

## Pigments in the Fruit of Red-Fleshed Kiwifruit (*Actinidia chinensis* and *Actinidia deliciosa*)

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Kiwifruit cultivars (*Actinidia chinensis* and *A. deliciosa*) generally have fruit with yellow or green flesh when ripe. A small number of genotypes also have red pigments, usually restricted to the inner pericarp but varying in intensity and in distribution within the fruit. Carotenoids, chlorophylls, and anthocyanins were extracted from the fruit pericarp of such red-fleshed kiwifruit selections. Pigments were analyzed by HPLC and identified by comparison with authentic standards and by liquid chromatography–mass spectroscopy to obtain a tentative identification of the major anthocyanins in red-fleshed kiwifruit. The yellow and green colors of the outer fruit pericarp are due to different concentrations and proportions of carotenoids and chlorophylls. The red color found mainly in the inner pericarp is due to anthocyanins. In the *A. chinensis* genotypes tested the major anthocyanin was cyanidin 3-*O*-xylo(1-2)-galactoside, with smaller amounts of cyanidin 3-*O*-galactoside. In the *A. deliciosa* genotypes analyzed, cyanidin 3-*O*-xylo(1-2)-galactoside was not detected; instead, the major anthocyanins identified were cyanidin 3-*O*-galactoside and cyanidin 3-*O*-glucoside. However, the two species did not differ consistently in anthocyanin composition.

**KEYWORDS:** *Actinidia chinensis*; *Actinidia deliciosa*; anthocyanins; carotenoids; chlorophylls; cyanidin 3-*O*-galactoside; cyanidin 3-*O*-glucoside; cyanidin 3-*O*-xylo(1-2)-galactoside; kiwifruit

### INTRODUCTION

The kiwifruits of international trade are the fruits of *Actinidia chinensis* Planch. and *Actinidia deliciosa* (A.Chev.) C.F.Liang et A.R.Ferguson (Actinidiaceae). When the fruits of *Actinidia* species are ripe, the fruit flesh can be green, red, purple, yellow, or orange (1). Until recently, the only *Actinidia* species grown commercially throughout most of the world was *A. deliciosa*, and this species, of which cv. Hayward is the best-known example, has green fruit flesh. However, in China, a number of different cultivars of *A. chinensis* and *A. deliciosa* are grown commercially. Most of these Chinese cultivars have fruit with green flesh, but some *A. chinensis* fruits have flesh that is lime green or bright yellow when ripe.

During the past decade, the possibility of kiwifruit with different flesh colors has created much interest, and *A. chinensis* cultivars with yellow fruit flesh are now being grown in countries other than China. Possibly even more exciting is the potential of the red-fleshed cultivars such as *A. chinensis* Hongyang (Red Sun), the first kiwifruit cultivar with red flesh to be cultivated on a commercial scale (2, 3). Red flesh was

first noted in the fruit of *A. chinensis* from a restricted part of Hubei Province (4), and its presence has been used to distinguish the variety *A. chinensis* var. *rufopulpa* (C.F.Liang et R.H.Huang) C.F.Liang et A.R.Ferguson, although red pigments in the inner pericarp are also sporadically found in *A. chinensis* from Henan Province (the source of the seed from which Hongyang originated) and from other parts of China. A variant of *A. deliciosa* with red pigments in the inner pericarp, described as *A. deliciosa* var. *coloris* T.H.Lin et X.Y.Xiong, appears to be restricted to the Dongshanfeng mountains in northern Hunan Province and southern Hubei Province (5).

Chlorophylls, carotenoids, and anthocyanins are the most important pigments responsible for color in fruits and vegetables and, as well as contributing to their appearance and attractiveness, may also provide nutritional value in the form of dietary antioxidants (6–8). Biochemical studies on the pigments in the fruits of *A. chinensis* and *A. deliciosa* have so far been limited to cultivars with yellow or green flesh. The pigments responsible are carotenoids and chlorophylls (9–12), and any non-anthocyanin phenolics present are in such low concentrations (13) that it is unlikely that they would affect fruit flesh color. Anthocyanins occur in some *Actinidia* fruit (e.g., fruit of the hybrid *A. arguta* × *A. melanandra*) (14) but have not previously been reported in the fruit of *A. chinensis* or *A. deliciosa*. This paper examines the amounts of individual carotenoids, chloro-

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phylls, and anthocyanins present in the fruit flesh of red-fleshed kiwifruit genotypes.

