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Varietal Difference in Vitamin C Content in the Fruit of Kiwifruit and Other *Actinidia* Species

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Vitamin C content in the fruit of various cultivars of kiwifruit and other *Actinidia* species was estimated by determination of L-ascorbic acid and L-dehydroascorbic acid using ion-pair reversed-phase high-performance liquid chromatography. Fruit of *A. deliciosa* cv. Hayward, the most common commercially available cultivar, contained 65.5 mg/100 g fresh weight (FW) vitamin C. Vitamin C content in *A. deliciosa* fruit varied from 29 mg/100 g FW to 80 mg/100 g FW. In most cultivars of *A. chinensis*, vitamin C content in fruit was higher than that of Hayward. In particular, vitamin C content in cv. Sanuki Gold fruit reached more than 3-fold that of Hayward on a weight for weight basis. In *A. arguta* fruit, there was wide variation in vitamin C content, with concentrations ranging from 37 to 185 mg/100 g FW. In cv. Gassan, Issai, and Mitsuko, vitamin C content of the fruit was much higher than that of Hayward. In *A. arguta* fruit, the ratio of L-ascorbic acid to total ascorbic acid tended to be higher than that of other species.

KEYWORDS: ***Actinidia* spp.; kiwifruit; vitamin C; ascorbic acid; dehydroascorbic acid**

1. INTRODUCTION

An increased consumption of fruit and vegetables has been associated with reduced risks of certain diseases, including cancer and cardio- and cerebrovascular diseases (*1*−*3*). These beneficial effects have been attributed to the various antioxidants in these foods (*4*−*6*). One of the major antioxidants in fruits and vegetables is L-ascorbic acid (AA), which is better known as vitamin C by most consumers. Therefore, vitamin C is an attractive index of the quality of these foods both as a vitamin and as an antioxidant.

AA, the most active form of vitamin C, is a labile substance that is easily oxidized to L-dehydroascorbic acid (DHAA), mainly due to the activity of L-ascorbate oxidase and reaction with oxygen in the presence of heavy metal ions and light (*7*, *8*). Although DHAA itself does not exhibit vitamin C activity, its biological activity has been considered to be equivalent to AA, because it can be readily converted into AA in the human body (*9*). Vitamin C activity is lost when DHAA is further oxidized to 2,3-diketo-L-gulonic acid, because of the irreversibility of this reaction (*10*). Therefore, vitamin C content in the food is usually expressed as the sum of AA and its partially oxidized form, DHAA. According to Ogiri et al. (*11*), however, the antiscorbutic activity of orally administered DHAA was almost 10% of that of AA in inherently scorbutic ODS rat, a human model animal for studies of vitamin C metabolism. These observations suggested that AA and DHAA should be measured separately to evaluate vitamin C activity in foods properly.

Fruit is one of the major dietary sources for vitamin C in humans. Fruit of *Actinidia* species, as well as *Citrus* fruit, are excellent sources of vitamin C. The most widely grown *Actinidia* crop is the *A. deliciosa* cv. Hayward. The commercial growing of this variety has spread to many countries because of its distinctive characteristics, including fruit size, high productivity, and sufficient storageability (*12*). Although *Actinidia* fruit sold on the world market is dominated by those of a single cultivar, Hayward, there are a considerable number of cultivars and selections in the genus. They show a wide diversity in size and shape of fruit, hairiness, color of flesh, actinidin content, flavor, and taste (*13*, *14*). These various cultivars and selections are of commercial potential and/or are useful genetic resources for the development of new cultivars. These fruits also show a wide variation in vitamin C content (*15*). The purpose of the present study was to estimate vitamin C content of the fruit of various kiwifruit and other *Actinidia* cultivars by determining AA and DHAA separately.

