Bioactive compounds and antioxidant activity in scalded Jalapeño pepper industrial byproduct (*Capsicum annuum*)

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Abstract Bioactive compounds and antioxidant activity were evaluated from industrial Jalapeño pepper byproducts and simulated non processed byproducts from two Mexican states (Chihuahua and Sinaloa) to determine their value added potential as commercial food ingredients. Aqueous 80% ethanol produced about 13% of dry extract of polar compounds. Total phenolic content increased and capsaicin and dihydrocapsaicin decreased on scalding samples (80 °C, 2 min) without affecting ascorbic acid. The major phenolic compounds, rutin, epicatechin and catechin comprised 90% of the total compounds detected by HPLC

of each Jalapeño pepper byproducts. ORAC analysis showed that the origin and scalding process affected the antioxidant activity which correlated strongly with capsaicin content. Although scalding decreased capsaicinoids (up to 42%), phenolic content by (up to 16%), and the antioxidant activity (variable). Jalapeño pepper byproduct is a good source of compounds with antioxidant activity, and still an attractive ingredient to develop useful innovative products with potential food/non-food applications simultaneously reducing food loss and waste.

Keywords Byproduct · Valorization · Jalapeño pepper · Antioxidant activity · Bioactive compounds

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Introduction

Jalapeño is one of the varieties of peppers most preferred by consumers in Mexico where 836,246 t are produced annually (SIAP 2015). Most of the harvest (60%) is pickled and canned, and about 20% is consumed raw (Galicia-Cabrera 2005). One of the most popular industrialized Jalapeño pepper product is the pickled non-fermented sliced and canned pepper and considerable amount (more than 500 t, only in the Northwest region of Mexico) of byproduct is generated during their preparation (Sandoval-Castro et al. 2014). This byproduct-consisting of seeds and placenta of Jalapeño pepper-is eliminated during the preliminary operation in pickling after scalding (Galicia-Cabrera 2005) and currently not exploited but discarded as waste. Such waste increases the contribution of vegetables as a commodity to the economic cost of food loss and waste (23% of total cost) with considerable carbon footprint (21% of total) (FAO 2013). Cost-effective and environmentally friendly reuse is one of the strategies

advocated to reduce food loss and waste due to processing from local facilities (FAO 2014). Therefore, adding value to the Jalapeño pepper byproduct is a viable solution to reduce food loss and waste by simultaneously developing useful innovative products.

Anatomically, pepper consists primarily of the pericarp that includes the exocarp (skin), mesocarp and endocarp and seeds that are attached to the placenta (Bosland and Votava 2012). The pericarp contains up to 20% (dry weight) cellulose and other fibrous material, whereas the skins, a by-product of canning green New Mexican pods contain 77% soluble fiber and 80% total dietary fiber. The dry pericarp and seeds contain 16–17 and 18% protein, respectively. The placenta consists of vesicles known for the production of capsaicinoids and oleoresin (Bosland and Votava 2012). Extracts from red pepper pericarp exhibit strong ferrous-chelating and high free radical (OH[•] and DPPH) scavenging activity, but weak superoxide dismutase (SOD) activity due to the presence of high total phenolic and flavonoid contents (Bosland and Votava 2012). Total phenolic content of ethanol (80%) extracts and their antiradical potential from the pericarp were reported to be higher than from placenta (2175 vs 2082 mg chlorogenic acid equivalents per kg of fresh pepper). However, phenolic acid derivatives predominated in the placenta, particularly the ten times higher trans-p-feruloyl- β -D glucoside levels than in the pericarp (Materska 2014).

Pepper seeds have been investigated as an alternative source of bioactive compounds in an effort to reduce/reuse byproduct or wastes of the pepper processing industry (Silva et al. 2013). A similar study examined utilization of seed waste as an antioxidant due to increased red pepper production in Korea and global consumption (Sim and Sil 2008). Our waste reduction research has focused on byproducts utilization from food industries by identifying them as sources of bioactive compounds with potential industrial application. In this regard, we demonstrated the potential use of tomato peel, seeds and byproducts from the tomato processing industry in neighboring region (Valdez-Morales et al. 2014). The current investigation aims to valorize Jalapeño industrial byproduct by characterizing the effects of the scalding process on hydrophilic compounds, phenolic, ascorbic acid and capsaicinoids contents and in vitro antioxidant capacity of industrial Jalapeño pepper byproducts from two different local sources/origins. This characterization of Jalapeño pepper byproducts may lead to their enhanced utilization and/or development of novel products for potential food/non-food applications.

