Phenolic composition of tomato varieties and an industrial tomato by-product: free, conjugated and bound phenolics and antioxidant activity

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Abstract The aim of this study was to isolate, identify and quantify soluble free phenolics, conjugated acid-hydrolysable phenolics (AHP) and alkaline-hydrolysable phenolics, and bound phenolics (BP) fractions from two tomato varieties (saladette and grape) and an industrial tomato by-product, as well as, to determine their antioxidant capacity. Phenolic composition was determined using Folin–Ciocalteu’s method and HPLC–DAD. AHP were predominant in grape and saladette tomato extracts (91.47 ± 17.28 mg gallic acid equivalents (GAE) per g dry extract (DE) and 57.41 ± 8.80 mg GAE per g DE, respectively), while BP form was predominant in tomato by-product (51.30 ± 10.91 GAE per g DE). AHP extract of grape tomato presented the highest antioxidant capacity by

DPPH assay (252.35 ± 42.55 μ mol trolox equiv (TE) per g DE). In the case of ORAC assay, AHP fractions from both grape (1005.19 ± 138.52 μ mol TE per g DE) and saladette tomatoes (804.16 ± 131.45 μ mol TE per g DE), and BP fraction from by-product (852.40 ± 71.46 μ mol TE per g DE) showed the highest ORAC values. Caffeic acid was the most abundant phenolic acid and it was found mainly in its conjugated forms. Naringenin was the most abundant flavonoid and it was mainly detected in bound form. Our analysis allowed a better characterization of phenolic compounds in whole tomato and by-product, remarking the importance of the fractionation. The valorization of the industrial tomato by-product, through the use of its different fractions of phenolic antioxidant compounds, could generate additional income to the tomato industry and reduce the waste disposal problem.

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Keywords Free phenolics · Conjugated phenolics · Bound phenolics · Phenolic profile · Tomato by-product · Antioxidant

Abbreviations

SFP	Soluble free phenolics
AHP	Acid-hydrolysable phenolics
AKHP	Alkaline-hydrolysable phenolics
BP	Bound phenolics
GAE	Gallic acid equivalents
CAE	Catechin equivalents
TE	Trolox equivalents
TPC	Total phenolic content
DE	Dry extract

Introduction

Tomato is one of the most widely used and versatile fruit crops. It is consumed fresh and processed into a wide range of manufactured products (de Sousa et al. 2008). Mexico ranks tenth worldwide among tomato-producing countries with approximately 2.7 million tons per year and the State of Sinaloa is the main tomato producer contributing with 27% of the national production (SIAP 2016). Epidemiologic studies suggest that consumption of tomato and tomato-based products reduces the risk of chronic diseases such as cardiovascular disease and certain types of cancer especially, prostate cancer (Willcox et al. 2003; Paur et al. 2016).

Industrial processing of tomato generates a considerable amount of by-products that are used mainly for animal feed or turn into compost. The management of tomato by-products is considered an important problem faced by tomato processing companies, as they cannot be discharged to the environment (Bennamoun et al. 2016). Industrial tomato by-products can be recovered in order to produce valuable metabolites via chemical and biotechnological processes (Ravindran and Jaiswal 2016). A relatively simple recovery technology could consist of production of powders or extracts to be directly incorporated into foods (Nour et al. 2015). Recently, studies have been conducted to incorporate extracts from tomato by-products as an ingredient in the formulation of antioxidant rich functional foods (Nour et al. 2015) as well as to improve the oxidative stability of dairy products (Abid et al. 2017).

Traditionally, the bioactivity of tomato and its products has been attributed to carotenoids. During the last years, tomato and tomato by-products have been the subject of significant research mainly focused on recovery of lycopene and β -carotene, as well as, evaluation of their bioactive potential (Strati and Oreopoulou 2011; Lavelli and Torresani 2011; Stajčić et al. 2015), however, also phenolic compounds have been reported widely as bioactive, like antioxidant, antimutagenic and cytotoxic activities in vitro, in fresh tomato, processed products and industrial by-products (Ferrerres et al. 2010; Valdez-Morales et al. 2014).

