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Phenolic composition and antioxidant capacity of yellow and purple-red Ecuadorian cultivars of tree tomato (*Solanum betaceum* Cav.)

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ABSTRACT

Tree tomato fruits from the yellow giant, giant purple and New Zealand purple cultivars, cultivated in Ecuador were analysed for their phenolic composition and antioxidant capacity. Twelve hydroxycinnamoyl derivatives and four anthocyanins (in the purple cultivars) were detected and identified. The hydroxycinnamoyl derivatives mostly derived from caffeic acid, being 3-*O*-caffeoylquinic acid and rosmarinic acid the majority compounds. Furthermore, various rosmarinic acid glucosides, caffeoyl glucoside, feruloyl glucoside and two ferulic acid dehydrodimers were tentatively identified. The presence of rosmarinic acid is particularly relevant as it constituted a majority phenolic compound in the four studied tree tomato cultivars and it had not been reported previously in this fruit. In the purple cultivars main anthocyanins were pelargonidin 3-*O*-rutinoside and delphinidin 3-*O*-rutinoside. The New Zealand purple cultivar was by far the richest sample in both hydroxycinnamates (421.6 mg/100 g dry pulp) and anthocyanins (168.9 mg/100 g dry pulp). Antioxidant capacity, as determined by FRAP, ABTS and ORAC assays, followed the same pattern as phenolic contents, with the New Zealand purple cultivar being the one with the highest and the yellow giant cultivar with the lowest values.

1. Introduction

The tree tomato (*Solanum betaceum* Cav.) is a neglected Andean crop, which nonetheless is quite popular in local markets especially of South America being consumed in juices and as a fresh fruit. This crop represents an important alternative for diversification of fruit production both in its region of origin and also in other areas of the world. Important efforts have been made for the development of the crop in Colombia, Ecuador and New Zealand, where production and export have increased markedly in the last decades (Biodiversity International, 2013). Also, it is considered as a promising crop for some regions with a Mediterranean climate (Prohens & Nuez, 2000).

S. betaceum Cav. is a small tree native to the Andean region. The common name is tree tomato, also being used the following names: *tomate extranjero*, *tomate cimarron*, *tomate de monte*, *tomate silvestre* and *tamarillo*. The fruit has an ovoid shape with around 6–8 cm of length and 4–5 cm of diameter (Fig. S1, supplementary material). The ripe fruit turns to orange or purple colour depending on the variety, and exhibits a slightly bitter, sour, and astringent taste with a characteristic aroma. It is generally consumed fresh, or blended together with water and sugar to make juices and desserts (Bohs & Nelson, 1997; Lester & Hawkes, 2001).

In Ecuador tree tomato cultivars are not preserved pure due to crosslinking among materials that are grown in farmer orchards, presenting a great genetic variability and giving as result fruits with a wide range of tones, between yellow and purple (León, Viteri, & Cevallos, 2004; Revelo, Perez, & Maila, 2004). Within genotypes grown in Ecuador the most representatives are cultivars of sharp-pointed yellow, yellow ball, yellow giant, giant purple, and New Zealand purple, being the yellow giant genotype the one of major production (León et al., 2004). The purple cultivars are different by the size of the fruits and the colour of the mucilage around the seeds with tones from dark red to purple (Fig. S1) (León et al., 2004).

Phenolic compounds are secondary plant metabolites with acknowledged antioxidant and radical scavenging properties that have been related to beneficial effects in human health, such as reducing the risk of cardiovascular disease, diverse cancers and other pathologies (Rodriguez-Mateos et al., 2014). Tree tomato

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fruits have been shown to possess high antioxidant capacity *in vitro*, which has been related with the presence of phenolic compounds (Hurtado, Morales, González-Miret, Escudero-Gilete, & Heredia, 2009; Mertz et al., 2009; Vasco, Ruales, & Kamal-Eldin, 2008). Among them, the presence of anthocyanins (delphinidin, cyanidin and pelargonidin glycosides) and hydroxycinnamoyl derivatives (e.g., caffeoylquinic acids, caffeoyl glucose and feruloyl glucose) has been described (Mertz et al., 2009; Osorio et al., 2012; Vasco, Avila, Ruales, Svanberg, & Kamal-Eldin, 2009). Anthocyanins are the largest group of water-soluble pigments in the plant kingdom. They can be used as colourants for the food industry, possess antioxidant capacity, which can protect food components against the oxidation, therefore maintaining the nutritional value, and have been associated to health benefits, namely prevention of cardiovascular diseases (de Pascual-Teresa, Moreno, & Garcia-Viguera, 2010). The hydroxycinnamoyl derivatives also show antioxidant properties and have also been related to protective effects on human health (Crozier, Jaganath, & Clifford, 2009; Del Rio et al., 2013). In particular, the caffeoyl ester rosmarinic acid has a number of interesting biological activities, such as antiviral, antibacterial, anti-inflammatory and antioxidant effects (Petersen, 2013).

With the aim of improving the knowledge about the composition and properties of tree tomato, in the present work, samples of the fruit belonging to the yellow giant, giant purple and New Zealand purple cultivars (Fig. S1), cultivated in Ecuador have been studied for their phenolic composition and antioxidant capacity. Data obtained are expected to contribute to the valorisation of this crop as a source of valuable phytochemicals with potential health benefits.