## MATERIALS AND METHODS

**Fruit.** Fruit samples were collected from one vine of each of 15 red-fleshed genotypes of *A. chinensis*, including the cultivar Hongyang, and of 10 red-fleshed genotypes of *A. deliciosa* growing at the HortResearch Orchard, Te Puke, Bay of Plenty, New Zealand. Genotypes of *A. chinensis* are identified by the prefix CH followed by a genotype number or name; those of *A. deliciosa* are identified by the prefix DE followed by a genotype number. Fruits of most *A. chinensis* genotypes were harvested after they had reached 10 °Brix and were then stored for ~2 months at 0 °C. Fruits of *A. chinensis* CH-06 were harvested in mid-June 2002. Fruits of *A. deliciosa* were also harvested in mid-June 2002. When analyzed, all fruits were at close to eating ripeness, that is, <1 kg/cm<sup>2</sup> firmness.

**Measurement of Outer Pericarp Color.** A 2 mm thick slice of skin and outermost pericarp was removed from the equatorial region of each of 10 fruit of each genotype, and the color of the exposed pericarp flesh was measured using a Minolta CR-300 chroma meter (Osaka, Japan) consisting of a measuring head using diffuse illumination and a 0° viewing angle geometry for color measurement of an 8 mm diameter measuring area. The instrument was first calibrated using a *D*<sub>65</sub> illuminant, and the measurements were operated on *L*\*, *C*\*, and *h*°, representing, respectively, lightness, which ranges over the scale from 0 for black to 100 for white; chroma (or saturation index), which gives the distance from the central chromatic axis along the corresponding radius [ $(a^{*2} + b^{*2})^{1/2}$ ]; and hue angle, which describes the hue of the color, indicating a sector on the color wheel [ $h^\circ = \tan^{-1}(b^*/a^*)$  when  $a^* > 0$  and  $h^\circ = 180^\circ + \tan^{-1}(b^*/a^*)$  when  $a^* < 0$ ] (15).

**Sampling for Pigment Analysis.** Sampling and pigment extractions were carried out in a dark room illuminated by a yellow light (Philips TLD 36W/16). An equatorial slice, 2–3 cm thick, was cut from each fruit. Samples for chlorophyll and carotenoid analysis were taken from the outer pericarp of the 3–4 fruits for which the color parameters measured were closest to the average for all 10 fruit of that particular genotype. Cylinders of fruit flesh were collected from the pericarp of the slice using an 8 mm diameter cork borer, the samples being then combined for each genotype. Samples for anthocyanin analysis were collected by combining about half of the slice taken from each of the 10 fruit of a particular genotype.

**Extraction of Chlorophylls and Carotenoids.** Fruit flesh, 1–2 g, was extracted in an Ultra-Turrax homogenizer (Rose Scientific Ltd., Edmonton, AB, Canada) with 5 mL of acetone containing 0.1% butylated hydroxytoluene (BHT) in the presence of 100 mg of Na<sub>2</sub>CO<sub>3</sub> and 500 mg of anhydrous Na<sub>2</sub>SO<sub>4</sub>. Canthaxanthin (100 ppm) was added as an internal standard. Homogenates were held overnight at 4 °C in the dark. The following day, 2 mL of supernatant was collected and extracted against 2 mL of diethyl ether and 8 mL of 10% (w/v) NaCl and then centrifuged at 3000g for 10 min. The ether phase was collected, and the remaining water phase was extracted twice against 2 mL of ether and recentrifuged. The combined ether phases were taken to dryness and flushed with N<sub>2</sub> at 30 °C, and the residue was dissolved in a minimal volume of acetone (800 μL).

**HPLC Analysis of Carotenoids and Chlorophylls.** Extracts were analyzed by reversed-phase HPLC (HPLC Alliance Waters 2960) using a Waters Spherisorb ODS2 column and the ternary gradient system as described by Wright et al. (16). Gradient elution was performed with solutions (A) 0.5 M ammonium acetate/methanol (20:80 v/v), (B) water/acetonitrile (10:90 v/v), and (C) ethyl acetate, delivered at a flow rate of 1.0 mL/min. The gradient commenced at 100% A and changed to 100% B over 4 min before changing to 20% B/80% C over the next 14 min. This solvent composition was maintained for a further 4 min before changing to 100% B over 4 min and then to 100% A over a further 4 min. The initial composition was maintained for 6 min before injection of the next sample.

