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Flavanol–anthocyanin condensed pigments in plant extracts

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Abstract

Pigments resulting from the direct condensation of anthocyanins and flavanols are usually associated with reactions taking place during processing and storage of plant-derived foods and beverages and have been particularly studied in aged red wines. In this paper, small amounts of flavanol–anthocyanin condensed pigments are found in different plant extracts. Structures are suggested for 10 such condensed pigments detected in extracts of strawberry, runner beans, purple corn and grape skins, based on their MS^n fragmentation patterns, following analyses by electrospray tandem mass spectrometry. All of them correspond to dimers containing a flavan-3-ol [either (epi)afzelechin, (epi)catechin or (epi)gallocatechin] as the upper unit carbon–carbon linked to a lower anthocyanin unit consisting of different delphinidin, cyanidin, pelargonidin, peonidin or malvidin derivatives. The detection of these pigments in plant extracts may suggest that they are natural pigments and not products exclusively formed during storage and ageing of processed foods and beverages, as was previously assumed.

Keywords: Flavanol–anthocyanin; Condensed pigments; Strawberry; Runner bean; Purple corn; Grape skin

1. Introduction

Anthocyanin-derived pigments constitute a group of compounds mostly associated with anthocyanin transformations taking place during the maturation and ageing of red wines. Basically, three types of anthocyanin-derived pigments have been described: ethyl-linked compounds resulting from acetaldehyde-mediated condensation between anthocyanins and flavanols, 4-substituted anthocyanin derivatives containing additional pyrano rings in the flavylium nucleus (i.e., pyranoanthocyanins), and products from the direct condensation between anthocyanins and another flavonoid moiety, namely a flavanol.

Pyranoanthocyanins (Bakker et al., 1997; Bakker & Timberlake, 1997; Francia-Aricha, Guerra, Rivas-Gonzalo, & Santos-Buelga, 1997; Fulcrand, Cameira dos Santos, Sarni-Manchado, Cheynier, & Bonvin, 1996) and pigments resulting from acetaldehyde-mediated condensation (Bakker, Picinelli, & Bridle, 1993; Dallas, Ricardo-da-Silva, & Laureano, 1996; Francia-Aricha et al., 1997; Rivas-Gonzalo, Bravo-Haro, & Santos-Buelga, 1995; Roggero, Coen, Archier, & Rocheville-Divorne, 1987; Timberlake & Bridle, 1976) have been extensively studied in model systems and their presence is also well documented in matured and aged red wines (Mateus, Pascual-Teresa, Rivas-Gonzalo, Santos-Buelga, & de Freitas, 2002; Mateus, Silva,

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Santos-Buelga, Rivas-Gonzalo, & de Freitas, 2002; Vivar-Quintana, Santos-Buelga, Francia-Aricha, & Rivas-Gonzalo, 1999; Vivar-Quintana, Santos-Buelga, Rivas-Gonzalo, 2002). Pigments from the anthocyanin–flavanol condensation involving aldehydes other than acetaldehyde have also been described (Es-Safi, Cheynier, & Moutounet, 2002; Pissarra, Mateus, Rivas-Gonzalo, Santos-Buelga, & De Freitas, 2003; Pissarra, Lourenço, et al., 2004).

Fewer studies exist about pigments from the direct condensation between anthocyanins and flavanols. Their structures and contribution to the colour in ageing red wines were hypothesized in the late 1960s (Jurd, 1967, 1969; Jurd & Somers, 1970; Somers, 1971). Two possible pathways were suggested for their formation: (1) nucleophilic addition of the hemiketal form of an anthocyanin through their C-8 or C-6 positions at C-4 of a carbocation resulting from the cleavage of the interflavanic linkage of a flavanol oligomer; further conversion of the hemiketal form of the anthocyanin to the corresponding flavylium would yield F–A^+ adducts; (2) electrophilic substitution of the anthocyanin flavylium form (C-4) by a flavanol (C-8 or C-6) to yield an A–F dimer, where the anthocyanin moiety would be in the flavene form; this latter could oxidize to the corresponding flavylium form (A^+–F adducts) or rearrange, either to a yellow xanthylium ion (Jurd & Somers, 1970; Liao, Cai, & Haslam, 1992; Santos-Buelga, Bravo-Haro, & Rivas-Gonzalo, 1995) or to a doubly-linked A–F colourless structure containing an A-type interflavonoid bond (Bishop & Nagel, 1984; Remy-Tanneau, Le Guernevé, Meudec, & Cheynier, 2003). Detection, in red wines, of pigments showing molecular ions corresponding to either A^+–F or F–A^+ adducts was first achieved by Vivar-Quintana et al. (1999) using LC–MS and further confirmed by Salas et al. (2004) who also found evidence in favour of the F–A^+ structure. The occurrence, in red wine, of doubly-linked A–F colourless dimers was also demonstrated by Cheynier's group (Remy, Fulcrand, Labarbe, Cheynier, & Moutounet, 2000).