2. Materials and methods

2.1. Standards and reagents

Delphinidin-3-*O*-glucoside, cyanidin-3-*O*-glucoside and pelargonidin-3-*O*-glucoside were purchased from Extrasynthese (Genay, France). Rosmarinic acid, caffeic acid, ferulic acid, sodium carbonate, potassium persulphate, fluorescein, 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH), 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), phosphate buffered saline (PBS) were purchased from Sigma–Aldrich (Madrid, Spain). Quinic acid was from Alfa Aesar (Karlsruhe, Germany). ABTS (2,2-azino-bis-(3-ethylbenzothiazolne-6-sulfonic acid) diammonium salt) and Trolox (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid) were from Fluka (Madrid, Spain). HPLC-grade acetonitrile was from Carlo Erba (Rodano, Italy), and analytical grade glacial acetic acid, methanol, formic acid and iron trichloride ($FeCl_3{\cdot}6H_2O$) were obtained from Panreac (Barcelona, Spain).

2.2. Plant material

Four samples of tree tomato fruit (*S. betaceum* Cav.) were studied. Two of them belonged to the yellow giant cultivar and were collected in 2014 in orchards of farmers located in two of the main producing areas: Pelileo – Province of Tungurahua in middle region of Ecuador (2572 masl of altitude); and Chaltura – Province of Imbabura in the north region of Ecuador (2351 masl of altitude). The other two samples belonged to the Giant purple and New Zealand purple cultivars, and were purchased in April 2013 in local markets from Ambato-Tungurahua and Salcedo-Cotopaxi in middle region on Ecuador, respectively. All the samples were authenticated by experts at the *Instituto Nacional de Investigaciones Agropecuarias* (INIAP).

For each of the studied cultivars between 50 and 100 units were collected. The average raw mass of the fruits was 102.51 ± 11.59, 117.16 ± 15.65 and 92.56 ± 9.56 g for the yellow giant, giant purple and New Zealand Purple varieties, respectively. The skin was manually separated and the seeds removed by sieving after soft crushing in a blender. The yields of pulp (edible fraction) ranged between 71.93 ± 6.15 g (yellow giant) and 78.47 ± 3.59 g (giant purple). The pulp was freeze-dried and stored in a closed amber vial and keep at 5 °C until analyses.

For phenolic analysis, the freeze-dried samples (5 mg) were extracted with 75% methanol (1 mL) by shaking for 30 min in an incubating mini shaker, then put in a ultrasonic bath for 30 min and further centrifuged at 6700*g* for 5 min; the supernatant was collected and the residue submitted to the same process twice. The three supernatants were combined and concentrated under reduced pressure to dryness. The residue was recovered in 1 mL of methanol:water (25:75, v/v), sonicated twice for 5 s, centrifuged (10,000*g*, 5 min) and analysed by HPLC–DAD–MS. For the analysis of anthocyanins in the purple cultivars, the same protocol of extraction was followed but using a mixture of methanol and 0.1% formic acid (75:25, v/v) as solvent for the extraction. In all cases three different samples of each of the collected tree tomato fruits were separately extracted and analysed.

2.3. HPLC–DAD–ESI/MS analyses

HPLC analyses were carried out in a Hewlett–Packard 1100 chromatograph (Agilent Technologies, Waldbronn, Germany) equipped with a quaternary pump and a diode array detector (DAD) coupled to an HP Chem Station (rev. A.05.04) data-processing station. The HPLC system was connected via the DAD cell outlet to an API 3200 Qtrap (Applied Biosystems, Darmstadt, Germany) mass spectrometer (MS) consisting of an ESI source and a triple quadrupole-ion trap mass analyzer, which was controlled by the Analyst 5.1 software.

2.3.1. Analysis of phenolic acid derivatives

An Agilent Poroshell 120 EC-C18 column (2.7 μm, 150 mm × 4.6 mm) thermostatted at 35 °C was used. The solvents were: (A) 0.1% formic acid, and (B) acetonitrile. The elution gradient established was isocratic 15% B for 5 min, 15–20% B over 5 min, 20–35% B over 10 min, 35–50% B over 10 min, 50–60% B over 5 min, isocratic 60% B for 5 min and re-equilibration the column to initial solvent conditions. The flow rate was 0.5 mL/min. Double online detection was carried out in the DAD at 280, 330 and 370 nm as preferred wavelengths and in the MS operated in the negative ion mode. Spectra were recorded between *m/z* 100 and *m/z* 1500. Zero grade air served as the nebulizer gas (30 psi) and as turbo gas (400 °C) for solvent drying (40 psi). Nitrogen served as the curtain (20 psi) and collision gas (medium). Both quadrupols were set at unit resolution and EMS and EPI analyses were also performed. The EMS parameters were: ion spray voltage 4500V, DP −50 V, EP −6 V, CE −10 V and cell exit potential (CXP) −3 V, whereas EPI settings were: DP −50 V, EP −6 V, CE −25 V and CES 0 V.

