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Effect of cooking on the capsaicinoids and phenolics contents of Mexican peppers

José de Jesús Ornelas-Paz [a,*], J. Manuel Martínez-Burrola [a,c], Saúl Ruiz-Cruz [b], Víctor Santana-Rodríguez [c], Vrani Ibarra-Junquera [d], Guadalupe I. Olivas [a], J. David Pérez-Martínez [e]

[a] Centro de Investigación en Alimentación y Desarrollo A.C. Unidad Cuauhtémoc, Av. Río Conchos S/N, Parque Industrial, C.P. 31570. Cd. Cuauhtémoc, Chihuahua, Mexico
[b] Departamento de Biotecnología y Ciencias Alimentarias, Instituto Tecnológico de Sonora, 5 de Febrero 818 Sur, C.P. 85000, Cd. Obregón, Sonora, Mexico
[c] Facultad de Ciencias Químicas, Universidad Autónoma de Chihuahua, Escorza 900, C.P. 31000, Chihuahua, Mexico
[d] Facultad de Ciencias Químicas, Universidad de Colima, Km. 9 carretera Coquimatlán-Colima, C.P. 28400, Coquimatlán, Colima, Mexico
[e] Facultad de Ciencias Químicas, Universidad Autónoma de San Luis Potosí, Manuel Nava 6, Zona Universitaria, C.P. 78210, San Luis Potosí, Mexico

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ABSTRACT

Raw and cooked (boiled and grilled) Poblano, Bell, Chilaca, Caribe, Jalapeño, Serrano, Habanero, and Manzano peppers were evaluated for tristimulus colour, capsaicinoids (capsaicin, dihydrocapsaicin, nordihydrocapscapsaicin), and total phenolic contents. Boiling (96 °C) and grilling (210 °C) was performed under household conditions according to typical procedures of the Mexican cuisine. Contents of capsaicin (0.6–913.8 μg/g), dihydrocapsaicin (0–756.9 μg/g), nordihydrocapsaicin (0–68.2 μg/g), and total phenolics (1150.5–2190.0 μg of gallic acid equivalents/g) in raw peppers varied widely between types of peppers. Moderate losses (1.1–28.1%) in capsaicinoids were induced by boiling while grilling caused a significant increase (2.6–924.9%) in the content of these compounds. Proportion of individual capsaicinoids was similar in raw and cooked peppers. Boiling and grilling sequentially increased (7.4–137.0%) the total phenolic content in pungent peppers. Total phenolic content in non-pungent Bell peppers was reduced by cooking (1.6–26.9%). Boiling induced smaller changes in colour values (L^*, a^*, and b^*) compared to grilling. The results demonstrated that Mexican raw peppers are rich in capsaicinoids and phenolic compounds. Household cooking can cause either decreases or increases in the content of such compounds in peppers.

1. Introduction

Peppers (*Capsicum* spp.) are consumed world-wide, imparting flavour, aroma, and colour to foods. Capsaicinoids are distinctive components of this vegetable. They are synthesized exclusively in the epidermal cells of the placenta in Capsicum fruit and are accumulated in blisters along the epidermis (Cisneros-Pineda et al., 2007). Capsaicinoids are biosynthesized by condensation reaction between an aromatic moiety and a C9–C11 branched-chain fatty acid. The aromatic moiety is vanillylamine (phenylalanine derivative) and the branched-chain fatty acids are derived from valine and leucine. The profile of capsaicinoids in peppers depends on many factors, including ripening stage, geographical origin, type, and cultivar (Collins, Wasmund, & Bosland, 1995; Garcés-Claver, Arnedo-Andrés, Abadía, Gil-Ortega, & Álvarez-Fernández, 2006). Fifteen capsaicinoids and several related compounds have been identified in red peppers by HPLC-MS studies, being capsaicin, dihydrocapsaicin, and nordihydrocapsaicin the major capsaicinoids (Schweiggert, Carle, & Schieber, 2006). Capsaicinoids produce a burning sensation by activating sensory neurons that alert the central nervous system of noxious stimuli. Thus, capsaicinoids, particularly capsaicin, and dihydrocapsaicin are responsible for 90% of the pungency of peppers (Garcés-Claver et al., 2006). Besides their sensory importance, capsaicinoids exert several biofunctional activities. Capsaicinoids are used to ease the pain associated with neuropathy, cluster headaches, arthritis, muscle strain, postherpetic neuralgia, and postmastectomy pain syndrome. Capsaicinoids exert hypolipidemic, hypocholesterolemic, antilithogenic, antioxidant, anti-inflammatory, anti-tumour, and, anti-teratogenic activities (Srinivasan, 2005).

Some types of peppers are also rich in flavonoids and other phenolic compounds, which may occur as glycosides and contribute highly to the antiradical activity of this vegetable (Lee, Howard, & Villalón, 1995). This activity of phenolic compounds is based on the redox properties of their hydroxyl groups and the structural relationships between different parts of their chemical structure (Oboh & Rocha, 2007). Many studies have demonstrated protective roles of flavonoids and other phenolic compounds against coronary heart disease, stroke, and some forms of cancer. These protective effects of phenolic compounds are attributed to their antiradical and signalling activities in the cells (Oboh & Rocha, 2007).

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* Corresponding author. Tel./fax: +52 625 5812920.
E-mail address: jornelas@ciad.mx (J. de J. Ornelas-Paz).

