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HPLC–PDA–MS/MS of Anthocyanins and Carotenoids from Dovyalis and Tamarillo Fruits

VERIDIANA VERA DE ROSSO AND ADRIANA Z. MERCADANTE*

Department of Food Science, Faculty of Food Engineering, State University of Campinas (UNICAMP), P.O. Box 6121, Campinas - SP - Brazil - CEP 13083-862

Anthocyanins and carotenoids are natural pigments responsible for the color of vegetables and fruits, and they are also bioactive compounds, both demonstrating important biological, therapeutic, and preventative properties. Considering the biodiversity of edible fruits, high performance liquid chromatography coupled to photodiode array and mass spectrometry detectors (HPLC–PDA–MS) was used to establish the composition of carotenoids and anthocyanins from dovyalis and tamarillo fruits. Ten anthocyanins and 26 carotenoids were found in dovyalis, whereas tamarillo showed 3 anthocyanins and 17 carotenoids. Higher contents of anthocyanins and carotenoids were found in dovyalis, 42.0 and 6.6 mg/100 g, respectively, as compared to tamarillo fruits with 8.5 and 4.4 mg/100 g, respectively. Although these fruits belong to different families, delphinidin 3-rutinoside and β-cryptoxanthin were found to be, respectively, the major anthocyanin and carotenoid in both fruits.

KEYWORDS: Anthocyanins; carotenoids; dovyalis; tamarillo; exotic fruits; HPLC–PDA–MS/MS

INTRODUCTION

Anthocyanins and carotenoids are not only natural pigments responsible for the color of vegetables and fruits, but they are also bioactive compounds, both demonstrating important biological, therapeutic, and preventative properties (*1, 2*). In fact, increased ingestion of vegetables and fruits containing carotenoids and anthocyanins has been correlated to decreased prevalence of several chronic-degenerative diseases, such as cancer, inflammation, cardiovascular disease, cataract, age-related macular degeneration, among others (*3–7*).

Although both anthocyanins and carotenoids are found in the same foods, such as in acerola (*8, 9*) and camu-camu (*10, 11*) fruits, studies related to the concomitant determination of both pigments in the food sample are not common. The chemical structures of these two pigment groups have nothing in common (**Figure 1**), and consequently the analytical systems employed are very different. Extraction and separation of anthocyanins are carried out in aqueous-based solvents under acidic pH, whereas the presence of acid can lead to carotenoid isomerization, rearrangement, and degradation, and for these pigments organic solvents are employed for analysis.

The genus Dovyalis, from the Flacourtiaceae family, is composed of 11 species, such as *Dovyalis caffra*, known as kei-apple or unkokolo, and *D. abyssinica* A. Rich, known as African gooseberry, both native to Africa. Ceylon gooseberry is another species (*D. hebecarpa*) native to India and Sri Lanka, and Florida gooseberry is a natural cross of *D. hebecarpa* and *D. abyssinica* (*12*). The hybrid, obtained through *D. abyssinica* Warb and D. *hebecarpa* Warb, is also known as dovyalis fruit and cultivated in Brazil. This hybrid produces round fruits of 2–3 cm in diameter, dark red-purple skin, and orange pulp (**Figure 2**), with high levels of ascorbic acid (102–140 mg/100 g) (*12*). As far as we know, there are no available data regarding the composition of pigments, anthocyanin, and carotenoids from dovyalis.

The subtropical fruit tamarillo (*Cyphomandra betaceae*) belongs to the Solanaceae family, and it is also known as tree tomato. The fruit has a shape and size similar to eggs, with reddish-brown peel, orange pulp, and dark red seeds (**Figure 2**). Tamarillo is generally believed to be native to the Andes region of Peru, Chile, Ecuador, and Bolivia, and it is commercially cultivated in other South American countries, such as Argentina, Brazil, Colombia, and Venezuela, and in New Zealand. Six anthocyanins from tamarillo were identified as pelargonidin 3-rutinoside, cyanidin 3-rutinoside, delphinidin 3-rutinoside, pelargonidin 3-glucoside, cyanidin 3-glucoside, and delphinidin 3-glucoside in fruits from Peru (*13*), whereas pelargonidin 3-glucosyl-glucose, peonidin 3-glucosyl-glucose, and malvidin 3-glucosyl-glucose were identified in tamarillo fruits from Brazil (*14*). Six carotenoids were identified in tamarillo, also from Brazil, β-carotene and β-cryptoxanthin being the major ones (*15*). α-Carotene, β-carotene, and β-cryptoxanthin were quantified in tamarillo collected in Australia (*16*), whereas α-carotene was not detected in these fruits from the United States (*17*). It is worth highlighting that the identity of anthocyanins and carotenoids from tamarillo was only based on chromatographic profiles, UV–vis spectra, and chemical tests; high performance liquid chromatography (HPLC) was used only in one study (*17*), and mass spectrometry (MS) techniques were not employed in all those reports (*13–17*).

* To whom correspondence should be addressed. E-mail: azm@fea.unicamp.br. Fax: 55-19-3521-2153.