Carotenoid profiles were monitored at 455 nm and were detected with a PDA 996 Waters detector, set at 300–700 nm, whereas chlorophylls were monitored by fluorescence (excitation wavelength,

440 nm; emission, 660 nm) using a 474 scanning fluorescence detector (Waters, Milford, MA).

**Identification and Quantification of Carotenoids and Chlorophylls.** Chromatographic peaks were identified by comparison with authentic standards of β-carotene, lutein, and chlorophylls *a* and *b* (Sigma, Sydney, Australia) and antheraxanthin, violaxanthin, and zeaxanthin (Dr. P. Molnár, University of Pécs). When standards were not available, carotenoids such 9'-*cis*-neoxanthin and β-cryptoxanthin were tentatively identified from retention times and spectra (17, 18). Concentrations for these compounds were estimated using the lutein calibration curve. The detection limit for chlorophylls and carotenoids was 0.02 μg/g of fresh weight.

**Extraction of Anthocyanins.** Pericarp tissue (from both inner and outer pericarps), ~20 g, was extracted with a 5 times volume (v/w) of ethanol/H<sub>2</sub>O/acetic acid (80:20:1 v/v/v) in an Ultra-Turrax homogenizer. Homogenates were held for 2 days at 4 °C in the dark. The supernatant was then collected, centrifuged at 3000g for 10 min, and retained for HPLC analysis.

**HPLC Analysis of Anthocyanins.** Extracts were analyzed by reversed-phase HPLC using an Alliance 2960 instrument equipped with a 996 photodiode array detector (Waters). Separation was achieved with a 250 × 4.6 mm i.d., 5 μm, C18 Aqua column (Phenomenex, Torrance, CA), and a binary solvent system: (A) 5% aqueous formic acid (v/v); (B) acetonitrile (19).

**Identification and Quantification of Anthocyanins.** Anthocyanins were monitored at 530 nm, and chromatographic peaks were identified by comparison with authentic standards of cyanidin 3-*O*-galactoside, cyanidin 3-*O*-glucoside, and cyanidin 3-*O*-arabinoside (Polyphenol Laboratories AS, Sandnes, Norway). Concentrations of cyanidin 3-*O*-xylo(1-2)-galactoside and peak 3 were calculated using the cyanidin 3-*O*-galactoside calibration curve. The detection limit for anthocyanin was 0.2 μg/g of fresh weight.

**Liquid Chromatography–Mass Spectrometry (LC-MS) Analysis.** LC-MS analysis of anthocyanin extracts was carried out as previously described (20). Briefly, the instrument used was an LCQ Deca ion trap mass spectrometer (ThermoQuest, Finnigan, San Jose, CA) coupled to a Surveyor HPLC. The analytical column used was a 250 mm × 2 mm i.d. LiChroCart Superpher 100 RP-18 end-capped column (Merck, Darmstadt, Germany), maintained at 35 °C. Gradient elution was performed using 15:3.75:81.25 methanol/formic acid/water v/v/v (solvent A) and 100% methanol (solvent B). The flow rate was 250 μL/min, and the injection volume was 10 μL. ESI-MS data were acquired in the positive mode using a data-dependent LC-MS<sup>n</sup> method. The ESI voltage, capillary temperature, sheath gas pressure, and auxiliary gas pressure were 39 V, 300 °C, 448 kPa, and 138 kPa, respectively.

## RESULTS AND DISCUSSION

**Color of the Outer Pericarp.** Red-fleshed kiwifruit differed from most other *Actinidia* fruit in that the innermost part of the pericarp surrounding the core was red and different in color from the remainder of the pericarp. The outer pericarp of fruit of the *A. deliciosa* genotypes was consistently green, varying only in intensity of color from light green to dark green, even when the fruit were fully ripe. The color measured (Table 1) had hue values similar to those usually found for *A. deliciosa* Hayward, the common green kiwifruit (21), whereas chroma and lightness values were considerably lower. These values might be affected by fruit ripening; for example, lightness (*L*\*) is affected by changes in starch content (22). In contrast, the outer pericarp of *A. chinensis* genotypes with red pigments largely confined to the inner pericarp varied in color from yellowish green to bright yellow (Table 1).

