oils extracted by Soxhlet, there was a significant difference (\( p < 0.05 \)) with increasing concentrations of sterols, when compared with those extracted by cold pressing.

The levels of total phytosterols ranged from 47 to 112 mg 100 g\(^{-1}\) among the oils analyzed. These values coincide with the range of total phytosterols found in coconut and palm oils (CODEX ALIMENTARIUS COMMISSION, 2009). In a study by Normén et al. (2007), the levels of total phytosterols found in walnuts and chestnuts ranged between 68 and 144 mg 100 g\(^{-1}\).

As expected, cholesterol was the sterol present in smaller amounts in the lipid fractions studied, with levels of approximately 2 mg 100 g\(^{-1}\). \( \beta \)-sitosterol was found in all samples, being the most abundant sterol, especially in Carina illinensis oil extracted by Soxhlet, in which content of 112 mg 100 g\(^{-1}\) was detected. Stigmasterol was found in all samples, except in Juglans regia oil extracted by Soxhlet, but in smaller amount than \( \beta \)-sitosterol.

The concentration of carotenoids in oils is affected by ripening stage of the fruit, extraction conditions, and storage facilities. Oils extracted from ripe fruits may contain higher amounts of carotenoid pigments, as those obtained from partially ripe fruits have higher concentrations of chlorophyll (RAMADAN; MORSEL, 2003). As shown in Table 2, the concentrations of total carotenoids expressed as \( \beta \)-carotene, obtained in the lipid fractions of nuts, ranged from 4.8 to 6.9 \( \mu \)g g\(^{-1}\). Tuberoso et al. (2007) quantified \( \beta \)-carotene in olive (6.9 \( \mu \)g g\(^{-1}\)), pumpkin (5.7 \( \mu \)g g\(^{-1}\)), and canola (1.7 \( \mu \)g g\(^{-1}\)) oils.

There is a significant difference (\( p < 0.05 \)) between the levels of total carotenoids found in oils extracted from nuts by Soxhlet, with a higher concentration of total carotenoids compared with those extracted by cold pressing.

The phenolic compounds are important, since they contribute to antioxidant activity. The extraction of these compounds from natural products is strongly influenced by the solvent used. It has been observed that the greater the polarity of the extracting solvent, the greater the amount of phenolic compounds (GAMÉZ-MEZA et al., 1999).

According to the results presented in Table 2, the concentrations of total phenolics did not differ significantly by Tukey test (\( p < 0.05 \)) in the oils analyzed, except in Juglans regia oil extracted by Soxhlet, which was significantly higher than the other.
Conclusion

The oil extraction by pressing, despite having significantly influenced the fatty acid profile, minimized the degradation of total tocopherols and isomers when compared to Soxhlet extraction. However, Soxhlet extraction was more efficient in dragging the bioactive compounds phytosterols, carotenoids, and phenolic compounds.

Furthermore, the lipid fractions of nuts are good sources of unsaturated fatty acids, especially oleic and linoleic acids, and viable sources of γ- and δ-tocopherol, β-sitosterol, carotene, and total phenolic compounds, suggesting application of this material for the food and pharmaceutical industries.

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