Recent evidence shows that certain anthocyanin-derived pigments occur, not only in red wines, but also

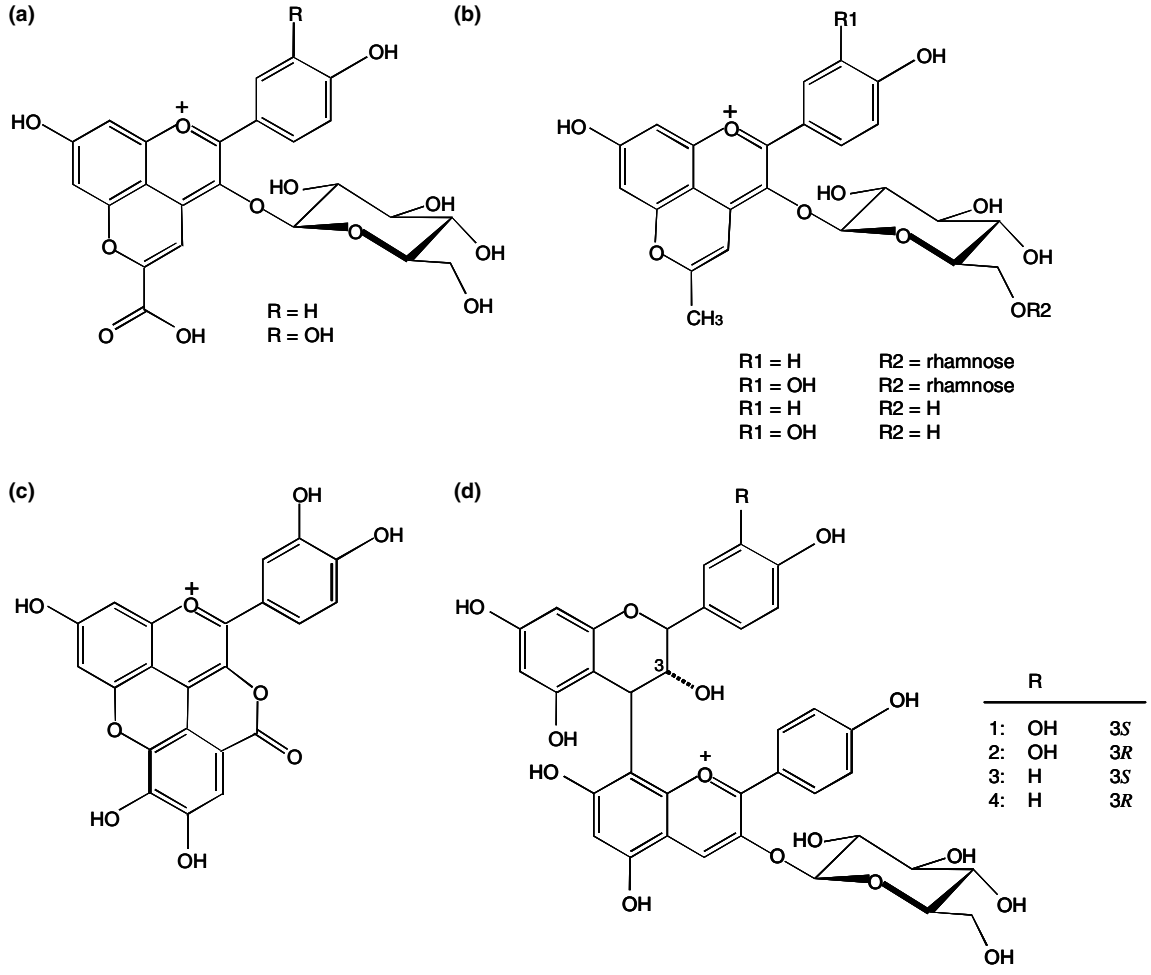


Fig. 1. Anthocyanin-derived pigments isolated from: (a) red onion (Fossen and Andersen, 2003) and strawberry (Andersen et al., 2004); (b) blackcurrant seeds (Lu et al., 2000); (c) petals of *Rosa hybrida* (Fukui et al., 2002); (d) strawberry (Fossen et al., 2004).

in small amounts in plants. Thus, 5-carboxypyranocyanidin and 5-carboxypyranopelargonidin derivatives (Fig. 1(a)) have been obtained from red onion (Fossen & Andersen, 2003) and strawberries (Andersen, Fossen, Torskangerpoll, Fossen, & Hauge, 2004), and 5-methylpyranocyanidin and 5-methylpyranodelphinidin derivatives (Fig. 1(b)) from blackcurrant seeds (Lu, Sun, & Foo, 2000). Also, a related 4-substituted cyanidin pigment containing two additional heterocycles attached to the anthocyanidin skeleton (Fig. 1(c)), derived from the C–C condensation between gallic acid and the anthocyanin, have been extracted from petals of *Rosa hybrida* (Fukui, Kusumi, Masuda, Iwashita, & Nomoto, 2002). Quite recently, four purple anthocyanin–flavanol complexes have also been found in strawberry extracts (*Fragaria ananassa*) and their structures elucidated by NMR, showing that they consist of pelargonidin 3-glucoside C–C linked to (epi)catechin and (epi)afzelechin moieties (Fig. 1(d)) with an F–A^+ structure (Fossen, Rayyan, & Andersen, 2004). In the present work, further evidence indicates this kind of pigment in different plant extracts.