2.3.2. Analysis of anthocyanins

An AQUA® (Phenomenex) reverse phase C18 column (5 μm, 150 mm × 4.6 mm) thermostatted at 35 °C was used. The solvents were: (A) 0.1% trifluoroacetic acid, and (B) acetonitrile. The elution gradient established was: isocratic 10% B for 3 min, 10–15% B in 12 min, isocratic 15% B for 5 min, 15–18% B over 5 min, 18–30% B over 20 min, 30–35% B over 5 min, and re-equilibration of the column to initial solvent conditions. The flow rate used was 0.5 mL/min. Double online detection was carried out in the DAD using 280 and 520 nm as preferred wavelengths, and in the MS operated in the positive ion mode. Spectra were recorded between *m/z* 100 and *m/z* 1500. Zero grade air served as the nebulizer gas

(40 psi) and as turbo gas (600 °C) for solvent drying (50 psi). Nitrogen served as the curtain (100 psi) and collision gas (high). Both quadrupols were set at unit resolution and the MS detector was programmed to perform a series of two consecutive analyses, a full scan of high sensitivity (Enhanced MS, EMS) and an Enhanced Product Ion analysis (EPI) to obtain the fragmentation pattern of the parent ion. The EMS mode parameters were the following: ion spray voltage 5000 V, declustering potential (DP) 41 V, entrance potential (EP) 7.5 V and collision energy (CE) 10 V. EPI mode was applied using the following settings: DP 41 V, EP 7.5 V, CE 10 V and collision energy spread (CES) 0 V.

Compounds were identified by their retention time, UV–vis spectra and mass spectra, and comparison with our data library and standards when available. The compounds were quantified from the areas of their chromatographic peaks recorded at 330 nm for hydroxycinnamoyl derivatives, and at 520 nm for anthocyanins. Calibration curves were constructed for the following compounds: rosmarinic acid, caffeic acid, ferulic acid, delphinidin-3-*O*-glucoside, cyanidin-3-*O*-glucoside, and pelargonidin-3-*O*-glucoside. For the identified phenolic compounds for which a commercial standard was not available, the quantification was performed through the calibration curve of a compound from the same group. The results were expressed in mg per 100 g of dry pulp.

2.4. Antioxidant capacity

Three different *in vitro* assays were performed: ferric reducing ability of plasma (FRAP assay), $ABTS^{\bullet+}$ radical cation scavenging activity (ABTS/persulphate assay) and oxygen radical absorbance capacity (ORAC assay).

2.4.1. FRAP assay

The method of Benzie and Strain (1996) with some modifications was used. The FRAP reagent contained 10 mM of TPTZ solution in 40 mM HCl, 20 mM $FeCl_3 \cdot 6H_2O$, and acetate buffer (300 mM, pH 3.6) (1:1:10, v/v/v). Serial dilutions of the samples were prepared in 15% methanol in the range 5–0.3125 mg/mL. The solutions (100 μL) were mixed with 3 mL of the FRAP reagent, vortexed for 5s, and the absorbance measured at 593 nm after incubation at room temperature for 6 min, using the FRAP reagent as a blank. The results were obtained by interpolating the absorbances on a calibration curve obtained with Trolox (0.03125–1 mM in 15% methanol).

2.4.2. ABTS/persulphate assay

The $ABTS^{\bullet+}$ radical was produced by the oxidation of 7 mM ABTS with potassium persulphate (2.45 mM) in water. The mixture was allowed to stand in the dark at room temperature for 16 h before use, and then the $ABTS^{\bullet+}$ solution was diluted with phosphate buffered saline (PBS) at pH 7.4 to give an absorbance of 0.7 ± 0.02 at 734 nm. Serial dilutions of the samples were prepared in 15% methanol in the range 5–0.3125 mg/mL. The solutions (50 μL) were mixed with 2 mL of the $ABTS^{\bullet+}$ diluted solution, vortex for 5 s, and the absorbance measured at 734 nm after 4 min of reaction at 30 °C. The results were obtained by interpolating the absorbances on a calibration curve obtained with Trolox (0.03125–1 mM in 15% methanol).

2.4.3. ORAC assay

It was carried out following the method reported by Prior, Wu, and Schaich (2005). A FLUOstar Omega Microplate Reader (BMG Labtech, Offenburg, Germany) with fluorescence filters for 485 nm (excitation) and 520 nm (emission) was used. The measurements were made in black flat-bottom 96-well microplates (Brand plates®, Werthein, Germany). Serial dilutions of Trolox (4–100 μM) and of the samples (0.031–0.25 mg/mL) were prepared in PBS ($NaH_2PO_4 \cdot H_2O$) (75 mM) at pH 7.4. A fluorescein sodium salt (1.4 μM) in PBS was also prepared and kept at 4 °C in the dark. 40 μL of extract, blank (PBS) or standard (Trolox solution) and 200 μL of fluorescein solution were placed in a well. The plate was heated to 37 °C for 30 min, afterwards 25 μL of the AAPH reagent (0.32 M in PBS) was added and fluorescence measurements were made every 90 s until the reading decreased to less than 5% of the initial value (≈60 cycles).

In all cases, two independent experiments were performed in triplicate for each of the assayed samples, and the results were expressed as TEAC (Trolox Equivalent Antioxidant Capacity) values, considered in this case as μmol of Trolox showing the same antioxidant capacity as a gram of dry pulp.