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In México, peppers represent a tradition, cultural identity, and an important source of income. They are highly consumed mainly as a consequence of their pungent flavour (Contreras-Padilla & Yahia, 1998). The consumption of Capsicum spices in México was reported to be 20 g/person/day while the daily intake of capsaicinoids in that country has been estimated to be 200 mg/person/day (Council of Europe, 2001). Sauces, salads, and many dishes in Mexican cuisine include raw, boiled and grilled pungent and non-pungent genotypes of peppers. Household heat treatments can affect the content of capsaicinoids and phenolic compounds of peppers and therefore, may alter their biofunctional and sensorial attributes. Suresh, Manjunatha, and Srinivasan (2007) reported losses of capsaicin in dried red peppers as a result of boiling and pressure cooking. Pasteurisation of red pepper pastes and heat treatment of canned Jalapeño peppers also caused losses in capsaicinoids (Harrison & Harris, 1985; Schweiggert, Schieber, & Carle, 2006). In contrast, the content of capsaicinoids in Jalapeño peppers and dried red pepper either did not change, or increased after domestic boiling (Harrison & Harris, 1985; Srinivasan, Sambaiah, & Chandrasekhara, 1992). Household heat treatments also alter the content of phenolic compounds in peppers and other horticultural commodities (Turkmen, Sari, & Velioglu, 2005). Undoubtedly, stability of capsaicinoids and phenolic compounds depends on intensity and duration of thermal treatment but genotype differences could be also involved. The objective of this work was to study the effect of household boiling and grilling on the contents of capsaicinoids and total phenols in the genotypes of peppers most common in México, the second largest pepper producer in the world.

2. Materials and methods

2.1. Plant material and cooking methods

Fresh peppers of several types were obtained in the local market (Poblano, Chilaca, Caribe, Bell, Habanero, and Manzano) or cultivated (Jalapeño and Serrano), in Chihuahua, México. Fruits of each pepper type were classified according to their ripening stage (green, yellow, and red) in order to be studied individually. Only fruits free of blemishes and defects were included in the study.

Samples (6 or 9) of six fruits each were formed from each pepper type at specifics ripening stages (green, yellow, and red). Peduncle of peppers was removed and then weight and size (length and major diameter) of the fruit were determined. Biometrical characteristics of tested peppers are shown in Table 1. Three samples were boiled at 96 °C in a covered pan and others three samples were grilled on a hot plate at 210 °C according to local procedures to make pepper sauces or dishes. The position of peppers on the hot plate was changed continuously during grilling. Poblano, Chilaca, and Caribe peppers were only used in the grilled form after removing of placenta, according to their local use. Each sample was individually cooked and the duration of heat treatment was recorded. Temperatures of boiling water and hot plate were also recorded three times during each cooking process. After cooling at room temperature fruit of each sample were weighted again in order to determine weight loss during cooking process. The remaining samples (3) were not thermally treated. Treated and non-treated samples were packed in polyethylene bags and stored at −20 °C until capsaicinoids and total phenolic analysis.

2.2. Colour measurements

Frozen samples were homogenised to puree in a kitchen blender. Triplicate samples of puree were immediately evaluated for colour using a Minolta colourimeter (Minolta Co. Ltd., Osaka, Japan) on the basis of the CIELAB colour system (L^*, a^*, and b^*). Puree of peppers was also evaluated for capsaicinoids and total phenolic contents.

2.3. Capsaicinoids analysis

Puree of peppers (5 g) was homogenised (Homogenizer PowerGen 700 D; Fisher Scientific Co., Norcross, GA) in the presence of methanol (25 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 AG, Hamburg, Germany) at 1610g 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 (25 μl) to a ProStar HPLC system (Varian Inc., Walnut Creek, CA), which was equipped with two 210 pumps and a 325 UV–Vis dual wavelength detector. Capsaicinoids were monitored at 236 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 water (containing 1% of acetic acid) and acetonitrile (50:50, v/v). The flow rate of the mobile phase was 1 ml/min.

Capsaicin and dihydrocapsaicin were identified in extracts of peppers by co-chromatography studies using standard compounds. Nordihydrocapsaicin was tentatively identified on the basis of its chromatographic behaviour on a C_{18} column, as reported in the literature (Cooper, Guzinski, & Fisher, 1991; Schweiggert, Carle, et al., 2006; Schweiggert, Schieber, et al., 2006). Quantifica-

Table 1
Bio-metrical characteristics of the tested peppers.

Pepper type (stage of ripening)	Abbreviated name	Genus and species	Length (cm)	Major diameter (cm)	Weight (g)
Habanero (green)	GH	*Capsicum chinense* J.	4.0 ± 0.1^{g}	3.4 ± 0.1^{h}	8.5 ± 0.3^{g}
Habanero (yellow)	YH	*Capsicum chinense* J.	4.4 ± 0.1^{g}	3.3 ± 0.1^{hi}	8.9 ± 0.4^{g}
Manzano (yellow)	M	*Capsicum pubescens* R and P.	4.9 ± 0.1^{f}	4.5 ± 0.1^{f}	31.7 ± 0.7^{ef}
Serrano (green)	GS	*Capsicum annuum* L.	8.6 ± 0.1^{c}	1.8 ± 0.0^{k}	13.9 ± 0.4^{g}
Serrano (red)	RS	*Capsicum annuum* L.	7.0 ± 0.1^{e}	1.6 ± 0.0^{k}	10.2 ± 0.3^{g}
Jalapeño (green)	GJ	*Capsicum annuum* L.	7.6 ± 0.2^{d}	3.0 ± 0.0^{ij}	31.5 ± 0.6^{ef}
Jalapeño (red)	RJ	*Capsicum annuum* L.	9.0 ± 0.1^{c}	2.9 ± 0.1^{j}	28.3 ± 1.0^{f}
Caribe (yellow)	C	*Capsicum annuum* L.	7.8 ± 0.1^{d}	4.9 ± 0.1^{e}	48.4 ± 1.4^{d}
Chilaca (green)	GC	*Capsicum annuum* L.	15.0 ± 0.2^{a}	4.1 ± 0.1^{g}	46.1 ± 0.9^{de}
Poblano (green)	GP	*Capsicum annuum* L.	12.7 ± 0.2^{b}	6.5 ± 0.1^{d}	103.6 ± 2.5^{c}
Bell pepper (green)	GBP	*Capsicum annuum* L.	8.9 ± 0.1^{c}	10.1 ± 0.1^{a}	237.5 ± 4.1^{a}
Bell pepper (yellow)	YBP	*Capsicum annuum* L.	8.9 ± 0.1^{c}	9.1 ± 0.1^{c}	214.2 ± 8.5^{b}
Bell pepper (red)	RBP	*Capsicum annuum* L.	9.0 ± 0.1^{c}	9.7 ± 0.1^{b}	223.8 ± 6.0^{ab}

