

Original Article

Anthocyanin composition in fig (*Ficus carica* L.)

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Abstract

The anthocyanin composition was analysed in fig fruit (*Ficus carica* L.) from five different varieties (Colar, Cuello de Dama (green), Cuello de Dama (dark purple), Granilla and Bursa Siyahi). Fifteen anthocyanin pigments were detected, most of them containing cyanidin (Cy) as aglycone; some pelargonidin (Pg) derivatives were also found. Rutinose and glucose were present as substituting sugars, as well as acylation with malonic acid. Minor levels of peonidin 3-rutinose (Pn 3-rutinoside) in the pulp were also detected. Other noticeable aspects in the pigment composition of the fig were the detection of anthocyanidin-derived pigments, namely 5-carboxypyranocyanidin-3-rutinoside, a cyanidin 3-rutinose dimer and five condensed pigments containing C–C linked anthocyanins (Cy and Pg) and flavanol (catechin and epicatechin) residues. Total anthocyanin content in the skin ranged between 32 and 97 $\mu\text{g g}^{-1}$ and between 1.5 and 15 $\mu\text{g g}^{-1}$ in the pulp. The main anthocyanin in both parts of the fruit was Cy 3-rutinoside (48–81% in skin and 68–79% in pulp) usually followed by Cy 3-glucoside (5–18% in skin and 10–15% in pulp). Malonyl derivatives were more abundant in the skin (1.2–6.5%) than in the pulp (1.0–2.6%).

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1. Introduction

The Common Fig (*Ficus carica* L.) is a tree native to southwest Asia and the eastern Mediterranean region, belong to botanical family *Moracea*. The Common Fig is one of the first plants that was cultivated by humans and is an important crop worldwide for dry and fresh consumption. Its edible part is commonly referred to as a “fruit” although it is a synconium, that is, a fleshy, hollow receptacle with a small opening at the apex partly closed by small scales.

Previous reports concerning the nutrient composition of dried figs have indicated that it has the best nutrient score among the dried fruit, being an important source of minerals and vitamins (USDA, 2002). The presence of phytosterols (433 mg/100 g dry basis) has also been reported in fig fruit (Jeong and Lachance, 2001). The fresh

and dried figs also present relatively high amounts of crude fiber (5.5%, w/w) and polyphenols (Vinson, 1999; Vinson et al., 2005).

Some recent works have reported that fig antioxidants can protect lipoproteins in plasma from oxidation and produce a significant increase in plasma antioxidant capacity for 4 h after consumption (Vinson et al., 2005). Also, Solomon et al. (2006) showed that the higher the polyphenols contents, especially anthocyanins, in fig fruit, the higher was their antioxidant activity.

According to our knowledge, there are no studies about the detailed pigment composition in fig. Early studies carried out by Robinson and Robinson (1932) identified cyanidin 3-glucoside (Cy 3-gluc). Furthermore, four anthocyanin were reported in the fig, with cyanidin 3-rhamnoglucoside (Cy 3-rut) accounting for about 75% of total pigments; other pigments were cyanidin 3,5-diglucoside (11%), cyanidin 3-glucoside (11%) and pelargonidin 3-rhamnoglucoside (3%) (Puech et al., 1975; Solomon et al., 2006; del Caro and Piga, 2007).

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The contents of total anthocyanins, polyphenols and flavonoids in the skin and pulp from commercial varieties of figs with different colour (black, red, yellow and green) have been recently analysed by Solomon et al. (2006). The fruit skin contributed most of the polyphenols and antioxidant activity compared to the pulp especially in darker varieties.

The aim of the present work was to characterise the qualitative and quantitative pigment composition, in the skin and flesh of five different fig varieties, using HPLC coupled to diode array and mass spectrometer (MS) detection.

2. Materials and methods

2.1. Samples

Fig fruit (*F. carica* L.) from five selected commercial varieties: cv. Colar, Cuello de Dama (green), Cuello de Dama (dark purple), Granilla and Bursa Siyahi, were sampled from five retail outlets in Salamanca (Spain). They were harvested in 2006 at the commercial mature stage in different Spanish regions excepting the Bursa variety from Turkey. Approximately 1 kg of each variety was randomly selected from bins in each retail outlet; 2–5 units were randomly taken for each extraction (three extractions for each variety). The figs were weighed and immediately peeled. Skin and pulp were weighed separately and extracted.

2.2. Sample preparation

The fruit (2–5 units) were manually peeled and the skin separated from the pulp and analysed separately. The yield of the skin versus the entire fruit was 5–6%. The skin (3–5 g) and pulp (20–30 g) were ground and homogenised in MeOH containing 0.5% trifluoroacetic acid (TFA), using a Polytron homogeniser (Kinematica, Littau, Switzerland) and macerated for 16 h at 4 °C. Subsequently, they were centrifuged for 20 min at 4000 × *g* at 5 °C in a refrigerated superspeed centrifuge (Sorvall RC 5B). This process was repeated twice at two intervals of 4 h, completing a total of 24 h of extraction. The extracts were combined and concentrated under vacuum at 30 °C until the methanol was removed. The final volume was adjusted to 50 mL. The aqueous extract was stored at –30 °C until purification and analysis. For purification, an aliquot (2 mL) of the aqueous extract was deposited onto a C₁₈ Sep-Pak cartridge (Waters, Milford, MA, USA), previously activated with methanol followed by water; sugars and more polar substances were removed by passing 10 mL of ultrapure water and anthocyanins pigments were eluted with 4 mL of 0.5% TFA in MeOH. Afterwards, the methanolic extract was evaporated under vacuum in a rotary evaporator (30 °C) and then dissolved in aqueous 0.1% TFA for HPLC analysis. For the skin and pulp of each variety,

three independent extracts were prepared, purified and analysed separately.