2.5. Statistical analysis

Statistical analyses were performed by one-way analysis of variance (ANOVA) using the PC software package, SPSS (version 18.0; SPSS Inc., Chicago). Significant differences were assessed with an LSD test at $p < 0.05$.

3. Results and discussion

3.1. Analysis of phenolic compounds

Similar phenolic composition was found in the four samples of tree tomato fruits but for the presence of anthocyanins, which were only found in the samples of the purple cultivars. In all cases, the phenolic composition mostly consisted of hydroxycinnamoyl derivatives. A representative HPLC profile recorded at 330 nm for a sample of yellow giant cultivar is shown in the Fig. 1a. Data obtained from the HPLC–DAD–MS analysis (retention time, λ_{max}, pseudomolecular ions and main fragment ions in MS^2) are presented in Table 1 together with tentative compounds identification.

But for peak 1, identified as quinic acid by comparison with a commercial standard, the remaining compounds were assigned as hydroxycinnamoyl derivatives. The presence of chlorogenic acid (i.e., caffeoylquinic acid) in the flesh of tamarillo fruits had been reported by Wrolstad and Heatherbell (1974), but they did not indicate about any other phenolic acid derivative. Vasco et al. (2009) detected five hydroxycinnamic acid derivatives in Spanish and Ecuadorian samples of golden-yellow and purple-red tamarillo, although they gave no information on the identities of the detected compounds, whereas Mertz et al. (2009) identified caffeoyl glucose, feruloyl glucose, and two caffeoylquinic acids in tree tomato fruits. The presence of peaks with UV and mass characteristics coherent with caffeoyl and feruloyl glucosides (peaks 2 and 7) and caffeoylquinic acids (peaks 4 and 6) was also detected in our samples. These latter were tentatively assigned as 3-*O*-caffeoylquinic (peak 4) acid and 5-*O*-caffeoylquinic acid (peak 6) based on their retention characteristics as compared with similar compounds identified in other plant materials previous analysed in our laboratory (Barreira et al., 2014; Martins et al., 2014; Pereira, Barros, Carvalho, Santos-Buelga, & Ferreira, 2015). Among the remaining compounds, peak 13 was identified as rosmarinic acid based on its retention, UV and mass characteristics compared with a commercial standard. Peaks 8, 9, 10 and 12 showed the same pseudomolecular ion $[M-H]^-$ at m/z 521, releasing in all cases a main MS^2 fragment ion at m/z 359 (rosmarinic acid) from the loss of a glucose moiety (−162 mu), suggesting that they may correspond to different isomers of rosmarinic acid glucoside. A possible explanation for the observation of four compounds could be that they corresponded to the substitution of the glucosyl moiety at each of the hydroxyl groups on the molecule of

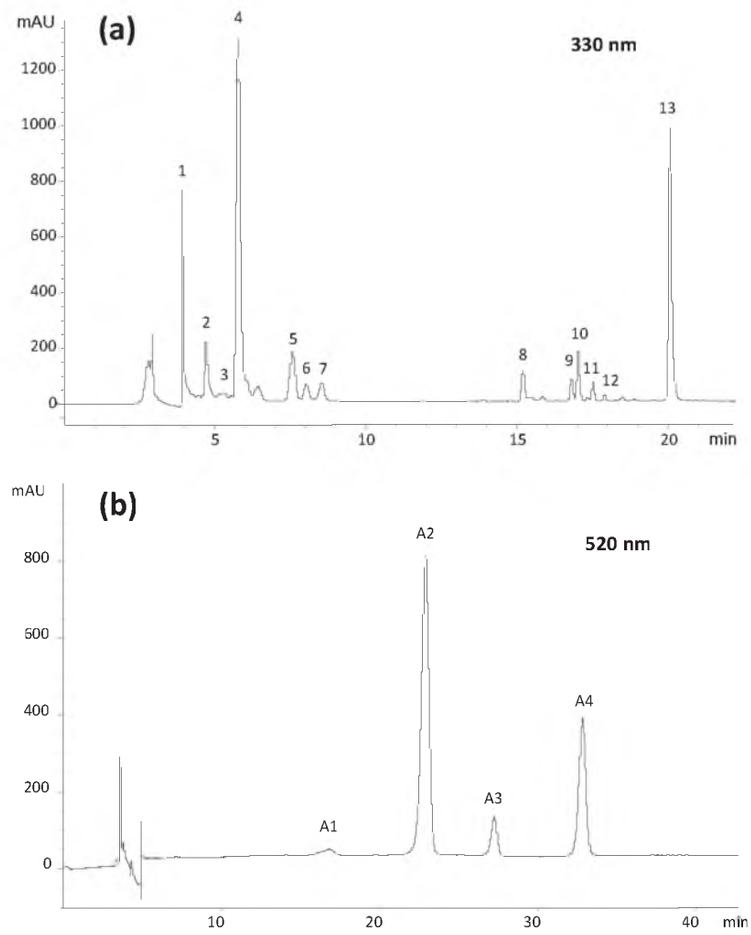

Fig. 1. HPLC chromatograms of tree tomato samples. (a) Giant yellow cultivar recorded at 330 nm for phenolic acid derivatives. (b) New Zealand purple cultivar recorded at 520 nm for anthocyanins. Peak identification is given in Tables 1 and 2.