Phenolic compounds have been extensively characterized in tomato varieties from different countries, including genetically modified tomatoes and by-products (Barros et al. 2012; Ćetković et al. 2012; Kalogeropoulos et al. 2012; Valdez-Morales et al. 2014). However, the chemical composition of tomatoes can vary among tissues of a single fruit and type of tomatoes, according to the cultivar, cultivation conditions, and handling and storage methods (Barros et al. 2012). Phenolic compounds in plants may exist in free, soluble conjugated (acid and alkaline

hydrolysable) and insoluble-bound forms (Wang et al. 2015). Recently, analysis and evaluation of bioactivity of different fractions of phenolic compounds from vegetable sources and by-products have attracted the attention of researchers because it allows a more complete characterization (Ambigaipalan et al. 2016; Ayoub et al. 2016).

Although many studies have been realized on tomato and tomato by-products, most studies have been conducted in crude phenolic extracts and there is no detailed identification of soluble, conjugated and bound phenolic compounds. There is therefore demand for a comprehensive analysis of these bioactive phenolic compounds. Thus, in this study, we have isolated, identified and quantified soluble free phenolics (SFP), acid-hydrolysable phenolics (AHP), alkaline-hydrolysable phenolic (AKHP) and bound phenolics (BP) fractions from grape and saladette tomato and industrial tomato by-product. The total and individual phenolic compounds in tomato extracts were identified and quantified by HPLC and the in vitro antioxidant capacity of the isolated fractions was also determined.

Materials and methods

Chemicals and reagents

Reagents including 2,2'-azobis-2-methyl-propanimidamide dihydrochloride (AAPH), Folin-Ciocalteu reagent, 2,2-diphenyl 1-picryl-hydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox) and phenolic standards (gallic acid, caffeic acid, ferulic acid, *p*-coumaric acid, chlorogenic acid, (+)-catechin hydrate, kaempferol, naringenin, quercetin and rutin) were purchased from Sigma-Aldrich (St. Louis MO, USA). ACS and HPLC-grade solvents were purchased from Karal (Leon, Guanajuato, Mexico).

Biological material and sample processing

Tomato fruits (saladette and grape varieties) grown in the State of Sinaloa were provided by a local farm during the first harvest of 2015. The fruits were stored at 25 °C until they reached the stage of maturity # 6 (last stage in USDA color scale). Tomatoes were cut into small cubes (1 cm³), frozen (− 20 °C) and lyophilized. The by-product sample (which is comprised by peel and seeds) was provided by a local company (La Costeña®, Guasave, Sinaloa Mexico), from industrial tomato processing line, operating to produce tomato paste. The industrial by-product was frozen and lyophilized. The samples were milled until obtaining a fine powder (TissueLyser II, Qiagen, Valencia, CA) and sieved (sieve number 35). Powdered samples were stored at − 20 °C protected from light for further analysis.

Color analysis

The color of fresh samples was recorded; ripe tomatoes was measured with a chroma meter CR-400 (Konica Minolta Sensing, Inc., Japan); 10 whole fruits of each type were evaluated in five points, three equidistant equatorial points and two axial. The color of fresh by-product and powdered samples were also recorded placing a representative samples in a petri plate. L^* , a^* , and b^* CIELab parameters were determined using a white standard CR-A43 and Chroma values were calculated.

Extraction of phenolic compounds from tomato and by-product

Different fractions of phenolic compounds were sequentially extracted from grape and saladette tomato powder and by-product powder. Previously, samples were defatted as follow: the fine powder (4 g) was transferred to an Erlenmeyer flask, 20 mL of hexane were added and the mixture was kept in a mechanical shaker during 1 h at 25 °C. The mixture was filtered and the final defatted flour was dried in a hood at room temperature (RT). In the case of saladette tomato, after filtered, the sample was dried in oven during 1 h at 45 °C. Three independent samples were obtained for at least three analytical replicates. SFP, AHP, AKHP and BP were extracted following the procedure reported by Kim et al. (2006) with some modifications, as described below (Supplementary figure 1).

