Effect of the smoking process and firewood type in the phytochemical content and antioxidant capacity of red Jalapeño pepper during its transformation to chipotle pepper

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1. Introduction

Jalapeño pepper (Capsicum annuum L.) is one of the most representative foods of the Mexican diet. It is consumed at the rate of 7–9 kg per year, per capita, mostly fresh; however it is also consumed in different forms such as pickled, dried and smoked (Álvarez-Parrilla, de la Rosa, Amarowicz, & Shahidi, 2011). Besides its sensory properties, Jalapeño pepper has a significant role in human health as it contains high concentrations of antioxidants and functional compounds (Ornelas-Paz et al., 2013). One growing market for Jalapeño, both in Mexico and USA, is as a chipotle pepper, which consists of red Jalapeño (last stage of maturation) that has undergone a process of smoking and drying (Ávila-Quezada, Islas-Valenzuela, Muñoz-Márquez, & Sánchez-Chávez, 2009).

The process of chipotle production involves the use of firewood to dry and smoke the red Jalapeño for a period of 6 days in an open smoker installation. The smoking process can affect structural, chemical, and nutritional properties of food (Cardinal, Cornet, Sérot, & Baron, 2006; Kjällstrand & Petersson, 2001; Vega-Gálvez et al., 2009) and wood type used in the smoking process has an impact on the resulting smoked food (Guillén & Ibargoitia, 1998; Guillén & Manzanos, 1996; Sérot, Baron, Knockaert and Vallet, 2004). Few studies have observed that total phenolics and antioxidant capacity of chipotle pepper are similar or higher than those of fresh Jalapeño peppers (Álvarez-Parrilla et al., 2011; Hervert-Hernández, Sáyago-Ayerdi, & Goñi, 2010). However, the effect of smoking on the phytochemical content of red Jalapeño pepper has not been studied. The aim of this study was to evaluate the phytochemical content and antioxidant activity of fresh red Jalapeño pepper and chipotle pepper smoked with traditional firewood (pecan) and alternative woods such as walnut–oak and oak–poplar. Also, the phytochemical content of red Jalapeño pepper in different stages of the smoking process (carried out with traditional wood) was evaluated.

2. Materials and methods

2.1. Samples

Samples from fresh red Jalapeño pepper, red Jalapeño at different stages of smoking and chipotle pepper (final product obtained with different firewood) were kindly supplied by the “Asociación de Chipotleros de Camargo” (Camargo Chipotle producers Association), Camargo Chihuahua, Mexico. To determine the effect of the smoking process, using the traditional firewood (pecan), on the phytochemical profile of peppers, 1 kg of fresh red Jalapeño pepper (day 0) and partially...
smoked pepper (days 1–6 at 65–75 °C) were randomly collected from the smoking installation during 6 days. In addition, to evaluate the effect of different firewood, one-kilogram samples of chipotle pepper (final product) smoked with two wood combinations (oak–pecan and oak–poplar) were collected and compared with the final product obtained with the traditional smoking process. All samples were kept in paper bags at 4 °C, transported to the laboratory, where they were sorted to eliminate damaged product; peduncles of peppers were removed, and peppers were cut in 4 pieces and frozen at −80 °C for 1 day. Samples were freeze-dried for 48 h (Labconco freeze/dry/shell freeze system, Labconco Corp., Kansas City, MO), milled in a laboratory miller and stored in vacuum at −80 °C. Moisture was determined from the difference in weight before and after lyophilization (Alvarez-Parrilla et al., 2011).

2.2. Extraction of phenolic compounds

25 g of milled freeze-dried samples were mixed with 80% methanol at a 1:10 (w/v) ratio, and sonicated (40 kHz) for 30 min in the dark. The extract was centrifuged (2000g) for 30 min at 4 °C and the supernatant was collected. The residues were re-extracted under the same conditions, and both supernatants were combined. The solvent was partially removed under vacuum at 40 °C, and then freeze-dried (−47 °C) for 48 h. Dried extracts were stored at −80 °C under vacuum for further analysis (Alvarez-Parrilla et al., 2011).

