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FOOD PRODUCTION

Drying and pickling on phenols, capsaicinoids, and free radical-scavenging activity in Anaheim and Jalapeño peppers

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ABSTRACT: *The effects of sun-drying, air-drying and pickling processes on phenol and capsaicinoid contents, and free radical-scavenging activity [2,2-diphenyl-1-picrylhydrazyl (DPPH), and 2,2'-azino-bis 3-ethylbenzthiazoline-6-sulphonic acid (ABTS)] in Anaheim (red) and Jalapeño peppers were evaluated. Sun-drying process in Anaheim pepper caused the highest phenols retention (100%), and the free radical-scavenging activity (100%) when compared with air-drying (80%). Pickling process in Jalapeño pepper caused a moderate reduction on the phenol content (24%) and the radical-scavenging activity by DPPH (35%). Processes studied did not cause variations in the capsaicinoid fractions neither in its radical-scavenging activity. Results suggested that dried and pickled peppers are a good source of phenolics and capsaicinoids with antioxidant activity.*
Key words: *Capsicum annuum, dried pepper, pickled pepper, jalapeño pepper, capsaicinoids.*

Secagem e conserva sobre os fenóis, capsaicinoides e neutralização de radicais livres em pimentas Anaheim e Jalapeño

RESUMO: *Os efeitos dos processos de secagem ao sol, secagem ao ar e conserva sobre o conteúdo dos fenóis e capsaicinoides, assim como a atividade de eliminação de radicais livres [2,2-difenil-1-picrilhidrazilo (DPPH), e ácido 2, 2'-azino-bis 3-etilbenztiazolino-6-sulfónico ABTS)] em pimentas Anaheim (vermelho) e Jalapeño foram avaliados. A secagem ao sol mostrou maior retenção de fenóis (100%) e atividade de eliminação dos radicais livres (100%) quando foi comparada com a secagem ao ar (80%). O processo de conserva mostrou uma redução moderada no teor de fenóis (24%) e na capacidade de remoção de radicais (35%). Os processos estudados não causaram alterações nas frações capsaicinoides nem na seu atividade de eliminação de radicais. Os resultados sugerem que as pimentas secas e em conserva são uma boa fonte de fenóis e capsaicinoides com atividade antioxidante.*
Palavras-chave: *Capsicum annuum, pimenta seca, pimenta conserva, pimenta jalapeño, capsaicinoides.*

INTRODUCTION

Peppers (*Capsicum annuum*) are widely consumed worldwide, in fresh and processed forms, because of their sensory attributes of color, aroma, and pungency. Peppers are also considered a source of polyphenolic compounds, including phenolic acids, flavonoids, and capsaicinoids (ORNELAS-PAZ et al., 2010; ÁLVAREZ-PARRILLA et al., 2011). Various studies have demonstrated protective roles of flavonoids and other phenolic compounds against coronary heart disease and some forms of cancer. These health-promoting properties can be attributed to antioxidant activity of compounds in cells (ORNELAS-PAZ et al., 2010). It has been reported that capsaicinoids also have antioxidant capacity (MATERSKA & PERUCKA, 2005). Pungency is due to the presence of capsaicinoids, which are alkaloids produced only by plants of the genus *Capsicum* (THIELE et al., 2008).

Peppers sweet, semi-pungent, and pungent are consumed fresh, dried, or canned (HERVERT-HERNÁNDEZ et al., 2010; ORNELAS-PAZ et al., 2010). Anaheim (red type) and Jalapeño peppers are two economically important varieties. Anaheim pepper is consumed fresh or dried; Jalapeño is consumed fresh and is frequently pickled (ÁLVAREZ-PARRILLA et al., 2011). Processing contributes to extending the shelf life of fruits and vegetables (JANJAI et al., 2012). Thermal processing has been associated with the depletion of natural antioxidants in foods (JONSSON,

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1991); however, studies have demonstrated that food processing also has some positive effects on phytochemicals. This is mainly due to the increased bioavailability of some compounds known to have antioxidant activity (KIM et al., 2014).