Materials and methods

Biological material

Raw Jalapeño pepper (*C. annuum*) and a Jalapeño byproduct after the scalding process were obtained from La Costeña—a Vegetable Processing Industry located in Sinaloa Mexico. The peppers were produced in August and January 2014, and harvested from two production states Chihuahua and Sinaloa respectively, in Mexico. The byproduct was obtained after the scalding (80 °C, 2 min) and slicing processes. In order to compare the effect of scalding process on metabolites and their antioxidant activity, the raw Jalapeño pepper was sliced; the seeds together with the placenta were removed and combined to obtain a simulated raw byproduct, in the same proportions than that generated in the industry (Sandoval-Castro et al. 2014). Simulated raw Chihuahua (RCh) and Sinaloa (RSin), and scalded Chihuahua (SCh) and Sinaloa (SSin) byproducts were lyophilized and stored in the dark at −20 °C. Samples were pulverized in a TissueLyser II (Quiagen, F = 30 1/s, during 3 min), stored in the dark at −20 °C until use. Lipids were removed from the dry milled pepper samples with hexane using the Soxhlet extraction.

Chemical compounds, standards and ACS solvents were purchased from Sigma-Aldrich (St. Louis, MO, USA) and HPLC-grade acetonitrile and methanol, were purchased from Karal (Leon, Guanajuato, Mexico).

Ethanol extraction

Dry defatted samples (500 mg) were extracted with 80% (v/v) aqueous ethanol (5 mL) 30 min at room temperature on a sonicator (Fischer Scientific FS20, Massachusetts, USA). Aqueous ethanol (15 mL) was added to the mixture, agitated (400 rpm, 2 h, room temperature, Corning Magnetic Stirrer, MP9I, New York, USA) and centrifuged (1610 g, 10 min, Sorvall ST 16R, Thermo Scientific, Massachusetts, USA). The residue was re-extracted with aqueous ethanol (20 mL) with repeated agitation and centrifugation. The ethanol extracts were pooled, ethanol removed (vacuum rotary evaporation, 40 °C, Yamato RE-300, Santa Clara, CA, USA), extraction yield calculated and expressed as g of dry extract per kg of dry defatted material. The powdered extracts were reconstituted with 80% aqueous ethanol (20 mg per mL) for total phenolic and antioxidant activity determination and with absolute methanol for phenolic HPLC analysis. Extracts were stored in the dark at −20 °C until analysis.

Total phenolics

The modified Folin–Ciocalteu assay (Nurmi et al. 1996) adapted for microplates was used to determine total phenolic content of extracts. Absorbance was monitored at 760 nm (Thermo Scientific Multiskan GO spectrophotometer, Waltham, MA) using gallic acid (0–200 µg per mL) and (+)-catechin (50–300 µg per mL) as standards. The results were expressed as g gallic acid equivalents (GAE) and (+)-catechin (CAE) per kg of dry defatted byproduct (ddb).

Ascorbic acid and capsaicinoids analyses

Samples (500 mg, ddb) were extracted with acidified (0.125% formic acid, v/v) methanol according to the procedure of Qin et al. (2014). Briefly, samples were sonicated (15 min, Fisher Scientific FS20), centrifuged (3550 g, 15 min, room temperature) to recover the supernatant. Ascorbic acid was determined by incubating 500 µL extract in the dark for 1 h with 5 µL 0.05% 2-mercaptoethanol. The mixture was recovered and filtered (0.2 µm PTFE filter) prior to chromatographic separation on a Supelco Discovery HS C18 column (150 mm × 2.1 mm i.d., 3 µm; Sigma Aldrich, Co. USA.) at 25 °C (Alós et al. 2013).

HPLC analysis was performed using a Dionex UltiMate 3000 liquid chromatograph with isocratic (water acidified with acetic acid [pH 2.4]) elution at 0.3 mL min^{−1} flow rate and 245 nm detection. Ascorbic acid (0.05–0.5 mg per mL) was used as reference standard and results expressed as g per kg ddb.