2.3. Determination of total phenolic content

Total phenolic content was measured following the method described by Alvarez-Parrilla et al. (2011) with slight modifications. An aliquot (50 μL) of pepper extract solution (2 mg/mL in methanol) was mixed with 100 μL of 7.5% sodium carbonate and incubated at room temperature. After 3 min of incubation, 250 μL of Folin–Ciocalteu’s reagent (1:10 v/v) was added to the mixture and incubated at 50 °C for 15 min and cooled to room temperature. Absorbance was read at 760 nm in a microplate reader (Microplate Spectrophotometer, Bio Rad Mexico). Gallic acid was used as standard, and results were expressed as mg of gallic acid equivalents (GAE)/g of dry weight (DW).

2.4. Determination of total flavonoid content

Total flavonoids were determined according to Alvarez-Parrilla et al. (2011). An aliquot (0.25 mL) of dissolved extract (2 mg/mL in methanol) was mixed with 2 mL of water and 125 μL of 5% NaNO2 and incubated at room temperature. After 5 min, 125 μL 10% AlCl3 was added and the content of the flask mixed thoroughly. After 3 min, 2 mL of 0.5 M NaOH was added and incubated at room temperature for 30 min. Absorbance was read at 510 nm in a microplate reader. Catechin was used as standard and results were expressed as milligrams of Catechin equivalents (GAE)/g of DW.

2.5. Extraction and quantification of ascorbic acid

Ascorbic acid (AA) content in pepper samples was determined according to Alvarez-Parrilla et al. (2011). Ascorbic acid was extracted from pepper samples by sonicating 0.2 g of the freeze-dried sample with 5 mL of metaphosphoric acid (5%) for 20 min in the dark, then samples were centrifuged at 3500 rpm for 10 min at room temperature and supernatant was collected. Ascorbic acid was quantified by mixing 300 μL of supernatant with 200 μL of 6.65% trichloroacetic acid and 75 μL of DPNH (dinitrophenylhydrazine) reagent (2 g dinitrophenylhydrazine, 230 mg of thiourea and 270 mg of CuSO4·5H2O in 100 mL of 5 M sulfuric acid). The mixture was incubated for 3 h at 37 °C before addition of 0.5 mL of 65% H2SO4. 250 mL of this mixture was placed in a microplate, and absorbance measured at 520 nm. Ascorbic acid was used as a standard, and results were expressed as mg AA/g of DW.

2.6. Extraction and quantification of total carotenoids

Carotenoids were determined according to the method described by López-Cervantes et al. (2014) with slight modifications. Briefly, 0.25 g of freeze-dried sample was sonicated for 20 min with 10 mL of acetone, centrifuged for 5 min at 2500g, and supernatant was recovered. The extraction was repeated, both supernatants were mixed in a 50 mL volumetric flask and the volume completed with acetone. 1 mL of this solution was mixed with 9 mL of acetone and 200 μL of this extract was placed in a microplate well. Absorbance was read at 454 nm in a microplate reader. β-carotene was used as a standard, and results were expressed as mg β-carotene/g of DW.

2.7. Antioxidant capacity

Antioxidant capacity of peppers samples was determined by different assays described below. For these assays, extracts were prepared as described for total phenolic compounds and total flavonoids.

2.7.1. Total antioxidant capacity by trolox equivalent antioxidant capacity (TEAC) assay

TEAC assay was performed according to the method described by Thaipong, Boonprakob, Crosby, Cisneros-Zevallos, and Byrne (2006) with slight modifications. 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation was prepared in 50 mL of 0.1 M saline phosphate buffer (PBS, pH 7.4, 0.15 M KCl) by mixing ABTS salt (7 mM, final concentration) with potassium persulfate (2.45 mM final concentration). This solution was kept in the dark at room temperature for 12–16 h before use. Afterwards, the ABTS+ solution was diluted with saline phosphate buffer to obtain an absorbance of 0.700 ± 0.1 at 734 nm and 285 μL was mixed with 12 μL of sample, blank (PBS) or trolox standard in a 96-well plate. The reaction was measured every 30 s for 6 min at 734 nm with a microplate reader (Microplate Spectrophotometer, Bio Rad Mexico). Inhibition percentage of radical scavenging activity was calculated using Eq. (1)

\[
\text{Inhibition} \% = \left( \frac{\text{Abs}_{\text{blank}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{blank}}} \right) \times 100.
\]

Where: Absblank is absorbance of ABTS+ (or DPPH+) at 6 min, and Abssample is absorbance of the radical plus sample or trolox standard at 6 min. The radical stock solution was freshly prepared daily, and all analyses were made in triplicate. A calibration curve was made by plotting Inhibition percentage against trolox concentration, and results were expressed as micromoles of trolox equivalents (TE)/g of DW.