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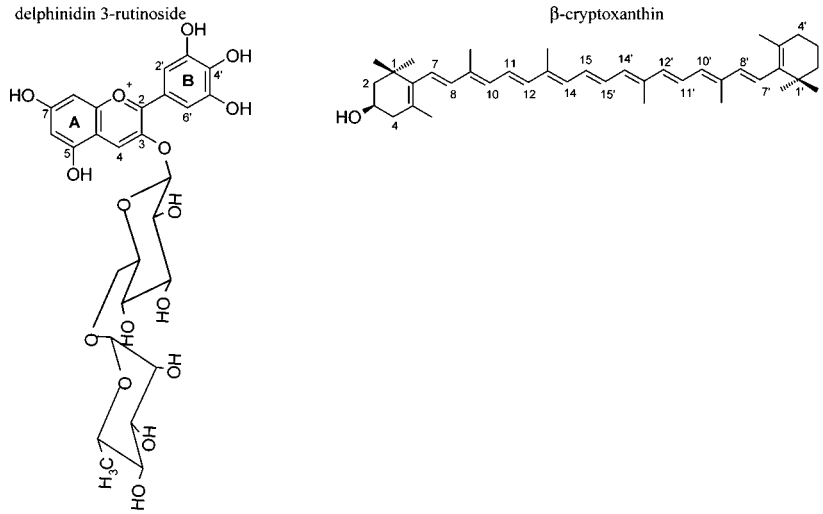

Figure 1. Structures of the main anthocyanin and carotenoid found in dovyalis and tamarillo.

Figure 2. Dovyalis (left) and tamarillo (right) fruits.

Considering the biodiversity of edible fruits and vegetables, the aim of this study was to use HPLC coupled to photodiode array (PDA) and MS detectors to establish the composition of carotenoids and anthocyanins from dovyalis and tamarillo fruits.

MATERIALS AND METHODS

Materials. Methanol, methyl *tert*-butyl ether (MTBE), and formic acid for HPLC were obtained from Merck (Darmstadt, Germany). The other reagents were all of analytical grade from Labsynth (Diadema, Brazil). The samples and solvents were filtered through Millipore (Billerica, MA) membranes (0.22 and 0.45 μm) prior to HPLC analysis.

Standards. Standards of (all-*E*)-lutein (β,ε-carotene-3,3′-diol), (all-*E*)-zeaxanthin (β,β-carotene-3,3′-diol), (all-*E*)-β-cryptoxanthin (β,β-caroten-3-ol), (all-*E*)-α-carotene (β,ε-carotene), (all-*E*)-β-carotene (β,β-carotene), (9*Z*)-β-carotene ((9*Z*)-β,β-carotene), (13*Z*)-β-carotene ((13*Z*)-β,β-carotene), and (15*Z*)-β-carotene ((15*Z*)-β,β-carotene) were provided by Dr. Werner Simon from DSM Nutritional Products (Basel, Switzerland), showing purity between 95 and 99 % measured by HPLC–PDA. Standards of cyanidin 3-glucoside, cyanidin 3-galactoside, cyanidin 3-rutinoside, cyanidin 3,5-diglucoside, cyanidin 3-rhamnoside, malvidin 3-glucoside, malvidin 3,5-diglucoside, pelargonidin 3-glucoside, pelargonidin 3,5-diglucoside, and peonidin 3-glucoside were obtained from Extrasynthèse (Genay, France), showing purity from 95 to 98%, demonstrated by HPLC–PDA.

Samples. About 2 kg each of dovyalis (*D. abyssinica* Warb × *D. hebecarpa* Warb *)* and tamarillo (*Cyphomandra betacea*) fruits at a ripe state were acquired in a supermarket in Campinas city, during their harvest season (February 2006) in São Paulo State, Brazil. The whole tamarillo fruits were homogenized since peel and pulp are eaten together. The dovyalis peel was manually removed, and peel and pulp were homogenized separated because in general, only the pulp is eaten when this fruit is consumed fresh, while the whole fruits are used to produce jam.

HPLC–PDA–MS/MS Equipment. The analysis of both pigments was carried out in a Shimadzu (Kyoto, Japan) HPLC equipment with quaternary pumps (model LC-20AD), a PDA detector (Shimadzu, model SPD-M20A), and a MS with an ion-trap analyzer (MS/MS), Esquire 4000 model, from Bruker Daltonics (Bremem, Germany). The equipment also included an on-line degasser and a Rheodyne injection valve with a 20 μL loop.

Carotenoid Determination. The carotenoids were exhaustively extracted with acetone from 5.0 g of tamarillo fruit and from 5.0 g of dovyalis pulp, transferred to petroleum ether/diethyl ether, saponified overnight at room temperature with 10 % methanolic KOH, washed until alkali free, and concentrated until dryness (*9, 11, 18, 19*). The experimental conditions for separation, identification, and quantification by HPLC–PDA–MS/MS were the same as previously described by de Rosso and Mercadante (*19*). The carotenoids were separated on a C_{30} YMC column (3 μm, 250 × 4.6 mm) using as the mobile phase a linear gradient of methanol/MTBE from 95:5 to 70:30 in 30 min, to 50:50 in 20 min, the latter proportion being maintained for a further 35 min, at 0.9 mL/min and a column temperature set at 22 °C (*19*).

Anthocyanin Determination. The anthocyanins were extracted from the whole tamarillo fruit (20.0 g) and from the peel of dovyalis (5.0 g) with 100 mL of 0.5% HCl in methanol, overnight at 5 °C in darkness. The slurry was filtered, and the solids were washed with additional 100 mL of 0.5 % HCl in methanol at room temperature. The acidic methanol extracts were combined and concentrated in a rotary evaporator (T < 38 °C) to yield the crude extract. Fifteen grams of each crude extract was acidified with 10% aqueous formic acid (5 mL), diluted with 50 mL of water, and washed twice with 150 mL of ethyl acetate to eliminate nonpolar compounds. The aqueous phase was applied on an open column packed with Amberlite XAD-7 resin (60 × 3 cm), and the anthocyanins were eluted with acetic acid/methanol (1:19) (*10, 20*). The efficiency of non-anthocyanic flavonoids removal by open column chromatography was verified by HPLC–PDA. The fraction containing anthocyanins was concentrated, yielding the partially purified extract.