Capsaicinoids were separated under an isocratic elution using the same HPLC system as above with two solvent systems: (a) acetonitrile: methanol (70:30, v/v; 0.14 mL min^{−1}) and (b) water (0.06 mL min^{−1}) at 0.2 mL min^{−1} and 25 °C. Capsaicin (20 ng per mL) and dihydrocapsaicin (40 ng per mL) in methanol solutions were used as standards and results expressed in g per kg ddb.

HPLC analysis of phenolic acids

Phenolic acids (5 mg per mL) in the reconstituted methanol extracts were analyzed on an HPLC (Dionex UltiMate 3000) system equipped with a titanium quaternary pump (LPG-3400AB), an autosampler (WSSIN-3000TBPL), a photodiode array detector (DAD-3000, Thermo Fisher Scientific, New York, NY) and Chromeleon 7.0 software (Dionex, Thermo Fisher Scientific, New York, NY). The separation was carried out with an Acclaim® 120 A C₁₈ column (250 mm × 4.6 mm i.d., 5 µm; Thermo Fisher Scientific, New York, NY, USA).

Chromatographic separation was performed according to established procedures (Espinosa-Alonso et al. 2006; Valdez-Morales et al. 2014) with 10 µL extract using two solvent systems: (a) water acidified with acetic acid (pH 2.8) and (b) acetonitrile at room temperature. A linear gradient solvent B 5–95% for 40 min was used for eluting the phenolic compounds followed by return to initial condition. The chromatograms were recorded at 260, 280, 300, 342, 350 and 375 nm. Phenolics were quantified using caffeic, chlorogenic, ferulic, gallic, 3,4-hydroxybenzoic, 2,5-hydroxybenzoic, *o*-coumaric, *p*-coumaric, sinapic, syringic, trans-cinnamic and vanillic acids; and flavonoids: (+) catechin hydrate, 6,7-dimethyl hydroxycoumarin, (−)-epicatechin, 7-hydroxycoumarin, naringenin, quercetin, quercetin 3-β-D-glucoside, and rutin hydrate as standards. Samples were filtered through a 0.2 µm PTFE filter prior to injection and results expressed in g per kg ddb.

Antioxidant activity

Trolox equivalent antioxidant capacity (TEAC) assay

TEAC assay based on ABTS·⁺ was performed according to the method of Pellegrinini et al. (2003). ABTS·⁺ work solution was diluted with 80% ethanol to obtain an absorbance value of 0.70 ± 0.02 at 734 nm. Aqueous 80% ethanol extract, blank and controls/standard (20 µL) was mixed with ABTS·⁺ work solution (200 µL), gently vortexed during 20 s, and absorbance monitored at 734 nm. Trolox (0–500 µM) was used as standard, and the results expressed as mmol TE per kg ddb.

DPPH method

Antioxidant activity was determined by the DPPH method adapted for use with microplates as previously described (Cardador-Martínez et al. 2006). Briefly, a solution of DPPH (200 µL, 0.24 µM) prepared in 80% methanol was mixed with sample extract (20 µL). Absorbance at 515 nm was measured with a spectrophotometer (Thermo Scientific Multiskan GO) from 0 to 60 min. Trolox (50–500 µM) was used as standards, and the results expressed as mmol TE per kg ddb.

IC₅₀ values were calculated for ABTS and DPPH assays using the semi-logarithmic ($y = a \ln(x) + b$) and linear ($y = mx + b$) equations, respectively, versus % ARA at different concentrations (0.1, 0.5, 1.5, 7.5, 10, 12.5 and 20 mg per mL) for samples; (0.05, 0.1, 0.15, 0.2, 0.25 and 0.3 mg per mL) ascorbic acid; (50, 100, 200, 300, 400, 500 µmol) Trolox, and (0.05, 0.1, 0.15, 0.2, 0.25, 0.3 mg per mL) BHT. IC₅₀ was expressed as mg per mL for ABTS and DPPH.

Oxygen radical absorbance capacity (ORAC)

Antioxidant activity was measured according to the ORAC_{FL} assay described previously (Prior et al. 2003). A DTX 880 Multimode Detector (Beckman Coulter, Brea, CA, USA) was used with excitation and emission wavelengths at 485 and 530 nm, respectively. Sample extracts and Trolox standards (0–400 μ M) were diluted with 75 mM phosphate buffer (pH 7.4) prior to transfer into a 96-well microplate. A peroxy radical was generated using AAPH [2,2'-azobis(2-methylpropionamide)dichloride] (70 mM) during measurement at 37 °C, and fluorescein (87 μ M) was used as substrate. Final ORAC values were calculated using a regression equation between the Trolox concentration (0–400 μ M) and the net area under the curve and expressed as mmol TE per kg ddb.