2. MATERIALS AND METHODS

2.1. Chemicals. All compounds were of the highest quality available. Standard AA, metaphosphoric acid, ethylenediamine-*N*,*N*,*N*′,*N*′-tetraacetic acid (EDTA), tetrabutylammonium hydroxide and high-performance liquid chromatography (HPLC) grade methanol were purchased from Wako (Tokyo, Japan). Tris [2-carboxyethyl] phosphine

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Table 1. *Actinidia* Cultivars and Species Examined

cultivar	species	color of flesh	density of hairs	fruit weight (g)[a]
Hayward	*A. deliciosa*	green	dense	99.6 ± 14.4
Bruno		green	dense	101.3 ± 16.8
Abbott		green	dense	70.9 ± 10.3
Elmwood		green	dense	113.1 ± 10.7
Koryoku		deep green	dense	102.2 ± 20.4
Sanryoku		yellow green	sparse or absent	105.0 ± 21.4
Jiangxi 79-1[b]	*A. chinensis*	yellow	sparse or absent	91.9 ± 8.6
Golden King		yellow	sparse or absent	138.8 ± 18.9
Kuimi[c]		yellow	sparse or absent	98.8 ± 10.2
Sanuki Gold[d]		deep yellow	sparse or absent	168.7 ± 19.5
Hongyang[e]		yellow, partly red	absent	77.6 ± 2.6
Kobayashi39		yellow	sparse or absent	107.7 ± 14.1
Hort16A[f]		yellow	sparse or absent	104.4 ± 34.8
Awaji	*A. rufa*	deep green	absent	10.2 ± 1.1
Nagano		deep green	absent	19.0 ± 2.5
Kosui[g]	*A. arguta*	deep green	absent	37.1 ± 5.1
Shinzan		deep green	absent	21.9 ± 3.3
Hirano		green	absent	6.7 ± 1.4
Gassan		green	absent	11.6 ± 2.9
Issai		green	absent	9.0 ± 2.0
Mitsuko		green	absent	11.5 ± 2.8

[a] Values are means ± SD. $n = 36$ (*A. deliciosa* and *A. chinensis*) or $n = 60$ (*A. rufa* and *A. arguta*). [b] Synonymous with Koshin. [c] Synonymous with Applekiwi or Kaimitsu. [d] Tentative name in the process of registration. [e] Synonymous with Rainbow red. [f] Known commercially as ZESPRI GOLD kiwifruit. [g] Recent study using RAPD analysis suggested *A. rufa*, not *A. arguta*, is involved in the parentage.

(TCEP) and L-ascorbate oxidase (EC 1.10.3.3) were from Fluka (Buchs, Switzerland).

2.2. Fruit Materials. Fruit samples at eating-ripe stage of six *A. deliciosa*, seven *A. chinensis*, two *A. rufa*, and six *A. arguta* cultivars were used in the experiments. Fruits of cv. Abbott and Elmwood were obtained from Johoku Farm in Saitama and Sawanobori Kiwifruit Ranch in Tokyo, respectively. Fruits of Hongyang and Kobayashi39 were from Kobayashi Farm in Shizuoka. Fruit of Hort16A, which was imported from New Zealand, was purchased from local markets in Tokyo. Fruits of all other cultivars were obtained from the experimental orchards in Kagawa Agricultural Experiment Station. Some characteristics of the fruit of each cultivar are described in **Table 1**.

2.3. Water Content. Water content of fruit was assessed on three batches each containing three (*A. deliciosa* and *A. chinensis*) or five fruits (*A. rufa* and *A. arguta*). The fruit samples of each cultivar were peeled, and the edible portion, including seeds and cores, was cut into small pieces. The tissue was homogenized in a Waring blender for 30 s, and the water content was determined gravimetrically by oven-drying at 100 °C to a constant mass.