Extraction of SFP

Defatted tomato powder was extracted twice with 80% (v/v) ethanol at a ratio 5:1 (v/w) during 1 h at RT. The mixture was centrifuged (3914g) at RT during 5 min, -the pellet obtained was preserved for extraction of BP- and the combined supernatant was concentrated in a vacuum evaporator at 37 °C and lyophilized to get a dry extract. The resulting dry extract was dissolved in 4 mL of acidified water (pH 2 with HCl) for each gram of defatted tomato powder used for extraction. Then, the mixture was partitioned with ethyl ether at a 1:1 (v/v) ratio and centrifuged (3914g) at RT during 5 min and the organic phase was recovered. The extraction was repeated twice more. Combined ether layer, which contain SFP, was dried under stream of gaseous nitrogen.

Extraction of AKHP and AHP

The water phase previously obtained was neutralized at pH 7 with 2 M NaOH and lyophilized. The residue was dissolved in 4 mL of 2 M NaOH and stirred during 4 h at RT. Solution was then acidified to pH 2 and extracted with

ethyl ether at 1:1 (v/v) ratio, two times as described above; resulting ether layer contained AKHP and water phase was used for the extraction of AHP. Conjugated phenolic compounds contained in the water phase were hydrolyzed with 3 mL of 6 M HCl and heated at 95 °C during 20 min. The mixture was partitioned one time with ethyl ether at 1:1 (v/v) ratio. The organic phases, which contain AHP and AKHP, were dried under stream of gaseous nitrogen.

Extraction of BP

The residue from SFP extraction equivalent to 1 g of sample was mixed with 2 M NaOH (40 mL) and stirred during 4 h at RT. After extraction, the mixture was acidified to pH 2 with HCl and BP were recovered one time with ethyl ether. BP fraction was dried using a vacuum evaporator.

Total phenolic content (TPC)

TPC of extracts was determined by the Folin–Ciocalteu's assay, according to Swain and Hillis (1959) with modifications and adapted to microplate. Briefly, 140 µL of distilled water, 10 µL of extract at 1 mg/mL in methanol, blank or standard and 10 µL of freshly prepared Folin–Ciocalteu reagent (1:1 v/v in water) were mixed and incubated at room temperature in dark during 3 min. Additionally, 40 µL of 7.5% (w/v) sodium carbonate was added, and the mixture was incubated at 45 °C during 15 min in the dark, then the absorbance was read at 760 nm. (+)-Catechin (0–300 µg/mL) and gallic acid (0–200 µg/mL) were used as standards and the results were expressed in mg (+)-catechin equivalents and gallic acid equivalents per g dry extract.

Antioxidant activity

DPPH assay

Antiradical activity of extracts of phenolic compounds was determined according to Cardador-Martínez et al. (2006) with minor modifications. A solution of 150 µM DPPH[•] was prepared in 80% (v/v) methanol. 20 µL of sample, blank, standard or control and 200 µL of DPPH solution were added to a 96 wells microplate. Absorbance was measured every 10 min from 0 to 30 min at 515 nm. Trolox (0–200 µg/mL) was used as standard and the results were expressed as µmol trolox equiv (TE)/g dry extract. All determinations were carried out by triplicate.

ORAC assay

The ORAC value of the different fractions of phenolic compounds from tomato and by-product was measured according Prior et al. (2003). For the assay, SFP, AHP, AKHP and BP extracts previously dissolved in methanol were diluted in ORAC buffer (potassium phosphate buffer, pH 7.4) and a trolox standard curve (0–30 mg/L) was prepared. At the day of analysis 87 μ M fluorescein and 70 mM AAPH solutions were prepared in ORAC buffer. A 96 wells black microplate was prepared containing 25 μ L of blank (ORAC buffer); standard, control or sample and 200 μ L of fluorescein solution were added. Then, 50 μ L of freshly prepared AAPH solution were quickly added. The plate was incubated at 37 °C. Readings of fluorescence were every 2 min during 76 cycles using a DTX 880 multimode detector, Beckman Coulter (Brea, CA, USA) (excitation wavelength of 485 nm and emission wavelength of 530 nm). The results were expressed as μ mol TE per g dry extract. Determinations were carried out by triplicate.

