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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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ABSTRACT

Chipotle pepper is a dry smoked red Jalapeño pepper well appreciated in the Mexican and American cuisines. Phytochemical content and antioxidant activity of red Jalapeño pepper has been previously reported, however, the effect of the smoking process in the content of phytochemicals, antioxidant capacity and capsaicinoids has not been previously reported. In the present study we found that the smoking process had a significant effect on the content of polyphenolic compounds and antioxidant capacity of red Jalapeño pepper. It induced a nearly 50% increase in total phenols and 15% increase in antioxidant capacity in chipotle pepper compared to fresh samples (in dry weight basis). Capsaicinoids and ascorbic acid content decreased in smoked samples, while carotenoids remained practically unchanged. The strongest effect of smoking was observed in the content of total flavonoids and catechin determined by HPLC. Therefore we can conclude that smoking process has a positive effect in the fruit because it raises its phytochemical properties. The type of wood used in the smoking process also had an effect on the phytochemical content and antioxidant capacity with traditional pecan wood being the best.

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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 (Alvarez-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 (Alvarez-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

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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 freezedry/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% $NaNO_2$ and incubated at room temperature. After 5 min, 125 μL 10% $AlCl_3$ 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 (CE)/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 DNPH (dinitrophenylhydrazine) reagent (2 g dinitrophenylhydrazine, 230 mg of thiourea and 270 mg of $CuSO_4 \cdot 5H_2O$ 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% H_2SO_4. 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 $ABTS^{\bullet+}$ 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)

$$\text{Inhibition } (\%) = \frac{(\text{Abs}_{\text{blank}} - \text{Abs}_{\text{sample}})}{\text{Abs}_{\text{blank}}} \times 100. \quad (1)$$

Where: Abs_{blank} is absorbance of $ABTS^{\bullet+}$ (or DPPH•) at 6 min, and Abs_{sample} 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 ($DPPH^{\bullet}$) 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 milimoles of TE/g of DW.

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