2.4. Extraction Method. Fruit extract for AA determination was prepared from 12 batches each containing three (*A. deliciosa* and *A. chinensis*) or five fruits (*A. rufa* and *A. arguta*). The edible portions of the fruit samples were homogenized as described above, and 10 g of the homogenate was combined with 20 mL of ice-cold 5% metaphosphoric acid containing 1 mM EDTA. The mixture was stirred in a Waring blender for 30 s to extract AA, and the suspension was centrifuged at 5000*g* at 4 °C for 10 min. The resultant supernatant was used for AA determination as described below. All the procedures for extraction of AA were carried out under chilled conditions to minimize undesirable oxidation of AA.

2.5. Sample Reduction. For the determination of total AA (TAA), the test solution was treated with TCEP to reduce DHAA to AA, as reported by Lykkesfeldt (*16*). In brief, 100 μL of sample solution was mixed with 400 μL of McIlvaine buffer (pH 4.5) containing 0.312 mM TCEP, and incubated in the dark at 20 °C for 90 min. The reduced samples were subjected to HPLC analysis as described below.

2.6. HPLC Analysis. AA was determined by ion-pair chromatography on an octadecylsilane (ODS) column according to the method of Daood et al. (*17*) 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 254 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. The isocratic mobile phase was a mixture of 1.55 mL of 0.5 M tetrabutylammonium hydroxide, 30 mL of methanol, and 970 mL of 0.01 M potassium dihydrogen phosphate solution (pH 4.0). The flow rate was 1.0 mL/min.

The test solution prepared as described above was diluted 1/5 or 1/10 with 1% metaphosphoric acid just before use, and 10 μL of the diluted sample was injected to an ODS column. The chromatographic peak corresponding to AA was identified by comparing the retention time with that of a standard. For confirmation, co-chromatography of standard AA with the sample was also applied. To check whether the AA peak contained any interfering materials, peak purity was tested by pretreating the fruit extract with 1 unit/mL L-ascorbate oxidase at 20 °C for 10 min. A calibration curve was prepared using the standard AA to determine the relationship between peak area and concentration. The AA standard solution was prepared daily in 5% metaphosphoric acid.

The AA concentration in the fruit was calculated in mg/100 g fresh weight (FW) according to the formula

$$AA_F = AA_E\,(10 \times W/100 + 20) \times 100/10$$

$$= AA_E\,(W + 200)$$

where AA_F = AA concentration in the fruit (mg/100 g FW), AA_E = AA concentration in the undiluted extract (mg/mL) and W = water content (%).

The concentration of DHAA in the fruit was calculated by subtraction of the measured AA concentration from that of TAA according to the following formula

$$DHAA = (TAA - AA) \times 174.11/176.13$$

where DHAA, TAA, or AA indicates the concentration of each molecule in the fruit (mg/100 g FW).

2.7. Statistical Analysis. The data were presented as mean ± SD of 12 determinations. Mann−Whitney *U*-test was used to compare AA or DHAA concentration in the fruit between each cultivar and Hayward, with P value less than 0.05 considered significant.

3. RESULTS AND DISCUSSION

3.1. Elution Profile of Fruit Extract. Figure 1a represents a chromatogram of Hayward fruit extract. AA was detected as a single peak with a retention time of 6.3 min. In other cultivars, the peak of AA was also clearly identified as in Hayward (data not shown). A reducing treatment using TCEP on the fruit extract did not produce any interfering peak in the chromatograms (**Figure 1b**). TCEP is a recently introduced reductant for reliable DHAA reduction and extended sample stability (*16*). Although dithiothreitol is a widely used reducing agent in biological studies, it is relatively unstable and inefficient as a reductant at low pH. TCEP is very stable and retains its reducing power even at pH 4 (*16*), where DHAA and AA are more stable than at higher pH.

An identical retention time was obtained for the authentic aqueous standard (**Figure 1c**). Co-chromatography of the sample with authentic AA also confirmed that the peak at 6.3 min found in the extract represents AA (**Figure 1d**). For confirmation of the peak identity, AA in the extract was enzymatically oxidized by incubating the samples with L-ascorbate oxidase, resulting in complete disappearance of the signal at 6.3 min (**Figure 1e**). The results demonstrated the homogeneity of the measured peak.

