JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Genotypic Differences in Chlorophyll, Lutein, and β-Carotene Contents in the Fruits of *Actinidia* Species

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Chlorophyll, lutein, and β-carotene contents in *Actinidia* fruits were determined by high-performance liquid chromatography in various genotypes, including five *Actinidia deliciosa*, seven *Actinidia chinensis*, two *Actinidia rufa*, five *Actinidia arguta*, and three interspecific hybrids. The concentrations of chlorophyll, lutein, and β-carotene in the fruit of *A. deliciosa* Hayward were 1.65, 0.418, and 0.088 mg/100 g fresh weight, respectively. Of *A. deliciosa* cultivars, Koryoku showed significantly higher concentrations in chlorophyll, lutein, and β-carotene than Hayward. In most cultivars of *A. chinensis*, although both chlorophyll and lutein contents were significantly lower than in Hayward, the β-carotene content tended to be slightly higher. In *A. rufa*, *A. arguta*, and their interspecific hybrids, the contents of chlorophyll, lutein, and β-carotene were much higher than in Hayward. In particular, these fruits were found to be the richest dietary source of lutein among commonly consumed fruits.

KEYWORDS: ***Actinidia*** **spp.; kiwifruit; chlorophyll; lutein; β-carotene; pigment composition; genotypic difference**

INTRODUCTION

Fruits and vegetables contain several types of pigments, including chlorophylls and carotenoids. The composition of these pigments affects the color of the food, which is an attractive index for consumers. These pigments also influence the health-promoting effects of the foods. It is well-known that β-carotene and lycopene have a provitamin A activity. Some carotenoids, including β-carotene, lycopene, lutein, and zeaxanthin, are potent antioxidants and free radical scavengers (*1*), and a higher dietary intake of such carotenoids is associated with a lower risk for heart disease and certain types of cancer (*2*−*6*). Lutein, together with its structural isomer zeaxanthin, is a predominant carotenoid that accumulates in the lens and the macular region of the retina (*7*−*9*). Although lutein and zeaxanthin do not have provitamin A activity, they are strongly implicated in protection against age-related macular degeneration and cataract formation (*4*, *10*). Possible mechanisms of action for these carotenoids in this regard include antioxidant activity and filtering of damaging blue light (*1*, *8*).

Kiwi fruits are a nutritious fruit distinguishable from other fruits by the attractive green color of their flesh. Previous studies using spectrophotometric procedures and thin-layer chromatography revealed that the green color of their flesh is mainly due to chlorophylls a and b (*11*−*13*). More recently, high-performance liquid chromatography (HPLC) analysis has enabled the more discrete separation of their pigments. Cano (*14*) has shown that kiwi fruit extract contains xanthophylls, including neoxanthin, violaxanthin, and lutein; chlorophylls and their derivatives; and only one hydrocarbon carotenoid, β-carotene.

Until recently, *Actinidia* fruits sold on the world market were dominated by those of a single cultivar, *Actinidia deliciosa* Hayward. Commercial production of a new yellow-fleshed cultivar, *Actinidia chinensis* Hort16A, was started in New Zealand several years ago and later spread to other countries including the United States and Japan. *Actinidia arguta* Ananasnaya is also cultivated mainly in the United States and Chile and is sold under the commercial name Baby kiwi. In addition, new cultivars of *A. arguta* are now under development in New Zealand (*15*). Besides these commercial cultivars, there are a considerable number of cultivars and selections in the genus *Actinidia* (*16*). They have a wide diversity in sizes and shapes of fruits, hairiness, flesh color, vitamin C content, flavor, and taste (*17*, *18*).

These various genotypes are of commercial potential and/or are useful genetic resources for the development of new cultivars. Although McGhie and Ainge (*19*) have reported considerable variation in the chlorophyll and carotenoid compositions in four genotypes representing four different *Actinidia* species, genotypic differences in the pigment content have not been fully investigated. In the present study, we determined the concentrations of chlorophyll, lutein, and β-carotene in the fruits of various *Actinidia* genotypes.