The crude extract was diluted in 5% formic acid/methanol (85:15) immediately before analysis by HPLC–PDA to obtain the relative anthocyanin distribution and concentration in the samples. For HPLC–PDA–MS/MS analysis, the partially purified extract dissolved in the same injection solvent was used. For all the samples, anthocyanin separation was carried out as previously described (*21*), on a 250 × 4.6 i.d. mm, 5 μm particle size, C_{18} Shim-pack CLC-ODS column (Shimadzu, Canby, Oregon), using as the mobile phase a linear gradient of methanol/5% formic acid (v/v) from 15:85 to 80:20 in 25 min, the latter proportion being maintained for a further 15 min, at a flow rate of 0.9 mL/min and a column temperature set at 26 °C. The chromatograms were processed at 280 and 520 nm, and the spectra were obtained between 250 and 600 nm. After the sample was passed through the flow cell of the PDA, the column eluate was split allowing only 0.15 mL/min into the electrospray ionization (ESI) interface. The MS/MS parameters were set as follows: positive mode, capillary voltage: 2500 V,

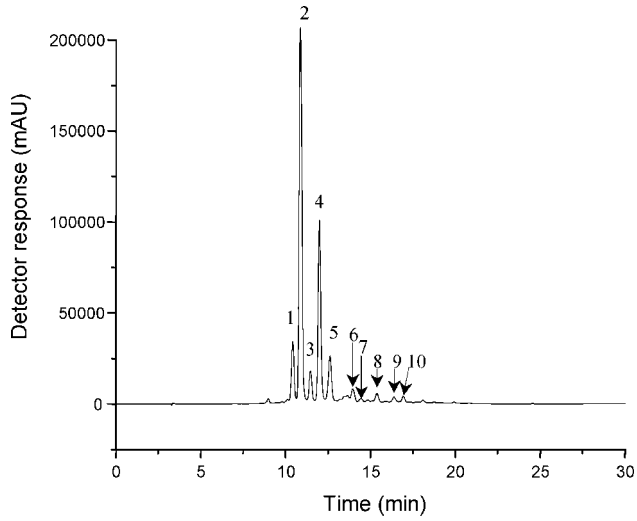

Figure 3. Chromatogram, obtained by HPLC–PDA–MS, of the anthocyanins from dovyalis. Chromatographic conditions: see text. Processed at 520 nm. Peak characterization is given in **Table 1**.

end plate offset: 2000 V, capillary exit: 110 V, skimmer 1: 20 V, skimmer 2: 10 V, dry gas (N_2) temperature: 325 °C and flow: 11 L/min, nebulizer: 30 psi, scan range from *m/z* 100 to 800. MS/MS was set in automatic mode applying fragmentation energy of 1.2 V.

The anthocyanins were identified based on the combined information provided by elution order in the reversed phase column, co-chromatography with standards, UV–vis and mass spectra compared to the literature data (*20, 22–26*). All anthocyanins were quantified by HPLC as cyanidin 3-glucoside, using an external calibration curve for cyanidin 3-glucoside with a minimum of five concentration levels. The concentration of the cyanidin 3-glucoside solution was calculated using a molar extinction coefficient of 34300 (*27*).

RESULTS AND DISCUSSION

Pigments from Dovyalis. The typical chromatogram of the anthocyanins from dovyalis is shown in **Figure 3**, and their characteristics are presented in **Table 1**. The MS spectra figures are available in Supporting Information Figure 1. The analysis performed by HPLC–PDA–MS/MS showed the separation of 10 anthocyanins.

Peaks 1 and 2 were identified as delphinidin 3-glucoside and delphinidin 3-rutinoside, respectively. The delphinidin 3-glucoside had the molecular ion at *m/z* 465 and a fragment with 303 u related to the loss of a hexose, and the identity of the hexose was assumed to be glucose according to the elution order (*25, 26*). The mass spectrum of delphinidin 3-rutinoside showed the molecular ion at *m/z* 611 and two mass fragments at *m/z* 465 and 303; the first one resulted from the loss of a deoxyhexose moiety (146 u), and the second fragment corresponded to the delphinidin moiety as a result of the loss of rutinose (308 u). Different from other disaccharides, where the ionization did not cleave the glucosyl linkage between the sugar unities, the 1,6-glucosyl linkage between the rhamnose and the glucose moieties allowed free rotation and more accessibility to the gas used to produce fragmentation (*28*). The identification was also confirmed by comparison of UV–vis spectrum and elution order with literature data (*22–26*).

The mass spectra of peak **3** showed a molecular ion at *m/z* 449 and the MS/MS fragment at *m/z* 287, which corresponds to the cyanidin moiety, resulting from the loss of a hexose. The identification of this anthocyanin was confirmed as cyanidin 3-glucoside by co-elution of peak **3** with the cyanidin 3-glucoside standard. Peak **4** was identified as cyanidin 3-rutinoside considering the mass spectrum features (**Table 1**) and confirmed by HPLC co-elution with the corresponding authentic standard.

Peaks **5**, **6**, **7**, and **10** were, respectively, identified as petunidin 3-rutinoside, peonidin 3-rutinoside, malvidin 3-rutinoside, and pelargonidin 3-rutinoside, considering the UV-vis and mass spectra, and chromatographic characteristics (retention time and elution order). The mass spectra of all these anthocyanins showed the corresponding expected molecular ion and two mass fragments as a result of losses of rhamnose and rutinose unities, verified in the MS/MS spectra. The t_R values of peaks **6**, **7**, and **10** were longer than those found for peonidin 3,5-diglucoside (t_R = 11.5 min), malvidin-3,5-diglucoside (t_R = 11.7 min), and pelargonidin 3-glucoside (t_R = 14.2 min) standards, demonstrating the presence of a disaccharide (rutinose) instead of two monosaccharides linked to the anthocyanidin. This elution behavior was previously reported in the literature (*25, 26*).