Contents of phenolic compounds in fruits can be highly influenced by the geographic region, environmental factors and genetic characteristics (DEL VALLE LEGUIZAMÓN et al., 2005). In the Northwest of Mexico, high-quality peppers are produced for domestic and export use. These cultivars and their processing have not been fully studied. Therefore, the aim of this study was to evaluate the effects of drying and pickling processes on phenol and capsaicinoid contents, and their free radical-scavenging activity in Anaheim (red) pepper, and Jalapeño pepper.

MATERIALS AND METHODS

DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2′-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid), Folin-Ciocalteu (FC) reagent, capsaicin, dihydrocapsaicin, myricetin, catechin, resveratrol, gallic acid, coumaric acid, caffeic acid, ferulic acid, sinapic acid, and chlorogenic acid were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). All solvents analytical and chromatographic grades were purchased from J.T. Baker (México City).

Samples

Fresh and processed fruits (*Capsicum annuum* L.) were evaluated. Two pepper varieties were used to study the drying process, because two different processes were evaluated: Anaheim (Sandía cv.) pepper was used to evaluate sun-drying effects, and Anaheim (Colegio 64 cv.) pepper was used to evaluate air-drying effects. Jalapeño pepper was used to assess pickling process.

Batches of 10kg of fresh and 3kg of Anaheim dried pepper (Sandía cv.) by a local producer (29°49′29″N, 110°13′28″W, Sonora, México) were provided in October 2010. Sun-drying consisted of direct exposure to the sun for 12 days at temperatures from 28 to 30°C. Conversely, batches of 10kg of fresh and 3kg of Anaheim dried pepper (Colegio 64 cv.) were kindly donated by Chiles Secos Andrade S.A. de C.V (29°05′56″N, 110°57′15″W, Sonora, México), in September 2010. Air-drying process consisted of a parallel flow of hot air at 70°C for 24 hours. Fresh peppers were cut into slices (5×0.5cm) and lyophilized. Samples were powdered using an Osterizer blender (Oster model 4122, México City, México) and passed through a 30-mesh sieve for homogeneity. Ground samples were stored at -20°C.

Batches of 10kg of fresh pepper and 30 cans of Jalapeño pickled pepper from the same lot by La Costeña S.A de C.V (Sinaloa, México) were provided in January 2011. Pickling conditions were as follows: pH 3.5, bleaching at 80-85°C, canning at 85-90°C, and commercial sterilization at 100°C. The pericarp was cut into slices (5×0.5cm) and lyophilized.

Extraction and quantification of phenolic compounds

Extraction was conducted according to MOLINA-QUIJADA et al. (2010). Total phenolic contents (mg g^{-1}) were determined using the Folin-Ciocalteu colorimetric method (SINGLETON et al., 1998), and values were expressed as chlorogenic acid equivalents (CAE) g^{-1} of dry sample. The flavonoid contents (mg g^{-1}) were measured spectrophotometrically (MOLINA-QUIJADA et al., 2010), and the values were expressed as quercetin equivalents (QE) (AGUSTIN-SALAZAR et al., 2014).

The identification of phenolic substances was determined according to CANTOS et al. (2000). Twenty µL of methanolic extract (0.025g dw mL^{-1} of methanol 70%) were analyzed by liquid chromatography (HPLC Pro Star 230; Varian, Palo Alto, California, USA). A Supelcosil TM LC-18 column (30×0.4cm×5µm particle size; Supelco, Bellefonte, Pennsylvania, USA) and a diode-array detector (Agilent G1315D 1260 DAD VL, Germany) were used. Solvents were formic acid (5%) in HPLC grade water (solvent A), and HPLC-grade methanol (solvent B). Elution was performed with a gradient starting with 2% of solvent B to reach 32% of solvent B at 30min, 40% of solvent B at 40min, and 95% of solvent B at 50min, followed by isocratic separation for 5min at a flow rate of 1.5mL min^{-1}. Mean peaks were identified by comparison with the retention times and absorption spectra of commercial standards. For quantification, calibration curves (0.002-0.07mg mL^{-1}) for each identified phenolic substance were prepared.