**Carotenoids and Chlorophylls in the Outer Pericarp.** The same chlorophylls and carotenoids were found in the fruit of *A. deliciosa* genotypes as in *A. deliciosa* Hayward (11, 12). Both chlorophyll *a* and lesser amounts of chlorophyll *b* were present. Fruit of the genotype DE-09, which had dark green flesh,

**Table 1.** Xanthophylls, Carotenes, and Chlorophylls in the Outer Pericarp of Fruit<sup>a</sup> of *A. deliciosa* and *A. chinensis* (Micrograms per Gram of Fresh Weight)

pigment	<i>A. deliciosa</i>		<i>A. chinensis</i>		
	DE-09	DE-18	CH-01	CH-26	CH-88
xanthophylls					
9'-cis-neoxanthin <sup>b</sup>	0.58	0.59	0.17	0.21	0.12
violaxanthin	0.48	0.64	0.15	0.27	0.12
antheraxanthin	0.05	0.06	0.04	0.08	0.06
lutein	1.83	1.84	0.76	1.24	0.75
zeaxanthin	0.03	0.03	0.02	0.04	0.06
$\beta$ -cryptoxanthin <sup>b</sup>	0.10	0.13	0.02	0.04	0.02
carotenes					
$\beta$ -carotene	0.91	0.80	0.13	0.23	0.22
total carotenoids	3.98	4.09	1.29	2.11	1.35
chlorophylls					
chlorophyll <i>a</i>	10.00	7.18	0.52	0.46	0.75
chlorophyll <i>b</i>	4.97	3.31	0.25	0.22	0.35
total chlorophylls	14.97	10.49	0.77	0.68	1.10

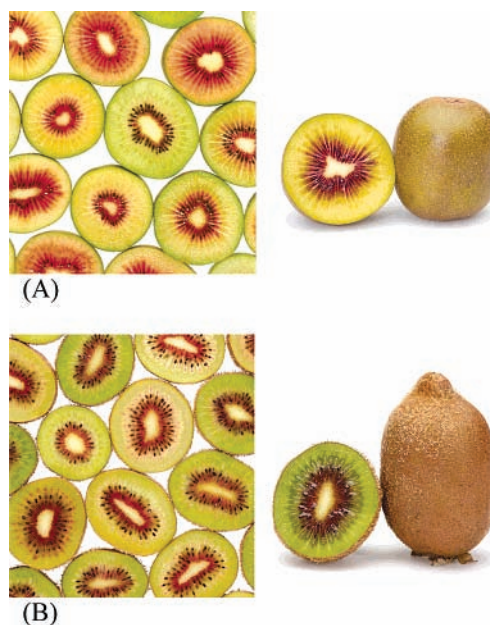
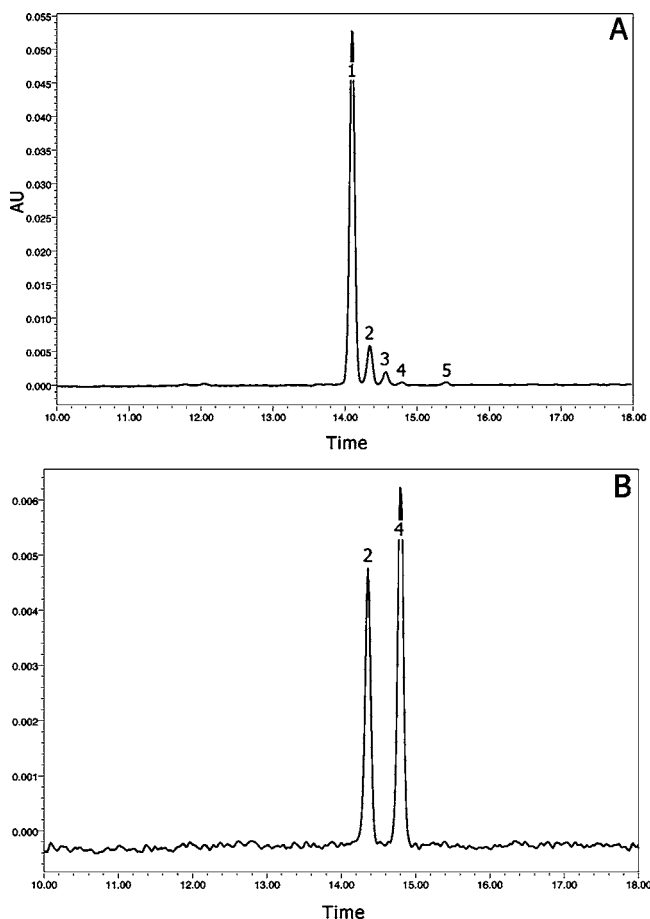
<sup>a</sup> Fruit color: *A. deliciosa* DE-09, dark green ( $L^* = 37.9$ ;  $C^* = 15.5$ ;  $h^\circ = 115.7$ ); DE-18, green ( $L^* = 39.41$ ;  $C^* = 17.12$ ;  $h^\circ = 114.5$ ); Hayward (20) ( $L^* = 61.81$ ;  $C^* = 42.59$ ;  $h^\circ = 112.8$ ). *A. chinensis* CH-01, pale yellow ( $L^* = 44.8$ ;  $C^* = 9.64$ ;  $h^\circ = 101.3$ ); CH-26, yellow ( $L^* = 37.65$ ;  $C^* = 10.94$ ;  $h^\circ = 102.9$ ); CH-88, green ( $L^* = 31.46$ ;  $C^* = 6.15$ ;  $h^\circ = 107.8$ ). <sup>b</sup> Evaluated as lutein equivalents.