The only acylated anthocyanin found in dovyalis (peak **8**) showed an aliphatic acid, indicated by the MS features, as the molecular ion at *m/z* 507 and a fragment at *m/z* 303, resulting from the loss of 204 u, which corresponded to an hexose plus acetyl moiety. Thus, peak **8** was tentatively identified as delphinidin 3-(6"-acetyl)-glucoside by comparison of spectroscopic data and elution order with the anthocyanins from Concord grape (*25*).

Peak **9** was not identified since the MS spectrum obtained did not determine without doubt the location of the molecular ion and important diagnostic mass fragments.

The anthocyanins from the crude extract of dovyalis peel were quantified by HPLC–PDA, in terms of cyanidin 3-glucoside, giving a total of 42.0 mg/100 g. The anthocyanin profile showed the preponderance of delphinidin 3-rutinoside (47.9%), followed by cyanidin 3-rutinoside (23.8%), delphinidin 3-glucoside (9.4%), petunidin 3-rutinoside (9.1%), and cyanidin 3-glucoside (5.8%). The other five minor anthocyanins, summing 4.0% of the total content, were found in less than 1.0% each.

The kei-apple juice (*D. caffra*) showed 215 mg of gallic acid equivalent (GAE)/L in a fraction containing procyanidins, catechins, and anthocyanin monomers, and 24 mg GAE/L of anthocyanin

Table 1. Characteristics and Concentration (mg/100 g of fresh weight) of Anthocyanins from Dovyalis

peak[a]	t_R (min)	compounds	λ_{max} (nm)[b]	MS/MS (*m/z*)	concn[c]
1	10.9	delphinidin 3-glucoside	277, 527	$465[M + H]^+$, $303[M + H - 162]^+$	3.94
2	11.5	delphinidin 3-rutinoside	276, 347, 530	$611[M + H]^+$, $465[M + H - 146]^+$, $303[M + H - 146 - 162]^+$	20.13
3	12.1	cyanidin 3-glucoside	275, 330, 521	$449[M + H]^+$, $287[M + H - 162]^+$	2.44
4	12.6	cyanidin 3-rutinoside	280, 522	$595[M + H]^+$, $449[M + H - 146]^+$, $287[M + H - 146 - 162]^+$	9.99
5	13.1	petunidin 3-rutinoside	277, 531	$625[M + H]^+$, $479[M + H - 146]^+$, $317[M + H - 146 - 162]^+$	3.81
6	14.6	peonidin 3-rutinoside	270, 531	$609[M + H]^+$, $463[M + H - 146]^+$, $301[M + H - 146 - 162]^+$	0.33
7	15.0	malvidin 3-rutinoside	270, 521	$639[M + H]^+$, $493[M + H - 146]^+$, $331[M + H - 146 - 162]^+$	0.18
8	15.4	delphinidin 3-(6″-acetyl)-glucoside[d]	273, 531	$507[M + H]^+$, $303[M + H - 204]^+$	0.41
9	16.4	not identified	280, 523	701, 657, 331	0.38
10	16.9	pelargonidin 3-rutinoside[d]	270, 430, 507	$579[M + H]^+$, $433[M + H - 146]^+$, $271[M + H - 146 - 162]^+$	0.35

[a] Numbered according to **Figure 3**. [b] Linear gradient of 5% formic acid/methanol. [c] Quantified as cyanidin 3-glucoside. [d] Tentatively identified.

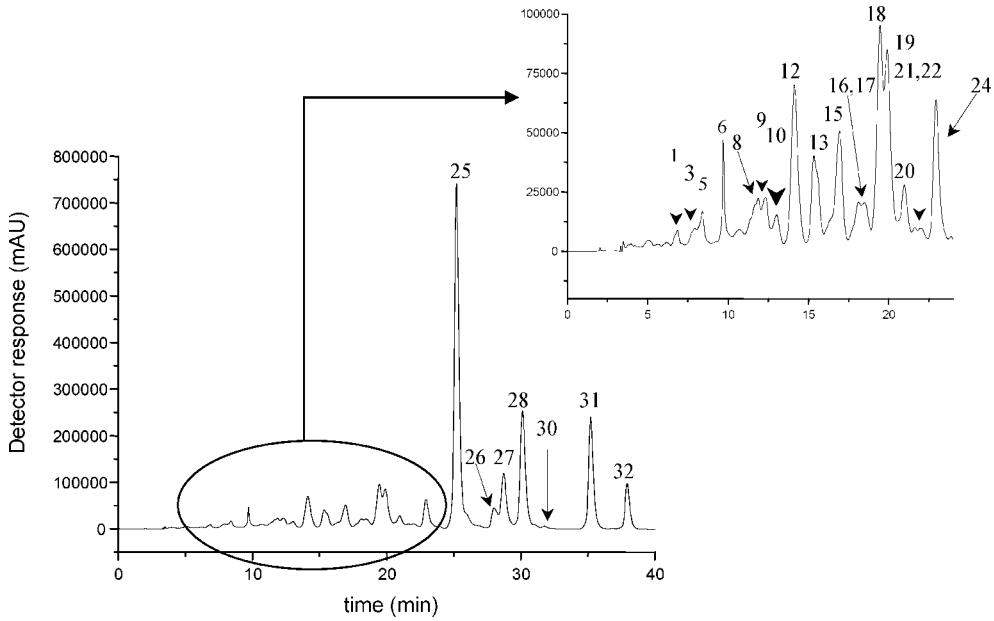

Figure 4. Chromatogram, obtained by HPLC–PDA–MS, of the carotenoids from dovyalis. Chromatographic conditions: see text. Processed at 450 nm. Peak characterization is given in **Table 2**.