rosmarinic acid (Fig. 2). This supposition seems supported by the different MS^2 fragmentation behaviour observed in peaks 8 and 9 (Fig. 2a) in relation to peaks 1o and 12 (Fig. 2b). Thus, in the mass spectra of these latter two additional fragment ions at *m/z* 341 and 323 were produced, indicating that the sugar moiety should be located on the hydroxyls at position 3 or 4, as depicted in Fig. 2c; those fragments were absent in the mass spectra of peaks 8 and 9, which would point to sugar substitution at positions 3′ or 4′. Peak 11 ($[M-H]^-$ at *m/z* 637) showed MS^2 fragment ions at *m/z* 521 (−116 mu; rosmarinic acid glucoside), 475 (−162 mu; loss the glucosyl moiety), 457 (−180 mu; loss the glucosyl moiety and a water molecule) and 359 (−116 to 162 mu; rosmarinic acid). The loss of 116 mu might match a malonyl residue; although no information about the position of substitution of that residue

Table 1
Retention time (Rt), wavelength of maximum absorption in the UV region (λ_{max}), mass spectral data and tentative identification of the compounds detected in the analysed samples of tree tomato.

Peak	Rt (min)	Pseudomolecular ion $[M-H]^-$ (*m/z*)	MS/MS (*m/z*)	λ_{max} (nm)	Tentative identity
1	4.0	191	173, 147, 129, 111	265	Quinic acid
2	4.8	341	179, 161, 135	330	Caffeoyl glucoside
3	5.2	385	223, 205, 191	269	Dehydrodiferulic acid (I)
4	5.9	353	191, 179, 161, 135	325	3-*O*-Caffeoylquinic acid
5	7.6	385	223, 205, 191	269	Dehydrodiferulic acid (II)
6	8.0	353	191, 179, 161, 135	325	5-*O*-Caffeoylquinic acid
7	8.6	355	193 175, 160, 135	326	Feruloyl glucoside
8	15.1	521	359, 197, 179, 161, 135	285, 320	Rosmarinic acid glucoside (I)
9	16.8	521	359, 197, 179, 161, 135	329	Rosmarinic acid glucoside (II)
10	17.0	521	359, 341, 323, 297, 197, 161	320	Rosmarinic acid glucoside (III)
11	17.5	637	521, 475, 457, 359, 197, 161	329	Malonyl derivative of rosmarinic acid glucoside
12	17.9	521	359, 341, 323, 297, 197, 161	320	Rosmarinic acid glucoside (IV)
13	20.1	359	197, 179,161, 135	329	Rosmarinic acid