2.3. HPLC–diode array detector (DAD)–MS analyses

The anthocyanin extracts were analysed using a Hewlett-Packard 1100 series liquid chromatograph (Agilent Technologies, Waldbronn, Germany). Separation was achieved on an AQUA[®] (Phenomenex, Torrance, CA) reverse-phase C₁₈ column (5 μm, 150 mm × 4.6 mm i.d.) thermostatted at 35 °C. The solvents used were: (A) 0.1% TFA in water and (B) 100% HPLC-grade acetonitrile. The gradient employed was: isocratic 10% B for 3 min, from 10% to 15% B for 12 min, isocratic 15% B for 5 min, from 15% to 18% B for 5 min, from 18% to 30% B for 20 min and from 30% to 35% for 5 min, at a flow rate of 0.5 mL min^{–1}. Detection was carried out in a DAD, using 520 nm as the preferred wavelength, and in a MS connected to the HPLC system via the DAD cell outlet.

LC–MS analyses were performed using a Finnigan[™] LCQ MS detector (Thermoquest, San Jose, CA) equipped with an API source, using an electrospray ionisation (ESI) interface. Both the sheath gas and the auxiliary gas were nitrogen at flow rates of 1.2 and 6 L min^{–1}, respectively. The capillary and source voltage were 4 V and 4.5 kV, respectively, and the capillary temperature was 195 °C. Spectra were recorded in positive ion mode between *m/z* 120 and 1500. The MS was programmed to carry out a series of three consecutive scans: a full mass, an MS² scan of the most abundant ion in the full mass, and an MS³ of the most abundant ion in the MS², using a normalised energy of collision of 45%.

2.4. Quantification

For the quantitative analysis of anthocyanins, a calibration curve was obtained by injection of different concentration of cyanidin 3-*O*-glucoside (for cyanidin-based anthocyanins), pelargonidin 3-*O*-glucoside (for pelargonidin-based anthocyanins) and peonidin 3-*O*-glucoside (for peonidin-based anthocyanins) standards purchased from Polyphenols Labs., Sandnes, Norway.

The limit of detection (LOD) and limit of quantification (LOQ), respectively, defined as a 3:1 and 10:1 peak to noise ratio, were determined for cyanidin 3-*O*-glucoside, pelargonidin 3-*O*-glucoside and peonidin 3-*O*-glucoside. The working concentration range was 0.001 and 0.1 mg mL^{–1} for three compounds. The calibration curves were calculated by plotting peak area ratio (*Y*) versus concentration (*X*, mg mL^{–1}) of the three anthocyanins in the standard solution at six different concentrations, with least-squares linear regression. Within this interval, the calibration curves were linear with correlation coefficient for cyanidin 3-*O*-glucoside, pelargonidin 3-*O*-glucoside and peonidin 3-*O*-glucoside (*R*² > 0.999). The limits of detection were calculated under the Glaser criteria (Glaser et al., 1981).

The limits of detection were $0.016 \mu\text{g mL}^{-1}$ for Cy 3-*O*-glucoside, $0.025 \mu\text{g mL}^{-1}$ for Pg 3-*O*-glucoside and $0.014 \mu\text{g mL}^{-1}$ for Pn 3-*O*-glucoside. Limits of quantification were $0.048 \mu\text{g mL}^{-1}$ for Cy 3-*O*-glucoside, $0.074 \mu\text{g mL}^{-1}$ for Pg 3-*O*-glucoside and $0.042 \mu\text{g mL}^{-1}$ for Pn 3-*O*-glucoside. The precision of the chromatographic method was satisfactory. Six replicate determinations of the standards were carried out on the same day. Relative standard deviations were calculated with results of coefficients of variation less than 7%.

2.5. Statistical analysis

The determination of anthocyanin contents in skin and pulp extracts of each variety were carried out in triplicate and the results are given as mean \pm standard deviation (S.D.). Data were analysed by one-way analysis of variance (ANOVA). Significant differences were assessed with an LSD test ($p < 0.05$). The statistical analysis was performed using PC software package SPSS (version 13.0; SPSS Inc., Chicago).

3. Results and discussion

3.1. Pigment identification

Up to 15 anthocyanin pigments were detected in the fig samples analysed. As an example, Fig. 1 shows the HPLC pigment profile in extracts from skin and pulp of Colar fig variety. Data (retention time, λ_{max} in the visible region, molecular ion and main fragment ions observed in MS² and MS³) obtained for the anthocyanin peaks in the HPLC–DAD–MS analyses are presented in Table 1, together with their occurrence in pulp and skin. Pigments were cyanidin (Cy) or pelargonidin (Pg) derivatives, as demonstrated from their UV–vis spectra and mass spectral data, only compound 14 showed data in concordance with peonidin as aglycone (λ_{max} at 520 nm and MS³ at m/z 301).

Peaks 4, 10, 11 and 13 were usually the major pigments identified in the skin, which were identified as Cy 3,5-diglucoside (Peak 4), Cy 3-glucoside (Peak 10), Cy 3-rutinoside (Peak 11) and Pg 3-rutinoside (Peak 13). The identity of these compounds was elucidated by comparison