Table 2. Characteristics and Concentration (mg/100 g of Fresh Weight) of Carotenoids from Dovyalis and Tamarillo[a]

peak[c]	carotenoid	t_R (min)[b]	λ_{max} (nm)[d]	% III/II	% A_B/II	$[M + H]^+$ (*m*/*z*)	MS/MS (*m*/*z*)	dovyalis	tamarillo
1	not identified 1	7.1	375, 391, 417	33	0	601	583, 565, 221	0.02	n.d.
2	(*Z*)-neoxanthin	7.6	412, 437, 467	44	40	601	583, 565, 547, 221	n.d.	0.02
3	(*Z*)-neochrome	7.9	325, 395, 419, 440	n.c.	45	601	583, 565, 547, 221	0.05	n.d.
4	(all-*E*)-neoxanthin	8.1	415, 442, 468	85	0	601	583, 565, 547, 509, 491, 393, 221	n.d.	0.09
5	mixture 1	8.4	380, 400, 420, 467	n.c.	n.c.	601	583	0.07	n.d.
6	(all-*E*)-neochrome	9.6–9.7	398, 421, 447	90	0	601	583, 565, 547, 509, 491, 221	0.13	0.03
7	not identified 2	11.4	420, 444, 472	62	0	585	565, 547, 491, 221	n.d.	0.13
8	(*Z*)-violaxanthin 1	11.8–11.9	328, 414, 435, 463	75	27	601	583, 565, 509, 491, 221	0.11	0.01
9	(*Z*)-violaxanthin 2	12.3	326, 411, 433, 463	61	18	601	583, 565, 509, 491, 221	0.11	n.d.
10	(*Z*)-luteoxanthin	13.0	310, 396, 416, 442	83	20	601	583, 221	0.03	n.d.
11	(all-*E*)-antheraxanthin	13.5	420, 444, 472	50	0	585	567, 549, 493, 475, 221	n.d.	0.18
12	mixture 2	14.1–14.7	400, 427, 451	n.c.	0	601	583, 565, 509, 491, 221	0.29	<0.01
13	(all-*E*)-mutatoxanthin	15.3	400, 426, 451	57–69	0	585	567, 549, 493, 475, 221	0.18	n.d.
14	(all-*E*)-lutein	15.4	420, 444, 472	62	0	569	551, 533, 463	n.d.	0.13
15	(all-*E*)-zeaxanthin	16.2–16.9	425, 450, 476	20–33	0	569	551, 533, 463	0.19	0.22
16a	(all-*E*)-phytoene or (*Z*)-phytoene 1	17.7–18.1	276, 286, 300	0	0	545	339	0.11	0.03
16b	(all-*E*)-5,6-epoxy-β-cryptoxanthin		420, 445, 471	25	0	569	551, 459, 221	0.09	0.13
17a	(all-*E*)-phytoene or (*Z*)-phytoene 2	18.5	276, 286, 300	0	0	545	450, 339	0.03	n.d.
17b	(all-*E*)-5,8-epoxy-β-cryptoxanthin		400, 427, 449	50	0	569	551, 459, 221	0.08	n.d.
18	(13*Z*)- or (13′*Z*)-β-cryptoxanthin	18.8−19.4	335, 415, 443, 470	12	50	553	535, 497, 461	0.33	0.07
19	(13*Z*)- or (13′*Z*)-β-cryptoxanthin	19.9–20.2	336, 415, 443, 470	14	47	553	535, 497, 461	0.26	0.06
20	(all-*E*)-zeinoxanthin	20.2–20.9	420, 444, 472	44–55	0	553	535	0.11	0.03
21a	(*Z*)-phytofluene 1	21.6	330, 347, 366	64	n.d.	543	406, 338	0.03	n.d.
21b	(*Z*)-zeinoxanthin or di-(*Z*)-β-cryptoxanthin		412, 438, 460	14	n.c.	553	535, 497, 461	0.03	n.d.
22a	(*Z*)-phytofluene 2	21.9	330, 347, 366	n.c.	n.d.	543	406, 338	0.01	<0.01
22b	(*Z*)-zeinoxanthin or di-(*Z*)-β-cryptoxanthin		410, 437, 460	11	n.c.	553	535, 497, 461	0.04	n.d.
23	(all-*E*)-5,6:5′,6′-diepoxy-β-carotene	21.9	417, 440, 469	100	0	569	551, 477, 205	n.d.	0.01
24	(all-*E*)-5,6-epoxy-β-carotene	22.9–23.0	420, 445, 471	50	0	553	535, 461, 205	0.19	0.02
25	(all-*E*)-β-cryptoxanthin	24.1–25.2	421, 450, 476	25–33	0	553	535, 497, 461	2.04	1.97
26	(13*Z*)-β-carotene	26.9–28.0	336, 420, 444, 470	14	40	537	444	0.06	0.04
27	(9*Z*)- or (9′*Z*)-β-cryptoxanthin	28.7	339, 418, 445, 472	43–50	16	553	535, 497, 461	0.37	n.d.
28	(9*Z*)- or (9′*Z*)-β-cryptoxanthin	30.1	339, 420, 445, 472	43–50	13	553	535, 497, 461	0.76	n.d.
29	not identified 3	31.0	330, 419, 443, 467	12–25	21	537	444	n.d.	0.02
30	(all-*E*)-α-carotene	31.7	415, 445, 473	60–66	0	537	n.d.	0.01	n.d.
31	(all-*E*)-β-carotene	33.7–35.2	421, 450, 476	25	0	537	444	0.62	1.13
32	(9*Z*)-β-carotene	35.1–37.9	338, 420, 447, 472	20	18	537	444	0.25	0.03

[a] n.d., not detected; n.c., not calculated. [b] Numbered according to **Figures 4** and **6**. [c] Elution time on the C30 column. [d] Linear gradient methanol/MTBE.

polymers, both determined by the Folin-Ciocalteu method (*29*). So far, no studies regarding the separation and identification of anthocyanins from dovyalis were found in the literature.