2. Materials and methods

2.1. Sample preparation

2.1.1. Strawberries

Strawberry samples (*Fragaria x ananassa cv* Camarosa) were homogenized in 0.1% HCl in methanol and later filtered through a Büchner funnel under vacuum. The solid residue was exhaustively washed with methanol and the filtrates obtained were centrifuged. After addition of water, the supernatant was concentrated under vacuum to total evaporation of the methanol and the aqueous extract obtained was washed with *n*-hexane to remove liposoluble substances. An aliquot of the aqueous phase was deposited onto a C-18 SepPak® Vac 3 cc cartridge (waters); sugars were removed by passing through 15 ml of water and anthocyanin pigments further eluted with 5 ml of MeOH: 0.1% TFA (95:5). The methanolic extract was concentrated under vacuum, redissolved in 2 ml of ultrapure water and filtered through a 0.45-μm membrane filter for High pressure liquid chromatography (HPLC) analysis.

2.1.2. Beans

The seed coat of scarlet red runner beans (*Phaseolus coccineus*) was manually separated and ground to obtain an homogeneous powder. Anthocyanin extraction was carried out by successive macerations in methanol containing 5% of 1 N HCl in an ultrasonic bath. The methanol phases were pooled and centrifuged and the supernatant was concentrated under vacuum to total evaporation of the methanol. The aqueous extract obtained was washed with *n*-hexane to remove liposoluble substances and further purified using a C-18 SepPak cartridge, as described for strawberry anthocyanins.

2.1.3. Purple corn

A commercial anthocyanin-rich powder, obtained after drying of purple corn cob (cv. Morado) by a Peruvian company, was used (de Pascual-Teresa, Santos-Buelga, & Rivas-Gonzalo, 2002). The corn powder was dissolved in 0.01 M HCl and filtered through a 0.45-μm membrane filter (Millipore, Bedford, Mass., USA) previous to injection in the HPLC system.

2.1.4. Grape skins

Pigment extraction was carried out as described in Heredia, Francia-Aricha, Rivas-Gonzalo, Vicario, and Santos-Buelga (1998). Briefly, grape skins (*Vitis vinifera* cv. Tempranillo) were macerated in methanol containing 5% of 1 N HCl; the methanol extract was evaporated under vacuum and the aqueous phase obtained washed with *n*-hexane to remove liposoluble substances. The aqueous extract was deposited onto a column loaded with a mixed stationary phase composed of 20% Polyclar AT and 80% Kieselgel 60; sugars and acids were eliminated by exhaustive washing with water and anthocyanin pigments further eluted with 0.1% HCl in methanol. The eluate was concentrated under vacuum, redissolved in water and filtered through a 0.45-μm membrane filter for HPLC analysis.

2.2. HPLC–DAD–MS analysis

HPLC analyses were performed in a Hewlett–Packard 1100 series liquid chromatograph, using the method described by Lopes da Silva, de Pascual-Teresa, Rivas-Gonzalo, and Santos-Buelga (2002). Separation was achieved on a 5-μm AQUA® C18 150 × 4.6 mm column (Phenomenex®, Torrance, CA) thermostatted at 35 °C. Solvents used were: (A) 0.1% trifluoroacetic acid (TFA) in water, and (B) gradient-grade acetonitrile, establishing the following gradient: isocratic 10% B for 5 min, 10–15% B over 15 min, isocratic 15% B for 5 min, 15–18% B over 5 min, and 18–35% B over 20 min, using a flow rate of 0.5 ml min^{-1}. Double on-line detection was carried out in a photodiode spectrophotometer, using 520 nm as the preferred wavelength, and in a mass spectrometer connected to the HPLC system via the UV cell outlet.

The mass spectrometer was a Finnigan LCQ (San Jose, CA) equipped with an ESI source and an ion trap mass analyser, which were controlled by the LCQ navigator software. Both the auxiliary and the sheath gas were a mixture of nitrogen and helium at flow rates of 6 and 1.2 l min^{-1}, respectively. The capillary voltage was 4 V and the capillary temperature 195 °C. Spectra were recorded in positive ion mode between 150 and

1500 *m/z*. The MS detector was programmed to perform a series of three consecutive scans: a full scan, an MS–MS scan of the most abundant ion in the first scan and a MS^3 of the most abundant ion in the MS^2, using a normalised collision energy of 45%.

3. Results and discussion

Fig. 2 shows chromatograms of the anthocyanin profiles of the plant extracts analyzed. Together with the major peaks corresponding to anthocyanins identified elsewhere (de Pascual-Teresa et al., 2002; Hebrero, Santos-Buelga, & Rivas-Gonzalo, 1988; Lopes da Silva et al., 2002; Macz-Pop, Rivas-Gonzalo, Pérez-Alonso, & González-Paramás, 2005) the presence of small peaks, showing UV–Vis spectra similar to anthocyanins, were also observed in the first part of the chromatograms. Molecular ions of those peaks obtained with the mass detector (Table 1) showed them to be anthocyanin–flavanol dimers, similar to those previously detected in red wines (Salas et al., 2004; Vivar-Quintana et al., 1999) and recently characterised by Fossen et al. (2004) in strawberry extracts.