Quantification of capsaicinoids

Extraction of capsaicinoids was performed according to ESTRADA et al. (2000). Quantification was carried out according to TANAKA et al. (2009). Fifty microliters of extract (0.1g mL^{-1} of acetonitrile) were analyzed by liquid chromatography using a Supelcosil TM LC-18 column, and an ultraviolet detector (model 9050; Varian, CA, USA). The mobile phase was methanol (70%) in HPLC-grade water at a flow rate of 1.0mL min^{-1}. The identification was accomplished by comparison of retention times of commercial standards (Sigma-Aldrich Co.; St. Louis, MO, USA). Capsaicin and dihydrocapsaicin calibration curves (0.001-0.05mg mL^{-1}) were prepared.

Free radical-scavenging activity evaluation

Free radical-scavenging activity was evaluated in phenolic and capsaicinoid extracts; and it was analyzed by two different methods, assessing different reaction characteristics and mechanisms using 2,2'-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS•+), and 2,2-diphenyl-1-picrylhydrazyl (DPPH•) radicals (MOLINA-QUIJADA et al., 2010). Free radical-scavenging activity was calculated in µmol Trolox equivalent (TE) g^{-1} of dry sample using a calibration curve of Trolox from 0.00 to 0.89µmol mL^{-1}.

Statistical analysis

One-way analysis of variance (ANOVA) was used to determined significant differences among processes. To identify the significant differences ($P<0.05$) the Tukey's test was used. The correlations among antioxidant activities with the different compounds by cultivar were determined. All the results were expressed as mean ± standard deviation (SD) of three replications. Statistical analyses were conducted using the Sigmastat 11 package (Systat Software Inc, San Jose, CA, USA).

RESULTS AND DISCUSSION

Phenolic compounds. Sun-drying.

Total phenols in Anaheim pepper (Sandía cv.) showed 100% of retention after sun-drying (Table 1). LOIZZO et al. (2013), reported comparable phenol content in both fresh and sun dried bell peppers.

Caffeic and sinapic acids reported in Anaheim pepper (Sandía cv.), were retained 64 and 62.5%, respectively, after sun-drying (Table 1). During the drying process, enzyme activity remains high for long periods of time when temperature is maintained among 55-60°C, whereas many enzymes are inactivated after short periods of exposure to temperatures of 75-80°C (MADRAU et al., 2009). Caffeic acid is susceptible to decarboxylation reactions, which can be promoted by either thermal conditions or enzymatic action through decarboxylases to generate vinylphenols (RIZZI & BOEKLEY, 1992). Decarboxylases are able to exert their activity at similar temperatures to the applied in the present study (HUANG et al., 1994). Flavonoids are not degraded by the same mechanism as the phenolic acids; they are not direct substrates for oxidases (BARUAH & SWAIN, 1959). Flavonols disappear proportionally to the temperature increase and by effect of drying conditions (MADRAU et al., 2009).

Air-drying

Total phenols in Anaheim pepper (Colegio 64 cv.) retained 80.8% after air-drying process (70°C, 24 hours) (Table 1). Caffeic, sinapic, chlorogenic, and ferulic acids were identified in fresh samples; with the exception of chlorogenic acid (77.3% retained), none of these acids were detected in dry peppers (Table 1). MADRAU et al. (2009), reported a similar retention (74%) for the same acid (HPLC) in apricot after air drying (75°C). HERAS-RAMÍREZ et al. (2012), reported a retention of 91% of chlorogenic acid in unbleached apple pomace dried at 50, 60, 70, and 80°C. Reduction of phenolic compounds during air-drying could be attributed primarily to the high temperatures. Degradation by polyphenol oxidase may have been minimal due to the enzyme is quickly inactivated at high temperatures (75°C) (MADRAU et al., 2009).