Figure 4 shows the chromatogram of the carotenoids from dovyalis, with the separation of 25 peaks, of which 4 peaks contained 2 different carotenoids each, 1 peak was not identified,

Table 3. UV–vis Characteristics of Geometrical Isomers of β-Cryptoxanthin

carotenoid	t_R (min)[a]	λ_{max} (nm)[b]	% III/II	% A_B/II
di-(*Z*)-β-cryptoxanthin	17.4	312, (400), 438, 460	0	n.c.[c]
(15*Z*)-β-cryptoxanthin	18.7	340, 423, 449, 474	10	63
(13*Z*)- or (13′*Z*)-β-cryptoxanthin	20.1	336, 415, 443, 470	16	48
(13*Z*)- or (13′*Z*)-β-cryptoxanthin	20.6	336, 415, 443, 470	16	48
(all-*E*)-β-cryptoxanthin	25.8	420, 451, 477	25	0
(9*Z*)- or (9′*Z*)-β-cryptoxanthin	29.3	339, 420, 447, 472	50	16
(9*Z*)- or (9′*Z*)-β-cryptoxanthin	30.7	339, 420, 447, 472	50	14

[a] Elution on the C_{30} column. [b] Linear gradient of methanol/MTBE. [c] n.c., not calculated.

2 were not identified mixtures, and 26 carotenoids were identified based on the combined information obtained from chromatographic elution, UV–vis and mass spectra characteristics (**Table 2**). The fragments obtained from MS/MS experiments confirmed the assignment of the protonated molecule ($[M + H]^+$) of all identified peaks, as can be seen in **Table 2**. Since a detailed description of carotenoid identification using the above information was already reported by de Rosso and Mercadante (*19*), only considerations regarding the carotenoids not identified in the previous report were discussed below.

To induce isomerization, (all-*E*)-β-cryptoxanthin standard was dissolved in MTBE/1% HCl in MeOH (1:1) and left at room temperature overnight in the dark, as recommended by Zechmeister (*30*). The relative amounts of seven isomers of β-cryptoxanthin separated on the C_{30} column were 0.4% (di-*Z*)-, 2.9% (15*Z*)-, 8.9% (13*Z*)- or (13′*Z*)-, 11.2% (13′*Z*)- or (13*Z*)-, 66.6% (all-*E*)-, 4.9% (9*Z*)- or (9′*Z*)-, and 5.0% (9′*Z*)- or (9*Z*)-. The peaks were tentatively identified based on the spectral features (**Table 3**) since the spectral fine structure (%III/II) decreases and the intensity of the *cis*-peak ($\%A_B/A_{II}$) increases as the (*Z*)-double bond gets closer to the center of the molecule (*30*). However, it was not possible to differentiate between the (13*Z*)- and (13′*Z*)-, and between the (9*Z*)- and (9′*Z*)- isomers of β-cryptoxanthin since there are no data available in the literature using nuclear magnetic resonance for exact determination of the (*Z*)- double bond positions in the β-cryptoxanthin structure for these compounds eluted on a C_{30} column. Unexpectedly, the spectral final structure values calculated for (9*Z*)- and (9′*Z*)-isomers of β-cryptoxanthin were higher than that obtained for the corresponding (all-*E*) standard.

The (13*Z*)- or (13′*Z*)-β-cryptoxanthin (peaks **18** and **19**), and (9*Z*)- or (9′*Z*)-β-cryptoxanthin (peaks **27** and **28**) were tentatively identified by comparison of their elution order and UV–vis characteristics with those isomers formed by acid-catalyzed isomerization (**Table 3**) and with data from the literature (*31*). Since (*Z*)-phytofluene co-eluted with monohydroxy carotenoids in peaks **21** and **22**, the UV–vis spectra did not show the cis-peak at 330–333 nm and at 335–339 nm corresponding to (*Z*)-isomers of zeinoxanthin and β-cryptoxanthin, respectively, due to the UV absorption of phytofluene at 330, 347, and 366 nm. Considering that β-cryptoxanthin and zeinoxanthin show identical mass spectra and that both (all-*E*)- isomers were found in dovyalis, with the present information it was not possible to ensure that the carotenoids that co-eluted in peaks **21** and **22** were (*Z*) isomers of β-cryptoxanthin or of zeinoxanthin.

The (all-*E*)-, peak **6**, and (*Z*)-neochrome (peak **3**) isomers showed similar MS spectra, the $[M + H]^+$ at *m*/*z* 601 was confirmed by MS/MS experiments with fragments at 583, 565, and 547 u due to respective elimination of one, two, and three water molecules, along with the fragment at *m*/*z* 509 corresponding to toluene elimination ($[M + H - 92]^+$). The fragment at *m*/*z* 221, also obtained by MS/MS, indicated the presence of an epoxy substituent in a β-ring with a hydroxyl group. These peaks were differentiated by their UV–vis spectra, (*Z*)-neochrome showed the presence of a *cis*-peak at 325 nm and an hypsochromic shift of 3 nm compared to the (all-*E*)-isomer (peak **6**).