Values represent the mean of at least 36 individual measurements ± the standard error. Values in the same column not connected by the same letter are significantly different ($p < 0.05$).

tion of capsaicin and dihydrocapsaicin was carried out by calibration curves, which were constructed by three independent sets of dilutions of pure capsaicinoids. Nordihydrocapsaicin was quantified as capsaicin. Total capsaicinoids content was determined as sum of the contents of capsaicin, dihydrocapsaicin, and nordihydrocapsaicin. Recovery studies were performed using Vanillin (10 μg) as an internal standard.

2.4. Determination of the total phenolic content

Samples of pureed peppers (1 g) were homogenised in 80% methanol containing 0.5% of sodium bisulfite (10 ml) and then sonicated and centrifuged as mentioned above for capsaicinoids analysis. Aliquots (0.1 ml) of the extract were mixed with 0.5 ml of 50% Folin–Ciocalteu reagent and 7.9 ml deionised water. After the reaction solution had been incubated at room temperature for 10 min, 1.5 ml of 20% sodium carbonate solution was added and mixed well. The mixture was incubated at room temperature for 60 min. The absorbance of the solution was determined at 750 nm using a 6405 Jenway UV–Vis pectrophotometer (Jenway Ltd., Essex, UK). Gallic acid was used as a standard reference. Five independent measurements were done for each sample of puree. Total phenolic content was expressed as μg gallic acid equivalents of each g fresh pepper (μg GAE/g).

2.5. Statistical analysis

Data were analysed in a completely randomized-design structure. Statistical significance of the difference between raw, boiled and grilled peppers for each pepper type was determined using ANOVA followed by Tukey–Kramer post hoc test taking 0.05 as the limit of significance. Correlation analyses were also carried out. Data analysis was performed using JMP statistical software (SAS Institute Inc., Cary, NC) or Microsoft Excel 2002.

3. Results and discussion

3.1. Weight loss and colour changes induced by cooking

In the present study, the duration of cooking was determined on the basis to the development of some changes in peppers. These changes were subjectively assessed in order to mimic real conditions of household cooking. Boiling was stopped when peppers developed a pale colour (less green, yellow or red) and became soft. Peppers were considered as grilled when they became soft and their entire surface was slightly burned (darker). Cooking time varied widely between types of pepper. Grilling temperature and times (210 °C, 8.8–19 min) were higher than those for boiling (96 °C, 7–13.5 min). This was probably the cause of the severe (10.7–39.2%) and minimal (1.04–4.6%) weight loss of peppers during boiling and grilling, respectively (Table 2). Since vegetables tend to absorb water during boiling, we infer that the weight loss in boiled peppers occurred during cooling at room temperature. The absence of water during grilling could also contribute to the weight loss of peppers in this cooking method. Initial weight of fruit (Table 1) did not correlate with cooking time and weight loss during cooking (Table 2) when data of all pepper types were considered. Interestingly, when data were analysed on the basis of the stage of ripening of the fruit (green, yellow or red stages) the correlation coefficients (R) for the relationships between initial weight of fruit and time of cooking (boiling and grilling) or weight loss during cooking increased significatively, reaching in some cases values above 0.9, suggesting that as the initial fruit weight is increased the cooking time increase and the total weight loss during cooking decrease. In this regard, Lownds, Banaras, and Bosland (1993) indicated that the weight loss rate in peppers depends on cultivar differences and the initial water content of the fruit. Initial water content of peppers differs widely between types of peppers and cultivars of the same type of pepper (Cisneros-Pineda et al., 2007; Lownds et al., 1993).

In green peppers, boiling consistently reduced a^* and b^* values, but caused a slight increase in L^* values (except in Green Bell Peppers; GBP) (Table 3). Similar colour changes have been reported for Green Jalapeño peppers (GJ) as a consequence of blanching, probably due to chlorophylls degradation (Quintero-Ramos, Bourne, Barnard, & Anzaldúa-Morales, 1998). Decreases in L^*, a^*, and b^* values were observed in boiled yellow peppers (except in Yellow Bell peppers) while in red peppers colour changes by boiling were not consistent. In general, boiling induced smaller colour changes in yellow and red peppers than in green ones. Similarly, Ismail and Revathi (2006) reported only small colour changes (L^*, a^*, and b^*) in puree of red peppers treated in a wide range of temperatures while Ahmed, Shivhare, and Debnath (2002) reported high colour changes in puree of green peppers during heat processing. This suggest that yellow (zeaxanthin, violaxanthin, anteraxanthin, β-cryptoxanthin, β-carotene, and curcubixanthin A) and red (capsanthin, capsanthin 5,6-epoxide, and capsorubin) carotenoids are more thermoresistant than chlorophylls in peppers. Chlorophylls were more susceptible to heat loss than some carotenoids in sweet potato leaves (Chen & Chen, 1993). In the other hand, grilling caused a significant decrease in all evaluated colour variables (Table 3).