Statistical analysis

At least three independent experiments were conducted for all assays with four experimental replicates. Analysis of variance by the general linear models (GLM) procedure, means comparison by Duncan's test and Pearson correlation were performed according to Statistical Analysis System, SAS 9.1 for Windows.

Results and discussion

Aqueous 80% ethanol produced significantly ($P < 0.05$) higher yield from RCh than other samples (Table 1). The yields were higher than those previously reported for ethanol extract of two Italian bell pepper cultivars (Loizzo et al. 2013). Furthermore, simulated raw byproducts yields were twice those reported for aqueous methanol (80%) extract of Jalapeño pepper (*cv.* Ixtapa) grown in Texas, thereby confirming the strong solvent extraction effect

(Bae et al. 2012). The scalding process significantly ($P < 0.05$) reduced extraction yield (13.4%) of simulated raw Chihuahua byproduct (RCh), although that reduction (5%) was insignificant for samples from Sinaloa. Similar reductions in extraction yields (15–36%) have been reported after boiling whole fresh yellow Cayenne and red chili peppers from five Italian cultivars for 10 min (Loizzo et al. 2015).

Total phenolics

Scalding significantly increased total phenolic compared to simulated raw Jalapeño byproduct (22 and 15% when expressed as GAE and CAE, respectively) (Table 1) and were similar to those reported previously (Alvarez-Parilla et al. 2011). Similar increase in total phenolic content (expressed as g GAE per kg) have been reported during smoking of red Jalapeño pepper (Moreno-Escamilla et al. 2015), and boiling (96 °C, 13.5 min) of Chihuahua Jalapeño peppers (Ornelas-Paz et al. 2010). RCh had significantly lower total phenolic content than those from Sinaloa, indicating environmental differences. Total phenolic content (g GAE per kg) of our simulated raw samples were 60% lower than those reported for the 80% methanol extract of whole Jalapeño pepper (Bae et al. 2012), although phenolic acids are presumed to predominate in the placenta (Materska 2014).

Ascorbic acid and capsaicinoids analyses

Ascorbic acid content (Table 1; Fig. 1) did not differ significantly among the samples suggesting its retention after the scalding process similar to those observed after blanching and pasteurization of Jalapeño peppers (Howard and Wildman 2006). However, ascorbic acid contents of

Table 1 Hydrophilic yield extraction, total phenols, ascorbic acid and capsaicinoids content (g per kg^a) of raw and scalded Jalapeño byproduct from Chihuahua and Sinaloa

Sample	Yield	Total phenolic		Ascorbic acid	Capsaicin	Dihydrocapsaicin
		GAE	CAE			
RCh	141.4	10.01 $\pm$ 0.61 ^b	12.00 $\pm$ 0.41 ^c	0.0496 $\pm$ 0.008	0.292 $\pm$ 0.304 ^a	0.1206 $\pm$ 0.013 ^b
SCh	123.7	13.09 $\pm$ 0.38 ^a	15.57 $\pm$ 1.05 ^a	0.0419 $\pm$ 0.003	0.165 $\pm$ 0.114 ^b	0.1002 $\pm$ 0.004 ^b
RSin	132.5	10.70 $\pm$ 0.27 ^b	13.92 $\pm$ 0.68 ^b	0.0394 $\pm$ 0.014	0.284 $\pm$ 0.105 ^a	0.1622 $\pm$ 0.017 ^a
SSin	125.9	12.12 $\pm$ 0.79 ^a	14.27 $\pm$ 0.76 ^{ab}	0.0436 $\pm$ 0.015	0.142 $\pm$ 0.123 ^b	0.1170 $\pm$ 0.006 ^b
Raw	136.9	10.35 ^y	12.96 ^y	0.0435	0.2881 ^x	0.1414 ^x
Scalded	124.8	12.60 ^x	14.92 ^x	0.0428	0.1534 ^y	0.1086 ^y

Mean $\pm$ SD (n = 3)

Different letters in the same column indicate significant differences between samples ($P < 0.05$)