3.2. Water Content. Table 2 shows water content in fruit of various *Actinidia* cultivars. Water content tends to be lower in *A. arguta* than in other *Actinidia* species tested. These values

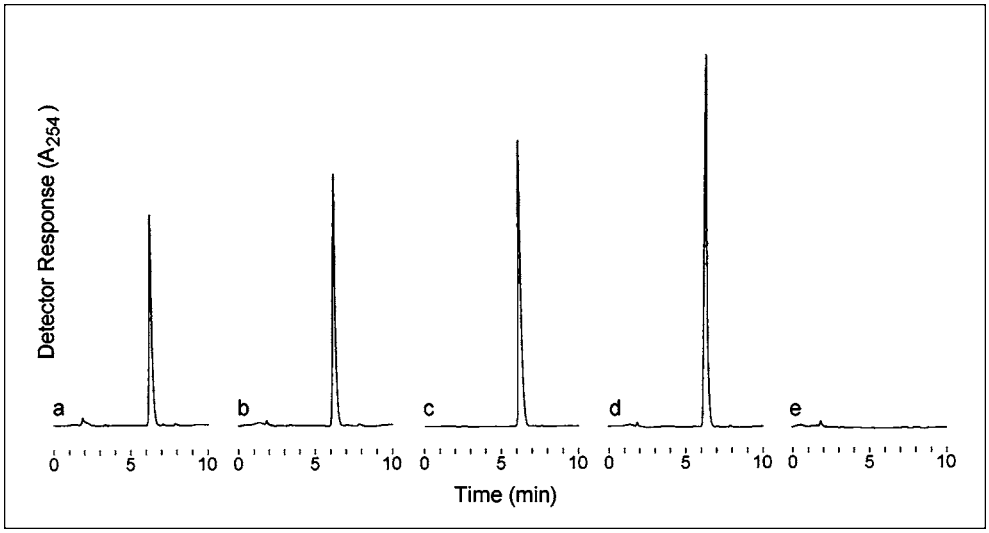

Figure 1. Chromatograms of fruit extract and standard: (**a**) Hayward fruit extract, (**b**) Hayward fruit extract reduced with TCEP, (**c**) aqueous standard of AA (20 μg/mL), (**d**) mixture of Hayward fruit extract and standard AA (10 μg/mL), and (**e**) Hayward fruit extract treated with ascorbate oxidase.

Table 2. Water Content in Fruit of *Actinidia* Cultivars

cultivar	water (%)[a]
Hayward	83.6 ± 0.33
Bruno	85.2 ± 0.37
Abbott	85.7 ± 0.21
Elmwood	86.8 ± 0.32
Koryoku	82.1 ± 0.71
Sanryoku	83.2 ± 042
Jiangxi 79-1	84.4 ± 0.63
Golden King	86.0 ± 0.41
Kuimi	82.9 ± 0.53
Sanuki Gold	86.1 ± 0.04
Hongyang	84.2 ± 0.34
Kobayashi39	86.3 ± 0.98
Hort16A	88.5 ± 1.38
Awaji	83.7 ± 0.50
Nagano	86.6 ± 0.47
Kosui	81.3 ± 0.61
Shinzan	88.1 ± 0.62
Hirano	77.3 ± 1.02
Gassan	78.5 ± 0.44
Issai	82.7 ± 0.77
Mitsuko	79.4 ± 0.66

[a] Values are means ± SD of three experiments.