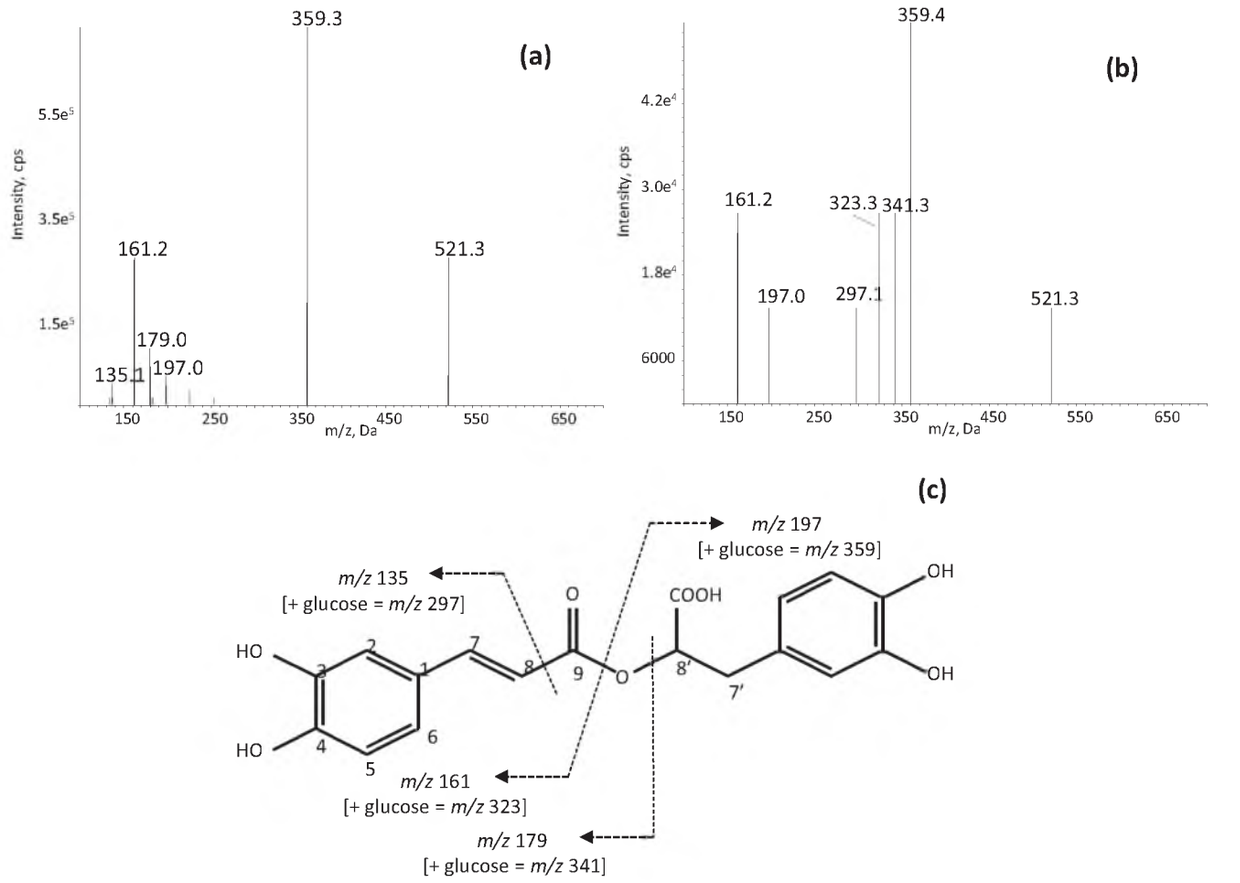

Fig. 2. MS^2 spectra of peaks 8/9 (a) and 10/12 (b), and structure of rosmarinic acid and interpretation of the fragments produced in the MS^2 spectra of those peaks (c).

was obtained, it should not be located on the glucose moiety, otherwise the MS^2 fragment at *m/z* 475 would not be produced. The compound was tentatively assigned as a malic acid derivative of rosmarinic acid glucoside. As far as we are aware, neither rosmarinic acid nor its detected derivatives have been previously reported in tree tomato.

Finally, peaks 3 and 5 showed the same pseudomolecular ion $[M-H]^-$ at *m/z* 385 and UV spectra coherent with ferulic acid dehydrodimers. Different dehydrodimers that would match the same molecular mass have been reported in other plant materials, in particular 8-*O*-4′-diferulic acid, 8-5′-diFA (linear and benzofuran forms), 5-5′-diFA and 8-8′-diFA (cyclic and non-cyclic forms), although the information obtained herein did not allow us to conclude about the actual structures of the detected compounds. This type of ferulic acid derivatives are usually found cereals (e.g., Andreasen, Christensen, Meyer, & Hansen, 2000; Bily et al., 2004; Bunzel, Ralph, Marita, Hatfield, & Steinhart, 2001; Hernanz et al., 2001), but as far as we know, they have not been described in tree tomato.

3.2. Analysis of anthocyanins

In addition to the discussed hydroxycinnamoyl derivatives, four anthocyanins were also detected in the two samples of the purple tree tomato cultivars (Fig. 1b and Table 2). They were identified based on their absorption and mass spectral characteristics and comparison with data reported in the literature (Osorio et al., 2012). The detected compounds derived from three aglycones: delphinidin (A1 and A2), cyanidin (A3) and pelargonidin (A4). In the cases of peaks A2 to A4 the same loss of 308 mu in the $[M]^+$ molecular ion to yield the corresponding aglycones (*m/z* at 303, 287 and 271, respectively) was observed, which can be attributed to the presence of one deoxyhexosyl and one hexosyl substituents (146 + 162 mu). Although the obtained information did not allow establishing the precise identity and location of the sugar moieties, they were tentatively assigned as the respective 3-*O*-rutinosides, owing to their previous identification by NMR by Osorio et al. (2012) in Colombian samples of purple tree tomato. The minority peak A1 was assigned as delphinidin 3-*O*-glucosylrutinoside based on the loss of 470 mu (146 + 162 + 162 mu) and previous identification of the same compound in tree tomato by Osorio et al.

Table 2
Retention time (Rt), wavelength of maximum absorption in the UV region (λ_{max}), mass spectral data and tentative identification of the anthocyanins detected in the purple samples of tree tomato fruits.

Peak	Rt (min)	$[M]^+$ (*m/z*)	MS^2 (*m/z*)	λ_{max} (nm)	Anthocyanin
A1	16.5	773	303	526	Delphinidin 3-*O*-glucosyl rutinoside
A2	23.6	611	303	526	Delphinidin 3-*O*-rutinoside
A3	27.8	595	287	516	Cyanidin 3-*O*-rutinoside
A4	33.4	579	271	502	Pelargonidin 3-*O*-rutinoside

Table 3
Concentrations of phenolic compounds determined in the four analysed samples of tree tomato.