UV–Vis spectra of compounds St1–St4, detected in strawberry extracts, were similar to those observed by Fossen et al. (2004) for the flavanol–pelargonidin pigments identified by them. They show λ_{max} in the visible region at 515–518 nm (Table 1), bathochromically shifted with regard to that of the precursor anthocyanin (pelargonidin 3-glucoside, $\lambda_{vis\text{-}max}$ at 502 nm). They also present increased absorption at 430–440 nm and, subsequently, an increased $A_{440\ nm}/A_{\lambda_{vis-max}}$ ratio. Similar spectral differences in relation to the parent anthocyanin have been found for the condensed pigments detected in the different plants analysed in this work.

Peaks St1 and St2 possessed an identical molecular ion $[M]^+$ at *m/z* 721 and similar fragmentation patterns. Fig. 3 shows the MS^2 and MS^3 spectra of pigment St1 together with the fragmentation scheme. The MS^2 fragment at *m/z* 559 (−162 amu) corresponds to the loss of a glucose moiety. MS^3 led to fragment ions at *m/z* 541 ($[M-18]^+$, loss of water), 407 ($[M-152]^+$, retro Diels–Alder fission of an (epi)catechin moiety), 313 ($[M-246]^+$, partial loss of an (epi)catechin unit), 433 ($[M-126]^+$, loss of $C_6H_6O_3$-A-ring-), and 271 ($[M-288]^+$, loss of an (epi)catechin unit). As was noticed by Friedrich, Eberhardt, and Galensa (2000) for the fragmentation of proanthocyanidin dimers and also observed by Salas et al. (2004) for $F\text{–}A^+$ adducts found in red wines, the loss of a $C_6H_6O_3$ fragment is characteristic of the upper flavanol unit. Furthermore, the loss of 288 amu is expected when (epi)catechin constitutes the upper unit (but −290 amu when it is the terminal one). Therefore, the production of fragments at *m/z* 433 and 271 reveals that the flavanol moiety (catechin

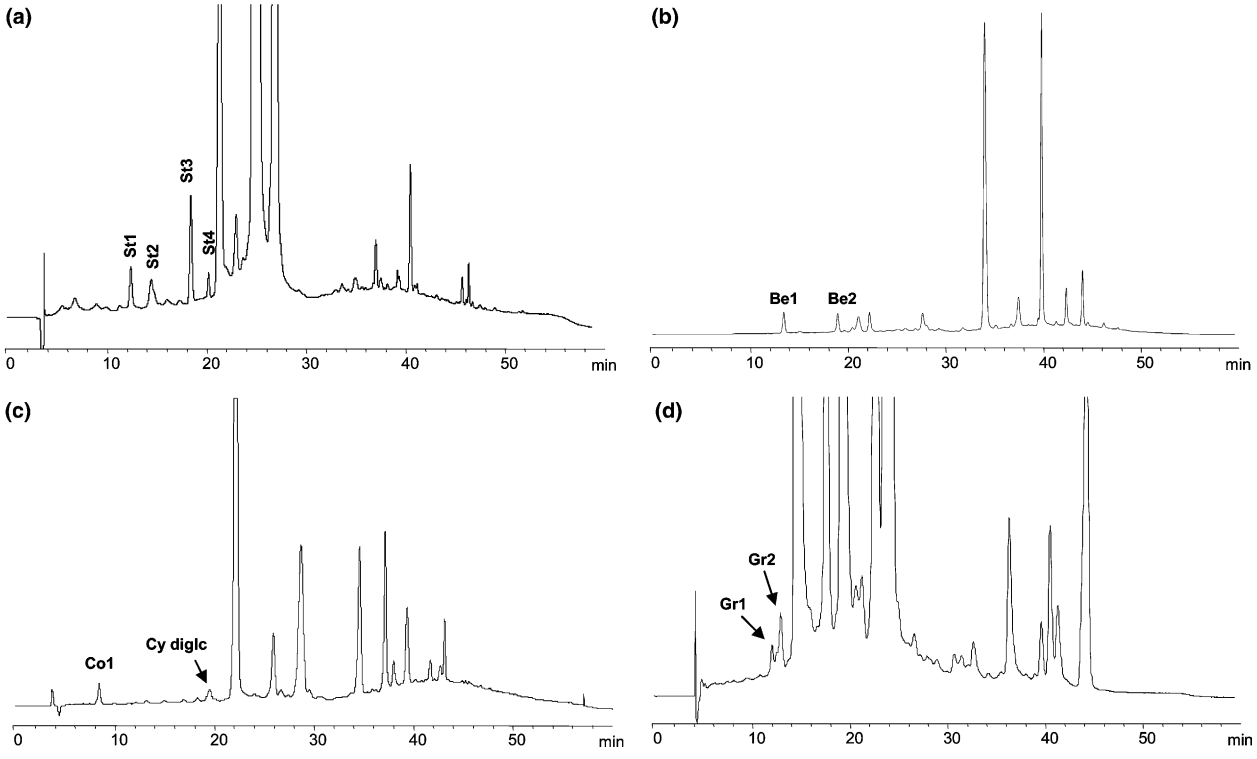


Fig. 2. HPLC chromatograms recorded at 520 nm of the different plant extracts: (a) strawberry; (b) runner beans; (c) purple corn; (d) grape skins. Peaks indicated correspond to flavanol–anthocyanin condensed pigments.