R raw, S scalded, Ch Chihuahua, Sin Sinaloa

^a ddb dry defatted byproduct

Fig. 1 HPLC-DAD chromatogram **a** ascorbic acid standard and **b** scalded Chihuahua extract. UV detection at 245 nm

our samples were lower than those reported earlier (Silva et al. 2013), probably due to the absence of pericarp where ascorbic acid accumulation is presumed to occur (Castro-Concha et al. 2012). Scalding significantly ($P < 0.05$) reduced capsaicin and dihydrocapsaicin contents of the Jalapeño byproducts (Table 1; Fig. 2) similar to those reported for boiling Jalapeño peppers (Ornelas-Paz et al. 2010), or cooked peppers due to capsaicin oxidation catalyzed by high temperature (Moreno-Escamilla et al. 2015). Furthermore, RSin had significantly ($P < 0.05$) higher dihydrocapsaicin resulting in its significantly higher retention upon scalding than those from Chihuahua.

HPLC analysis of phenolic compounds

Total phenolic compounds were most abundant in simulated raw Chihuahua than Sinaloa Jalapeño pepper byproducts (19%) and reduced by scalding (22 and 13%, respectively) (Table 2, Fig. 3). The phenolic composition according to the HPLC analysis was not significantly different among the samples, except for caffeic and ferulic acids and rutin ($P > 0.05$). Thus, RCh and SSin had the highest and the lowest caffeic acid and rutin contents, respectively, whereas ferulic acid content was most and the least abundant in RCh and RSin, respectively. Differences

Fig. 2 HPLC-DAD chromatogram of **a** capsaicinoids standards (peaks: 1 capsaicin; 2 dihydrocapsaicin) and **b** scalded Chihuahua extract. UV detection at 210 nm

in caffeic acid have been reported with no changes in ferulic acid content of fresh peppers (Troconis-Torres et al. 2012). The major phenolic compounds, rutin, epicatechin and catechin accounted for 90% with rutin comprising 1/3 of the total peak area for all samples. Their high concentration in our samples suggested that these Jalapeño pepper byproducts were excellent sources of flavonoids similar to red pepper pericarp and seed extracts (Sim and Sil 2008). Phenolic compounds in RCh were generally higher than those in RSin, except for catechin. For example, RCh had significantly ($P < 0.05$) higher caffeic, ferulic and coumaric acids, and lower catechin contents than RSin,

indicating thereby differences in Jalapeño origin. Similarly, SCh had significantly ($P < 0.05$) higher caffeic acid and lower 3,4-dihydroxybenzoic and coumaric acids than SSin. Scalding significantly ($P < 0.05$) reduced both catechin and rutin and increased coumaric acid contents only in Sinaloa Jalapeño. Coumaric acid content also decreased significantly ($P < 0.05$) during scalding of Chihuahua Jalapeño. Overall, scalding significantly ($P < 0.05$) reduced chlorogenic acid when simulated raw Jalapeño was compared to scalded byproducts. Similar reduction in chlorogenic acid (22%) has been reported in Jalapeño pepper due to smoking (Moreno-Escamilla et al. 2015).

Table 2 Phenolic content (g per kg^c) of raw and scalded Jalapeño byproduct from Chihuahua and Sinaloa, determined by HPLC

Phenolic compounds	RCh	SCh	RSin	SSin
Gallic acid	1.002 ± 0.097 ^a	0.982 ± 0.090 ^a	0.971 ± 0.072 ^a	0.952 ± 0.072 ^a
Caffeic acid	0.449 ± 0.028 ^a	0.437 ± 0.020 ^a	0.214 ± 0.015 ^b	0.187 ± 0.017 ^b
Ferulic acid	1.145 ± 0.081 ^a	1.037 ± 0.183 ^{ab}	0.814 ± 0.027 ^b	0.934 ± 0.108 ^{ab}
3,4-Dihydroxybenzoic acid	0.087 ± 0.028 ^b	0.094 ± 0.005 ^b	0.107 ± 0.021 ^b	0.148 ± 0.015 ^a
Chlorogenic acid	0.682 ± 0.102 ^a	0.559 ± 0.050 ^a	0.713 ± 0.119 ^a	0.569 ± 0.032 ^a
<i>o</i> -Coumaric acid	0.150 ± 0.020 ^a	0.091 ± 0.026 ^b	0.071 ± 0.016 ^b	0.141 ± 0.010 ^a
Catechin	6.848 ± 0.107 ^{ab}	6.357 ± 1.056 ^b	7.907 ± 0.327 ^a	7.026 ± 0.192 ^{ab}
Epicatechin	12.614 ± 3.936 ^a	10.544 ± 2.036 ^a	8.849 ± 0.751 ^a	9.256 ± 2.755 ^a
Rutin	15.611 ± 2.563 ^a	12.488 ± 2.064 ^{ab}	11.441 ± 0.772 ^b	9.263 ± 0.692 ^b
Kaempferol	0.098 ± 0.034 ^a	0.101 ± 0.014 ^a	0.083 ± 0.004 ^a	0.090 ± 0.008 ^a
Total	38.686	32.358	31.168	28.575