Table 2
Boiling and grilling times and weight loss of peppers during cooking.

Pepper type[A]	Boiling time (min)	Weight loss during boiling (%)	Grilling time (min)	Weight loss during grilling (%)
GH	7.5 ± 0.6^{c}	2.5 ± 0.5^{a}	8.8 ± 0.3^{e}	39.2 ± 0.9^{a}
YH	7.0 ± 0.3^{c}	1.04 ± 0.3^{a}	10.0 ± 0.3^{de}	30.8 ± 5.0^{ab}
M	8.0 ± 0.6^{bc}	4.6 ± 0.6^{a}	11.0 ± 0.3^{cde}	22.8 ± 5.9^{bcde}
GS	11.8 ± 2.2^{abc}	3.0 ± 0.9^{a}	9.8 ± 0.5^{e}	17.9 ± 0.5^{cdef}
RS	8.5 ± 0.9^{abc}	1.5 ± 0.6^{a}	9.3 ± 0.3^{e}	21.5 ± 0.2^{bcdef}
GJ	13.5 ± 1.8^{a}	2.2 ± 0.9^{a}	18.5 ± 0.9^{ab}	23.6 ± 1.0^{bcd}
RJ	8.8 ± 1.1^{abc}	1.8 ± 0.3^{a}	18.8 ± 1.7^{a}	25.9 ± 1.3^{bc}
C	NB[B]	NB[B]	14.3 ± 1.0^{bcd}	20.0 ± 2.6^{bcdef}
GC	NB[B]	NB[B]	11.0 ± 0.9^{cde}	18.6 ± 0.4^{bcdef}
GP	NB[B]	NB[B]	15.0 ± 1.0^{abc}	17.9 ± 0.9^{cdef}
GBP	11.5 ± 0.7^{abc}	3.6 ± 0.7^{a}	16.8 ± 0.8^{ab}	13.0 ± 0.8^{def}
YBP	13.0 ± 0.8^{ab}	2.4 ± 0.4^{a}	19.0 ± 1.4^{a}	10.7 ± 1.9^{f}
RBP	11.8 ± 0.5^{abc}	1.4 ± 0.4^{a}	17.0 ± 0.7^{ab}	11.9 ± 1.3^{ef}

Values represent the mean of at least 36 individual measurements ± the standard error. Values in the same column not connected by the same letter are significantly different ($p < 0.05$).

[A] Abbreviated and full names of tested pepper types are shown in Table 1.

[B] NB = not boiled.

Table 3
Colour changes of peppers as affected by cooking.

Pepper type[A]	Colour values								
	L^*			a^*			b^*		
	Raw	Boiled	Grilled	Raw	Boiled	Grilled	Raw	Boiled	Grilled
GH	45.8 ± 0.5^{b}	48.0 ± 0.2^{a}	35.4 ± 0.2^{c}	-16.6 ± 0.1^{c}	-8.0 ± 0.1^{b}	-1.6 ± 0.1^{a}	29.7 ± 0.5^{a}	28.2 ± 0.4^{a}	15.1 ± 0.1^{b}
YH	63.9 ± 0.5^{a}	62.8 ± 0.9^{a}	37.4 ± 0.3^{b}	10.8 ± 0.2^{a}	8.8 ± 0.2^{b}	3.6 ± 0.0^{c}	43.9 ± 0.4^{a}	38.0 ± 0.6^{b}	14.6 ± 0.1^{c}
M	50.8 ± 0.6^{a}	47.9 ± 0.6^{b}	41.3 ± 0.5^{c}	7.5 ± 0.2^{a}	6.0 ± 0.2^{b}	4.9 ± 0.2^{c}	43.2 ± 0.8^{a}	41.8 ± 0.8^{a}	30.7 ± 0.7^{b}
GS	44.1 ± 1.1^{a}	45.0 ± 0.4^{a}	36.0 ± 0.5^{b}	-16.0 ± 0.3^{c}	-9.8 ± 0.1^{b}	-3.5 ± 0.1^{a}	30.0 ± 0.9^{a}	29.0 ± 0.3^{a}	18.6 ± 0.3^{b}
RS	45.4 ± 0.5^{b}	48.3 ± 0.5^{a}	37.7 ± 0.4^{c}	40.8 ± 1.1^{a}	37.7 ± 0.5^{b}	19.7 ± 0.2^{c}	41.0 ± 0.6^{a}	45.7 ± 1.1^{a}	29.1 ± 3.8^{b}
GJ	46.4 ± 1.1^{a}	47.7 ± 0.4^{a}	42.3 ± 0.4^{b}	-20.0 ± 0.3^{c}	-7.6 ± 0.1^{b}	-3.6 ± 0.0^{a}	33.6 ± 1.1^{a}	31.9 ± 0.5^{a}	23.7 ± 0.3^{b}
RJ	44.3 ± 0.4^{a}	43.6 ± 0.8^{a}	37.6 ± 0.9^{b}	41.3 ± 0.6^{b}	45.4 ± 0.3^{a}	20.6 ± 0.6^{c}	40.9 ± 0.7^{a}	39.9 ± 0.9^{a}	25.5 ± 1.3^{b}
C	71.9 ± 0.7^{a}	NB[B]	42.3 ± 0.5^{b}	-4.0 ± 0.0^{b}	NB[B]	1.8 ± 0.0^{a}	34.4 ± 0.5^{a}	NB[B]	15.1 ± 0.3^{b}
GC	62.5 ± 0.3^{a}	NB[B]	60.3 ± 0.7^{b}	-18.9 ± 0.1^{b}	NB[B]	-1.3 ± 0.1^{a}	38.6 ± 0.3^{a}	NB[B]	36.9 ± 0.6^{a}
GP	48.6 ± 0.9^{a}	NB[B]	46.3 ± 0.7^{a}	-18.3 ± 0.3^{b}	NB[B]	-5.7 ± 0.1^{a}	32.5 ± 0.7^{a}	NB[B]	30.3 ± 0.4^{a}
GBP	62.4 ± 1.0^{a}	55.5 ± 1.0^{b}	53.4 ± 0.3^{b}	-20.9 ± 0.2^{c}	-6.8 ± 0.1^{b}	-5.8 ± 0.0^{a}	38.7 ± 0.6^{a}	33.8 ± 0.6^{b}	29.4 ± 0.1^{c}
YBP	79.6 ± 0.6^{a}	70.5 ± 0.6^{b}	59.3 ± 0.9^{c}	2.3 ± 0.2^{b}	8.0 ± 0.3^{a}	1.7 ± 0.2^{b}	74.0 ± 1.4^{a}	74.5 ± 0.7^{a}	47.2 ± 0.6^{b}
RBP	40.4 ± 0.4^{b}	43.5 ± 0.5^{a}	33.9 ± 0.4^{c}	46.6 ± 0.6^{a}	44.4 ± 0.5^{b}	26.7 ± 0.3^{c}	37.5 ± 0.3^{a}	37.6 ± 1.5^{a}	22.5 ± 0.4^{b}