Phenolic profile analysis by HPLC–DAD

The phenolic compounds profile was determined by HPLC–DAD. SFP, AHP, AKHP and BP fractions of each sample were obtained as previously described and dissolved in methanol for the analysis. Extracts were filtered (nylon membrane, 0.45 μ m and 25 mm diameter) and analyzed in a Dionex UltiMate 3000 liquid chromatograph equipped with a titanium quaternary pump (LPG-3400AB), an autosampler (WPS-3000TBPL), a column oven, and a photodiode array detector DAD-3000(RS) (Thermo Fisher Scientific, New York, NY, USA). An Acclaim 120A (C18, 5 μ m, 120 Å, 4.6 $\times$ 250 mm) column was used for separation of phenolics according to the analytical method described by Valdez-Morales et al. (2014). The phenolic compounds were identified and quantified using pure standards, using retention time and absorption spectral characteristics. The fixed wavelengths were 270 nm (gallic acid), 280 nm [(+)-catechin], 300 nm (ferulic acid, *p*-coumaric acid and naringenin), 320 nm (caffeic and chlorogenic acids) and 360 nm (kaempferol, quercetin and rutin). The analysis was performed in triplicate. The data were processed using Chromeleon 7.0 software (Dionex, Thermo Fisher Scientific).

Statistical analysis

Analysis of variance and multiple ranges test (Tukey, $p \leq 0.05$) were done with Statgraphics® Plus (Statistical Graphics Corp., Herndon, VA, USA).

Results and discussion

Color determination

Color (CIELAB scale) of whole fruits and the fresh by-product were determined (Fig. 1A). Significant differences ($p \leq 0.05$) were found in the parameter L^* (luminosity) between samples, being grape fruit the most lightness. The fresh by-product shown both high redness (parameter a^*) and yellowness (parameter b^*) index, as well being the sample with the most striking color (Chroma) ($p \leq 0.05$). In addition, the color parameters of the flours constituting a mixture of the components of the whole fruit and by-product, i.e., pulp, peel and seed (Fig. 1B), were determined. In this case, the grape tomato is the most luminous, but remains the furthest from the redness index ($p \leq 0.05$). The mixture of saladette tomato components provided a high redness index to their flour, while the by-product powder is high yellowness index.

Total phenolic content (TPC)

In order to obtain a more complete phenolic characterization from tomato samples, SFP, BP, AHP, and AKHP were sequentially obtained from grape, saladette tomatoes, and by-product. Significant differences ($p \leq 0.05$) in extraction yields per fraction were observed (Table 1). SFP from grape tomato, SFP from saladette tomato and AKHP from industrial by-product showed a significantly higher yield ($p \leq 0.05$) than the other fractions obtained from different samples. In addition, it was observed that the AHP fraction of all samples generated lowest yields ($p \leq 0.05$).

When analyzing the TPC of each extract (Table 1), it was observed that SFP fractions of the three samples showed the lowest concentrations ($p \leq 0.05$) in comparison to the other fractions. Results are expressed as mg GAE per g defatted sample (multiplying extraction yield by TPC per mg of DE) (Table S1), SFP represent $\sim 30\%$ of TPC in saladette and grape tomato, meanwhile in by-product it represent only $\sim 19\%$. This means that SFP fraction contains other compounds co-extracted along with phenolics compounds, supporting our strategy to perform different process to release phenolics compounds from the matrix in which they are contained. We consider that other water-soluble compounds, such as some sugars, may have been extracted, increasing the yield of dry extract.