MATERIALS AND METHODS

Chemicals. Standard chlorophylls a and b were purchased from Sigma (St. Louis, MO). Lutein and β-carotene were from Fluka (Buchs,

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10.1021/jf050785y CCC: $30.25
Published on Web 07/09/2005

Table 1. *Actinidia* Genotypes Examined[a]

species	genotype	color of flesh	density of hairs	fruit weight (g)
A. deliciosa	Hayward	green	dense	100.3 ± 7.9
	Bruno	green	dense	113.7 ± 12.1
	Abbott	green	dense	73.9 ± 10.0
	Elmwood	green	dense	118.7 ± 14.6
	Koryoku	deep green	dense	87.9 ± 6.9
A. deliciosa × *A. chinensis*	Sanryoku	yellow green	sparse or absent	101.7 ± 16.8
A. chinensis	Jiangxi 79-1[b]	yellow	sparse or absent	92.1 ± 9.9
	Golden king	yellow	sparse or absent	135.2 ± 16.1
	Kuimi[c]	yellow	sparse or absent	100.0 ± 9.1
	Sanuki gold	deep yellow	sparse or absent	169.8 ± 20.7
	Hongyang[d]	yellow, partly red	absent	78.1 ± 3.1
	Kobayashi39	yellow	sparse or absent	98.7 ± 10.8
	Hort16A[e]	yellow	sparse or absent	118.4 ± 4.1
A. rufa	Awaji	deep green	absent	9.3 ± 0.8
	Nagano	deep green	absent	12.7 ± 1.5
A. arguta	Hirano	green	absent	5.8 ± 0.8
	Gassan	green	absent	9.7 ± 1.7
	Issai	green	absent	8.8 ± 2.3
	Mitsuko	green	absent	9.0 ± 1.6
	Ananasnava[f]	green	absent	7.0 ± 1.2
A. arguta × *A. deliciosa*	Kosui	deep green	absent	35.9 ± 5.6
	Shinzan	deep green	absent	21.5 ± 3.3

[a] Values are means ± SD; $n = 24$. [b] Synonymous with Koshin or Red princess. [c] Synonymous with Applekiwi or Kaimitsu. [d] Synonymous with Rainbow red. [e] Known commercially as Zespri Gold Kiwifruit. [f] Known commercially as Baby kiwi.

Switzerland). HPLC grade acetonitrile and ethyl acetate were purchased from Wako (Tokyo, Japan).

Fruit Materials. Samples of ripe fruit from five *A. deliciosa*, seven *A. chinensis*, two *Actinidia rufa*, five *A. arguta*, and three interspecific hybrid genotypes were examined. Some of the characteristics of the fruit for each genotype are described in **Table 1**. Fruits of Abbott and Elmwood were obtained from Johoku Farm in Saitama and Sawanobori Kiwifruit Farm in Tokyo, Japan, respectively. Fruits of Hongyang and Kobayashi39 were from Kobayashi Farm in Shizuoka, Japan. Fruits of Hort16A imported from New Zealand and fruits of Ananasnaya imported from Chile were purchased from local markets in Tokyo. Fruits of all other genotypes were obtained from the experimental orchards at Kagawa Agricultural Experiment Station, Japan.

Extraction Method. The concentration of each pigment was determined in eight batches of three fruits each. Pigment extraction was performed according to the method by Mizda et al. (*20*) with slight modifications. The fruit samples of each genotype were peeled, and the edible portion, including seeds and cores, was cut into small pieces. The tissue was homogenized for 30 s in a Waring blender with 2 g Na_2CO_3/100 g fresh weight (FW). A portion of the homogenate (5.0 g) was ground for 1 min in a mortar with 15 mL of cold acetone (−20 °C) and quartz sand. The extract was filtered through a sintered glass funnel and then transferred to a volumetric flask. The extraction procedure was repeated five times. After all of the filtrate was collected in the volumetric flask, the volume was brought up to 100 mL with cold acetone. The extract was centrifuged at 15000*g* at 4 °C for 1 min, and the resulting supernatant was subjected to HPLC analysis as described below. All of the procedures for pigment extraction were carried out under dim light.