Values represent the mean of nine individual measurements ± the standard error. Values in the same row, for each colour variable, not connected by the same letter are significantly different ($p < 0.05$).

[A] Abbreviated and full names of tested pepper types are shown in Table 1.

[B] NB = not boiled.

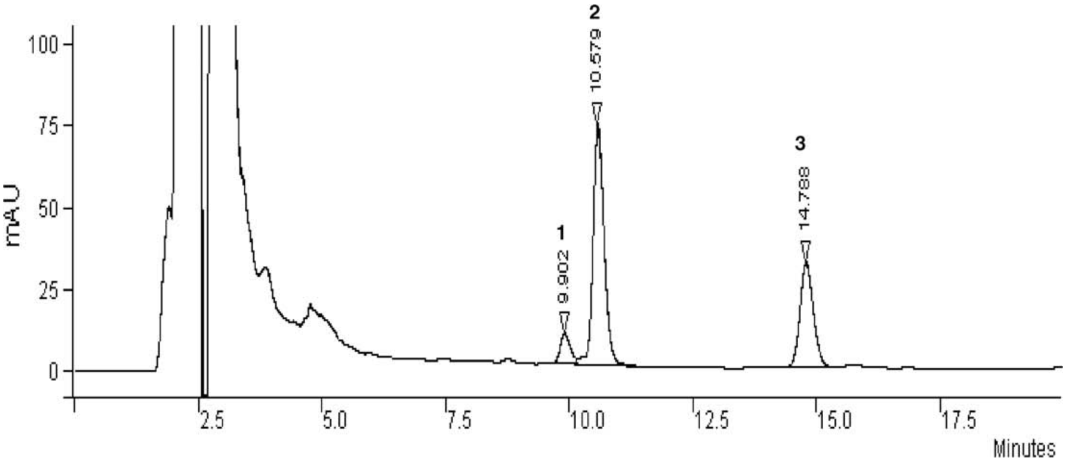

Fig. 1. HPLC separation of nordihydrocapsaicin (1), capsaicin (2) and dihydrocapsaicin in methanolic extracts of grilled Red Serrano peppers. Capsaicinoids were monitored at 326 nm. Nordihydrocapsaicin was tentatively identified on the basis of its chromatographic behaviour on a C_{18} column as reported by others (Cooper et al., 1991; Schweiggert, Carle, et al., 2006; Schweiggert, Schieber, et al., 2006).

3.2. Capsaicinoids content in raw and cooked peppers

Capsaicin and dihydrocapsaicin were clearly identified by using commercially available standard compounds. However, nordihydrocapsaicin was only tentatively identified on the basis of its chromatographic behaviour, since it was not available as reference compound. Elution order of capsaicinoids on C_{18} columns is well characterised. In the present study, capsaicinoids eluted from the C_{18} column in order of molecular weight (Fig. 1), as previously reported (Cooper et al., 1991; Schweiggert, Carle, et al., 2006). Schweiggert, Carle, et al. (2006) demonstrated by HPLC-MS studies (using a C_{18} column and a mobile phase similar to that of the present study) that nordihydrocapsaicin is a major capsaicinoid eluting immediately before (closely) capsaicin. Our separation of nordihydrocapsaicin, capsaicin, and dihydrocapsaicin was virtually identical to those reported by others using similar C_{18} columns (Supelco, Rad-Pak, Shimadzu, Synergi Hydro-RP) and mobile phases (Cooper et al., 1991; Schweiggert, Carle, et al., 2006; Schweiggert, Schieber, et al., 2006). This allowed us to infer that peak 1 in Fig. 1 was nordihydrocapsaicin. Undoubtedly, UV–Vis spectra and MS data are required to allow unequivocal identification of nordihydrocapsaicin.