Mean ± SD (n = 3)

Different letters in the different columns indicate significant differences between samples ($P < 0.05$)

R raw, S scalded, Ch Chihuahua, Sin Sinaloa

^c *ddb* dry defatted byproduct

The three major flavonoids, catechin, epicatechin and rutin in our samples have been reported only once before in Jalapeño peppers (Medina-Juárez et al. 2012) (Fig. 4), together with gallic, caffeic and chlorogenic acids. Catechin, rutin, and gallic, caffeic, ferulic and coumaric acids have been reported in Mexican chili peppers (Troconis-Torres et al. 2012). However, the reported values were low since they were expressed in g per kg fresh fruit weight. Rutin content in our samples were 5–9 and 4–7 folds higher than those determined in red pepper and red chili pepper, respectively by the AOAC official methods (Atanassova and Bagdassarian 2012). Chlorogenic and coumaric acids were abundant in the pericarp, placenta and stalk of Jalapeño pepper, although caffeic, ferulic, and 3,4-dihydroxybenzoic acids and kaempferol were also present in the placenta (Chen and Kang 2013). High epicatechin content together with gallic and 3,4-dihydroxybenzoic acids have also been reported in seven Chinese pepper cultivars (Zhuang et al. 2012). The Jalapeño byproducts rich in flavonoids may provide the same coronary disease protection conveyed by red wine flavonoids (Lagiou et al. 2004).

Antioxidant activity

Trolox equivalent antioxidant capacity (TEAC) and DPPH assay

Jalapeño pepper byproducts exhibited antioxidant activity (ABTS and DPPH expressed in mmol Trolox per kg) not significantly different among the samples (Table 3). Byproduct displaying lower antioxidant activity compared to whole fresh Jalapeño fruit has previously been reported;

ABTS and DPPH values were between 7–8 and 5–6 times lower, respectively than those reported earlier for whole Jalapeño peppers (Alvarez-Parrilla, et al. 2011; Cervantes-Paz et al. 2012; Moreno-Escamilla et al. 2015). This inferior antioxidant activity of our samples is due to the predominance of seeds, placenta and pericarp known to exhibit low activity compared to whole and/or fresh Jalapeño fruit (Ornelas-Paz et al. 2010). However, the antioxidant activity of the Jalapeño byproducts was 5–10 times higher than those found in tomato byproduct using the same ABTS and DPPH methods (Valdez-Morales et al. 2014). Processing reduced the ABTS and DPPH radical scavenging activities by 7 and 19% after scalding of simulated raw Chihuahua Jalapeño byproduct. Similar reductions in antioxidant activities were reported for South Korean peppers after stir-frying (Hwang et al. 2012). However, this reduction was not observed in processing of simulated raw Jalapeño byproduct from Sinaloa suggesting differential effects due to source/origin of pepper. Furthermore, ABTS values were approximately 37% higher than those of DPPH in accordance with previous reports (Alvarez-Parrilla et al. 2011; Moreno-Escamilla et al. 2015).