Table 1
UV–Vis absorption maxima obtained on-line with the diode array detector and molecular ions of the flavanol–anthocyanin condensed pigments detected in different plant samples

Compound	$\lambda_{vis\text{-}max}$ (nm)	$[M]^+$ (*m/z*)
St1	515,430	721
St2	517,436	721
St3	515,430	705
St4	518,433	851
St5	n.a.	705
Co1	528	899
Be1	536	607
Be2	532	737
Gr1	530	751
Gr2	534	781

n.a., not available.

or epicatechin) is located in the upper unit of the condensed pigment, whereas the pelargonidin constitutes the lower one.

Mass spectra do not allow conclusions about the position of the interflavonoid linkage. In the case of proanthocyanidins, a C4–C8 linkage is more likely to occur than a C4–C6 one (Haslam, 1998). Based on this and the previous pigment identification made by Fossen et al. (2004), using NMR, a C4–C8 linkage between flavanol and anthocyanin moieties has been assumed for pigments St1 and St2. Thus, St1 and St2 were tentatively identified as catechin-(4,8)-pelargonidin 3-glucoside (d1 in Fig. 1) and epicatechin-(4,8)-pelargonidin 3-glucoside (d2 in Fig. 1), respectively. Similarly, no information to differentiate between catechin and epicatechin units can

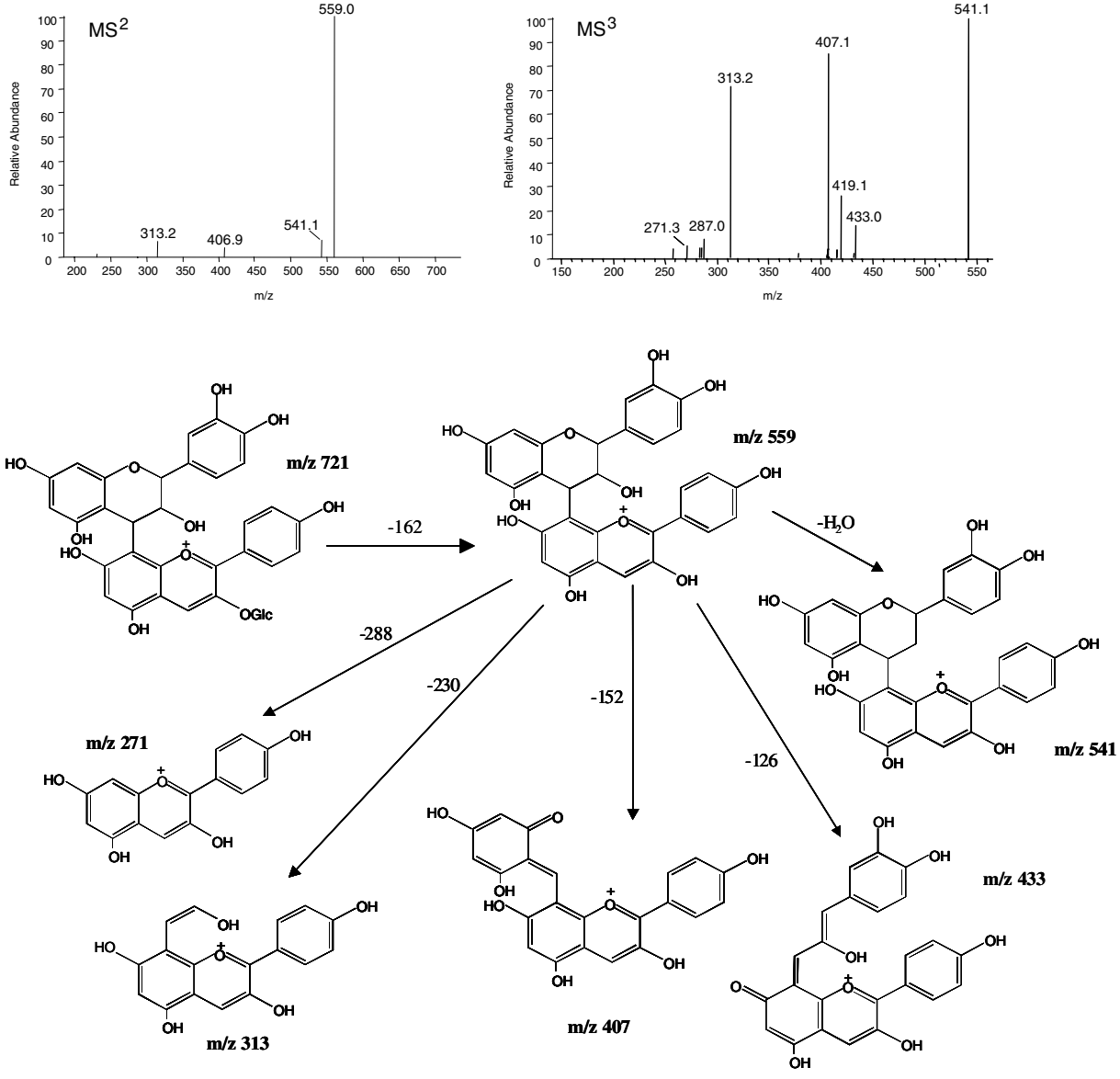


Fig. 3. MS^2 and MS^3 spectra of pigment St1 (*m/z* 721) and scheme of fragmentation.