Capsaicinoids were not detected in raw and cooked Green, Yellow, and Red Bell peppers (GBP, YBP, and RBP). The total content of capsaicinoids (sum of capsaicin, dihydrocapsaicin, and nordihydrocapsaicin contents) was higher in yellow and red peppers than in green peppers. Colour change is a ripeness index for peppers. The green colour of unripe peppers changes to yellow–orange–red when ripening process is completed (Sun et al., 2007). Several studies have demonstrated that the capsaicinoids content is higher in ripe than in unripe peppers (Cisneros-Pineda et al., 2007), although capsaicinoids content tends to decrease during senescence (Contreras-Padilla & Yahia, 1998). Raw Green and Yellow Habanero peppers (GH and YH) were the richest in capsaicinoids of tested types of peppers (Table 4). Concentrations of capsaicin (716.6–913.8 μg/g) and dihydrocapsaicin (463.4–756.9 μg/g) in raw Habanero peppers were similar to those previously reported (771 and 1187 μg/g, respectively) by Kozukue et al. (2005). Considering the mean moisture content of Habanero peppers previously reported (Cisneros-Pineda et al., 2007), the individual and total capsaicinoids contents found in the present study for this pepper type were similar to those reported in the literature (Garcés-Claver et al., 2006; Pino et al., 2007). Capsaicinoids content was higher in

Table 4
Capsaicin, dihydrocapsaicin and nordihydrocapsaicin contents (μg/g fresh tissue) and their proportion in relation to total capsaicinoids content (%, in parenthesis) of raw, boiled and grilled peppers.

Pepper type[A]	Capsaicinoids content (μg/g)								
	Capsaicin			Dihydrocapsaicin			Nordihydrocapsaicin		
	Raw	Boiled	Grilled	Raw	Boiled	Grilled	Raw	Boiled	Grilled
GH	716.6 ± 58.9^{b} (60%)	685.9 ± 59.1^{b} (62%)	1088.8 ± 55.5^{a} (60%)	463.4 ± 48.1^{b} (39%)	419.0 ± 51.0^{b} (38%)	727.3 ± 39.0^{a} (40%)	6.5 ± 0.2^{a} (1%)	8.2 ± 0.4^{a} (1%)	8.4 ± 1.0^{a} (0.5%)
YH	913.8 ± 18.6^{b} (54%)	903.6 ± 27.9^{b} (58%)	1360.5 ± 137.4^{a} (59%)	756.9 ± 60.9^{b} (45%)	648.2 ± 29.8^{b} (42%)	942.0 ± 70.2^{a} (41%)	9.4 ± 0.8^{a} (1%)	6.5 ± 0.7^{b} (0.4%)	10.0 ± 0.4^{a} (0.4%)
M	158.4 ± 6.7^{a} (21%)	145.4 ± 24.1^{a} (21%)	187.1 ± 11.0^{a} (21%)	514.4 ± 30.2^{a} (69%)	485.4 ± 88.9^{a} (70%)	602.3 ± 36.1^{a} (69%)	68.2 ± 5.0^{a} (9%)	60.9 ± 10.2^{a} (9%)	86.3 ± 9.6^{a} (10%)
GS	161.7 ± 10.9^{b} (50%)	129.4 ± 3.5^{b} (51%)	215.2 ± 17.7^{a} (52%)	137.6 ± 14.7^{ab} (43%)	107.5 ± 4.1^{b} (42%)	176.9 ± 17.7^{a} (42%)	22.3 ± 3.5^{a} (7%)	16.1 ± 1.0^{a} (6%)	25.2 ± 3.5^{a} (6%)
RS	288.3 ± 15.2^{a} (50%)	261.0 ± 22.2^{a} (51%)	306.0 ± 56.5^{a} (51%)	259.7 ± 11.0^{a} (45%)	230.5 ± 20.8^{a} (45%)	266.5 ± 63.0^{a} (44%)	27.8 ± 2.5^{a} (5%)	21.9 ± 1.9^{a} (4%)	30.4 ± 6.8^{a} (5%)
GJ	145.9 ± 10.3^{a} (52%)	111.9 ± 16.8^{a} (55%)	105.8 ± 5.7^{a} (55%)	123.6 ± 6.0^{a} (44%)	84.0 ± 12.4^{b} (42%)	78.2 ± 9.7^{b} (41%)	13.3 ± 0.7^{a} (5%)	6.3 ± 1.0^{b} (3%)	8.1 ± 0.7^{b} (4%)
RJ	184.4 ± 22.8^{b} (41%)	164.9 ± 18.6^{b} (49%)	329.8 ± 23.1^{a} (49%)	237.8 ± 21.4^{b} (53%)	157.4 ± 18.9^{c} (47%)	321.0 ± 19.0^{a} (47%)	24.4 ± 3.5^{ab} (5%)	14.3 ± 2.5^{b} (4%)	26.4 ± 2.0^{a} (4%)
C	60.3 ± 1.3^{a} (65%)	NB[B]	42.3 ± 2.0^{b} (82%)	27.4 ± 2.1^{a} (29%)	NB[B]	6.5 ± 1.7^{a} (13%)	5.6 ± 0.7^{a} (6%)	NB[B]	3.0 ± 0.1^{b} (6%)
GC	1.0 ± 0.2^{b} (51%)	NB[B]	9.8 ± 1.8^{a} (77%)	ND[C]	NB[B]	ND[C]	1.0 ± 0.0^{a} (49%)	NB[B]	2.9 ± 0.7^{a} (23%)
GP	0.6 ± 0.1^{b} (100%)	NB[B]	6.5 ± 1.5^{a} (62%)	ND[C]	NB[B]	ND[C]	ND[C]	NB[B]	4.0 ± 0.8 (38%)

Values represent the mean of nine individual measurements ± the standard error. Values in the same row, for each capsaicinoid, not connected by the same letter are significantly different ($p < 0.05$).