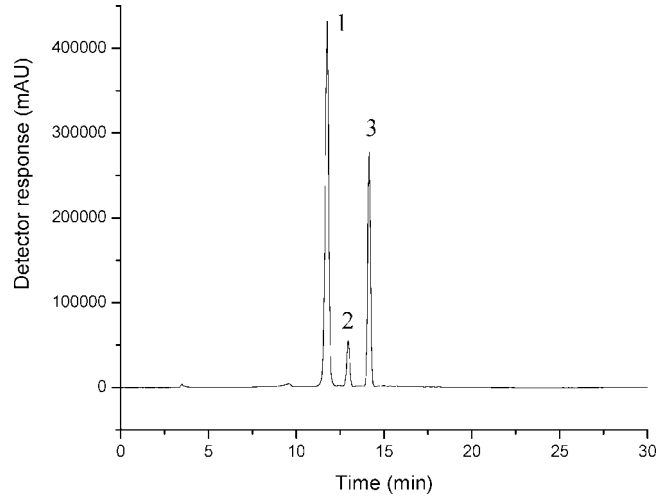

Figure 5. Chromatogram, obtained by HPLC–PDA–MS, of the anthocyanins from tamarillo. Chromatographic conditions: see text. Processed at 520 nm. Peak characterization is given in **Table 4**.

Since the high ionization temperature itself can promote the epoxide-furanoid rearrangement, the mass spectrum of antheraxanthin (5,6-epoxy-5,6-dihydro-β,β-carotene-3,3′-diol) was very similar to that of mutatoxanthin (5,8-epoxy-5,8-dihydro-β,β-carotene-3,3′-diol), both with $[M + H]^+$ at *m*/*z* 585. The MS/MS spectra of both compounds showed the same fragmentation profile with losses of one and two hydroxyl groups (567 and 549 u), toluene (493 u), and toluene + water (475 u), along with the fragment at *m*/*z* 221. Because the presence of a 5,8-furanoid group, mutatoxanthin (peak **13**) showed an hypsochromic shift of 18 nm as compared to antheraxanthin (peak **11**). The identification of these carotenoids was confirmed by comparison with data from the literature (*32*). Mutatoxanthin was only found in dovyalis, whereas antheraxanthin was detected in tamarillo.

As expected, peak **10** showed an MS spectrum similar to those from both (*Z*)-violaxanthin isomers, and a lower spectral fine structure than that reported for luteoxanthin, %III/II = 100 (*19*). Considering these data and the presence of a *cis*-peak at 310 nm, peak **10** was assigned as a (*Z*)-isomer of luteoxanthin (5,6:5′,8′-diepoxy-5,6,5′,8′-tetrahydro-β,β-carotene-3,3′-diol).

Since the lowest wavelength of the PDA detector is 190 nm, it was not possible to observe the *cis*-peak of phytoene. In addition, phytoene isomers, (all-*E*) and (*Z*), do not present spectral fine structure (%III/II = 0); therefore, it was not possible to assign the geometrical isomer configuration of peaks **16a** and **17a**.

The assignment of peaks **21a** and **22a** was carried out by comparison with data reported in (*19*), that showed (all-*E*)-phytofluene eluting at 24.4 min with %III/II = 94 and (*Z*)-phytofluene with t_R = 20.4–21.0 min and %III/II = 66, using the

Table 4. Characteristics and Concentration (mg/100 g of Fresh Weight) of Anthocyanins from Tamarillo

peak[a]	t_R (min)	compounds	λ_{max} (nm)[b]	MS/MS (*m/z*)	concentration[c]
1	11.4	delphinidin 3-rutinoside	277, 347, 530	611$[M + H]^+$, 465$[M + H\text{-}146]^+$, 303$[M + H - 146 - 162]^+$	5.26
2	12.5	cyanidin 3-rutinoside	280, 522	595$[M + H]^+$, 449$[M + H - 146]^+$, 287$[M + H - 146 - 162]^+$	0.55
3	14.1	pelargonidin 3-glucoside-5-rhamnoside[d]	270, 431, 506	579$[M + H]^+$, 433$[M + H - 146]^+$, 271$[M + H - 146 - 162]^+$	2.67

[a] Numbered according to **Figure 5**. [b] Linear gradient of 5% formic acid/methanol. [c] Quantified as cyanidin 3-glucoside. [d] Tentatively identified.

same conditions as in the present study. Peak **21a** showed similar chromatographic and UV–vis features as (*Z*)-phytofluene (*19*), whereas peak **22a** eluted earlier than (all-*E*)-phytofluene and therefore was assigned as (*Z*)-phytofluene. However, the exact position of the (*Z*)-double bonds can only be given by NMR.

The total carotenoid content found in dovyalis pulp was 6.6 mg/100 g, the major carotenoid being (all-*E*)-β-cryptoxanthin, responsible for 33.5% of the total content, followed by its (9*Z*) + (9′*Z*) isomers (18.7%), (all-*E*)-β-carotene (10.2%), (13-*Z*)- + (13′-*Z*)-β-cryptoxanthin (9.7%), and (9-*Z*)-β-carotene (4.1%). Studies regarding the carotenoid composition in dovyalis were not found in the literature.

Pigments from Tamarillo. The typical chromatogram of the anthocyanins from tamarillo showed the separation of three anthocyanins (**Figure 5**); the characteristics are presented in **Table 4**. The MS spectra figures are available in Supporting Information Figure 2.

Peaks **1** and **2** were identified as delphinidin 3-rutinoside and cyanidin 3-rutinoside, respectively, considering the information described above for the same pigments found in dovyalis. Cyanidin 3-rutinoside was definitively identified by its HPLC co-elution with standard. The UV–vis and mass spectra features of peak **3** (**Table 4**) were very similar to those of peak **10** from dovyalis identified as pelargonidin 3-rutinoside (**Table 1**); however, peak **3** from tamarillo (t_R = 14.1 min, **Figure 5**) eluted earlier than peak **10** from dovyalis (t_R = 16.9 min, **Figure 3**). Taking into consideration that anthocyanins with monosaccharides glycosylated at both positions 3 and 5 eluted earlier on the reversed phase column than their corresponding disaccharide at 3 position (*25*, *26*), peak **3** was tentatively assigned as pelargonidin 3-glucoside-5-rhamnoside.

