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Effect of heat treatment on the content of some bioactive compounds and free radical-scavenging activity in pungent and non-pungent peppers

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ABSTRACT

Raw and heat-treated (boiled and grilled) pungent and non-pungent peppers (poblano, bell, chilaca, caribe, jalapeño, serrano, habanero and manzano) were evaluated for their content of some bioactive compounds and free radical-scavenging activity. Boiling and grilling were performed under household conditions. Ascorbic acid content in raw peppers varied from 306 to 3438 μg/g. This content was reduced 15–87% by heat treatments. Total carotenoids content in raw peppers ranged between 1 and 156 μg/g. β-Carotene represented 3–78% of total carotenoids in raw peppers. β-Carotene content was reduced (1–45%) by heat treatments. Free radical-scavenging activity varied widely (7–101 μmol TE/g) in raw peppers. Boiling and grilling reduced (6–93%) sequentially the antiradical activity of pungent peppers. In contrast, gradual increases of antiradical activity in non-pungent peppers were induced by boiling and grilling. Household heat treatment altered highly the content of bioactive/antioxidant compounds in tested peppers.

1. Introduction

Peppers (*Capsicum spp*) are consumed worldwide, imparting flavor, aroma and color to foods. Besides their sensory importance, peppers play a relevant role on human health since they contain high concentrations of many biofunctional and antioxidant compounds, including ascorbic acid (AA; vitamin C) and carotenoids. Carotenoids are the pigments responsible for the yellow, orange and red color of many pepper types, being β-carotene one of the most abundant carotenoids in this vegetable (Sun et al., 2007). β-Carotene is a precursor of vitamin A and exerts protective effects against cardiovascular diseases and some forms of cancer (Yahia & Ornelas-Paz, 2010). AA prevents allergies, reduces the levels of circulating proinflammatory cytokines, modulates gene expression and cell cycle progression, prevents some forms of cancer and neurological (Alzheimer, Parkinson, Huntington and cerebral ischemia) and cardiovascular diseases (Davey et al., 2000; Harrison & May, 2009). AA is involved in neural maturation, neuronal transmission, learning/memory and locomotor activity (Harrison & May, 2009). Biological actions of AA, carotenoids and other compounds commonly found in peppers have been discussed elsewhere (Davey et al., 2000; Ornelas-Paz, Martínez-Burrola, et al., 2010; Ornelas-Paz, Yahia, & Gardea-Béjar, 2010; Yahia & Ornelas-Paz, 2010).

Free radicals may be harmful, leading to inflammation, tissue damage and development of diseases. Free radicals are involved on the pathogenesis of at least 100 different diseases, including cancer, atherosclerosis, rheumatoid arthritis, inflammatory and cataracts (Yahia & Ornelas-Paz, 2010). Humans use several lines of defense against free radicals, including endogenous enzymes and proteins as well as dietary antioxidants. The latter are commonly found in fruits and vegetables and therefore their consumption has been associated with protection against several non-communicable diseases (Davey et al., 2000; Harrison & May, 2009; Yahia & Ornelas-Paz, 2010). Peppers are rich in free radical scavengers, including chlorophylls, carotenoids, phenolics, AA, capsaicinoids and tocopherols (Ornelas-Paz, Martínez-Burrola, et al., 2010); however, the free radical-scavenging activity of peppers has been mainly attributed to their contents of polyphenols and AA (Materska & Perucka, 2005). The contribution of AA to the antioxidant capacity in fresh peppers was estimated to be between 72% and 88% (Greco, Riccio, Bergero, Del Re, & Trevisan, 2007). A positive correlation between phenols content and the antioxidant activity in peppers has been reported by Howard, Talcott, Brenes, and Villalon (2000). More specifically, Lee, Howard, and Villalón (1995) observed a linear relationship between flavonoid concentration and antioxidant activity in pungent peppers. β-Carotene showed only a minor participation on the lipophilic reducing capacity of peppers (Greco et al., 2007).

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In Mexico, peppers represent a tradition, cultural identity and an important source of income. Consumption of *Capsicum* spices in Mexico was reported to be 20 g/person/day (Ornelas-Paz, Martínez-Burrola, et al., 2010). Sauces, salads and many dishes in Mexican cuisine include raw, boiled and grilled pungent and non-pungent pepper types. These cooking methods alter positively the sensory attributes of peppers but can greatly affect their contents of biofunctional compounds and their free radical-scavenging activity. AA disappears quickly from foods during cooking as a consequence of its thermolability and solubility in water (Davey et al., 2000). AA content in sweet peppers was reduced 28%–73% by several processing methods, including grilling (Greco et al., 2007). Similar AA losses (75%) were observed in pungent peppers (Jalapeño) as a consequence of heating (Howard, Smith, Wagner, Villalon, & Burns, 1994). β-Carotene content in Italian peppers was reduced (16%–60%) by different styles of thermal processing (Greco et al., 2007). In contrast, the content of β-carotene in other pepper types was unaffected or even increased by heat treatment (Bernhardt & Schich, 2006; Greco et al., 2007). On the other hand, high decrements (23%–36%) on the antiradical activity have been reported in green and red peppers after boiling for 5–30 min (Chuah et al., 2008). Similar findings have been reported for several vegetables during aquathermal processing (Sikora, Cieslik, Leszczynska, Filipiak-Florkiewicz, & Pisulewski, 2008). The stability of AA, carotenoids and antiradical activity in peppers depend on the conditions of the thermal treatment, genotype differences, ripening stage, geographical origin and other factors (agricultural practices, season and postharvest handling). Information about the changes on AA and carotenoids content and free radical-scavenging activity in Mexican peppers as a consequence of household heat treatment is scarce. The objective of this work was evaluate the effect of household boiling and grilling on the free-radical-scavenging activity and the contents of AA and carotenoids in the pepper types most consumed in Mexico.