2.7.2. 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity

The DPPH assay was performed according to the method described by Thaipong et al. (2006) with some modifications. 50 μL of sample or trolox standard was mixed with 200 μL of DPPH radical (190 μM in methanol) into each well of a 96-well plate, and absorbance was measured at 515 nm every 30 s for 10 min with a microplate reader. The inhibition percentage of the radical scavenging activity was calculated using Eq. (1) and a calibration curve prepared as described in the previous section. Results were expressed as micromoles of TE/g of DW.

2.7.3. Ferric reduction antioxidant power (FRAP)

FRAP was conducted following the method described by Benzie and Strain (1996) with slight modifications. 180 μL of FRAP reagent (0.3 M acetate buffer (pH 3.6), 10 mM TPTZ–HCl (2,4,6-Tripyridyl-s-Triazine; HCl 40 mM), and 20 mM ferric chloride 10:1:1, v/v/v, heated at 37 °C for 30 min) was mixed with 24 μL of sample or trolox standard into each well of a 96-well plate, and absorption was measured at 595 nm every 60 s during 30 min. Results were expressed in millimoles of TE/g of DW.
2.8. Identification and quantification of phenolic compounds by HPLC–DAD

Identification and quantification of phenolic compounds in samples of pepper were carried out following the method described by de la Rosa, Alvarez-Parrilla, and Shahidi (2011) with slight modifications. Before HPLC analysis, samples were hydrolyzed, by mixing 15 mg of each sample with 10 mL of acidified methanol (25%; 2 M HCl) and heating to 95 °C for 60 min. Samples were cooled and extracted with 10 mL of ethyl ether in triplicate. The organic phase was recovered, solvent evaporated and dry solids suspended in methanol (1 mg/mL). 15 μL of this solution was filtered through a 0.45 μm filter and injected into a Perkin Elmer model 200 series HPLC equipment with diode array detector (DAD). Separation was achieved using a Supercosil C18 column (250 × 4.6 mm, 5 µm, Phenomenex Inc., Torrence CA), a binary mobile phase was used (solvent A, acetonitrile 5% in methanol; solvent B, formic acid 1% in water) with the following gradient program: 0 min, 100% B; 2.5 min, 100% B; 3 min, 50% B; 17 min, 100% A and 5 min, 100% B. A flux rate of 1 mL/min was used and detection wavelengths were 280 and 320 nm. Identification and quantification of phenolic compounds was achieved by comparison of retention time (rt) and UV spectra with those of pure standards (gallic acid, catechin, quercetin, naringenin, chlorogenic acid, caffeic acid, p-coumaric acid).

2.9. Identification and quantification of capsaicinoids by HPLC–DAD

Capsaicinoids in red Jalapeño and chipotle peppers were identified and quantified according to the method described by Alvarez-Parrilla et al. (2011). Briefly, 0.5 g of freeze-dried samples was extracted with 10 mL of methanol by sonication in the dark for 20 min. Then samples were centrifuged at 2000g for 5 min and supernatant was collected. The extraction was repeated, and both supernatants were combined and stored at −20 °C until analysis. Identification and quantification of capsaicinoids were carried out in the HPLC equipment formerly described with a Supercosil LC-18 reverse-phase column (5 μm particle size, 250 × 4.6 mm i.d., Phenomenex, Torrence CA), a binary mobile phase was used (solvent A, acetonitrile 5% in methanol; solvent B, acetonitrile 1% in water) with the following gradient program: 0 min, 100% B; 2.5 min, 100% B; 3 min, 50% B; 17 min, 100% A and 5 min, 100% B. A flux rate of 1 mL/min was used and detection wavelengths were 280 and 320 nm. Identification and quantification of phenolic compounds was achieved by comparison of retention time (rt) and UV spectra with those of pure standards (gallic acid, catechin, quercetin, naringenin, chlorogenic acid, caffeic acid, p-coumaric acid).