Compound	Concentration (mg/100 g dw)*			
	Yellow giant cultivar A (Chaltura)	Yellow giant cultivar B (Pelileo)	Giant purple cultivar	New Zealand purple cultivar
Caffeoyl glucoside	1.35 ± 0.317[a]	3.90 ± 1.054[b]	3.64 ± 0.412[b]	29.26 ± 0.471[c]
Dehydrodiferulic acid (I)	0.12 ± 0.068[a]	0.06 ± 0.019[b]	3.27 ± 0.324[c]	21.14 ± 7.309[d]
3-*O*-Caffeoylquinic acid	25.04 ± 2.463[a]	42.73 ± 7.720[b]	50.33 ± 4.361[b]	163.62 ± 10.227[d]
Dehydrodiferulic acid (II)	7.50 ± 1.084[a]	8.46 ± 1.996[a]	10.36 ± 1.537[a]	22.09 ± 4.957[b]
5-*O*-Caffeoylquinic acid	1.27 ± 0.180[a]	2.62 ± 0.540[b]	0.51 ± 0.020[c]	1.97 ± 0.659[d]
Feruloyl glucoside	1.44 ± 0.303[a]	3.01 ± 0.680[b]	0.21 ± 0.040[c]	0.40 ± 0.566[c]
Rosmarinic acid glucoside (isomer I)	3.27 ± 0.273[a]	4.49 ± 0.853[b]	9.37 ± 0.240[c]	14.85 ± 2.877[d]
Rosmarinic acid glucoside (isomer II)	2.01 ± 0.214[a]	2.98 ± 0.915[a]	7.50 ± 0.160[b]	14.62 ± 2.317[c]
Rosmarinic acid glucoside (isomer III)	3.56 ± 0.437[a]	5.19 ± 1.125[b]	10.27 ± 0.400[c]	19.71 ± 2.557[d]
Malonyl derivative of rosmarinic acid glucoside	1.61 ± 0.229[a]	2.52 ± 0.660[b]	2.78 ± 0.402[b]	6.30 ± 3.652[c]
Rosmarinic acid glucoside (isomer IV)	0.86 ± 0.100[a]	1.42 ± 0.365[b]	4.76 ± 0.439[c]	5.70 ± 0.559[d]
Rosmarinic acid	12.22 ± 1.956[a]	32.85 ± 6.998[b]	29.57 ± 2.571[b]	121.89 ± 11.067[c]
Total hydroxycinnamoyl derivatives	60.25 ± 0.635	110.23 ± 1.910	132.57 ± 0.909	421.55 ± 3.082
Delphinidin 3-*O*-rutinoside			21.79 ± 0.130[a]	87.43 ± 2.356[b]
Cyanidin 3-*O*-rutinoside			2.49 ± 0.197[a]	4.49 ± 0.526[b]
Pelargonidin 3-*O*-rutinoside			78.07 ± 1.469[a]	76.96 ± 5.094[a]
Total anthocyanins			102.35 ± 1.469	168.88 ± 2.658

* Values expressed as mean ± SD ($n = 3$). Values in the same row followed by different letters are significantly different by ANOVA test ($p < 0.05$).

Table 4
Antioxidant capacity in the pulp extracts of the four analysed samples of tree tomato fruits.

Sample	Antioxidant capacity (TEAC values)[a]
FRAP	
Giant purple	15 ± 0.2[a]
New Zealand purple	50 ± 0.8[b]
Yellow giant A (Chaltura)	10 ± 0.4[c]
Yellow giant B (Pelileo)	17 ± 0.5[d]
ABTS	
Giant purple	70 ± 0.8[a]
New Zealand purple	89 ± 1.2[b]
Yellow giant A (Chaltura)	22 ± 0.4[c]
Yellow giant B (Pelileo)	45 ± 0.6[d]
ORAC	
Giant purple	202 ± 1.4[a]
New Zealand purple	325 ± 3.8[b]
Yellow giant A (Chaltura)	220 ± 2.2[c]
Yellow giant B (Pelileo)	240 ± 2.3[d]

[a] TEAC values: μmol Trolox showing the same antioxidant capacity as a gram of dry pulp. Each value represents the mean ± SD ($n = 6$). Values for the same antioxidant capacity assay followed by different letters are significantly different by ANOVA test ($p < 0.05$).

(2012). The same four anthocyanins detected in our samples were also reported by Mertz et al. (2009) in Ecuadorian samples of tree tomato, whereas De Rosso and Mercadante (2007) identified delphinidin 3-rutinoside, cyanidin 3-rutinoside, and pelargonidin 3-glucoside-5-rhamnoside (tentatively) in fruits from Brazil, in both cases using HPLC–MS analyses. Previously, Wrolstad and Heatherbell (1974) had described the presence of six main anthocyanins in the juice of tree tomato, i.e., the 3-glucosides and 3-rutinosides of pelargonidin, cyanidin and delphinidin. According to those authors, different composition existed in the peel and pulp, being cyanidin-3-rutinoside the major pigment in the peel and delphinidin-3-rutinoside in the purple-coloured jelly surrounding the seeds, this latter was also a major pigment in the pulp of the New Zealand purple cultivar analysed herein. Minority amounts of other anthocyanins, tentatively identified as diglucosides of pelargonidin, cyanidin or delphinidin (in the pulp) and acylated derivatives with *p*-coumaric, caffeic and ferulic acids (in the peel), were also tentatively identified by Wrolstad and Heatherbell (1974).