Insoluble BP represent $\sim 18\%$ of TPC in whole tomato samples, whereas in by-product sample, BP represent $\sim 67\%$ of TPC indicating that the major phenolic compounds in tomato waste were not extractable by aqueous ethanol but released upon alkaline hydrolysis. This result may be due to the fact that by-product, came from a

Fig. 1 CIELAB parameters of color of whole tomato fruit (a) and fresh tomato by-product and parameters of color of samples powder (b). $n = 10$ for tomato fruits and $n = 5$ for by-product; $n = 6$ for sample powder. Different letters indicate statistically significant

difference between treatments (multiple-range test; Tukey, $p \leq 0.05$). Bars represent standard deviation (SD). Grape tomato (■), Saladette tomato (▨), By-product (□)

Table 1 Total phenolic content and antioxidant activity of different fractions of phenolic compounds of whole tomato fruits and industrial by-product

Sample	Fraction	Yield (mg DE per g defatted sample)	Total phenolic content ^a		Antioxidant activity	
			mg GAE per g DE	mg CAE per g DE	DPPH (μmol TE per g DE)	ORAC (μmol TE per g DE)
Grape tomato	SFP	49.97 ± 4.05a	9.04 ± .21f	6.09 ± 1.16 g	38.55 ± 6.56d	131.64 ± 0.73de
	BP	10.63 ± 2.09ef	32.87 ± 2.4 cd	28.79 ± 3.46de	87.86 ± 13.87c	635.88 ± 28.01bc
	AHP	3.93 ± 0.59f	91.47 ± 17.28a	86.02 ± 17.06a	252.35 ± 42.55a	1005.19 ± 138.52a
	AKHP	14.40 ± 5.53def	36.84 ± 5.75c	33.89 ± 5.71 cd	86.41 ± 23.65c	653.82 ± 190.83bc
	Total	78.93	170.22	154.79	465.17	2426.53
Saladette tomato	SFP	33.80 ± 5.98b	11.08 ± 1.37ef	7.74 ± 2.60 g	35.86 ± 9.64d	107.75 ± 12.87e
	BP	9.10 ± 0.54ef	21.85 ± 2.37de	18.25 ± 2.99efg	60.79 ± 6.80 cd	430.83 ± 53.54 cd
	AHP	5.90 ± 1.07ef	57.41 ± 8.80b	53.54 ± 8.94b	150.59 ± 39.92b	804.16 ± 131.45ab
	AKHP	17.90 ± 3.56cde	25.04 ± 8.47cde	22.51 ± 7.77def	60.56 ± 7.45 cd	288.65 ± 62.41de
	Total	66.7	115.38	102.04	307.8	1631.39
By-product	SFP	29.60 ± 2.66bcd	13.40 ± 3.66ef	10.25 ± 2.28 fg	25.79 ± 6.47d	141.44 ± 47.67de
	BP	28.67 ± 5.11bcd	51.30 ± 10.91b	46.36 ± 10.71bc	83.56 ± 10.26c	852.40 ± 71.46ab
	AHP	5.65 ± 0.78ef	32.23 ± 5.77 cd	29.49 ± 5.89de	88.17 ± 19.87c	468.25 ± 139.14 cd
	AKHP	31.65 ± 10.68bc	7.33 ± 0.60ef	5.72 ± 0.29 fg	nd	64.09 ± 4.98e
	Total	95.57	104.26	91.82	197.52	1526.18

Values are the mean of three replicates of three independent experiments ($X \pm SD$, $n = 3$). Values marked by the same letter within the same column are not statistically significantly different (multiple-range test; Tukey, $p \leq 0.05$)

SFP soluble free phenolics, BP bound phenolics, AHP acid-hydrolysable phenolics, AKHP alkaline-hydrolysable phenolic, DE dry extract, TE Trolox equivalents, GAE gallic acid equivalents, CAE-(+) catechin equivalents, nd no detected

^aTotal phenolic content was quantified with the Folin–Ciocalteu method

process that eliminate most of pulp from tomato fruit, resulting in a sample composed mainly of peel and seeds. By that reason phenolics in the insoluble forms are rich in

this samples, since they are covalently bound to cell wall structural components such as cellulose, hemicellulose, lignin, pectin and structural proteins (Wong 2006) It is

important note that bound forms of phenolic compounds can be released and absorbed in the organism during digestion process (Acosta-Estrada et al. 2014) and high contribution of BP to the antioxidant activity, even higher than free and esterified fractions, has been reported (Liyana-Pathirana and Shahidi 2006).