be obtained from the fragmentation pattern. The assignment of the flavanol unit to pigments St1 and St2 was done according to their order of elution in the HPLC chromatogram, consistent with that observed by Fossen et al. (2004) and with the retention characteristics expected for catechins in reversed-phase columns, with *2R,3S* compounds (i.e., catechin) eluting earlier than *2R,3R* (i.e., epicatechin) (Santos-Buelga, García-Viguera, & Tomás-Barberán, 2003). Nevetherless, it is necessary to indicate that the relative polarities of catechin and epicatechin may not be retained in all their derivatives and, in fact, the reverse order of elution was observed by Salas et al. (2004) for (epi)catechin-malvidin 3-glucoside adducts whose formation was induced in model solutions.

Pigment St3 showed a molecular ion $[M]^+$ at *m/z* 705 with a similar fragmentation pattern to St1 and St2. In addition, when the molecular ion at *m/z* 705 was extracted from the total ion current chromatogram, the presence of a second peak (St5) was noticed, eluting at 22 min (Fig. 4). That peak was not observed in the HPLC–DAD chromatograms of the strawberry extract since it was overlapped by the peak of pelargonidin 3-glucoside (major anthocyanin in strawberry). MS^2 fragmentation of the molecular ion at *m/z* 705 yields a signal at *m/z* 543 (−162 amu, loss a glucose moiety) that released MS^3 fragments at *m/z* 525 (−18 amu, loss of water), 407 (−136 amu, RDA fission of an (epi)afzelechin moiety), 313 (−230 amu, partial loss of (epi)afzelechin), and 271 (−272 amu, loss of an upper (epi)afzelechin unit). As indicated above, no conclusion can be obtained about the position of the interflavonoid linkage and the nature of the flavanol unit from the mass spectra and fragmentation pattern. As for St1 and St2, an assignment of those structural features was made, based on the identifications and elution order of the pigments previously characterised by Fossen et al. (2004). Thus, peak St3 was tentatively identified as afzelechin-(4,8)-pelargonidin 3-glucoside and St5 as epiafzelechin-(4,8)-pelargonidin 3-glucoside (structures d3 and d4 in Fig. 1, respectively). Additional support for the identity of St3 was provided by its λ_{max} in the visible region (515 nm), closer to that obtained by Fossen et al. (2004) for the catechin adduct (516 nm). Maximum wavelength of visible absorption at 520 nm was found by those authors for the equivalent epiafzelechin derivative, which could not be corroborated in our case due to the overlapping of St5 with the peak of pelargonidin 3-glucoside.

A molecular ion $[M]^+$ at *m/z* 851 was found for pigment St4, with a major MS^2 fragment at *m/z* 543 (−308 amu, loss a rutinose moiety) that showed a MS^3 fragmentation pattern identical to that of pigment St3. According to these characteristics, the peak was tentatively identified as the dimer afzelechin-(4,8)-pelargonidin 3-rutinoside (Fig. 5). The presence of afzelechin as flavanol unit and a C4–C8 interflavonoid linkage were assumed, based on the same considerations as for the other condensed pigments found in the strawberry extracts. There was no detection of another peak that could correspond to the epiafzelechin derivative equivalent to St4 when the total ion current chromatogram was scanned for the molecular ion *m/z* 851. Such a compound, if present, would be in very small amounts,

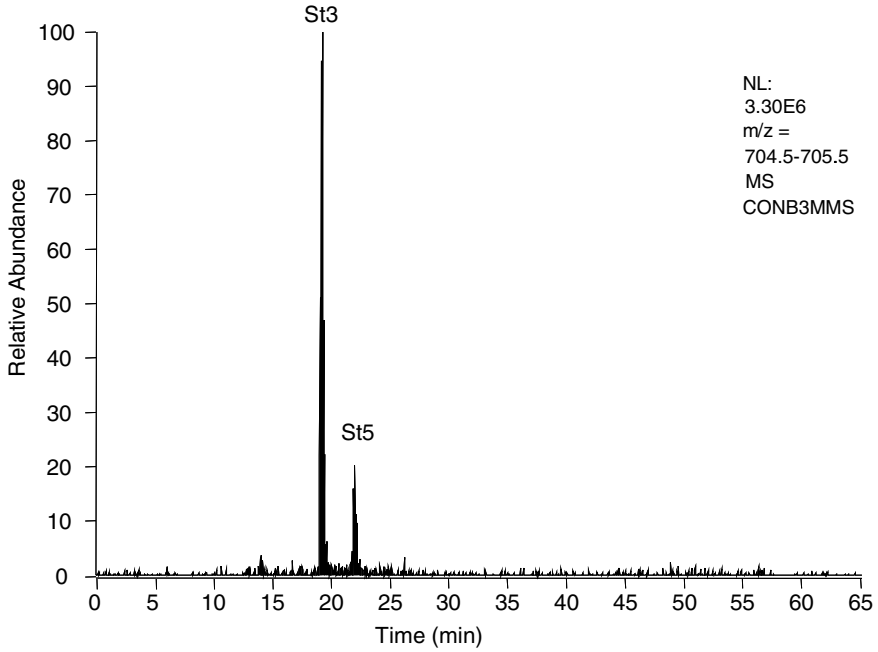


Fig. 4. Mass chromatogram for the molecular ion *m/z* 705 extracted from the total ion current chromatogram of the strawberry extract.