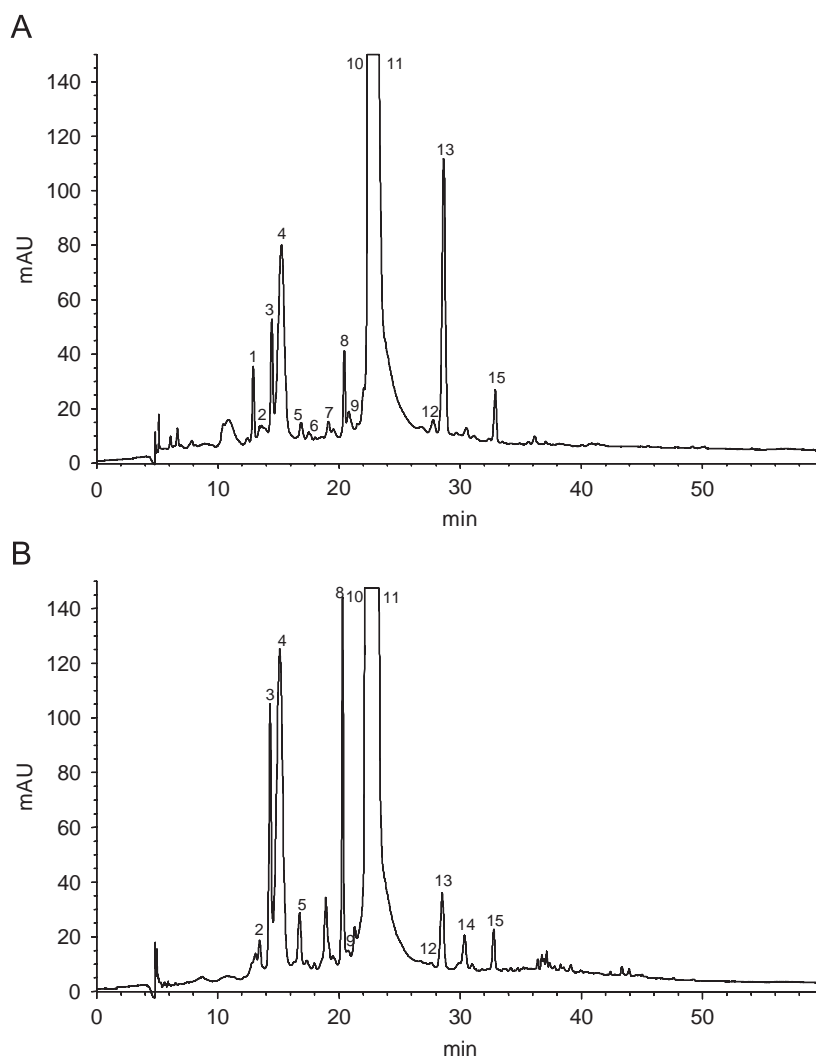


Fig. 1. Chromatograms (zoom) recorded at 520 nm showing the anthocyanin profiles of fig fruit (variety: Colar). (A) Skin fig, (B) pulp fig.

Table 1
Retention time (Rt), wavelengths of maximum absorption in the visible region (λ_{\max}), mass spectral data and tentative identification of anthocyanin pigments in fig skin and pulp

Peak	Rt (min)	λ_{\max} (nm)	Molecular ion $[M+H]^+$ (m/z)	MS ² (m/z)	MS ³ (m/z)	Tentative identification
1	12.9	528	1189	881	735, 573, 421, 287	Cy 3-rutinoside dimer
2	13.6	528	737	575, 557	557, 449, 423, 329, 287	(Epi)catechin-(4→8)-Cy 3-glucoside
3	14.4	528	883	737, 575	557, 449, 423, 329, 287	(Epi)catechin-(4→8)-Cy 3-rutinoside
4	15.2	516	611	449, 287	287	Cy 3, 5-diglucoside
5	16.8	528	883	737, 575	557, 423, 329, 287	(Epi)catechin-(4→8)-Cy 3-rutinoside
6	17.5	524	867	721, 559	559, 541, 433, 407, 312	(Epi)catechin-(4→8)-Pg 3-rutinoside
7	19.1	525	867	721, 559	559, 541, 433, 407, 312	(Epi)catechin-(4→8)-Pg 3-rutinoside
8	20.4	506	663	355	327, 311, 299	5-Carboxypyranocyanidin-3-rutinoside
9	20.8	520	697	535, 449, 287	287	Cy 3-malonylglicosyl-5-glucoside
10	22.2	516	449	287	287	Cy 3-glucoside
11	22.8	518	595	449, 287	287	Cy 3-rutinoside
12	27.7	n.a.	433	271	–	Pg 3-glucoside
13	28.6	506	579	433, 271	271	Pg 3-rutinoside
14	29.9	520	609	463, 301	301	Pn 3-rutinoside
15	32.9	518	535	287	287	Cy 3-malonylglucoside

n.a.: not available.

of their chromatographic characteristics and absorption spectra with data in our library and confirmed by mass analysis (Table 1). Compounds 4, 10 and 11 showed λ_{\max} at 516–518 nm characteristic of cyanidin derivatives and compound 13 at 506 nm together with a spectrum shape characteristic of pelargonidin derivatives (Santos-Buelga et al., 2003). The presence of cyanidin and pelargonidin as anthocyanidins in these peaks was confirmed by their mass spectra, which showed MS² signals at m/z [M]⁺ 287 and 271, respectively. The MSⁿ fragmentation profiles were in accordance with the proposed identities of the compounds. These four anthocyanins had been described in fig by other authors (Robinson and Robinson, 1932; Puech et al., 1975; Solomon et al., 2006, del Caro and Piga, 2007). However, to the best of our knowledge, the rest of identified pigments that will be discussed below are here described in fig for the first time.

Compound 12 was identified as Pg 3-glucoside by comparison of its chromatographic, spectral and MS characteristics with those of a commercial standard. Peak 14 was only detected in the pulp and showed a molecular ion at m/z 609. Its MS² gave two fragments at m/z 463 ([M–146]⁺, loss of a rhamnose moiety) and at m/z 301 ([M–308]⁺ loss of rhamnoglucoside moiety). This fragmentation pattern is characteristic of rutinosides (Giusti et al., 1999) and the compound was identified as peonidin 3-*O*-rutinoside.