Table 3. Concentration of Ascorbic Acid and Dehydroascorbic Acid in Fruit of *Actinidia* Cultivars

	concentration (mg/100g fresh weight)[a]			TAA ratio to	
cultivar	AA	DHAA	TAA	Hayward	AA/TAA
Hayward	55.0 ± 14.3	10.4 ± 3.5	65.5 ± 14.2	1.00	0.84
Bruno	69.4 ± 24.4*	10.5 ± 9.8	80.0 ± 16.8**	1.22	0.87
Abbott	14.0 ± 4.2**	15.0 ± 5.0	29.2 ± 5.5**	0.45	0.48
Elmwood	21.7 ± 10.2**	25.1 ± 7.1	47.0 ± 4.4**	0.72	0.46
Koryoku	21.9 ± 4.5**	17.8 ± 5.4	39.9 ± 4.0**	0.61	0.55
Sanryoku	50.8 ± 13.4	23.8 ± 5.6	75.0 ± 9.0	1.15	0.68
Jiangxi 79-1	25.9 ± 22.4**	47.3 ± 7.6	73.7 ± 19.9	1.13	0.35
Golden King	72.9 ± 25.1	70.5 ± 19.7	144.2 ± 16.5**	2.20	0.51
Kuimi	55.8 ± 46.9	100.0 ± 19.4	157.1 ± 36.4**	2.40	0.36
Sanuki Gold	156.2 ± 31.4**	49.0 ± 24.5	205.8 ± 19.8**	3.14	0.76
Hongyang	38.3 ± 18.2**	25.8 ± 10.4	64.4 ± 10.0	0.98	0.59
Kobayashi39	55.3 ± 36.3	72.9 ± 24.6	129.1 ± 16.2**	1.97	0.43
Hort16A	75.1 ± 17.0**	28.2 ± 14.3	103.7 ± 13.1**	1.58	0.72
Awaji	19.7 ± 3.7**	5.7 ± 2.4	25.5 ± 3.3**	0.39	0.77
Nagano	41.3 ± 8.0*	5.7 ± 3.0	47.1 ± 6.4**	0.72	0.88
Kosui	33.9 ± 9.9**	6.9 ± 2.1	40.9 ± 9.4**	0.62	0.83
Shinzan	87.9 ± 43.7*	11.7 ± 12.1	99.8 ± 32.1**	1.52	0.88
Hirano	29.3 ± 10.8**	7.9 ± 1.4	37.3 ± 10.9**	0.57	0.79
Gassan	129.7 ± 22.7**	11.1 ± 4.7	141.0 ± 24.2**	2.15	0.92
Issai	171.0 ± 18.5**	13.4 ± 10.8	184.6 ± 23.4**	2.82	0.93
Mitsuko	139.8 ± 28.6**	10.6 ± 8.4	150.6 ± 33.0**	2.30	0.93

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

were used for calculation of AA and DHAA concentration in the fruit of each cultivar, as described in Materials and Methods.

3.3. Varietal Difference in AA Content. There is wide variation in TAA content between and within species. *Actinidia* fruit tested in the present study contained 26−206 mg TAA/100 g FW (**Table 3**). These data have confirmed that *Actinidia* fruit are an excellent source of vitamin C. Fruit of *A. deliciosa* contained 29−80 mg TAA/100 g FW. Fruit of Hayward, the most common commercially available cultivar, contained 65.5 mg/100 g FW TAA, including 55.0 mg/100 g FW AA, for an AA/TAA ratio of 0.84. The 2000 Dietary Reference Intake values for vitamin C are 75 mg/day for adult females and 90 mg/day for adult males. Therefore, intake of an average-sized Hayward fruit a day supplies 87% for females or 72% for males of required vitamin C.

In this species, fruit of Abbott contained the lowest concentration of TAA, and fruit of Bruno contained the highest. In Abbott, Elmwood and Koryoku, AA/TAA ratios were much lower than that in Hayward.

In most fruits and vegetables, it is well-known that TAA content declines during storage; however, Ferguson and MacRae (*15*) reported that TAA content in *Actinidia* fruit, including *A. deliciosa*, *A. chinensis*, and *A. arguta*, showed little or no decline during post-harvest ripening. Therefore, the difference in TAA content shown here reflects varietal difference more so than difference in maturity or storage period.