2. Materials and methods

2.1. Chemicals and solvents

High-performance liquid chromatography (HPLC) grade methanol, *tert*-butyl methyl ether and water were purchased from J.T. Baker (Baker-Mallinckrodt, Mexico). L-AA (purity of 99%), β-carotene (purity ~97%), 6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) and 2,2-di(4-*tert*-octylphenyl)-1-picrylhydrazyl (DPPH$^{\bullet}$) were obtained from Sigma-Aldrich (St. Louis, MO). Other reagents and materials were obtained from J.T. Baker, unless stated otherwise.

2.2. Plant material and heat treatments

Several pepper types were included in the work, according to their availability in the market or commercial orchards. Poblano, chilaca, caribe, bell, habanero and manzano peppers were purchased in the local market. Jalapeño and serrano peppers were cultivated on open field in Chihuahua, Mexico. These peppers were collected from the field containers during harvesting. Buy and cultivated peppers were freshly harvested. All fruits were physiologically ripe; however, fruits of each pepper type were classified in a more precise ripening stage based on their color (green, yellow or red) to be evaluated separately. The number of stages of ripening studied for each pepper type also was determined by their availability in the market or orchard. Our previous results about capsaicinoids content in these pepper types (Ornelas-Paz, Martínez-Burrola, et al., 2010) were used to classify the fruits in pungent and non-pungent (Table 1). Only fruits free of blemishes and defects were included in this study.

Samples (6 or 9) of six fruits each were formed from each pepper type at specific ripening stages (green, yellow or red). Three samples were boiled at 96 °C in a covered pan (containing water in an approximate ratio fruit to water of 1:4, v/v) and other three samples were grilled on a hot plate at 210 °C according to local procedures to make pepper sauces or dishes. Boiling (7–13.5 min) and grilling (8.8–19 min) times were based on those previously reported for these pepper types (Ornelas-Paz, Martínez-Burrola, et al., 2010). Fruit position on the hot plate was changed continuously during grilling. Poblano (GP), chilaca (GC) and caribe (YC) peppers were not boiled and were used in the grilled form after removing of placenta, according to the local use. Each sample was individually cooked. The remaining three samples were used as not thermally treated controls. Pepper samples were weighted before and after heat treatment in order to determine gravimetrically the weight loss during cooking. Cooked and raw samples were immediately analyzed for AA and free radical-scavenging activity. Remaining samples were packed in polyethylene bags and stored at −20 °C for 5 days until carotenoids analysis. Preliminary studies demonstrated that carotenoids were completely stable during such short-time storage.

Table 1
Pepper types analyzed in the present study.

Pepper type (stage of ripening)	Abbreviated name	Genus and specie	Sensorial classification
Habanero (green)	GH	*Capsicum chinense* J.	Pungent
Habanero (yellow)	YH	*Capsicum chinense* J.	Pungent
Manzano (yellow)	YM	*Capsicum pubescens* R and P.	Pungent
Serrano (green)	GS	*Capsicum annuum* L.	Pungent
Serrano (red)	RS	*Capsicum annuum* L.	Pungent
Jalapeño (green)	GJ	*Capsicum annuum* L.	Pungent
Jalapeño (red)	RJ	*Capsicum annuum* L.	Pungent
Caribe (yellow)	YC	*Capsicum annuum* L.	Pungent
Chilaca (green)	GC	*Capsicum annuum* L.	Pungent
Poblano (green)	GP	*Capsicum annuum* L.	Pungent
Bell pepper (green)	GBP	*Capsicum annuum* L.	Non-pungent
Bell pepper (yellow)	YBP	*Capsicum annuum* L.	Non-pungent
Bell pepper (red)	RBP	*Capsicum annuum* L.	Non-pungent

2.3. Color measurements

Pepper samples were evaluated for tristimulus color, previous to bioactive compounds and free radical-scavenging activity analysis. Samples of whole peppers were homogenized to puree in a kitchen blender (Taurus, Model Robot 180) during 2 min. Three subsamples of puree were immediately evaluated for color using a Minolta colorimeter (Minolta, Co. Ltd., Osaka, Japan) on the basis of the CIELAB color system (L^*, a^* and b^*), as reported previously (Ornelas-Paz, Martínez-Burrola, et al., 2010). These three subsamples of pureed peppers were also individually evaluated for their content of bioactive compounds (AA and carotenoids) and free radical-scavenging activity.

2.4. Ascorbic acid analysis

AA was determined according to the method of Ruiz-Cruz et al. (2010). Aliquots of pureed peppers (2 g) were homogenized (Homogenizer Ika T18 Basics; IKa Works Inc., Wilmington, NC) in the presence of 4% metaphosphoric acid (15 mL). The mixture was sonicated (Sonicator VWR model 150 D; VWR International., West Chester, PA) for 5 min and centrifuged (Centrifuge Eppendorf model 5418; Eppendorf A.G., Hamburg, Germany) at 1610×*g* for 10 min at room temperature. The extract was filtered through a polyethylene membrane of 0.45 μm of pore size (Millipore Corp., Bedford, MA) and manually injected (20 μL) into a ProStar HPLC system (Varian Inc., Walnut Creek, CA), which was equipped with two pumps (Model 210) and a UV-Vis detector (Model 325). AA was monitored at λ = 254 nm. The chromatographic system included a Microsorb MV 100 C_{18} (4.6×250 mm, 5 μm) reversed-phase column (Varian Inc., Walnut Creek, CA), which was kept at 25 °C. The mobile phase (isocratic system) was composed of 0.2 M KH_2PO_4, adjusted to final pH value of 2.3 with 85% *o*-phosphoric acid. The flow rate of the