Pickling process

Total phenols and flavonoids in Jalapeño pepper after pickling process were retained 75.9 and 82.2%, respectively (Table 1). ÁLVAREZ-PARRILLA et al. (2011), reported 78% and 55% retentions of total phenol content for Jalapeño and Serrano peppers, respectively, after pickling. It has been reported that significant losses of phenolic compounds occur among 65-75°C (NWANGUMA & EZE, 1996). Gallic, chlorogenic, and coumaric acids in fresh pepper were identified in addition to catechin and resveratrol; however, the later was not detected in pickled pepper. These results can be explained by the fact that lixiviation of phenolic compounds occurs during pickling, and canning (CHUAH et al., 2008; ÁLVAREZ-PARRILLA et al., 2011). The loss of catechin during pickling can be attributed to epimerization reactions that occur at higher drying temperatures (CHUAH et al., 2008).

Capsaicinoids. Drying process

There was no reduction in the content of capsaicinoids in Anaheim peppers (Sandía cv and Colegio 64 cv) after sun-drying and air-drying (Table 1). TOPUZ et al. (2011), reported that drying processes did not affect residual capsaicinoids concentrations in paprika puree. TUNDIS et al. (2012), reported higher contents of capsaicin (1400µg g^{-1}) and dihydrocapsaicin (500µg g^{-1}) in *C. annuum* var. *acuminatum* medium (Serrano pepper) than those reported in our study.

Table 1 - Phenolic and capsaicinoid contents in dried red pepper (sun-drying and air-drying methods) and pickled jalapeño pepper.

Sun-drying			
	Red pepper	Dried pepper	Retention (%)
Total phenols (mg CAE g^{-1} DM)	14.42 ± 1.12^a	13.68 ± 0.25^a	100.00
Total flavonoids (mg QE g^{-1} DM)	8.39 ± 0.12^a	8.29 ± 0.14^a	100.00
Caffeic acid (mg g^{-1} DM)	0.25 ± 0.01^b	0.16 ± 0.01^a	64.00
Sinapic acid (mg g^{-1} DM)	0.24 ± 0.01^b	0.15 ± 0.01^a	62.50
Chlorogenic acid (mg g^{-1} DM)	Nd	Nd	
Ferulic acid (mg g^{-1} DM)	Nd	Nd	
Myricetin (mg g^{-1} DM)	0.07 ± 0.00	Nd	Nr
Capsaicin (µg g^{-1} DM)	31.05 ± 0.91^a	32.29 ± 1.36^a	100.00
Dihydrocapsaicin (µg g^{-1} DM)	12.65 ± 0.42^a	14.49 ± 1.68^a	100.00
Air-drying			
	Red Pepper	Dried pepper	Retention (%)
Total phenols (mg CAE g^{-1} DM)	14.86 ± 0.30^b	12.00 ± 0.05^a	80.8
Total flavonoids (mg QE g^{-1} DM)	7.69 ± 0.41^a	7.04 ± 0.25^a	100.0
Caffeic acid (mg g^{-1} DM)	0.09 ± 0.00	Nd	Nr
Sinapic acid (mg g^{-1} DM)	0.16 ± 0.00	Nd	Nr
Chlorogenic acid (mg g^{-1} DM)	0.64 ± 0.01^b	0.49 ± 0.01^a	77.3
Ferulic acid (mg g^{-1} DM)	0.12 ± 0.01	Nd	Nr
Myricetin (mg g^{-1} DM)	Nd	Nd	
Capsaicin (µg g^{-1} DM)	17.91 ± 2.84^a	18.49 ± 2.80^a	100.0
Dihydrocapsaicin (µg g^{-1} DM)	9.80 ± 1.62^a	9.73 ± 1.64^a	100.0
Pickled			
	Jalapeño Pepper	Pickled pepper	Retention (%)
Total phenols (mg CAE g^{-1} DM)	8.66 ± 0.13^b	6.57 ± 0.26^a	75.9
Total flavonoids (mg QE g^{-1} DM)	4.89 ± 0.04^b	4.02 ± 0.15^a	82.2
Catechin (mg g^{-1} DM)	3.13 ± 0.01^b	2.01 ± 0.06^a	64.2
Gallic acid (mg g^{-1} DM)	0.86 ± 0.00^b	0.44 ± 0.01^a	51.3
Chlorogenic acid (mg g^{-1} DM)	0.07 ± 0.00^b	0.03 ± 0.00^a	48.7
Coumaric acid (mg g^{-1} DM)	0.05 ± 0.00^b	0.03 ± 0.00^a	66.2
Resveratrol (mg g^{-1} DM)	0.02 ± 0.00	Nd	Nr
Capsaicin (µg g^{-1} DM)	76.71 ± 3.39^a	75.86 ± 1.84^a	100.0
Dihydrocapsaicin (µg g^{-1} DM)	25.36 ± 0.57^a	23.25 ± 1.53^a	100.0