contained nearly 50% more total chlorophyll than did fruit of the genotype DE-18, the flesh of which appeared light green. The carotenoids detected (**Table 1**) were those generally associated with chlorophyll in the chloroplasts, where they act as accessory pigments and photoprotectants (23). Lutein was the most abundant carotenoid, followed by  $\beta$ -carotene.

The same chlorophylls and carotenoids were also present in the outer pericarp of the fruit of *A. chinensis* genotypes with red pigments in the inner pericarp (**Table 1**), but the chlorophyll concentrations were much lower. The bright yellow, outer pericarp of fruit of *A. chinensis* CH-26 contained  $<1/20$  the amount of chlorophyll found in DE-09, the *A. deliciosa* genotype with a dark green outer pericarp; even the yellow-green outer pericarp of *A. chinensis* CH-88 contained  $<1/10$  the amount of chlorophyll found in the outer pericarp of DE-09. The total amounts of carotenoids present in the outer fruit pericarp of the *A. chinensis* genotypes studied were about a third to a half those of the *A. deliciosa* genotypes. As concluded by McGhie and Ainge (12), who analyzed different genotypes of *Actinidia*, variation in outer pericarp color from dark green through yellow-green to yellow seems to be due primarily to differences in the amount of chlorophyll present, not to any variation in the carotenoid composition or total amount. If chlorophylls have been completely degraded, then the yellow color of the fruit flesh is likely to be affected by the different amounts of carotenoids present (**Table 1**).

**Red Color in the Fruit Flesh.** In the genotypes of *A. deliciosa* examined, the red color was restricted to a ring around the core, largely confined to the inner part of the locules where the seed occur (**Figure 1B**). Individual genotypes of *A. chinensis* showed more variation: their fruits all had a similar ring of red tissue around the core, but the intensity of the color and the distribution of the red pigments varied with genotype. In some, the red color was restricted to the inner part of the locules; in others, the entire locules were red, so that a circle of red rays formed around the core, and in a few genotypes, the red coloration extended to the outer pericarp (**Figure 1A**).

**Anthocyanin Analysis.** A small number of anthocyanins were detected in the HPLC chromatograms of extracts of red-

**Figure 1.** Sections and whole fruit of red-fleshed *A. chinensis* (A) and *A. deliciosa* (B).**Figure 2.** HPLC chromatogram traces of anthocyanins from fruit of *A. chinensis* CH-01 (A) and *A. deliciosa* DE-18 (B). Detection was at 530 nm. Peaks: cyanidin 3-O-xylo(1-2)-galactoside, 1; cyanidin 3-O-galactoside, 2; unknown, 3; cyanidin 3-O-glucoside, 4; cyanidin 3-O-arabinoside, 5.