ORAC assay

ORAC was the only antioxidant activity methodology that clearly differentiated the samples with the simulated raw and scalded Chihuahua Jalapeño displaying the highest and lowest ORAC values, respectively (Table 3). Both the origin of the Jalapeño pepper and the scalding process affected the ORAC antioxidant activity significantly ($P < 0.05$) in contrast to previous report (Alvarez-Parrilla

Fig. 3 HPLC-DAD chromatogram **a** phenol acids standards (peaks: 1 gallic acid; 2 3,4-dihydroxy benzoic acid; 3 chlorogenic acid; 4 caffeic acid; 5 vanillic acid; 6 syringic acid; 7 ferulic acid; 8 *o*-coumaric acid; 9 *trans*-cinnamic acid) and **b** scalded Sinaloa extract. UV detection at 280 nm

et al. 2011), where no significant differences were observed among peppers. Scalding significantly ($P < 0.05$) reduced ORAC (47% and 10% for SCh and SSin) of their respective simulated raw Jalapeño byproduct values. This concurs with those observed by Loizzo et al. (2015), supporting the claim that peppers differs in their sensitivity to processing.

IC₅₀ values based on ABTS and DPPH radical scavenging activity also showed significant ($P < 0.05$) differences among samples (Table 3). Furthermore, scalding significantly ($P < 0.05$) reduced scavenging activity compared to simulated raw Jalapeño byproduct with SCh exhibiting the lowest

DPPH scavenging activity. RSin displayed higher scavenging activity (lower IC₅₀ for both ABTS and DPPH) than those from Chihuahua. These IC₅₀ values discriminated samples based both on Jalapeño origin and processing similar to those of ORAC. Antiradical scavenging activity of our samples determined by IC₅₀ values were lower than those reported previously for whole pepper fruit and/or their extracts (Tundis et al. 2011; Loizzo et al. 2013), suggesting that they contained more bioactive compounds than those present in our simulated raw byproducts. Scalding reduced the unit contribution of the total phenolics (g GAE per kg) to antioxidant activity (DPPH)

Fig. 4 HPLC-DAD chromatogram **a** flavonoids standards (peaks: 1 catechin; 2 epicatechin; 3 rutin; 4 hydroxycoumarin; 5 quercetin; 6 kaempferol; 7 dimethyl hydroxycoumarin) and **b** flavonoids identified in scalded Sinaloa extract. UV detection at 280 nm

expressed as PAOXI, the antioxidant potential relative to phenolic content (Oomah et al. 2008).

Correlation analysis

The comparison of the byproduct constituents revealed strong correlation with antioxidant activity (Table 4). Total phenolic content (expressed as g GAE or CAE per kg ddb) was inversely associated with capsaicin and ORAC values and positively correlated with IC_{50} DPPH with higher significance for GAE compared to CAE ($P < 0.005$ vs

<0.05). Similar correlation has been reported between IC_{50} DPPH and phenolic contents of eight pepper genotypes (Carvalho et al. 2015). Capsaicin content was positively correlated with dihydrocapsaicin content and ORAC values and inversely associated with IC_{50} (ABTS and DPPH) values. Similarly, dihydrocapsaicin content and IC_{50} values were inversely related suggesting strong ($P < 0.0001$) IC_{50} ABTS dependence on dihydrocapsaicin. The antioxidant activities determined by ABTS and DPPH were strongly ($P < 0.0001$) related, but not associated with total phenolic and capsaicinoid contents. Similar relationship between

Table 3 Antioxidant activity and IC₅₀ of raw and scalded Jalapeño byproduct from Chihuahua and Sinaloa

Byproduct	ABTS (mmol TE per kg ^c)	IC ₅₀ ABTS (mg per mL)	DPPH (mmol TE per kg ^c)	IC ₅₀ DPPH (mg per mL)	ORAC (mmol TE per kg ^c)
RCh	6.627 ± 0.941 ^a	0.601 ± 0.013 ^b	4.383 ± 0.616 ^a	6.78 ± 0.11 ^c	16.138 ± 0.941 ^a
SCh	6.190 ± 0.775 ^a	0.727 ± 0.005 ^a	3.681 ± 0.635 ^a	9.39 ± 0.57 ^a	8.591 ± 0.399 ^b
RSin	6.657 ± 1.042 ^a	0.621 ± 0.043 ^b	4.061 ± 0.525 ^a	6.45 ± 0.06 ^c	12.181 ± 0.668 ^{ab}
SSin	6.872 ± 1.273 ^a	0.717 ± 0.021 ^a	4.523 ± 0.748 ^a	7.83 ± 0.20 ^b	10.978 ± 0.973 ^b

Mean ± SD (n = 3)

Different letters in the same column indicate significant differences between samples ($P < 0.05$)