Values are expressed as means±standard deviation. CAE: chlorogenic acid equivalents. QE: quercetin equivalents. DM: dry material. Nr: not retained. Nd: not detected. Detection limits: chlorogenic acid, 0.00156mg; ferulic acid, 0.00138mg; myricetin, 0.00356mg; caffeic acid, 0.00178mg; sinapic acid, 0.00177mg; resveratrol: 0.00277mg. Values in the same row followed by the different superscript letter are significantly different ($P<0.05$).

Pickling process

After the pickling process, capsaicin content was retained (100%) in Jalapeño pepper (Table 1). The fact that concentration of capsaicinoids remained constant after pickling could be attributed to the permeability of peppers cells membranes may change during heat processing resulting in release of capsaicinoids and spreading from pericarp throughout the pepper thus contributing to the capsaicinoid concentration remain unchanged. Concentration of capsaicinoids did not change with boiling and grilling processes (VICTORIA-CAMPOS et al., 2015).

Free radical-scavenging activity. Drying process.

The free radical-scavenging activity of phenolic extracts from Anaheim pepper (Sandía cv.) was retained 100% after sun-drying. In addition to phenolic compounds, the constituents with moieties possessing antioxidant behavior that are bound to different components of food/plant matrix can be released from cell walls during thermal processes allowing them to exhibit their antioxidant activity (WANGCHAROEN & GOMOLMANEE, 2013).

During the air-drying process, the antioxidant activity of phenolic extracts from

Anaheim pepper (Colegio 64 cv.) was retained 82.28% for ABTS and 78.07% for DPPH. ÁLVAREZ-PARRILLA et al. (2011), reported that antioxidant capacity in Jalapeño pepper was retained after smoking and drying (52% and 53% by ABTS and DPPH assays, respectively). Decrease in antioxidant activity that occurs during hot air-drying could be the result of oxidation of phenolic compounds (WANGCHAROEN & GOMOLMANEE, 2013). Air-drying proved to be more oxidizer than sun-drying.

Significant correlations (R>0.97) between ABTS assay and total phenolics, and DPPH assay with total phenolics (R>0.88) in dried peppers were found. These results indicated that the presence of phenolic compounds in dried pepper contributes significantly to their antioxidant potential. High correlations between capsaicin contents and free radical scavenging activity measured by ABTS and DPPH were also reported (R=0.99).

Pickling process

After the pickling process, the free radical-scavenging activity of phenolic extracts was retained 37.26 and 65.43% by ABTS and DPPH assays, respectively. ORNELAS-PAZ et al. (2013), reported that boiling between 7 and 13.5 minutes retained 94% of free radical-scavenging activity in Jalapeño pepper, while only 32% activity was retained in Serrano pepper by DPPH assay.

Antioxidant activity of capsaicinoid extracts did not decrease with the processes studied. Side chain of capsaicin contains a polar amide (−NHCO−) group, which gives low volatility, enabling its antioxidant activity to be maintained during heating (SI et al., 2012). The information presented in this study can help promote the consumption of peppers in fresh and processed form. Further studies on this topic should be conducted.

CONCLUSION

Sun-drying process in Anaheim pepper caused the highest phenolics retention, and free radical scavenging activity when compared with the air-drying. Pickling processes in Jalapeño pepper caused a moderate reduction on the phenolic content and the radical-scavenging activity. Sun-drying, air-drying and pickling processes did not cause changes in the capsaicinoid fractions neither in its radical-scavenging activity. Results suggested that dried and pickled peppers are a good source of phenolics and capsaicinoids with antioxidant activity.

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