HPLC Analysis. The extracted pigments were analyzed by reversed phase chromatography on an octadecylsilane column according to the method of Taylor and McDowell (*21*) with slight modifications. The instrument used was a Hitachi (Tokyo, Japan) model L-2000 liquid chromatograph equipped with a Hitachi model L-2420 UV−vis detector set at 445 nm and a Hitachi model D-2500 data processor. An analytical LiChroCART 250-4 LiChrospher 100 RP-18e (5 μm) column (Merck, Darmstadt, Germany) was used.

Gradient elution was performed with solution A (acetonitrile−water, 90:10 v/v) and solution B (ethyl acetate) delivered at a flow rate of 1.5 mL/min. The gradient was initiated at 0% B, linearly increased to 60% B over 15 min, and then maintained at 60% B for 5 min. Duplicate 10 μL samples were injected for each extract.

The chromatographic peaks corresponding to chlorophylls a and b, lutein, and β-carotene were identified by comparing the retention times with those of authentic standards. For confirmation, cochromatography of each sample with the standard pigment was also applied. For quantification, an external standard was used to prepare calibration curves for each pigment over the ranges of 0.10−2.00 μg/mL (chlorophyll a or b) and of 0.05−1.00 μg/mL (lutein or β-carotene), yielding resolutions with correlation coefficient values of greater than 0.995.

Statistical Analysis. The data are presented as means ± SD of eight determinations. Nonrepeated measures analysis of variance was used to compare the concentrations of chlorophylls, lutein, and β-carotene between genotypes. Differences between Hayward and other genotypes were examined by a Dunnett test. Differences were considered significant at $P < 0.05$. The correlations among pigment concentrations in green-fleshed genotypes were examined by Pearson correlation analysis.

RESULTS AND DISCUSSION

Elution Profiles of Pigments Extracted from *Actinidia* Fruit. A chromatogram of pigments extracted from Hayward fruit is shown in **Figure 1a**. The elution profile was nearly the same as those reported by Cano (*14*) and McGhie and Ainge (*19*), although the relative height of each peak was different from that in the previous reports because of the differences in the detector wavelength. Comparison of the retention times of the peaks with those of authentic standards suggested that peaks 1, 2, 3, and 4 represent lutein, chlorophyll b, chlorophyll a, and β-carotene, respectively. The peak identification was confirmed by the spectral data (**Table 2**) and cochromatography of the extract with authentic pigments (data not shown). The elution profiles of pigments extracted from other *A. deliciosa* fruits were very similar to that of Hayward.

The chromatographic profiles of *A. chinensis* fruit extracts were also similar to that of Hayward except that the peaks corresponding to chlorophylls a and b were much smaller (**Figure 1b**). In *A. rufa*, *A. arguta*, and their interspecific hybrids, the elution profiles were basically the same as that of Hayward,

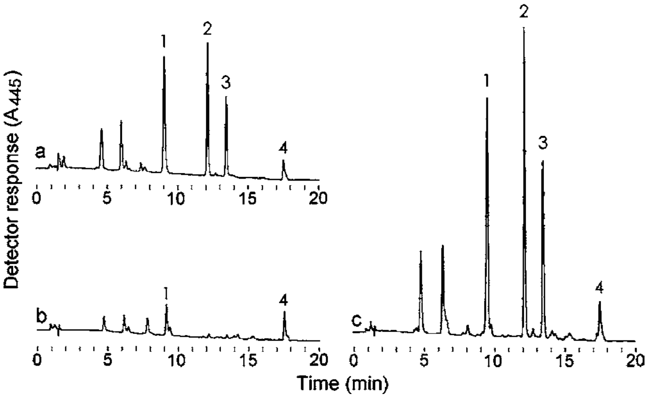

Figure 1. HPLC elution profile of carotenoids and chlorophylls extracted from *Actinidia* fruit of Hayward (**a**), Jiangxi 79-1 (**b**), and Ananasnaya (**c**). Peaks: lutein, 1; chlorophyll b, 2; chlorophyll a, 3; and β-carotene, 4.

Table 2. Spectral Data of Chlorophylls and Carotenoids Detected in *Actinidia* Fruit

peak no.	retention time (min)	spectral data maxima (nm)			pigment
1	9.2	424	447	474	lutein
2	12.2	457	597	646	chlorophyll b
3	13.5	430	616	662	chlorophyll a
4	17.6	454	476		β-carotene

but all of the peaks were markedly larger than those of Hayward. An elution profile of the pigments extracted from Ananasnaya fruits is shown in **Figure 1c**.