Peaks 9 and 15 corresponded to acyl derivatives of cyanidin. Peak 15 showed λ_{\max} at 518 nm and spectra molecular ion at m/z 535 releasing a unique MS² fragment at m/z 287 ([M–248]⁺, loss of a malonylglicoside moiety). These characteristics allowed its identification as Cy 3-*O*-malonylglucoside. For peak 9, the molecular ion at m/z 697, releasing three fragments MS² at m/z 535 (–162 amu, loss of an hexose), m/z 449 (–162–86 amu, loss of a

malonyl-hexoside residue) and m/z 287 (cyanidin), was consistent with Cy 3-malonylglucosyl-5-glucoside. Further confirmation of the identity of compounds 9 and 15 was provided by comparison of their characteristics with those of the same compounds previously identified in our laboratory in purple corn and strawberry (de Pascual-Teresa et al., 2002; Lopes da Silva et al., 2007) and available in our anthocyanin library.

Compounds 2, 3, 5, 6 and 7 were assigned to condensed pigments containing C–C linked anthocyanin (Cy or Pg) and catechin residues. Pigments with similar structural characteristics were found in other sources as beans (Macz-Pop et al., 2006; González-Paramás et al., 2006), purple corn extracts (de Pascual-Teresa et al., 2002), strawberries (Fossen et al., 2004; Lopes da Silva et al., 2007), red wine (Salas et al., 2004) and black currant (McDougall et al., 2005). These compounds presented bathochromic shifts of about 12 nm in the visible region of their absorption spectra with regard to the parent anthocyanins (see Table 1), as also found by other authors (Fossen et al., 2004; Salas et al., 2004; Lopes da Silva et al., 2007).

Peak 2 showed a positive molecular ion [M]⁺ at m/z 737, with a major MS² fragment at m/z 575 ([M–162]⁺, loss of an hexose moiety). Its MS³ fragmentation led several fragments ions at m/z 557 ([M–18]⁺, loss of water), m/z 449 ([M–126]⁺, loss of a C₆H₆O₃ residue from A ring), m/z 423 ([M–152]⁺ retro Diels-Alder cleavage (RDA) of a flavanol unit), m/z 329 ([M–246]⁺ partial loss of (epi)catechin, m/z at 287 ([M–288]⁺, loss of an upper (epi)catechin unit. This type of fragmentation profile was consistent with the one previously described for anthocyanin-catechin condensed pigments (González-Paramás et al., 2006).

Peaks 3 and 5 showed identical molecular ions (m/z [M]⁺ at 883) and fragmentation patterns. Fig. 2 shows the MS²