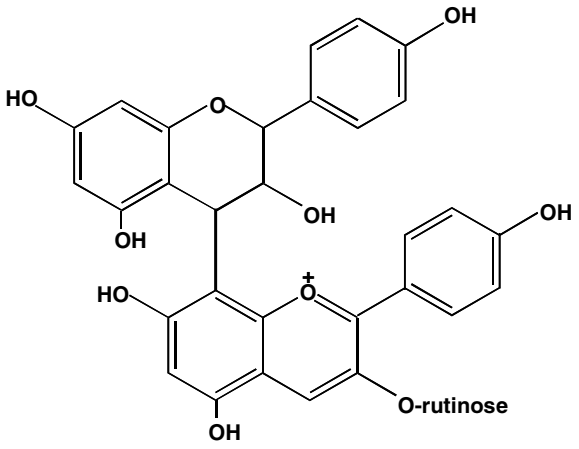


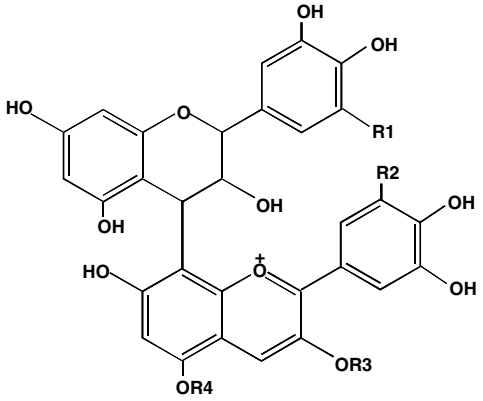
Fig. 5. Structure suggested for pigment St4 detected in the strawberry.

which is not surprising taking into account the disproportion existing between the corresponding condensed pigments derived from pelargonidin 3-glucoside (i.e., St1/St2 and St3/St5). Pigment St4 was not mentioned by Fossen et al. (2004) and, according to our knowledge, it is here detected for the first time. It is notable that proportions between peaks St3 and St4 in the extract are similar to those existing between pelargonidin 3-glucoside and pelargonidin 3-rutinoside, the major anthocyanins in strawberry and precursors of those condensed pigments. There were no detection of peaks showing a molecular ion at *m/z* 867 (i.e., catechin–pelargonidin 3-rutinoside dimers).

In the anthocyanic profile of runner bean extracts (Fig. 2(b)) two minor peaks (Be1 and Be2), corresponding to condensed pigments, were detected. Peak Be1 gave a molecular ion at *m/z* 607 with a fragmentation scheme similar to that shown in Fig. 3. Major MS^2 signals were observed at *m/z* 439 (−168 amu, RDA fission of an (epi)gallocatechin moiety), 345 (−262 amu, partial loss of (epi)gallocatechin), 589 (−18 amu, loss of water), 481 (−126 amu, loss of $C_6H_6O_3$), and 303 (−304 amu, loss of an upper (epi)gallocatechin unit). Interestingly, no loss of a fragment corresponding to a sugar was observed, indicating that the anthocyanidin residue (cyanidin) is not glycosylated. In this respect, it is notable that major peaks detected in the chromatogram of the beans corresponded to anthocyanidins (delphinidin, cyanidin, petunidin, pelargonidin and peonidin, peaks located at 35–45 min in the chromatogram of Fig. 2(b)), whereas only minor peaks due to anthocyanin glycosides were detected. The presence of free anthocyanin aglycones seems to constitute a feature in the pigment composition of beans from different *Phaseolus* species (Macz-Pop et al., 2005). All in all, peak Be1 was associated with (epi)gallocatechin–delphinidin (Fig. 6).

Peak Be2 showed a molecular ion at *m/z* 737 that gave a major MS^2 fragment at *m/z* 575 (−162 amu, loss of a hexose), releasing MS^3 fragment ions at *m/z* 423 (−152 amu, RDA fission of an (epi)catechin moiety), 329 (−246 amu, partial loss of an (epi)catechin unit), and 449 (−126 amu, loss of $C_6H_6O_3$). This fragmentation allowed us to tentatively identify it as to (epi)catechin–cyanidin 3-glucoside (Fig. 6).

The pigment composition of purple corn was characterised by the presence of different anthocyanins based on cyanidin, pelargonidin and peonidin (de Pascual-Teresa et al., 2002). Besides them, a small peak was also observed in the HPLC chromatograms (Co1 in Fig. 2(c)) corresponding to a condensed pigment. Pigment Co1 had a molecular ion at *m/z* 899 that released two MS^2 fragments at *m/z* 737 and 575 (successive loss of two hexose residues). The MS^3 fragmentation pattern of the ion at *m/z* 575 was similar to that shown in Fig. 3, showing signals at *m/z* 423 (−152 amu, (epi)catechin RDA fission), 329 (−246 amu, partial loss of (epi)catechin), 557 (−18 amu, loss of water), 449 (−126 amu, loss of $C_6H_6O_3$) and 287 (−288 amu, loss of an upper (epi)catechin unit). The appearance of two signals in the MS spectrum corresponding to the loss of two hexose residues indicated that they were located at different positions in the anthocyanidin moiety since, if they were constituting a disaccharide only one signal should have been observed, corresponding to the aglycone (Giusti, Rodríguez-Saona, Griffin, & Wrolstad, 1999). Thus,