[A] Abbreviated and full names of tested pepper types are shown in Table 1.

[B] NB = not boiled.

[C] ND = not detected.

raw Serrano than in raw Jalapeño peppers, contrasting with previous reports (Krajewska & Powers, 1987). Capsaicin and dihydrocapsaicin contents in raw Jalapeño peppers were similar to those reported in other studies (165.2 and 204.2 μg/g, respectively) (Krajewska & Powers, 1987). In the present study, the capsaicinoids contents in raw non-dried Caribe (C), Manzano (M), Green Chilaca (GC), and Green Poblano (GP) peppers were measured as the first time. GC and GP presented the lowest capsaicinoids content, probably due to removal of placenta, as it is commonly done locally. Capsaicinoids are accumulated preferentially in placenta rather than in pericarp and in seeds (Cisneros-Pineda et al., 2007). If data of raw GC and GP are not considered, since placenta and seeds were removed in these pepper types, the individual contents of capsaicin, dihydrocapsaicin, and nordihydrocapsaicin represented the 21.4–64.6%, 29.4–69.4%, and 0.5–9.2% of total capsaicinoids in the rest of studied raw types of peppers, respectively. In dried raw-fruits of other Mexican pepper types (Cascabel, Chipotle, Jalapeño, Guajillo Puya, Costeño, Pico de Pájaro, Piquín, Serrano Criollo de Morelos, Habanero, and Chilpaya) these capsaicinoids represented 49.1–88.2%, 11.8–53.5%, and 1.9–9.5% of total capsaicinoids, respectively (Cisneros-Pineda et al., 2007; Collins et al., 1995; Contreras-Padilla & Yahia, 1998; Harrison & Harris, 1985; Krajewska & Powers, 1987) while in dried raw-fruits of some Cayenne, Italian, and Turkish peppers the individual content of major capsaicinoids varied between 0.3% and 59% of total capsaicinoids content (Kirschbaum-Titze, Mueller-Seitz, & Petz, 2002; Topuz & Ozdemir, 2007). In all raw and cooked peppers capsaicin was the major capsaicinoid, except in M peppers where capsaicin only represented the 21% of total capsaicinoids. In this regard, M peppers (*Capsicum pubescens*) showed an atypical capsaicinoids profile. Garcés-Claver et al. (2006) reported lower levels of capsaicin compared to dihydrocapsaicin in some cultivars of *Capsicum annuum* (Sincap, Perennial, and Nigrum). Collins et al. (1995) found that capsaicin and dihydrocapsaicin represent 21% and 35% of total capsaicinoids content, respectively, in some cultivars of *C. pubescens*, respectively. Similar unusual capsaicinoids profiles have been reported in others cultivars of *C. annuum* and *C. pubescens*.

Boiling moderately decreased (1.1–28.1%) the initial content of the three evaluated capsaicinoids, except in Jalapeño peppers (Table 4), where boiling caused a severe loss (10.6–52.2%) of these compounds. The extent of capsaicinoids losses varied between tested pepper types. Suresh et al. (2007) reported similar capsaicin losses (18.3–36.2%) in dried red peppers during boiling and pressure cooking. Capsaicinoids losses have also been reported in heat-treated Indian and Thai red peppers (0–30%) and in sun- and oven-dried Turkish paprika (21.5–100%) (Schweiggert, Schieber, et al., 2006; Srinivasan et al., 1992; Topuz & Ozdemir, 2004). However, our data for Jalapeño peppers contrast with those reported by others (Harrison & Harris, 1985; Huffman, Schadle, Villalon, & Burns, 1978), who observed a slight increase of capsaicin, dihydrocapsaicin, and nordihydrocapsaicin in Jalapeño peppers after boiling or canning. Pasteurisation also increased capsaicinoids levels in Yellow Banana peppers (Lee & Howard, 1999). In the present study, the proportion of individual capsaicinoids (%) in raw peppers was virtually maintained in boiled peppers (Table 4), suggesting that the thermostability of the evaluated capsaicinoids is similar during boiling, as demonstrated in others studies (Schweiggert, Schieber, et al., 2006; Topuz & Ozdemir, 2004).

Excepting GJ and C peppers, grilling caused significant increases in capsaicin (6.1–924.9%), dihydrocapsaicin (2.6–57%) and nordihydrocapsaicin (6.6–206.8%) contents (Table 4). Low correlation coefficients ($R = 0.3–0.7$) were observed for the relationships between the increases in the capsaicinoids content in grilled peppers and the weight loss during grilling, suggesting that besides dehydration of peppers other factors were involved in such increases. Increases of capsaicinoids content in thermally-treated peppers have been attributed to several factors such as dehydration of the food matrix, improved extractability of these compounds by cell disruption during thermal process, liberation of conjugated capsaicinoids and inactivation of capsaicinoids-destroying enzymes such as peroxidases (Harrison & Harris, 1985; Huffman et al., 1978; Lee & Howard, 1999; Schweiggert, Schieber, et al., 2006). In the present study, the proportion of individual capsaicinoids (%) was similar in raw and grilled peppers (except in C, GC,

Table 5
Total phenolic content (μg GAE/g fresh tissue) of raw and cooked peppers.