2.10. Statistical analysis

All analyses were carried out by triplicate. Values were expressed as mean ± standard deviation (SD). One way analysis of variance (ANOVA), and Tukey analyses were performed in order to determine statistical differences (p < 0.05) between the samples. All data were analyzed using the commercial software SPSS 21 (SPSS Inc. Headquarters, Chicago, IL, USA).

3. Results and discussion

3.1. Effect of traditional firewood smoking process

Chipotle is a smoked dried red Jalapeño pepper highly consumed in Mexico, traditionally produced by smoking red Jalapeño pepper in an open smoker, with a temperature maintained between 65–75 °C, using pecan (Carya illinoinensis) firewood. In order to analyze the effect of the smoking process on the phytochemical content and antioxidant capacity of peppers, samples of partially dried peppers were randomly selected each day of the process (days 0–6). Moisture of red Jalapeño peppers slightly decreased from 88 to 81% during the first three days, and once the outer cuticle was broken moisture drastically decreased to a final value of 6%.

Fig. 1A depicts the change in total phenolics and flavonoid contents in red Jalapeño samples during its transformation into chipotle. In order to avoid confusion due to the effect of water loss during the smoking process, all values are reported in dry weight (DW) basis. Initial total phenolic content was 13.91 mg GAE/g DW, this value was higher than those reported for fresh green Jalapeño pepper (Alvarez-Parrilla et al., 2011), because the content of phenolics in peppers increases with the ripening (Ornelas-Paz et al., 2010). Other studies also showed that Jalapeño at its maximum state of maturation (red) contains higher phenolic content than green stage Jalapeño pepper (Adedayo, Oboh, & Akindahunsi, 2010; Marín, Ferreres, Tomás-Barberán, & Gil, 2004). This increase in phenolic content in red peppers compared with green peppers could be due to an increment in PAL (Phenylalanine Ammonia Lyase) activity during fruit ripening which induces the accumulation of several compounds, like phenols and flavonoids, which play a major role in pigmentation and protection of certain fruits (Rupinder, Smita, & Upendra, 2010). Total phenolic content remained practically unchanged during the first three days, and then steadily increased reaching a final value of 18.19 mg GAE/g DW, resulting in a 42% increase compared to initial level in fresh red Jalapeño pepper. This increase could be due to adsorption of phenolic compounds found in the firewood, like phenolic acids, which are part of the wood structure (lignin) and can be liberated during its combustion (Kjällstrand & Petersson, 2001; Nascimento, Santana, Maranhão, Oliveira, & Bieber, 2013), once
the outer cuticle was broken. This increase could also be explained as a result of disruption of the food matrix, which allows for a better extractability of phenolic compounds in agreement with Ornelas-Paz et al. (2010) and Németh, Takácsöva and Piskula (2003), who found an increase in phenolic compounds and flavonoids in cooked peppers and onions, respectively, compared to raw products. Another explanation could be heat-inhibition of polyphenol oxidase (PPO) and other enzymes involved in phenolic compound degradation (Vicente, Martínez, Chaves, & Civello, 2006).

Total phenolic content of dry chipotle pepper (18.19 mg GAE/g DW) was similar to that reported by Hervert-Hernández et al. (2010) for extractable phenolic content in chipotle pepper and higher than that reported by Alvarez-Parrilla et al. (2011), for canned chipotle pepper, implying that food processing, such as canning, may decrease phenolic content of chipotle.