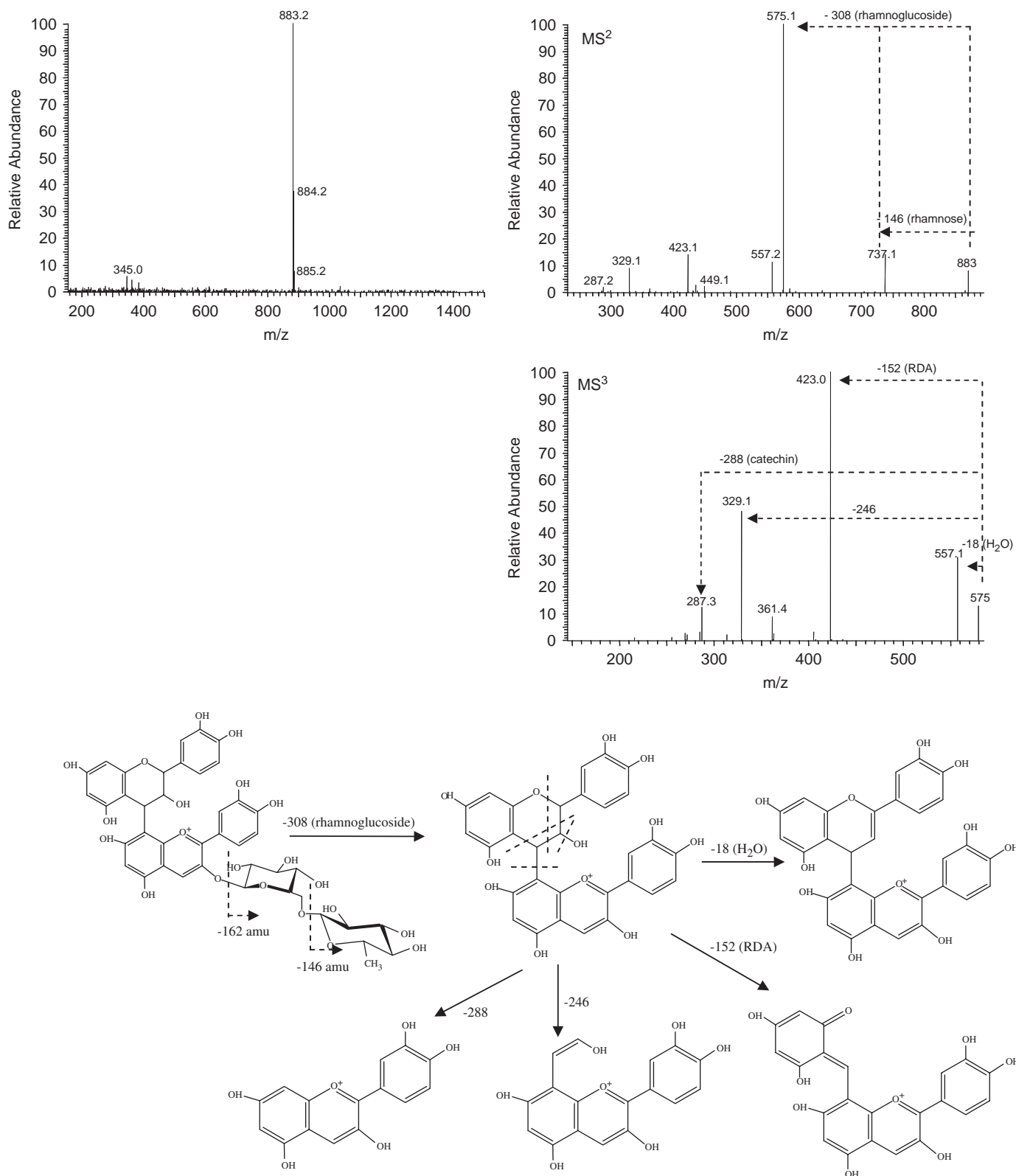


Fig. 2. MS² and MS³ of peak 3: (epi)catechin-(4→8)-Cy 3-rutinoside (*m/z* [M⁺] at 883).

and MS³ spectra of pigment 3 together with its fragmentation scheme. The MS² fragments at *m/z* 737 ([M-146]⁺) and 575 ([M-308]⁺) corresponded to the loss of rhamnose

and rhamnoglucoside moieties. MS³ fragmentation of the ion at *m/z* 575 gave the same fragments as detected for pigment 2 (Table 1). Thus, these compounds were

identified as pigments resulting from the direct condensation of Cy 3-*O*-rutinoside and either catechin or epicatechin.

Peaks 6 and 7 showed λ_{\max} in the visible region at 524 nm, bathochromically shifted with regard to that of the precursor anthocyanin (Pg 3-*O*-glucoside, λ_{\max} at 506 nm). These compounds showed a positive molecular ion $[M]^+$ at m/z 867, with an MSn fragmentation characteristic of anthocyanin–catechin condensed pigments. MS² were found at m/z 721, ($[M-146]^+$, loss of a rhamnose moiety), and 559 ($[M-308]^+$, loss of a rhamnoglucoside moiety). MS³ fragmentation of the ion at m/z 559 gave signals at m/z 541 ($[M-18]^+$, loss of water), m/z 433, ($[M-126]^+$, loss of a C₆H₆O₃ residue), m/z 407 ($[M-152]^+$, RDA of the flavanol unit), m/z 312 ($[M-246]^+$, partial loss of (epi)catechin, m/z 271 ($[M-288]^+$, loss of (epi)catechin unit. Thus, compounds 6 and 7 were identified as (epi)catechin-Pg 3-*O*-rutinoside condensed pigments. Compounds consisting of (epi)afzelechin-Pg 3-*O*-rutinoside have been described in strawberry (Lopes da Silva et al., 2007), but according to our knowledge this is the first time that similar compounds containing (epi)catechin as flavanol moiety are reported.

Peak 1, showed a positive molecular ion $[M]^+$ at m/z 1189 and its MS² fragmentation led one fragment at m/z 881 ($[M-308]^+$) corresponding to the loss of a rhamnohexoside moiety. MS³ fragmentation of this ion gave fragments at m/z 735 and 573 corresponding to successive losses of rhamnose (–146 amu) and rhamnoglucoside (–308 amu) moieties from a Cy 3-rutinoside unit. Further information to conclude about the identity of compound 1 was provided by other fragment detected at m/z 421, (–152 amu) corresponding to the RDA fission of a cyanidin unit. This fragmentation pattern is similar to the one obtained by Vidal et al. (2004) for malvidin 3-glucoside dimer isolated from grape skins. Thus, the compound was tentatively interpreted as a cyanidin 3-rutinoside dimer, for which two structures could be proposed, an A-type flavanflavylium and a B-type flavene-flavylium (Fig. 3).

Peak 8 showed a molecular ion at m/z 663. Its MS² fragmentation gave one fragment at m/z 355 ($[M-308]^+$, loss of a rhamnohexoside moiety). The MS³ fragmentation of this ion gave, among others, a minor fragment at m/z 311 (–44 amu) corresponding to a loss of a carboxyl residue, allowing its tentative identification as carboxypyranocyanidin 3-*O*-rutinoside. This compound showed λ_{\max} in the visible region at 506 nm, hypsochromically shifted with regard to that of the precursor anthocyanin (Cy 3-*O*-glucoside, λ_{\max} at 516 nm).

The same compound was described in processed black olives (del Caro et al., 2006). This type of pyranoanthocyanin pigments have been mostly associate to reactions involving anthocyanins during processing of plant-derived products, such as red wines (Fulcrand et al., 1998; Bakker et al., 1997), although they have also been reported in non-processed fruit like strawberry (Lopes da Silva et al., 2007; Andersen et al., 2004).

3.2. Content and distribution of anthocyanin pigments in fig varieties

The content and distribution of anthocyanins were investigated in five fig varieties. One of them a green variety, in which the content was only investigated in its brown–pink pulp. The other varieties presented an intense violet–blue colour in the skin and dark pink colour in the flesh. In these, both pulp and skin were analysed.

Significant differences in the anthocyanin content were found between pulp and skin and also among some varieties. Table 2 shows the individual concentration of the anthocyanin pigments ($\mu\text{g g}^{-1}$ fresh weigh) found in the skin of the fig varieties and Table 3 those of the pulp. The concentration of anthocyanins in the skin ranged from 32 and 97 $\mu\text{g g}^{-1}$ fresh weight. Granilla, Cuello de Dama (dark purple) and Bursa Siyahi were the varieties with the higher anthocyanin content without significant differences between them. Colar variety had the minor content. In the pulp, the anthocyanin contents ranged from approximately 14 $\mu\text{g g}^{-1}$ for Colar and Cuello de Dama (dark purple) varieties to 1.5 $\mu\text{g g}^{-1}$ for Cuello de Dama (green), this later expected according with its fair red colour. Solomon et al. (2006) found contents of the same order for the variety Bursa, 41 $\mu\text{g g}^{-1}$ for skin and 1 $\mu\text{g g}^{-1}$ for pulp.