Genotypic Difference in Chlorophyll Content. There was wide variation in chlorophyll content between species. The chlorophyll content in the *Actinidia* fruit tested in the present study ranged from a trace amount to 4.39 mg/100 g FW (**Table 3**). Fruits of Hayward, the most common commercially available cultivar, contained 1.65 mg/100 g FW chlorophylls, including 1.12 and 0.53 mg/100 g FW of chlorophylls a and b, respectively. These values are almost in the same range as those previously reported by Cano (*14*). Of the *A. deliciosa* cultivars, Koryoku was only one that showed a significantly higher chlorophyll concentration than Hayward.

In contrast to *A. deliciosa*, the fruits from most *A. chinensis* cultivars are bright to deep yellow in flesh color. As expected from the color, the chlorophyll content in *A. chinensis* fruit is below the detection limit or is significantly lower than in Hayward (**Table 3**). The low chlorophyll concentration in *A. chinensis* fruits is considered to reflect degradation of the pigment during fruit maturation and ripening, which induces a transition of chloroplasts to chromoplasts (*19*).

In *A. rufa*, *A. arguta*, and their interspecific hybrids, the concentrations of both chlorophylls a and b were significantly higher than those in Hayward (**Table 3**). The chlorophyll content in the fruits ranged from 2.91 to 4.39 mg/100 g FW, which was 1.76−2.66-fold that in Hayward in a weight for weight basis (**Table 3**).

Genotypic Difference in Lutein and β-Carotene Content. Hayward fruits contained 0.418 mg/100 g FW lutein and 0.088 mg/100 g FW β-carotene (**Table 4**). In *A. deliciosa* cultivars, the fruit of Koryoku was significantly higher than Hayward in both lutein and β-carotene concentrations. Other cultivars in this species had pigment concentrations that were not statistically different from those in Hayward.

In *A. chinensis*, the lutein content was much lower than that in Hayward and other *A. deliciosa* cultivars except for Hongyang (**Table 4**). In contrast, the β-carotene content in the fruits of most *A. chinensis* cultivars tended to be higher than that in Hayward fruits, although the difference was not statistically significant except in the case of Sanuki gold. In agreement with the previous report by McGhie and Ainge (*19*), these results suggest that the yellow flesh color of *A. chinensis* is due mainly to the absence of chlorophyll from the fruit instead of an abundance of these carotenoids.

Table 3. Concentration of Chlorophylls in Fruit of *Actinidia* Genotypes[a]

species	genotype	concentration (mg/100 g FW)[b] chlorophyll a	chlorophyll b	chlorophylls a + b	chlorophylls a + b ratio to Hayward
A. deliciosa	Hayward	1.12 ± 0.20	0.53 ± 0.11	1.65 ± 0.31	1.00
	Bruno	1.02 ± 0.18	0.44 ± 0.07	1.46 ± 0.25	0.88
	Abbott	0.92 ± 0.15	0.41 ± 0.10	1.33 ± 0.24	0.81
	Elmwood	1.28 ± 0.15	0.59 ± 0.08	1.87 ± 0.22	1.13
	Koryoku	1.84 ± 0.57*	0.90 ± 0.23**	2.74 ± 0.80*	1.66
A. deliciosa × *A. chinensis*	Sanryoku	1.59 ± 0.19	0.74 ± 0.06	2.33 ± 0.25	1.41
A. chinensis	Jiangxi 79-1	traces[c]	traces		
	Golden king	0.10 ± 0.03**	traces		
	Kuimi	0.20 ± 0.10**	0.07 ± 0.04**	0.27 ± 0.14**	0.16
	Sanuki gold	0.07 ± 0.02**	traces		
	Hongyang	0.53 ± 0.17*	0.20 ± 0.07**	0.73 ± 0.24*	0.44
	Kobayashi39	0.26 ± 0.09**	0.08 ± 0.05**	0.34 ± 0.14**	0.21
	Hort16A	0.07 ± 0.05**	traces		
A. rufa	Awaji	2.83 ± 0.20**	1.37 ± 0.08**	4.20 ± 0.28**	2.55
	Nagano	2.41 ± 0.18**	1.18 ± 0.09**	3.59 ± 0.28**	2.18
A. arguta	Hirano	2.55 ± 0.89**	1.07 ± 0.28**	3.62 ± 1.17**	2.19
	Gassan	2.41 ± 0.65**	0.98 ± 0.19**	3.39 ± 0.83**	2.05
	Issai	2.32 ± 0.78**	0.99 ± 0.29**	3.31 ± 1.06**	2.01
	Mitsuko	3.00 ± 0.90**	1.21 ± 0.29**	4.21 ± 1.19**	2.55
	Ananasnaya	2.68 ± 0.67**	1.20 ± 0.16**	3.88 ± 0.80**	2.35
A. arguta × *A. deliciosa*	Kosui	1.99 ± 0.22**	0.92 ± 0.19**	2.91 ± 0.40*8	1.76
	Shinzan	3.15 ± 0.55**	1.24 ± 0.23**	4.39 ± 0.79**	2.66