Initial total flavonoid content, determined by the Aluminum complexation method, for fresh red Jalapeño pepper was 1.16 mg CE/g DW (Fig. 1A), about half of the reported values for green Jalapeño peppers using the same methodology (Alvarez-Parrilla et al., 2011). Smoking, total carotenoids) were able to preserve carotenoids from oxidative degradation (Daoud, Kaptiány, Biacs, & Albrecht, 2006). Antioxidant capacity of red Jalapeño peppers, determined by FRAP, DPPH and TEAC methods, during the smoking process is presented in Fig. 2. Results from the three methods showed a similar behavior. Antioxidant capacity of the samples decreased during the first 3 to 4 days; and thereafter increased until the end of the smoking period, reaching values statistically higher than the initial ones (for DPPH and TEAC assays). When comparing results of antioxidant capacity obtained in this study for fresh red Jalapeño pepper, with those reported by other authors, it is possible to observe that our results are slightly lower than those reported with DPPH, for green (Alvarez-Parrilla et al., 2011) and red peppers (Ghasemnezhad, Sherafati, & Payvast, 2011) and in the range of those reported with TEAC (ABTS+ scavenging) for green Jalapeño peppers (Alvarez-Parrilla et al., 2011). Dry chipotle pepper showed higher DPPH and TEAC values compared to those reported by Alvarez-Parrilla et al. (2011) and Hervert-Hernández et al. (2010), but similar FRAP values compared to those reported by Hervert-Hernández et al. (2010). The behavior observed in Fig. 2 could be explained in terms of the content of all the phytochemicals during the drying process. Vitamin C decreased during the whole smoking process, but similar FRAP values compared to those reported by Hervert-Hernández et al. (2010).

### Table 1

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total phenols a</th>
<th>Flavonoids b</th>
<th>Ascorbic acid c</th>
<th>Total carotenoids d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jalapeño pepper</td>
<td>13.91 ± 0.5 g</td>
<td>1.16 ± 0.37 g</td>
<td>14.14 ± 0.46 g</td>
<td>2.04 ± 0.09 g</td>
</tr>
<tr>
<td>Pecan wood</td>
<td>18.19 ± 0.7 g</td>
<td>15.73 ± 0.48 g</td>
<td>8.17 ± 0.54 g</td>
<td>2.12 ± 0.22 g</td>
</tr>
<tr>
<td>P-O woods</td>
<td>15.31 ± 0.5 g</td>
<td>17.07 ± 1.61 g</td>
<td>7.26 ± 0.16 g</td>
<td>2.40 ± 0.07 g</td>
</tr>
<tr>
<td>O-Po woods</td>
<td>16.24 ± 0.42 g</td>
<td>16.21 ± 1.59 g</td>
<td>7.50 ± 0.26 g</td>
<td>1.84 ± 0.03 g</td>
</tr>
</tbody>
</table>

Values represent the mean of three or four measurements (±SD). P-O (pecan–oak firewood combination); O-Po (oak–poplar firewood combination). Values in the same column with different letters are significantly different (Tukey test, P < 0.05).

### Table 2

<table>
<thead>
<tr>
<th>Sample</th>
<th>ABTS‡</th>
<th>DPPH‡</th>
<th>FRAP‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jalapeño pepper</td>
<td>50.0 ± 3.3 mg</td>
<td>25.2 ± 1.4 mg</td>
<td>51.1 ± 7.6 mg</td>
</tr>
<tr>
<td>Pecan wood</td>
<td>70.7 ± 7.7 mg</td>
<td>34.5 ± 2.6 mg</td>
<td>51.8 ± 6.1 mg</td>
</tr>
<tr>
<td>P-O woods</td>
<td>42.2 ± 1.9 mg</td>
<td>16.2 ± 1.1 mg</td>
<td>47.0 ± 6.3 mg</td>
</tr>
<tr>
<td>O-Po woods</td>
<td>48.4 ± 3.0 mg</td>
<td>22.4 ± 2.0 mg</td>
<td>48.3 ± 6.3 mg</td>
</tr>
</tbody>
</table>

Values represent the mean of three measurements (±SD). P-O (pecan–oak firewood combination); O-Po (oak–poplar firewood combination). Values in the same column with different letters are significantly different (Tukey test, P < 0.05).