Regarding anthocyanin distribution, both in skin and in pulp, the cyanidin derivatives were the most abundant. Cy 3-rutinoside was the predominant compound in (48–81% in the skin and 68–79% in the pulp), followed by Cy 3-glucoside (5–18% in the skin and 10–15% in the pulp) except in the skin of Collar variety where Cy 3,5-diglucoside and Pg 3-rutinoside were more abundant (11–12%). Percentages of 75% for Cy 3-rutinoside, 11% for Cy 3,5-diglucoside, 11% for Cy 3-glucoside and 3% for Pg 3-rutinoside were found by Puech et al. (1975) in a study carried out with the variety Mission (pulp and skin).

Malonyl derivatives were also found being usually more abundant in skin (1.2–6.5%) than in pulp (1.0–2.6%). They were particularly abundant in the skin of the Granilla variety.

It was noticeable the presence of Pg derivatives in the skin extracts of Colar variety which percentages around 13% (Pg 3-glucoside + Pg 3-rutinoside). In other varieties, these compounds ranged between 0.8% and 2.2%. Peonidin 3-rutinoside was observed in pulp extracts of all the studied varieties, however in the skin no evidences of this compound were found when the extracts were screened for its ion (m/z 609) in the full spectra of the LC–MS chromatograms.

The levels of Cy 3-rutinoside dimer were in general scarce. This compound was not detected in the pulp except in the variety Cuello de Dama (dark purple). Colar skin showed the greatest content of this compound with almost 3% of the anthocyanins identified in this variety. Furthermore, this variety presented the greatest content in flavanol–anthocyanin condensed pigments (2.6 $\mu\text{g g}^{-1}$ that represents 8.2% of total anthocyanins in the skin, and 0.8 $\mu\text{g g}^{-1}$

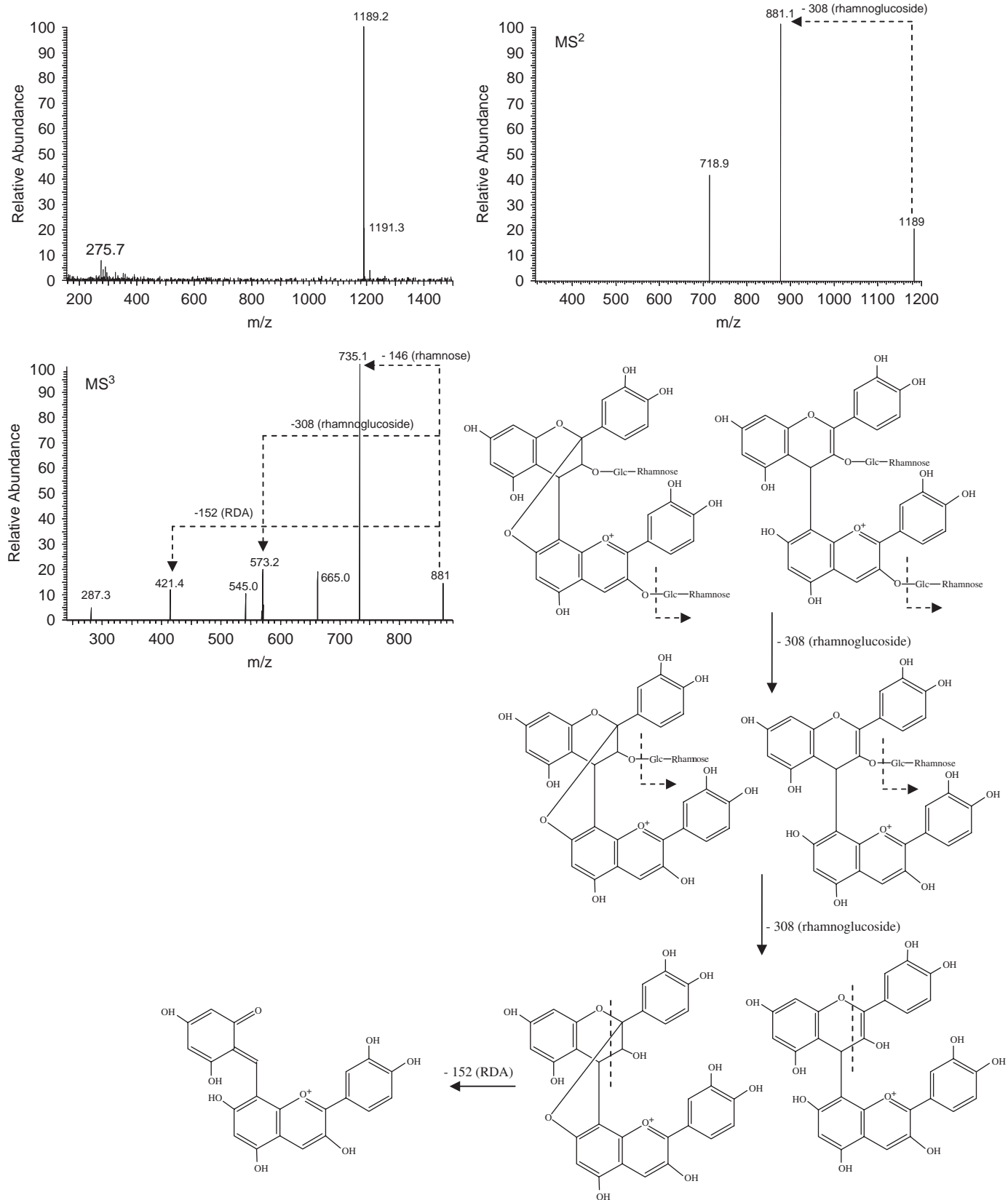


Fig. 3. MS² and MS³ of peak 1 Cy 3-rutinoside dimer at *m/z* 1189.