	R1	R2	R3	R4
Be1	OH	OH	H	H
Be2	H	H	Glc	H
Co1	H	H	Glc	Glc

Fig. 6. Structures suggested for pigments Be1 and Be2 detected in runner beans, and for pigment Co1 of purple corn.

the pigment was tentatively identified as the condensed dimer (epi)catechin–cyanidin 3-glucoside-5-glucoside (Fig. 6). It should be noted that the precursor anthocyanin (i.e., cyanidin-3,5-diglucoside) for that pigment was not mentioned in our previous paper about pigment composition of purple corn (de Pascual-Teresa et al., 2002). However, it was further detected as a minor peak at in the chromatograms of different corn extracts (peak marked as *Cy diglc* in Fig. 2(c)).

The anthocyanin profile in grape skins of *V. vinifera* varieties has been throughly studied and it is very well known that it is based on the 3-glucosides of five anthocyanidins (delphinidin, cyanidin, petunidin, peonidin and malvidin) that may be acylated with acetic, *p*-coumaric or caffeic acids (Hebrero et al., 1988). Besides these anthocyanins, minor peaks, corresponding to flavanol–anthocyanin condensed pigments, are also usually detected in the first part of the HPLC chromatograms. Analysis of those pigments by ESI–MS revealed molecular ions and fragmentation patterns similar to that shown in Fig. 3. In Fig. 2(d) the chromatogram of a grape skin extract, where two of such pigments (marked as Gr1 and Gr2) were detected, is shown as an example. Molecular ions of pigments Gr1 and Gr2 were found at *m/z* 751 and 781, yielding MS^2 fragments at *m/z* 589 and 619 (−162 amu, loss of an hexose residue), respectively. In both cases, the MS^3 fragmentation was similar to that observed for the condensed pigments previously discussed, showing signals corresponding to the (epi)catechin RDA fission (−152 amu; signals at *m/z* 437 and 467 for Gr1 and Gr2, respectively), partial loss of (epi)catechin (−246 amu, *m/z* at 343 and 373), loss of water (−18 amu, *m/z* at 571 and 601), loss of $C_6H_6O_3$ (−126 amu, *m/z* at 463 and 493), and loss of an upper (epi)catechin unit (−288 amu, *m/z* at 301 and 331). According to these mass characteristics, they were identified as (epi)catechin–peonidin 3-glucoside (Gr1) and (epi)catechin-malvidin 3-glucoside (Gr2) (Fig. 7). It is interesting to point out that the bathochromic shift in $\lambda_{vis\text{-}max}$ of pigment Gr2 (534 nm, Table 1) with regard to the precursor anthocyanin (malvidin 3-glucoside, λ_{max} at 527 nm in our chromatograms), was not as high as those observed for the other condensed pigments here discussed (12–16 nm). A similar λ_{max} (535 nm) for the same pigment was found by Salas et al. (2004). Condensed pigments similar to Gr1 and Gr2, derived from other grape anthocyanins, have also been occasionally detected in our laboratory in the regular analyses carried out with grapes and wines (Alcalde-Eon, Escribano-Bailón, Santos-Buelga, & Rivas-Gonzalo, 2004).

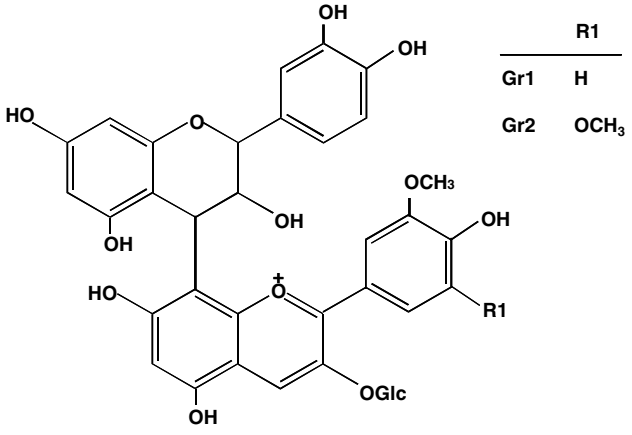


Fig. 7. Structures suggested for two flavanol–anthocyanin condensed pigments detected in grape skins.

In conclusion, the results obtained suggest that flavanol–anthocyanin condensed pigments may occur in plants and they are not products exclusively formed during storage and ageing of processed food and beverages, as was previously assumed. Nevertheless, it is still possible that they may be formed in vitro during the extraction process, as a result of the reaction of the carbocations released after acid cleavage of proanthocyanidins with anthocyanins. In this respect, it is notable that, in extracts of beans obtained with water or diethyl ether without acid addition (Macz-Pop et al., 2004), the condensed pigments continued to be detected in the HPLC chromatograms in spite of a limited proanthocyanidin cleavage being expected. This observation indicates their natural presence in plants, although additional studies are necessary to obtain further confirmation.

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