The anthocyanins from the crude extract of tamarillo were quantified by HPLC–PDA, in terms of cyanidin 3-glucoside, giving a total of 8.5 mg/100 g. Delphinidin 3-rutinoside was the major anthocyanin, representing 62.0% of the total anthocyanin content in this fruit, pelargonidin 3-glucoside-5-rhamnoside accounted for 31.5%, while cyanidin 3-rutinoside was 6.5%.

Delphinidin 3-rutinoside and cyanidin 3-rutinoside were also detected in this fruit (*13*), whereas the other anthocyanins previously reported in tamarillo (*13*, *14*) were not found in the present study. This fact can be attributed to different sample anthocyanin rearrangements since a faster method was used for separation in the present study (HPLC) as compared to paper chromatography (*13*, *14*), and also to mis-identification. In addition, Wrolstad and Heatherbell (*13*) reported that considerable difficulty was encountered in the isolation of anthocyanins from tamarillo fruits compared to black currant fruits. The anthocyanin contents were not given in these studies.

Figure 6 shows the chromatogram of the carotenoids from tamarillo, with the separation of 20 peaks, of which 17 were identified based on chromatographic, UV–vis and mass characteristics (**Tables 2** and **3**). Peak **23**, 5,6:5′6′-diepoxy-5,6,5′6′-tetrahydro-β,β-carotene, was identified considering the hypsochromic shift of 10 nm and much higher spectral fine structure than that of (all-*E*)-β-carotene. The mass spectrum showed the $[M + H]^+$ at *m/z* 569, and its MS/MS showed the presence of fragment ions at *m/z* 551 and 477, corresponding to the losses of the hydroxyl group and toluene, respectively, and at *m/z* 205, that indicated the presence of a β-ring with an epoxy group.

β-Cryptoxanthin was the major carotenoid (45.3%), followed by β-carotene (26.1%), zeaxanthin (5.1%), and antheraxanthin (4.0%). The presence of β-cryptoxanthin (major), β-carotene, lutein, zeaxanthin, and 5,6-epoxy-β-carotene was already reported in this fruit (*15*), whereas this is the first time that neoxanthin, neochrome, antheraxanthin, phytoene, zeinoxanthin, 5,6:5,6-diepoxy-β-carotene, and (*Z*)-isomers of β-cryptoxanthin and of β-carotene were found as minor carotenoids in tamarillo.

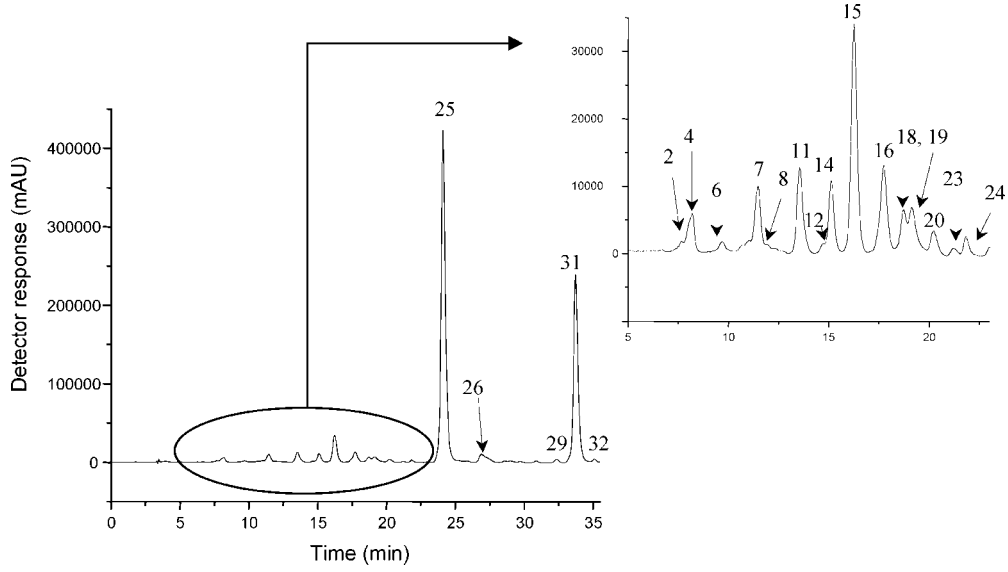

Figure 6. Chromatogram, obtained by HPLC–PDA–MS, of the carotenoids from tamarillo. Chromatographic conditions: see text. Processed at 450 nm. Peak characterization is given in **Table 2**.

α-Carotene found only in tamarillo from Australia (*16*) was not detected in the present study.

The total carotenoid content found in the present study, 4.4 mg/100 g, was in the range of those previously found in tamarillo (3.1–5.9 mg/100 g) also harvested in Brazil (*15*). Lower values were reported for provitamin A carotenoids in this fruit harvested in Australia (1.5 mg/100 g) (*16*) and in the USA (0.8–1.4 mg/100 g) (*17*).

In summary, higher contents of anthocyanins and carotenoids were found in dovyalis, 42.0 and 6.6 mg/100 g, respectively, as compared to tamarillo fruits with 8.5 and 4.4 mg/100 g. Although these fruits belong to different families, delphinidin 3-rutinoside and β-cryptoxanthin were found to be, respectively, the major anthocyanin and carotenoid in both fruits. In addition, considering the qualitative and quantitative composition of pigments in these fruits, the anthocyanins were the pigments that most contributed to the red-violet color of dovyalis and tamarillo.

Supporting Information Available: MS and MS/MS spectra of anthocyanins from dovyalis and tamarillo. This material is available free of charge via the Internet at http://pubs.acs.org.

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