The AHP fraction generated extracts rich in phenolic compounds. Grape tomato extract showed an AHP content significantly higher than the other extracts ($p \leq 0.05$), followed by saladette tomato and finally the industrial by-product extract. In defatted tomato powder AHP represent approximately from 24 to 30% of TPC while in by-product AHP content decreased to 10% of TPC. We consider that, in whole tomato, AHP possesses high bioactive potential. Kim et al. (2006) reported highest phenolic compounds in alkaline hydrolyzed fraction compared to acidic hydrolyzed phenolic in wheat bran. We are reporting highest concentration of AHP than AKHP, contrasting with that report.

Our results of TPC of all fractions from tomato by-product are similar to those reported by Peschel et al. (2006), who performed a solvent fractionation of crude phenolic extract from tomato canning industry by-product. El-Malah et al. (2015) optimized the extraction conditions of phenolic compounds from tomato waste by assisted methods (ultrasound and microwave), achieving lower yields of TPC (101.5–162.5 mg GAE/100 g dry weigh) than those found in this study (212.88 mg GAE/100 g of sample). Recently, Robles-Ramírez et al. (2016) reported lower yields of TPC in tomato waste extracts from tomato cultivated in Mexico.

Phenolic compounds profile by HPLC–DAD

We obtain the phenolic compounds profile of SFP, AHP, AKHP and BP fractions from grape and saladette tomatoes and an industrial tomato by-product by HPLC–DAD (Table 2). Ten compounds were identified and quantified (five phenolic acids and five flavonoids). Caffeic acid was the most abundant phenolic acid in the analyzed samples in concordance with that reported before in whole tomato fruit and different tomato by-products (Barros et al. 2012; Ćetković et al. 2012; Valdez-Morales et al. 2014) and this phenolic acid is also found mainly in its conjugated forms. Also, significant amounts of ferulic acid were detected in all fractions tested. The AHP fractions of all samples are rich in p -coumaric acid. Flavonoids analysis indicates that by-product present the highest concentrations of quercetin and catechin in both bound and conjugated forms. Naringenin was the most abundant flavonoid in accordance with previously reported (Vallverdú-Queralt et al. 2012) and it was mainly detected in bound form in whole tomatoes and by-product. Naringenin concentration in BP fraction of by-

product was significantly higher ($p \leq 0.05$) than those found in different fractions of whole tomato. Ćetković et al. (2012) reported lower concentrations of naringenin-derivative in tomato by-product. This may be due, at least in part, to the fact that quantification was performance on soluble phenolic fraction. Naringenin has been previously investigated for its ability to inhibit growth of breast, colon, gastric and prostate cancer cells and it causes apoptosis in human pancreatic cancer cells (Park et al. 2017).

Distribution patterns of phenolic acids and flavonoids as free, conjugated, and insoluble-bound forms in grape tomato, saladette tomato, and by-product are shown in Fig. 2. In grape tomato, total phenolic acids (as the sum of the individual phenolic acids) were predominantly in the conjugated acid hydrolysable fraction, whereas in saladette and by-product, which comes from the processing of saladette tomato, phenolic acids were mainly in the alkaline-hydrolysable fraction. Phenolic acids were detected in low concentration as soluble free form. Flavonoid contents of grape and by-product followed the same order BP > SFP > AKHP > AHP while in saladette the order was BP > AKHP > SFP > AHP. It is very interesting to see that the highest amount of flavonoids in tomato samples was detected as insoluble-bound form. These findings highlight the importance of extracting the insoluble-bound fraction during total phenolic characterization. Pomegranate by-products has similar flavonoids distribution pattern (Ambigaipalan et al. 2016).