### Table 3

<table>
<thead>
<tr>
<th>Sample</th>
<th>Chlorogenic acid</th>
<th>Catechin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jalapeño pepper</td>
<td>834.1 ± 265.9 mg</td>
<td>652.1 ± 166 mg</td>
</tr>
<tr>
<td>Pecan wood</td>
<td>959.7 ± 164.4 mg</td>
<td>4712.6 ± 1251.3 mg</td>
</tr>
<tr>
<td>P-O woods</td>
<td>272.6 ± 87.6 mg</td>
<td>3646.9 ± 1105.8 mg</td>
</tr>
<tr>
<td>O-Po woods</td>
<td>680.2 ± 345.5 mg</td>
<td>2820.3 ± 1064.6 mg</td>
</tr>
</tbody>
</table>

Values represent the mean of three or four measurements (±SD). P-O (pecan–oak firewood combination); O-Po (oak–poplar firewood combination). Values in the same column with different letters are significantly different.
however, flavonoids and phenols increased during this process, so it is possible to assume that the antioxidant capacity of the smoked chipotle samples improved due to the combined effect of their own phytochemical compounds (phenolic compounds and flavonoids) and those provided by the combustion of wood (Howard, Talcott, Brenes, & Villalon, 2000; Materska & Perucka, 2005).

Table 4
Capsaicin, dihydrocapsaicin (DHC), and nordihydrocapsaicin (Nor-DHC) (μg/g DW) and their proportion in terms of total capsaicinoid content of fresh red Jalapeño and Chipotle peppers. Values represent the mean of two measurements (±SD). P–O (pecan–oak firewood combination); O–Po (oak–poplar firewood combination). Values in the same column with different letters are significantly different (Tukey test, P < 0.05).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Capsaicin</th>
<th>DHC</th>
<th>Nor–DHC</th>
<th>Total capsaicinoids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jalapeño PEPPER</td>
<td>579.61 ± 154.85 (43.80%)</td>
<td>595.9 ± 166.5 (45.75%)</td>
<td>135.99 ± 37.63 (10.4%)</td>
<td>1302.50 ± 359.02a</td>
</tr>
<tr>
<td>Pecan wood</td>
<td>376.41 ± 39.11 (45.1%)</td>
<td>372.9 ± 24.96 (44.6%)</td>
<td>85.30 ± 8.18 (10.3%)</td>
<td>834.61 ± 72a</td>
</tr>
<tr>
<td>P–O wood</td>
<td>103.52 ± 31.6 (46.81%)</td>
<td>117.61 ± 24.05 (53.18%)</td>
<td>n/d</td>
<td>221.13 ± 55.64b</td>
</tr>
<tr>
<td>O–Po wood</td>
<td>507.94 ± 133.54 (41.1%)</td>
<td>623.06 ± 112.5 (50.5%)</td>
<td>103.92 ± 19.02 (8.4%)</td>
<td>1234.93 ± 265.07a</td>
</tr>
</tbody>
</table>

Fig. 3. HPLC-DAD chromatogram of (A) capsaicinoid standards (peaks: 1, nordihydrocapsaicin; 2, capsaicin; 3, dihydrocapsaicin), (B) fresh Jalapeño pepper and (C) oak–poplar chipotle pepper. UV detection at 280 nm.
3.2. Phytochemicals and antioxidant capacity in chipotle peppers smoked with different types of firewood

In order to evaluate the effect of different types of firewood on phytochemical profile of chipotle pepper, two combinations of wood were used: oak–pecan and oak–poplar, and compared to the results obtained with pecan wood. Table 1 summarizes phytochemical content of fresh red Jalapeño and chipotle peppers produced with the traditional wood (pecan) and the two wood combinations. As expected, all three chipotle samples showed higher total phenolic content than fresh red Jalapeño pepper. The sample smoked with pecan wood (traditional) presented the highest total phenolic content, followed by the one smoked with oak–poplar and finally with oak–pecan. For flavonoids, a large increase (approximately 13–14 times) was observed in all chipotle peppers, independently of the wood used for smoking. These results are in agreement with those reported by Choi, Lee, Chun, Lee, and Lee (2006) who independently of the wood used for smoking. These results are in agreement with those reported by Choi, Lee, Chun, Lee, and Lee (2006) who observed an increase in the flavonoid content of smoked shitake mushrooms, in comparison with fresh shitake.