Table 2
Anthocyanin contents ($\mu\text{g/g}$ fresh weight) in the skin of different fig varieties

Compounds	Colar	%	Granilla	%	Cuello de Dama (dark purple)	%	Bursa Siyahi	%
Cy 3-rutinoside dimer	0.93 ± 0.03 ^{ckj}	2.9	0.54 ± 0.04 ^{bi}	0.6	0.46 ± 0.03 ^{ai}	0.5	0.44 ± 0.01 ^{ai}	0.5
(epi)catechin-(4 → 8)-Cy 3-glucoside	t ^{ai}	0.0	t ^{ai}	0.0	0.48 ± 0.04 ^{ci}	0.5	0.38 ± 0.02 ^{bi}	0.4
(epi)catechin-(4 → 8)-Cy 3-rutinoside	1.23 ± 0.04 ^{bklm}	3.9	0.62 ± 0.08 ^{ai}	0.7	0.59 ± 0.12 ^{ai}	0.6	0.63 ± 0.04 ^{ai}	0.7
Cy 3,5-diglucoside	3.80 ± 0.06 ^{abn}	11.9	5.23 ± 1.96 ^{bj}	6.1	1.99 ± 0.20 ^{ai}	2.2	2.44 ± 0.26 ^{ai}	2.5
(epi)catechin-(4 → 8)-Cy 3-rutinoside	0.93 ± 0.04 ^{ckj}	2.9	0.65 ± 0.06 ^{bi}	0.8	0.53 ± 0.06 ^{abi}	0.6	0.44 ± 0.01 ^{ai}	0.5
(epi)catechin-(4 → 8)-Pg 3-rutinoside	0.24 ± 0.01 ⁱ	0.8	nd	0.0	t	0.0	t	0.0
(epi)catechin-(4 → 8)-Pg 3-rutinoside	0.20 ± 0.04 ⁱ	0.6	nd	0.0	nd	0.0	nd	0.0
Carboxypyran-Cy 3-rutinoside	1.26 ± 0.13 ^{dlim}	4.0	nd ^a	0.0	0.63 ± 0.08 ^{ci}	0.7	0.48 ± 0.06 ^{bi}	0.5
Cy 3-malonylglycosyl-5-glucoside	1.04 ± 0.05 ^{bjkl}	3.3	1.39 ± 0.27 ^{ci}	1.6	0.63 ± 0.10 ^{ai}	0.7	0.53 ± 0.03 ^{ai}	0.5
Cy 3-glucoside	1.51 ± 0.03 ^{am}	4.7	15.38 ± 3.42 ^{ck}	18.0	10.98 ± 0.15 ^{bj}	12.0	10.33 ± 0.76 ^{bj}	10.7
Cy 3-rutinoside	15.42 ± 0.53 ^{ao}	48.5	57.16 ± 0.53 ^{bl}	66.9	73.36 ± 7.90 ^{ck}	79.9	78.32 ± 7.53 ^{ck}	80.9
Pg 3-glucoside	0.68 ± 0.09 ^{bj}	2.1	0.34 ± 0.01 ^{ai}	0.4	0.29 ± 0.05 ^{ai}	0.3	0.60 ± 0.09 ^{bi}	0.6
Pg 3-rutinoside	3.52 ± 0.01 ^{dn}	11.1	0.47 ± 0.06 ^{ai}	0.5	0.74 ± 0.16 ^{bi}	0.8	1.55 ± 0.15 ^{ci}	1.6
Pn 3-rutinoside	nd	0.0	nd	0.0	nd	0.0	nd	0.0
Cy 3-malonylglucoside	1.03 ± 0.04 ^{ajkl}	3.2	3.51 ± 0.83 ^{bj}	4.3	1.10 ± 0.07 ^{ai}	1.2	0.67 ± 0.03 ^{ai}	0.7
Total anthocyanin	31.79 ± 0.08 ^a		85.29 ± 4.26 ^b		91.78 ± 9.39 ^b		96.81 ± 6.91 ^b	

^{a,b,c,d}Means values in the same column with different letters are significantly different: LSD ($p < 0.05$).

^{ij,k,l,m,n,o}Means values in the same row with different letters are significantly different: LSD ($p < 0.05$). t: traces; nd: not detected.

Table 3
Anthocyanin contents ($\mu\text{g g}^{-1}$ fresh weight) in the pulp of different fig varieties