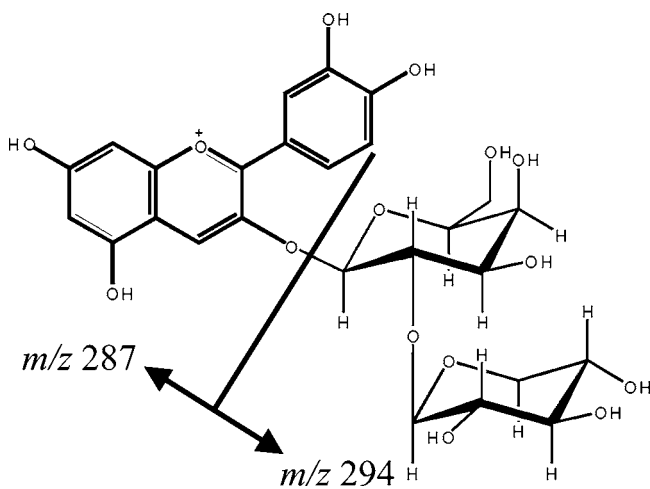
fleshed kiwifruit (**Figure 2**). Tentative identification of the anthocyanins detected was achieved by comparing retention times with authentic standards and ESI-MS data. Almost all genotypes (*A. chinensis* and *A. deliciosa*) contained two peaks



**Table 2.** Anthocyanins (Micrograms per Gram of Fresh Weight) in Fruit Pericarp of *A. chinensis* and *A. deliciosa*

genotype	total anthocyanins	cyanidin 3- <i>O</i> -xylo(1-2)-galactoside <sup>a</sup>	cyanidin 3- <i>O</i> -galactoside	cyanidin 3- <i>O</i> -glucoside	cyanidin 3- <i>O</i> -arabinoside	peak 3 <sup>a</sup>
<i>A. chinensis</i>						
CH-01	136.5	115.6	13.9	0.9	1.5	4.6
CH-02	139.9	115.4	17.9	0.6	1.5	4.5
CH-03	8.7	8.7	nd <sup>b</sup>	nd	nd	nd
CH-26	22.5	17.4	4.4	nd	nd	0.7
CH-42	27.6	nd	26.7	0.9	nd	nd
CH-51	13.4	11.7	1.7	nd	nd	nd
CH-54	15.3	9.5	5.8	nd	nd	nd
CH-59	61.6	49.2	11.1	0.3	nd	1.0
CH-69	2.9	2.9	nd	nd	nd	nd
CH-77	67.5	44.7	20.1	0.8	nd	1.9
CH-79	6.2	5.0	1.2	nd	nd	nd
CH-88	32.1	29.4	2.1	nd	nd	0.6
CH-89	13.6	10.9	2.7	nd	nd	nd
CH-90	0.9	0.9	nd	nd	nd	nd
Hongyang	29.9	29.4	nd	0.5	nd	nd
<i>A. deliciosa</i>						
DE-08	6.7	nd	3.1	3.6	nd	nd
DE-09	4.0	nd	2.0	2.0	nd	nd
DE-10	5.8	nd	3.3	2.5	nd	nd
DE-11	6.2	nd	1.5	4.7	nd	nd
DE-12	3.2	nd	1.5	1.7	nd	nd
DE-13	2.6	nd	1.1	1.5	nd	nd
DE-14	4.3	nd	1.8	2.5	nd	nd
DE-15	7.2	nd	3.5	3.7	nd	nd
DE-18	23.9	nd	11.6	12.3	nd	nd
DE-01	nd	nd	nd	nd	nd	nd

<sup>a</sup> Evaluated as cyanidin 3-*O*-galactoside equivalents. <sup>b</sup> Below the detection limit.