Antioxidant capacity

Table 1 shows the results of antioxidant capacity obtained for each fraction evaluated. In the case of the DPPH assay, the AHP extract of grape tomato presented the highest antioxidant capacity, followed by the AHP of saladette tomato. In the case of the by-product, high antioxidant capacity was observed in the AHP and BP fractions. The extracts of AHP fraction showed the highest antioxidant capacity in this study, while SFP showed lower antioxidant capacity. The antioxidant capacity of samples evaluated using the ORAC methodology was higher than that determined using the DPPH methodology. AHP fractions from both grape and saladette tomatoes, and BP fraction from by-product showed the highest ORAC values ($p > 0.05$). Previously, good contribution of BP to the antioxidant activity, even higher than free and esterified fractions (alkaline hydrolysable fraction), has been reported by Liyana-Pathirana and Shahidi (2006). Kim et al. (2006) and Wang et al. (2013) reported higher antioxidant activity of AKHP than AHP fraction for bran samples, unlike to our results, this behavior could be attribute to different type of samples. On the other hand, Valdez-Morales et al. (2014) reported lower values of antioxidant activity in peel and

Table 2 Profile of individual phenolic acids and flavonoids in tomato and tomato by-product extracts determined by HPLC–DAD

Sample	Fraction	Phenolic acids and flavonoids (mg per g DE)						Total phenolic acid per sample
		Caffeic acid	Chlorogenic acid	Ferulic acid	Gallic acid	p-coumaric acid	Total phenolic acids per fraction	
Grape tomato	SFP	0.25 ± 0.00d	0.49 ± 0.04a	1.59 ± 0.10bc	nd	nd	2.33	158.80
	BP	15.73 ± 0.44b	nd	1.08 ± 0.20d	nd	nd	16.81	
	AHP	22.34 ± 2.25a	nd	2.36 ± 0.19a	2.32 ± 0.30a	5.86 ± 0.62ab	32.88	
	AKHP	21.91 ± 3.52a	0.37 ± 0.04b	2.53 ± 0.00a	1.25 ± 0.00b	1.32 ± 0.06cd	27.38	
	Total	60.23	0.86	7.56	3.57	7.18	79.4	
Saladette tomato	SFP	0.89 ± 0.14d	0.13 ± 0.02c	0.54 ± 0.5e	nd	0.15 ± 0.02de	1.71	94.04
	BP	3.84 ± 0.28 cd	nd	1.59 ± 0.06bc	nd	1.12 ± 0.03de	6.55	
	AHP	15.50 ± 1.57b	nd	1.54 ± 0.13c	nd	5.38 ± 0.68b	22.42	
	AKHP	14.22 ± 1.55b	0.12 ± 0.00c	1.2 ± 0.13d	nd	0.80 ± 0.10e	16.34	
	Total	34.45	0.25	4.87	nd	7.45	47.02	
By-product	SFP	0.28 ± 0.01d	0.08 ± 0.00c	0.24 ± 0.02f	nd	0.12 ± 0.01e	0.72	52.22
	BP	1.82 ± 0.15d	nd	0.45 ± 0.01ef	nd	2.33 ± 0.02c	4.60	
	AHP	7.28 ± 0.72c	nd	1.85 ± 0.08b	1.43 ± 0.22b	6.72 ± 0.70a	17.28	
	AKHP	2.41 ± 0.63 cd	nd	0.70 ± 0.04e	nd	0.40 ± 0.05de	3.51	
	Total	11.79	0.08	3.24	1.43	9.57	26.11	
		Catechin	Kaempferol	Naringenin	Quercetin	Rutin	Total flavonoids per fraction	
Grape tomato	SFP	nd	0.048 ± 0.00e	0.22 ± 0.03f	0.27 ± 0.01 cd	0.12 ± 0.01c	0.66	97.94
	BP	1.23 ± 0.01b	Nd	13.24 ± 0.57b	nd	0.55 ± 0.03a	15.02	
	AHP	1.39 ± 0.05b	0.31 ± 0.02a	nd	0.79 ± 0.25c	nd	2.49	
	AKHP	nd	nd	nd	0.10 ± 0.01d	0.27 ± 0.01b	0.37	
	Total	2.62	0.36	13.46	1.16	0.94	18.54	
Saladette tomato	SFP	nd	0.04 ± 0.00e	0.19 ± 0.01ef	0.20 ± 0.01d	0.08 ± 0.01de	0.51	57.16
	BP	nd	0.19 ± 0.00c	4.54 ± 0.42c	0.85 ± 0.12c	nd	5.58	
	AHP	nd	nd	1.57 ± 0.18de	0.83 ± 0.09c	nd	2.40	
	AKHP	1.03 ± 0.06bc	0.24 ± 0.03b	nd	0.08 ± 0.01d	0.30 ± 0.01b	1.65	
	Total	1.03	0.47	6.3	1.96	0.38	10.14	
By-product	SFP	0.18 ± 0.01d	0.04 ± 0.00e	1.74 ± 0.16d	0.26 ± 0.01 cd	0.10 ± 0.00 cd	2.32	73.72
	BP	0.54 ± 0.01 cd	0.08 ± 0.00d	25.22 ± 1.26a	5.89 ± 0.14a	0.05 ± 0.00e	31.78	
	AHP	4.39 ± 0.33a	0.19 ± 0.00c	1.28 ± 0.16def	3.09 ± 0.34b	nd	8.95	
	AKHP	nd	nd	nd	4.56 ± 0.06 cd	nd	4.56	
	Total	5.11	0.31	28.24	13.80	0.15	47.61	