[a] *,**, Significantly different vs Hayward at $P < 0.05$ and $P < 0.01$, respectively. [b] Values are means ± SD of eight experiments. [c] Concentration below the limit of detection (<0.05).

Table 4. Concentration of Lutein and *β*-Carotene in Fruit of *Actinidia* Genotypes[a]

species	genotype	lutein mg/100 g FW[a]	lutein ratio to Hayward	β-carotene mg/100 g FW[b]	β-carotene ratio to Hayward
A. deliciosa	Hayward	0.418 ± 0.082	1.00	0.088 ± 0.013	1.00
	Bruno	0.434 ± 0.063	1.04	0.094 ± 0.014	1.07
	Abbott	0.398 ± 0.101	0.95	0.085 ± 0.009	0.97
	Elmwood	0.569 ± 0.104	1.36	0.093 ± 0.017	1.06
	Koryoku	0.897 ± 0.138**	2.15	0.150 ± 0.036**	1.70
A. deliciosa × *A. chinensis*	Sanryoku	0.691 ± 0.056**	1.65	0.110 ± 0.015	1.25
A. chinensis	Jiangxi 79-1	0.107 ± 0.026**	0.26	0.115 ± 0.015	1.31
	Golden king	0.087 ± 0.015**	0.21	0.121 ± 0.017	1.38
	Kuimi	0.152 ± 0.032**	0.36	0.097 ± 0.019	1.10
	Sanuki gold	0.117 ± 0.012**	0.28	0.150 ± 0.037**	1.70
	Hongyang	0.404 ± 0.072	0.97	0.123 ± 0.016	1.39
	Kobayashi39	0.144 ± 0.025**	0.34	0.081 ± 0.007	0.92
	Hort16A	0.155 ± 0.030**	0.37	0.066 ± 0.008	0.75
A. rufa	Awaji	0.926 ± 0.050**	2.22	0.177 ± 0.012**	2.01
	Nagano	0.876 ± 0.061**	2.10	0.145 ± 0.017**	1.65
A. arguta	Hirano	0.786 ± 0.209**	1.88	0.224 ± 0.049**	2.54
	Gassan	0.746 ± 0.140**	1.78	0.227 ± 0.048**	2.58
	Issai	0.799 ± 0.178**	1.91	0.247 ± 0.019**	2.80
	Mitsuko	0.933 ± 0.214**	2.23	0.245 ± 0.033**	2.78
	Ananasnaya	0.762 ± 0.132**	1.82	0.285 ± 0.041**	3.23
A. arguta ×	Kosui	0.736 ± 0.074**	1.76	0.143 ± 0.007**	1.62
A. deliciosa	Shinzan	1.082 ± 0.172**	2.59	0.269 ± 0.022**	3.05

[a] *,**, Significantly different vs Hayward at $P < 0.05$ and $P < 0.01$, respectively. [b] Values are means ± SD of eight experiments.