TE trolox equivalents, R raw, S scalded, Ch Chihuahua, Sin Sinaloa

^c ddb dry defatted byproduct**Table 4** Correlation coefficients (r) between total phenols, capsaicinoids and antioxidant activity

	TPC GAE	TPC CAT	Capsaicin	Dihydrocapsaicin	ABTS	DPPH	ORAC	IC ₅₀ ABTS	IC ₅₀ DPPH
TPC CAT	0.775**								
Capsaicin	−0.843**	−0.668***							
Dihydrocapsaicin	−0.567	−0.216	0.606***						
ABTS	−0.012	0.111	0.038	0.107					
DPPH	−0.153	−0.067	0.044	0.099	0.925*				
ORAC	−0.891**	−0.847**	0.776**	0.325	0.153	0.358			
IC ₅₀ ABTS	0.565	0.272	−0.737***	−0.913*	−0.052	0.036	−0.321		
IC ₅₀ DPPH	0.857**	0.662***	−0.756**	−0.761**	−0.235	−0.372	−0.778**	0.737***	
PAOXI	−0.700**	−0.519	0.554	0.364	0.670***	0.802**	0.802**	−0.275	−0.741***

TPC GAE total phenols expressed as g equivalents of gallic acid per kg, TPC CAT total phenols expressed as g equivalents of (+)-catechin per kg; PAOXI phenol antioxidant index defined as unit contribution of total phenolics to antioxidant activity

Values with single asterisk (*), double asterisk (**) and triple asterisk (***) are significant at $P < 0.0001$, $P < 0.005$, $P < 0.05$, respectively

total phenols and ABTS has been reported previously in fresh and processed Jalapeño peppers (Alvarez-Parrilla et al. 2011). The Pearson correlation was low between the two methodologies (DPPH and ORAC) used to determine antioxidant capacity and similar to those reported previously (Kevers et al. 2007). IC₅₀ DPPH was inversely associated with ORAC but correlated positively with IC₅₀ ABTS. Ascorbic acid content showed no association with any analytes or antioxidant activities, although it was moderately correlated ($r = 0.646$, $P < 0.05$) with extraction yield (data not shown) contrary to earlier studies (Alvarez-Parrilla et al. 2011). Furthermore, catechin one of the major phenolic compounds, was positively ($r = 0.657$, $P < 0.03$) correlated with dihydrocapsaicin and inversely associated ($r = -0.665$, $P < 0.02$) with IC₅₀ ABTS. The correlation data suggests that ORAC and IC₅₀ values best represent the association of bioactive compounds with antioxidant activity. Our results contrast with studies where DPPH radical scavenging activity was strongly correlated with total bioactive compounds (capsaicinoids,

carotenoids, flavonoids and total phenolics) in pepper cultivars (Bae et al. 2012), and red pepper byproducts (Sim and Sil 2008). The significant correlation between epicatechin and rutin with the free radical ABTS and/or DPPH scavenging activity reported earlier was not substantiated in our study (Medina-Juárez et al. 2012). However, correlation ($r = 0.665$, $P < 0.02$) was significant between epicatechin and rutin contents, but not with antioxidant activities in our study.

The abundance of rutin, epicatechin and catechin in Jalapeño byproducts suggests that it can be an excellent source of vegetable flavonoids. These flavonoids are known to exhibit strong antioxidant potency by inhibiting lipid peroxidation (Lagiou et al. 2004). Similar lipid inhibition and protection of LDL cholesterol oxidation have been demonstrated for processed Jalapeño (Alvarez-Parrilla et al. 2012). The control of lipid oxidation was not investigated in our antioxidant activity; however, the similarity of our Jalapeño byproducts with those of processed Jalapeño (Alvarez-Parrilla et al. 2012) suggests that they

have strong potential as ingredients for human health. Furthermore, the byproducts and/or their extracts may have other useful applications as previously described (Johnson 2007).

Conclusion

Aqueous 80% ethanol produced a solid byproduct extract with similar content of total phenolics reported in whole fresh and processed pepper fruit. This extract is an excellent source of flavonoids. Although scalding decreased capsaicinoids (up to 42%), HPLC phenolic content (up to 16%) and the antioxidant activity (variable), the Jalapeño pepper byproducts is still an attractive ingredient to develop useful innovative products for potential food/non-food applications simultaneously reducing food loss and waste.

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