In most cultivars of *A. chinensis*, TAA content is higher than that in Hayward (**Table 1**). In particular, Sanuki Gold, which was quite recently developed by the Fuchu Branch of Kagawa Agricultural Experiment Station, is extraordinary high in TAA. The TAA content reached more than 3-fold that in Hayward. Because the fruit of Sanuki Gold is very large (**Table 1**), the average-sized fruit contains 5.3-fold more TAA compared with Hayward (calculated from **Tables 1** and **2**). The TAA contents in fruit of Golden King, Kuimi, Kobayashi39, and Hort16A were also significantly higher than that of Hayward. In cultivars

of *A. chinensis*, AA/TAA ratios tended to be lower than that of Hayward. Therefore, AA content in fruit of Golden King, Kuimi and Kobayashi39 was not significantly higher than that of Hayward. At present, the reason for the low AA/TAA ratios in *A. chinensis* fruit is unknown. It may be that these fruits contain a significant amount of endogenous L-ascorbate oxidase, which catalyzes oxidation of AA to DHAA. Further experiments will be required for a solution to this problem.

In two *A. rufa* cultivars, both AA and TAA content were significantly lower than those in Hayward (**Table 3**); however, they were still excellent sources of vitamin C among commercially available fruit.

TAA content in *A. arguta* fruit showed wide variation within species (**Table 3**). While TAA content was relatively low in Kosui and Hirano, that in Gassan, Issai, and Mitsuko was much higher than that in Hayward. TAA content in these cultivars reached 2.2−2.8-fold that in Hayward on a weight for weight basis. Because these fruit are much smaller than those of *A. deliciosa* or *A. chinensis* (**Table 1**), intake of 5−6 average-sized fruit is required to supply enough vitamin C for a day. AA/TAA ratios in these three cultivars were also higher than those in *A. deliciosa*, and AA content reached 2.4−3.1-fold that in Hayward.

Although the biological activity of DHAA in vivo has been considered to be equivalent to that of AA, Ogiri et al. reported that the nutritional activity of orally ingested DHAA is no more than 10% that of AA (*11*). If this is the case, fruits of Gassan, Issai, and Mitsuko are much superior to *A. deliciosa* and to most of *A. chinensis* fruit as a source of vitamin C.

Recently, *A. arguta* fruits have become commercially available. They are sold by popular names such as "baby kiwi" or "grape kiwi", because they are completely fuzzless grape-sized fruit. Their skin is edible and they are easily eaten without peeling, producing no waste. *A. arguta* fruits are generally highly aromatic and taste much sweeter than Hayward. The high vitamin C content shown in the present study, together with the characteristics described above, make *A. arguta* fruit a very promising crop.

Commercial production of *A. arguta* fruit is now underway on a small scale in the United States, Europe, New Zealand, and South America. New selections of *A. arguta* are currently being commercialized in New Zealand (*18*). They are also produced on a small scale in Japan. Most of the fruit is consumed locally for eating raw; some of the fruit however, is processed into juice, wine, jam, and soft ice cream in some areas in North Japan.

The genus *Actinidia* contains more than 60 species, but only three of them have been successfully domesticated. At present, only a few cultivars are commercially available. Other cultivars or selections have commercial potential. High vitamin C content is one of the important and attractive quality parameters of *Actinidia* fruit; therefore, proper evaluation of vitamin C content is required for selection or development of new cultivars. Because it was demonstrated that interspecific hybridization techniques are available for *A. arguta* and *A. deliciosa* (*19*), some *A. arguta* cultivars, including Gassan, Issai, and Mitsuko, may be useful genetic resources for the development of new kiwifruit varieties with a higher content of vitamin C.

ABBREVIATIONS USED

AA, L-ascorbic acid; DHAA, L-dehydroascorbic acid; EDTA, ethylenediamine-*N*,*N*,*N*′,*N*′-tetraacetic acid; HPLC, high-performance liquid chromatography; TAA, total L-ascorbic acid; TCEP, Tris [2-carboxyethyl] phosphine

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