**Figure 3.** Structural formula and suggested fragmentation pattern of cyanidin 3-*O*-xylo(1-2)-galactoside.  $M^+ = m/z$  581.

that were identified as cyanidin 3-*O*-glucoside and cyanidin 3-*O*-galactoside by considering both retention times and ESI-MS data. The dominant anthocyanin peak in the genotypes of *A. chinensis* had a molecular ion of  $m/z$  581 and a fragment ion of  $m/z$  287, indicating that this is a cyanidin-pentose-hexose (**Figure 3**) (24). This is consistent with the previous identification of cyanidin 3-*O*-xylo(1-2)-galactoside in *Actinidia* (R. F. Webby, personal communication). A trace of an unidentified anthocyanin appeared in some fruit extracts as a small peak that eluted between cyanidin 3-*O*-galactoside and the small peak of cyanidin 3-*O*-glucoside. Spectroscopy analysis showed that all five peaks had their  $\lambda_{\max}$  around 520 nm. The absence of peaks in the 300–350 nm region indicated that none of them was acylated with aromatic acids (25), and this is consistent with the assigned identities.

In the *A. chinensis* genotypes examined, total anthocyanin concentrations varied enormously, from 0.92 to 139.97  $\mu\text{g/g}$  of

fresh weight of pericarp tissue (**Table 2**). Cyanidin 3-*O*-xylo(1-2)-galactoside predominated, accounting for at least 60%, and usually much more, of the total anthocyanin content. In several genotypes, generally those containing the least anthocyanin, it was the only anthocyanin detected; even in Hongyang, which contains more anthocyanins than two-thirds of the *A. chinensis* genotypes tested, it was also by far the most abundant. In one genotype (CH-42) no cyanidin 3-*O*-xylo(1-2)-galactoside was detected; instead, the most abundant anthocyanin was cyanidin 3-*O*-galactoside, usually the second major anthocyanin when more than one was detected. This particular genotype comes from the same populations as most of the other *A. chinensis* genotypes studied and morphologically would be classified as *A. chinensis* not *A. deliciosa*. This result was unexpected but has been confirmed by analyzing fruit collected from the same vine the following season. The other anthocyanins detected, cyanidin 3-*O*-arabinoside, cyanidin 3-*O*-glucoside, and that represented by peak 3, were present only in trace amounts, at most ~5% of the total, and are probably not important in determining the color of the fruit flesh. Although there were large differences in anthocyanin concentration between genotypes, these measurements were on fruit collected during one growing season. Other crop production and management practices could affect the amount of red pigment in the fruit of particular genotypes.

Anthocyanin analyses were carried out on fruit that had been in storage for several months. Storing Hongyang fruit for a further 3 months did not result in any major change in total or relative amounts of anthocyanins present (results not shown). Fruit of the various red-fleshed genotypes of *A. deliciosa* generally contained lower amounts of anthocyanin, 2.58–23.92  $\mu\text{g/g}$  of fresh weight of pericarp tissue (**Table 2**). Cyanidin 3-*O*-xylo(1-2)-galactoside was not detected in any of the genotypes examined: only two individual anthocyanins were detected, with cyanidin 3-*O*-glucoside being usually somewhat more abundant

than cyanidin 3-*O*-galactoside. No anthocyanins could be detected in extracts of DE-01, although traces of red pigment were visible around the core.

Anthocyanins have often been used in taxonomic studies (26). The anthocyanin composition of red-fleshed fruit of *A. chinensis* was, with the exception of the genotype CH-42, very different from that of red-fleshed fruit of *A. deliciosa*. However, this inconsistency means that fruit anthocyanin composition does not reliably distinguish the two species.

**Anthocyanins and Kiwifruit Improvement.** The total amounts of anthocyanins extracted from red-fleshed fruit of *A. chinensis* and *A. deliciosa* are much lower than in many berry fruits. The two genotypes of *A. chinensis* with the highest concentrations contained ~14 mg of anthocyanin/100 g of fresh weight of pericarp tissue, just  $1/10$  the concentration found in some whole blueberries (130 mg/100 g of fresh weight) (27),  $1/15$  that in another blueberry cultivar (197 mg/100 g of fresh weight) (28), or  $<1/40$  the concentration in black raspberries (589 mg/100 g of fresh weight) (29). It is therefore unlikely that the presence of anthocyanins would make a significant contribution to the already high antioxidant capacity of kiwifruit (30).

The main benefit of anthocyanins in red-fleshed kiwifruit is thus likely to be aesthetic, adding to the commercial appeal of the fruit. The bright red pigments are most attractive, especially when superimposed on a yellow background as in most of the red-fleshed *A. chinensis* genotypes, rather than the green flesh of *A. deliciosa*.

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