Three independent extractions were carry on and analyzed by HPLC–DAD ($X \pm SD$, $n = 3$). Values marked by the same letter within the same column of phenolic acids or flavonoids are not statistically significantly different (multiple-range test; Tukey, $p \leq 0.05$)

SFP soluble free phenolics, *BP* bound phenolics, *AHP* acid-hydrolysable phenolics, *AKHP* alkaline-hydrolysable phenolic, *DE* dry extract, *nd* no detected, *TPC* total phenolic compounds (phenolic acids and flavonoids) determined by HPLC

seed of an industrial tomato by-product by both methods DPPH (0.514 $\mu\text{mol TE per g}$ and 0.836 $\mu\text{mol TE per g}$, respectively), and ORAC (7.053 $\mu\text{mol TE per g}$ and 10.283 $\mu\text{mol TE per g}$, respectively) than those found in this study (3.53 $\mu\text{mol TE per g}$ by DPPH assay and 35.64 $\mu\text{mol TE per g}$ by ORAC assay) (Supplementary

Table 1). Even, the BP fraction from by-product showed greater antioxidant activity than that reported by Valdez-Morales et al. (2014). These results may be due that the fractionation method used in this study effectively released a greater amount of antioxidant compounds.

Fig. 2 Distribution pattern of phenolic acids (a) and flavonoids (b) as free, conjugated, and insoluble-bound forms in grape tomato, saladette tomato, and by-product determined by HPLC–DAD. Soluble free phenolics (SFP ■), bound phenolics (BP ▨), acid-hydrolysable phenolics (AHP ▩), alkaline-hydrolysable phenolic (AKHP □)

Conclusion

From the results, we can conclude that tomato by-product is rich in phenolic compounds with high antioxidant activity, and these are mainly bound to structural components of cell walls. Considering this information, it is important to take into account extraction methodologies of these compounds for the analysis of phenolic bioactivity, as well as studies of their potential applications, to achieve maximum utilization of the by-product as well characterization of other tomato varieties. The valorization of the industrial tomato by-product, through the use of its different fractions of phenolic antioxidant compounds, could generate additional income to the tomato industry and reduce the waste disposal problem. Finally, to the best of our knowledge, this is the first report of identification of soluble free, conjugated (acid and alkaline hydrolysable) and bound phenolic compounds from industrial tomato by-product as well in whole tomato fruit.

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