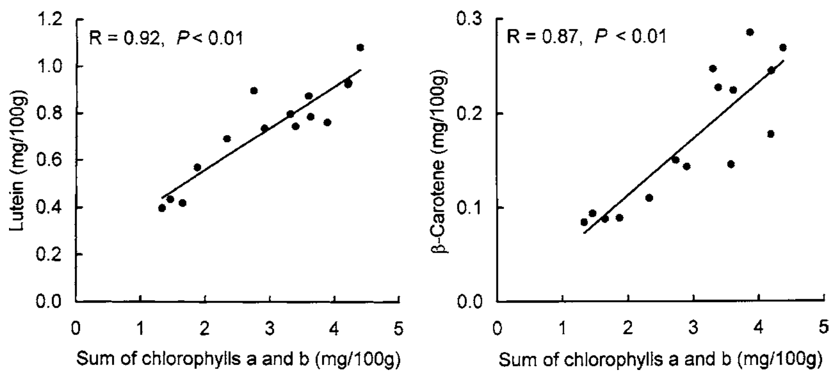

Figure 2. Lutein and *β*-carotene concentrations as a function of chlorophyll concentration in green-fleshed *Actinidia* genotypes.

In *A. rufa*, *A. arguta*, and their interspecific hybrids, both lutein and *β*-carotene contents were much higher than those in Hayward (**Table 4**). These fruits contained 0.736−1.082 mg/100 g FW lutein and 0.143−0.285 mg/100 g FW *β*-carotene. Lutein and *β*-carotene contents reached 1.76−2.59-fold and 1.62−3.23-fold of that in Hayward, respectively.

Figure 2 shows the correlation between chlorophyll and carotenoid contents in green-fleshed *Actinidia* genotypes. There was a significant positive correlation between the concentration of each carotenoid and that of chlorophylls ($P < 0.01$). It is possible that both chlorophyll and carotenoid contents depend mainly on the chloroplast density in these fruits. It may be possible that the lutein and *β*-carotene contents in the green-fleshed *Actinidia* fruits can be partially estimated by a visual inspection of the depth of the green color of the flesh.

Recent studies have strongly suggested that a higher dietary intake of lutein and/or zeaxanthin is associated with protection against age-related macular degeneration and cataract formation (*4*, *10*). Because lutein concentrations of commonly consumed fruits are estimated to be <0.15 mg/100 g FW (*22*, *23*), *A. arguta* fruits are considered to be the richest dietary source of lutein among commercially available fruits. Moreover, *A. arguta* fruits contain appreciable amounts of *β*-carotene, which is converted in vivo into vitamin A, a factor essential for normal function of the retina. The abundance of lutein, together with the presence of a substantial amount of *β*-carotene, suggests that *A. arguta* fruits may be good for eye health.

Recently, *A. arguta* fruits have become commercially available. In particular, Ananasnaya fruits are sold by popular names such as Baby kiwi, Kiwi berry, or Grape kiwi because they are completely hairless grape-sized fruits. They have a soft, edible skin and can be eaten whole without producing waste. The ripe fruits have a rich flavor and taste sweeter than Hayward. The high carotenoid content shown in the present study, together with the characteristics described above, make *A. arguta* fruits a very promising crop despite its relatively short storage life. Besides being eaten raw, *A. arguta* fruits can be processed into products such as jams and juices. During the processing, chlorophylls in the fruit are rapidly converted into pheophytins by the natural acidity of the fruit and thermal treatment, which results in a loss of the attractive green color (*24*). On the other hand, lutein is heat stable, and it is considered not to be seriously affected during processing (*25*). Therefore, processed products from these fruits are promising as a dietary source of lutein.

Each genotype in *A. rufa* and *A. arguta* is also important as a genetic resource for cultivar improvement. A high carotenoid content is an attractive attribute and one of the important quality parameters for *Actinidia* fruits. *A. rufa*, *A. arguta*, and their interspecific hybrids may be valuable genetic resources for the development of new kiwi fruit varieties that have higher carotenoid contents because interspecific hybridization techniques are available for *A. arguta* and *A. deliciosa* (*26*).

ACKNOWLEDGMENT

We are grateful to the staffs at Sawanobori Kiwifruit Farm and Kobayashi Farm for their generous gifts of the fruit samples.

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Received for review April 7, 2005. Revised manuscript received June 14, 2005. Accepted June 17, 2005.

JF050785Y