Pepper type[A]	Total phenolic content (μg of gallic acid equivalents/g)		
	Raw	Boiled	Grilled
GH	1186.2 ± 51.2^{b}	2492.3 ± 247.2^{a}	2773.8 ± 63.9^{a}
YH	1150.5 ± 124.6^{b}	2307.8 ± 152.6^{a}	2730.0 ± 120.4^{a}
M	1321.7 ± 23.7^{b}	1745.9 ± 16.3^{a}	1933.6 ± 114.7^{a}
GS	1643.8 ± 152.8^{b}	1765.8 ± 79.3^{b}	2291.1 ± 163.0^{a}
RS	1746.5 ± 64.1^{b}	1997.2 ± 38.5^{ab}	2129.6 ± 119.2^{a}
GJ	1609.4 ± 53.0^{b}	1759.5 ± 70.9^{b}	3746.3 ± 197.5^{a}
RJ	1782.4 ± 36.4^{c}	2549.7 ± 34.3^{b}	3121.7 ± 49.2^{a}
C	1287.3 ± 56.5^{b}	NB[B]	1996.1 ± 19.0^{a}
GC	1222.7 ± 41.2^{b}	NB[B]	2346.4 ± 49.3^{a}
GP	2190.0 ± 60.0^{b}	NB[B]	3677.0 ± 130.0^{a}
GBP	1152.8 ± 48.0^{a}	1134.0 ± 26.1^{a}	1068.4 ± 41.8^{a}
YBP	1450.9 ± 42.0^{a}	1308.1 ± 22.7^{b}	1061.1 ± 41.4^{c}
RBP	1345.7 ± 7.0^{a}	1237.2 ± 88.1^{ab}	1064.2 ± 103.6^{b}

Values represent the mean of 15 individual measurements ± the standard error. Values in the same row not connected by the same letter are significantly different ($p < 0.05$).

[A] Abbreviated and full names of tested pepper types are shown in Table 1.

[B] NB = not boiled.

and GP peppers), supporting that all evaluated capsaicinoids possess similar thermostability (Schweiggert, Schieber, et al., 2006; Topuz & Ozdemir, 2004).

3.3. Total phenolic content in raw and cooked peppers

The total phenolic contents in raw pungent peppers (1150.5–2190.0 μg/g) were in the range previously reported (200–7820 μg/g) for Mexican type peppers (Howard, Talcott, Brenes, & Villalon, 2000; Lee et al., 1995; Menichini et al., 2008), being GP peppers the richest in these compounds (Table 5). Interestingly, raw Habanero peppers were the poorest in phenolic content, being the content of these compounds higher in YH than in GH peppers, as reported by Menichini et al. (2008). Values for Habanero peppers in the present study were lower than those previously reported (4042–7820 μg/g) for this type of pepper (Howard et al., 2000; Menichini et al., 2008), probably due to differences between cultivars and ripening stages (Pino et al., 2007). Total phenolic content was similar for raw Jalapeño and Serrano peppers, as stated previously by Lee et al. (1995). The content of these compounds in raw Jalapeño peppers (1609.4–1782.4 μg/g) was virtually identical to that previously reported (1782 μg/g) for Jalapeño peppers cv. Veracruz (Lee et al., 1995). Raw M peppers presented moderate total phenolic content (1321.7 μg/g), which was similar to those reported for cultivars of *C. pubescens* (∼1100 μg/g) (Oboh & Rocha, 2007). Specific data for phenolic content in fresh M, C, GP, and GC peppers are unavailable in the literature. Total phenolic contents (1152.8–1450.9 μg/g) for raw non-pungent Bell peppers (GBP, YBP, and RBP) were similar to those reported (1344.8 μg/g) by Turkmen et al. (2005). The content of these compounds in GBP was higher than those of YBP and RBP, in agreement with other studies (Chuah et al., 2008; Sun et al., 2007). Total phenolic content is often higher in RBP than in YBP (Sun et al., 2007). However, the opposite was observed in the present study, agreeing with Lin and Tang (2007).

Boiling and grilling caused a sequential increase in total phenolic content in all pungent peppers (Table 5). Increases of phenolic content in pungent peppers by cooking ranged from minimal to large (7.4–137%). In this regard, Turkmen et al. (2005) found that several cooking methods (including boiling) caused increases (2–26%) in the phenolic content of peppers. Boiling also increased three fold the antioxidant capacity of peppers (Shobana & Naidu, 2000). Similar increases in total phenolic content have been reported for green beans, broccoli, spinach, and other vegetables (Turkmen et al., 2005). Increments of phenolic content of vegetables by cooking have been attributed to dehydration of food matrix and an improved extractability of phenolics from the food (Harrison & Harris, 1985; Huffman et al., 1978; Lee & Howard, 1999; Schweiggert, Schieber, et al., 2006). Cooking is able to inactivate the polyphenol oxidase enzyme during heating, leading to the inhibition of polyphenolics degradation (Chuah et al., 2008). In the present work, low correlation coefficients ($R = 0.5$) were observed for the relationships between the increases in the content of total phenolic compounds in cooked peppers and the weight loss during cooking, suggesting that besides dehydration of peppers others factors were involved in such increases.

On the other hand, cooking reduced total phenolic content (1.6–26.9%) in Bell peppers (GBP, YBP, and RBP) (Table 5). Significant losses of phenolic compounds by household cooking or industrial heat processing have been reported in peppers (green, yellow, and red), squash, leek, and other vegetables (Chuah et al., 2008; Lee & Howard, 1999; Turkmen et al., 2005). Reduction of total phenolic content in boiled vegetables has been attributed to the dissolution of phenolics compounds into the cooking water (Chuah et al., 2008).

4. Conclusions

Boiling reduced moderately the content of capsaicinoids in the tested peppers but grilling increased largely such content. Cooking increased the total phenolic content in pungent peppers while in non-pungent peppers such content was reduced by cooking. Weigh losses during cooking did not correlated with the increases in capsaicinoids and total phenolic contents after cooking; suggesting that besides dehydration of peppers others factors could be involved in such increases.

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