3.3. Concentrations of phenolic compounds

Different concentrations of phenolic compounds were found in each of the analysed cultivars, with significant differences among them (Table 3). Higher contents were determined in the purple samples, and especially the New Zealand purple cultivar. Thus, concentrations of total hydroxycinnamoyl derivatives ranged between 60.25 and 110.23 mg/100 g dry weight in samples of the yellow giant cultivar collected in the regions of Chaltura and Pelileo, respectively, and between 132.57 and 421.55 mg/100 g dw for the samples of the giant purple and New Zealand purple cultivars; this latter was by far the richest sample in phenolic compounds. Although the proportions of the different hydroxycinnamoyl derivatives varied among samples, in all cases 3-*O*-caffeoylquinic acid was the majority compound followed by rosmarinic acid. The presence of higher amounts of hydroxycinnamoyl derivatives in red-coloured samples of tree tomato compared to yellow ones was also found by Mertz et al. (2009), although only four individual compounds were quantified by those authors, being also a caffeoylquinic acid the majority compound (32.8–54.8 mg/100 g dw). Curiously none of the consulted authors cited the presence of rosmarinic acid or its derivatives in tree tomato, despite eudicotyledonous plants and mainly rosids and asterids, to which *S. betaceum* belongs, are considered major sources of this compound (Petersen et al., 2009).

The concentrations of anthocyanins in the two purple cultivars ranged between 102 and 169 mg/100 g dw, for the giant purple and New Zealand purple cultivars, respectively, although the proportions of the different anthocyanins varied in both of them. Pelargonidin-3-*O*-rutinoside (78 mg/100 g) was the main anthocyanin in the giant purple and delphinidin-3-*O*-rutinoside (87 mg/100 g) in the New Zealand purple cultivar. The concentration of anthocyanins was in the same range of the one determined by Mertz et al. (2009) in Ecuadorian samples of tree tomato fruits (165 mg/100 g dw). Total anthocyanin contents of 8.5 mg/100 g fresh weight were reported by De Rosso and Mercadante (2007)

in Brazilian tamarillo samples, and average concentrations of 38 ± 0.2 mg/100 g fw by Vasco et al. (2009) in Spanish and Ecuadorian samples of the purple-red variety.

3.4. Antioxidant capacity

The *in vitro* antioxidant capacity of the four analysed samples of tree tomato was assessed by the FRAP, ABTS and ORAC assays. Table 4 shows the obtained results expressed for the three assays as TEAC values (μmol of Trolox equivalents/g of dry pulp). Significant differences have been found in the antioxidant capacity of the different studied cultivars, which could be related to their phenolic composition and content. The highest antioxidant capacity was found for the extract of the New Zealand purple variety, and the lowest one in the sample of the yellow giant variety harvested in the Chaltura region, which was related to their different contents of phenolic compounds. Greater values of antioxidant capacity were also obtained by Vasco et al. (2008), Mertz et al. (2009) in purple-red tree tomato samples compared to yellow ones using the DPPH and ORAC assays, respectively, whereas Acosta-Quezada et al. (2015) found similar antioxidant values (DPPH assay) in samples of orange and red tree tomato cultivars. Nevertheless, it is not possible to compare our results with those obtained by those authors, due to the different assays, ways of expression and/or type of samples considered.

Despite the concentrations of phenolic compounds in tree tomato are similar or lower than other fruits, it possesses greater antioxidant capacity than many antioxidant-rich commonly consumed fruits such as orange, kiwifruit, red grape, pear or apple (see, e.g., Garcia-Alonso, de Pascual-Teresa, Santos-Buelga, & Rivas-Gonzalo, 2004; Pellegrini et al., 2003; Wang, Cao, & Prior, 1996). This might indicate that its phenolic compounds or other components are stronger antioxidants. Actually, tree tomato fruits are also rich in other antioxidants like vitamin C and carotenoids (Acosta-Quezada et al., 2015), which should contribute to its antioxidant capacity. Nevertheless, phenolic compounds seem main contributors to that activity, as concluded by Mertz et al. (2009) comparing the antioxidant capacity of crude extracts and of phenolic or carotenoid rich extracts from tree tomato. Indeed, hydroxycinnamoyl acids and rosmarinic acid, which are majority phenolic compounds in tree tomato, have been shown to possess higher scavenging activity than ascorbic acid and tocopherol, respectively (Alamed, Chaiyasit, McClements, & Decker, 2009). Also, delphinidin 3-rutinoside has been indicated as highly efficient anthocyanin at capturing the ABTS radical (Hurtado et al., 2009).

In summary, the phenolic composition of tree tomato fruits was characterised by the presence of hydroxycinnamoyl derivatives, among which 3-*O*-caffeoylquinic acid and rosmarinic acid were the majority compounds. Other compounds were tentatively identified as different rosmarinic acid glucosides, caffeoyl glucoside, feruloyl glucoside and ferulic acid dehydrodimers. Pelargonidin 3-*O*-rutinoside and delphinidin 3-*O*-rutinoside were the main anthocyanins in purple cultivars of tomato fruit. As far as we know, the presence in tree tomato of rosmarinic acid and its glucosides is reported for the first time herein, as also that of the ferulic acid dehydrodimers. The identification of rosmarinic acid is to be emphasised, as it constitutes a majority phenolic compound in the four studied tree tomato cultivars and it is recognised as a powerful antioxidant to which relevant biological activities have been attributed. The analysed samples showed relatively high values of antioxidant capacity, as determined by FRAP, ABTS and ORAC assays, which might be related to their phenolic composition.

Notes

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2015.07.131.

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