Vitamin C content largely decreased (42–48%) in all chipotle samples, compared with fresh Jalapeño pepper. Carotenoids were highest in pecan–oak smoked chipotle peppers, followed by pecan wood smoked chipotle peppers, which were similar to fresh Jalapeño pepper, and finally samples smoked with oak–poplar wood had the lowest carotenoid content. These results could be explained considering that, even though temperature was not measured, producers stated that poplar burned faster and produced higher temperatures during smoking process, and consequently a degradation of carotenoids may happen (Topuz et al., 2011). Further studies under control conditions are carried out to better understand the effect of different temperatures and smoking conditions on the phytochemical content of chipotle peppers.

Type of wood used in the preparation of chipotle peppers significantly affected their phytochemical content and therefore their antioxidant capacity (Table 2). Chipotle obtained with pecan wood presented the highest antioxidant capacity, measured by the three methods compared to Pecan–Oak and Oak–Poplar woods. DPPH and TEAC values obtained with pecan wood were statistically higher than control, while no statistical differences were observed with FRAP. Oak–poplar samples showed no statistical differences in antioxidant capacity (measured by the three methods) compared to control, while pecan–oak presented the lowest values of antioxidant capacity (DPPH and FRAP) compared to control.

The main phenolic compounds identified and quantified by HPLC in fresh Jalapeño and chipotle peppers were chlorogenic acid and catechin (Table 3). Jalapeño pepper showed a chlorogenic acid content of 83.41 mg/100 g DW, which was lower than that reported by Chen and Kang (2013) for red peppers (170 mg/100 g DW). Sample smoked with pecan and oak–poplar firewood showed chlorogenic acid values similar to fresh peppers. However, pecan–oak smoked chipotle showed the lowest chlorogenic acid content. If, as previously discussed, increment in total phenolic compounds in smoked peppers is due to heat-induced matrix disruption and better extractability, and the combustion temperature of each wood combination is different (Chung & Spearpoint, 2007), we may hypothesize that the pecan–oak combination combustion temperature was worst for liberating phenolic compounds such as chlorogenic acid. This result is supported by the fact that total phenolic compounds were lowest in pecan–oak smoked samples than in the samples smoked with the other firewood.

Catechin content in fresh Jalapeño peppers was similar to that reported by Zhuang, Chen, Sun, and Cao (2012) for different hot peppers. This flavonoid increased between 4 and 7 times in all chipotle samples compared with fresh Jalapeño pepper (Table 3). These results are consistent with those of total flavonoids, where a significant increase was also observed during the smoking process, and suggest that the burning temperature of all firewood didn’t have any effect on the extractability of flavonoids.

The main capsaicinoids responsible for pungent flavor (capsaicin, dihydrocapsaicin, and nordihydrocapsaicin) were identified and quantified by HPLC in fresh red Jalapeño pepper and smoked samples. Fig. 3A shows the chromatogram of standards, while Fig. 3B and C shows chromatograms of fresh Jalapeño and oak–poplar smoked chipotle, respectively. The content of capsaicinoids in fresh and smoked peppers is presented in Table 4. Dihydrocapsaicin was the most abundant, ranging from 44 to 54% of total capsaicinoids in all samples, followed by capsaicin and nordihydrocapsaicin, respectively. These results are in agreement with those previously published (Alvarez-Parrilla et al., 2011; Ornelas-Paz et al., 2010). Nordihydrocapsaicin was not detected in the sample smoked with poplar–oak. Total capsaicinoid content was highest in fresh Jalapeño pepper, followed by oak–poplar and pecan smoked samples, but without significant differences among the three groups. Finally, the pecan–oak smoked samples showed the lowest total capsaicinoid concentration. These results agree with those reported by Ornelas-Paz et al. (2010), who proposed several causes for a reduction of capsaicinoids in cooked peppers, among them, the effect of high temperature, which catalyzes fragmentation of and oxidation of capsaicin to produce vanillin (Bernal et al., 1993).

4. Conclusion

Total phenolic compounds flavonoids, catechin and antioxidant capacity were significantly increased in chipotle peppers produced by the three firewood used in this study; however peppers smoked with pecan firewood, which is the traditionally used wood, presented the highest values. In contrast, ascorbic acid was reduced in all smoked samples, and total carotenoids, capsaicinoids and chlorogenic acid were differentially affected by the firewood type. Results suggest that smoking has a little effect on degradation of phytochemicals, except ascorbic acid, and pecan wood is probably the best firewood for chipotle production.

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