Compounds	Colar	%	Granilla	%	Cuello de Dama (green)	%	Cuello de Dama (dark purple)	%	Bursa Siyahi	%
Cy 3-rutinoside dimer	nd	0.0	nd	0.0	nd	0.0	0.08 ± 0.01 ⁱ	0.6	nd	0.0
(Epi)catechin-(4 → 8)-Cy 3-glucoside	0.13 ± 0.05 ^{bi}	0.9	0.06 ± 0.01 ^{ai}	1.3	0.03 ± 0.00 ^{ai}	2.0	0.07 ± 0.00 ^{ai}	0.5	0.05 ± 0.00 ^{ai}	0.9
(Epi)catechin-(4 → 8)-Cy 3-rutinoside	0.59 ± 0.05 ^{dij}	4.0	0.07 ± 0.01 ^{bi}	1.5	0.02 ± 0.00 ^{ai}	1.3	0.16 ± 0.03 ^{ci}	1.2	0.14 ± 0.04 ^{ci}	2.5
Cy 3, 5-diglucoside	0.11 ± 0.02 ^{ai}	0.8	0.14 ± 0.03 ^{ai}	3.0	0.09 ± 0.02 ^{aij}	5.9	0.57 ± 0.08 ^{bi}	4.3	0.15 ± 0.02 ^{ai}	2.7
(Epi)catechin-(4 → 8)-Cy 3-rutinoside	0.13 ± 0.03 ^{ci}	0.9	0.05 ± 0.00 ^{ai}	1.1	0.02 ± 0.00 ^{ai}	1.3	0.09 ± 0.00 ^{bi}	0.7	0.06 ± 0.00 ^{ai}	1.1
(Epi)catechin-(4 → 8)-Pg 3-rutinoside	t	0.0	nd	0.0	nd	0.0	nd	0.0	t	0.0
(Epi)catechin-(4 → 8)-Pg 3-rutinoside	nd	0.0	nd	0.0	nd	0.0	nd	0.0	t	0.0
Carboxypyran-Cy 3-rutinoside	0.94 ± 0.12 ^{cj}	6.4	0.09 ± 0.01 ^{ai}	1.9	0.04 ± 0.00 ^{ai}	2.6	0.16 ± 0.03 ^{bi}	1.2	0.07 ± 0.00 ^{ai}	1.2
Cy 3-malonylglycosyl-5-glucoside	0.09 ± 0.01 ^{di}	0.6	0.06 ± 0.01 ^{ci}	1.3	0.02 ± 0.00 ^{ai}	1.3	0.07 ± 0.00 ^{ci}	0.5	0.04 ± 0.00 ^{bi}	0.7
Cy 3-glucoside	2.15 ± 0.25 ^{dk}	14.7	0.54 ± 0.14 ^{bi}	11.4	0.19 ± 0.02 ^{aij}	12.5	1.67 ± 0.39 ^{cj}	12.6	0.51 ± 0.04 ^{bi}	9.6
Cy 3-rutinoside	10.16 ± 1.21 ^{cl}	69.5	3.58 ± 1.11 ^{bj}	75.8	1.04 ± 0.44 ^{ak}	68.4	10.15 ± 1.62 ^{ck}	78.6	4.46 ± 1.57 ^{bj}	79.2
Pg 3-glucoside	0.09 ± 0.01 ^{ci}	0.6	0.01 ± 0.00 ^{bi}	0.2	t ^{ai}	0.0	0.02 ± 0.00 ^{bi}	0.2	0.01 ± 0.00 ^{bi}	0.2
Pg 3-rutinoside	0.08 ± 0.00 ^{bi}	0.5	0.04 ± 0.00 ^{ai}	0.8	0.02 ± 0.00 ^{ai}	1.3	0.08 ± 0.01 ^{bi}	0.6	0.04 ± 0.00 ^{ai}	0.7
Pn 3-rutinoside	0.06 ± 0.00 ^{ci}	0.4	0.02 ± 0.00 ^{ai}	0.4	0.03 ± 0.00 ^{abi}	2.0	0.03 ± 0.00 ^{bi}	0.2	0.02 ± 0.00 ^{ai}	0.4
Cy 3-malonylglucoside	0.09 ± 0.00 ^{ci}	0.6	0.06 ± 0.00 ^{bci}	1.3	0.02 ± 0.00 ^{ai}	1.3	0.07 ± 0.00 ^{ci}	0.5	0.05 ± 0.01 ^{bi}	0.9
Total anthocyanin	14.62 ± 1.10 ^c		4.72 ± 1.24 ^b		1.52 ± 0.40 ^a		13.22 ± 1.75 ^c		5.6 ± 1.56 ^b	

^{a,b,c}Means values in the same column with different letters are significantly different: LSD ($p < 0.05$).

^{ij,k,l}Means values in the same row with different letters are significantly different: LSD ($p < 0.05$). t: traces; nd: not detected.

corresponding to 5.8% of total anthocyanins in the pulp). This was the only variety in which the flavanol-pelargonidin condensed pigments could be quantified, in accordance with its relatively high levels of Pg 3-rutinoside.

In Colar variety was also noticeable the relative high proportion of the pyrananthocyanin (carboxypyranocya-

nidin 3-rutinoside) which reached 6.4% in the pulp and 4% in the skin. In the other varieties its levels were around 2% in the pulp and 0.5% in the skin, except in Granilla skin where this pigment was not found. It is curious to indicate that this pigment was in greater percentage in pulp than in skin, even if its absolute concentration were higher in this latter.

4. Conclusions

The use of HPLC–DAD–MS allowed us to detect up to 15 different anthocyanin pigments in fig fruit from five different varieties (Colar, Cuello de Dama (green), Cuello de Dama (dark purple), Granilla and Bursa Siyahi), most of them were reported in this fruit for the first time according to our knowledge. Anthocyanins showed mainly Cy as aglycone (99–85%), although one Pn and some Pg (1–15%) derivatives were also detected. Rutinose was the most usual substituting sugar, but glucose was also found. A relevant aspect was the presence of anthocyanidin-derived pigments whose detection in natural plant sources was very recently identified, namely 5-carboxypirano-cyanidin-3-rutinoside, a cyanidin 3-rutinose dimer and five condensed pigments containing C–C linked anthocyanins (Cy or Pg) and flavanol (catechin or epicatechin) residues. Pn 3-rutinose was only detected in the pulp at very minor levels. There were important quantitative differences between pulp and skin, being this latter the part that presented the highest contents (32–97 $\mu\text{g g}^{-1}$ of total anthocyanins). Granilla, Cuello de Dama (dark purple) and Bursa Siyahi were the varieties with the greater anthocyanin content.

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