Ascorbic Acid Content in Five Yellow-Flesh Kiwifruit Genotypes

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Abstract. In this study, the content of ascorbic acid (AsA) in five kinds of yellow-flesh kiwifruit genotypes was determined by high performance liquid chromatography. Content of AsA and GSH involved in AsA-GSH cycle were compared in different yellow flesh kiwifruit genotypes. The results indicated that the AsA levels changed remarkably in different yellow flesh kiwifruit genotypes, and the AsA levels in ‘Fengyue’ and ‘Guihai 4’ were higher.

1. Introduction

Kiwifruit belongs to Actinidiaceae Actinidia Lindl, originated in China. Kiwifruit riches in Vc and has high nutritional value, known as “the king of fruits” [1]. Ascorbic acid (AsA) is a high-abundance small molecule antioxidant that is commonly found in plant tissues [2-4]. AsA is not only an essential substance for maintaining human health, but also has important physiological functions for the plant itself. Ascorbic acid is an important antioxidant and a cofactor of many enzymes in organisms and plays an important role in plant growth and development and its resistance to stress [3]. The AsA content of plants is highly regulated by its own biosynthetic capacity, and it has been confirmed that L-galactose pathway is the main pathway for AsA synthesis [5-7]. In this study, we determined the AsA content of five yellow flesh kiwifruit genotypes using high performance liquid chromatography (HPLC).

2. Materials and methods

2.1. Plant material

Five yellow kiwifruit genotypes used in this study were harvested from Kiwifruit Resource Orchard in Shifang (104°16’N, 31°13’E), Chengdu, China. Guihai 4, Jinshi 2, Fengyue, Jinnong and Hort 16A kiwifruit all belong to A.chinensis. Fruits were selected according to the uniformity of the shape when samples have reached physiological maturity (total soluble solid content was 7-8%). At least 10 fruits were harvested for every sample. Prior to preparation of the test samples, the fruit samples were exposed to room temperature to reach easting maturity (total soluble solid content was 10-11%). These fruits were chopped and homogenised under liquid nitrogen in a high-speed blender for 1 min, then immediately frozen in liquid nitrogen and stored at -80°C until use.
2.2. Assays for AsA
Frozen tissue (0.5g) was added to 3ml of 0.2% metaphosphoric acid and ground. The homogenate was centrifuged and the supernatant was diluted with 0.2% metaphosphoric acid to 10ml and used for AsA determination. To determine the total AsA (T-AsA) level, method as described by Li et al was used. [8-9]. Thus, a 1000μl aliquot of supernatant was incubated for 4h in the dark with10μl of 200mM dithiothreitol (DTT). AsA was determined as described by Huang et al. [10] and Zhang et al. [11] via a high performance liquid chromatography (HPLC) with system with a photodiode array detector, Chromeleon software (Dinex), and a reverse C18 column. The mobile phase was composed of 15% methanol and 85% metaphosphoric acid aqueous solution, pH2.5. The column temperature was set at 35℃. Spectra were acquired at wavelengths between 200 and 400nm and AsA quantification was performed at 243nm.

3. Results and discussion

3.1. T-AsA and AsA levels
As is shown in Figure 1, T-AsA and AsA levels showed great differences in flesh of 5 kiwifruit genotypes. Values for T-AsA and AsA ranged from 14.23 (Hort 16A) to 31.51 μmol/g FW (Guihai 4) and 6.49 (Hort 16A) to 15.28 μmol/g FW (Guihai 4). The T-AsA and AsA content of ‘Guihai 4’ was significant higher than that of other genotypes, while the lowest value for this parameter was found in ‘Hort 16A’. Meanwhile, the results indicated that T-AsA and AsA levels of ‘Guihai 4’ kiwifruit were higher than ‘Hort 16A’ kiwifruit. The ratio of AsA/DHA in ‘Fengyue’ kiwifruit was more than one, but that in other genotypes was less than one. The ratio of AsA/DHA results showed that the AsA in ‘Fengyue’ kiwifruit flesh was mainly in the reduced state, while that in other kiwifruit genotypes was chiefly in the oxidation state (Figure 1).

Figure 1 Content of T-AsA, AsA and ratio of AsA/DHA in 5 yellow flesh kiwifruit genotypes.
3.2. T-GSH and GSH levels
Values for T-GSH and GSH differed significantly among kiwifruit genotypes, ranging from 0.41 (Jinshi 2) to 0.92 μmol/g FW (Fengyue) and 0.21 (Jinshi 2) to 0.54 μmol/g FW (Fengyue) in flesh (Figure 2). ‘Fengyue’ contained the highest contents of T-GSH and GSH in flesh whereas the lowest values were measured from ‘Jinshi 2’. The ratios of GSH/GSSG of ‘Fengyue’, ‘Jinnong’ and ‘Hort 16A’ kiwifruit were all more than one, while that of ‘Jinshi 2’ kiwifruit was less than one, and that of ‘Guihai 4’ kiwifruit was 1.0015. The ratio of GSH/GSSG results showed that the GSH in ‘Fengyue’, ‘Jinnong’ and ‘Hort 16A’ kiwifruit flesh were mainly in the reduced state, while that in ‘Jinshi 2’ kiwifruit was chiefly in the oxidation state (Figure 2). There was no correlation between the contents of T-AsA, AsA, T-GSH and GSH, as discovery in apple [12] and persimmon [13]. These results indicated that GSH content would not be a key factor of controlling AsA content.

Figure 2 Content of T-GSH, GSH and ratio of GSH/GSSG in 5 yellow-flesh kiwifruit genotypes.

4. Conclusion
The content of AsA and GSH involved in AsA-GSH cycle were compared in different yellow flesh kiwifruit genotypes. The results indicated that the AsA levels changed vary remarkably in different yellow flesh kiwifruit genotypes, and the AsA levels of ‘Fengyue’ and ‘Guihai 4’ kiwifruit were higher. The AsA content of different kiwifruit genotypes is also different.

References
[1] Possingham J V 1991 Kiwifruit science and management Scientia Horticulture vol 1-2, ed I J Warrington and G C Weston p 171
[2] Noctor G and Foyer C H 1998 Ascorbate and glutathione: keeping active oxygen under control Annu. Rev. Plant Physiol. Plant Mol. Biol. vol 49 p 249-79
[3] Davey M W, Monatgu M V and Sammatin M 2000 Plant L-ascorbic acid: chemistry, function, metabolism, bioavailability and effects of processing Journal Science of Food and Agriculture vol 80 p 825-60
[4] Smirnoff N 1996 The function and metabolism of ascorbic acid in plants Ann. Bot. vol 78 p 661-9
[5] Wheeler G L, Mark A and Smirnoff N 1998 The biosynthetic pathway of vitamin C in higher plants Nature vol 393 p 365-9
[6] Hancock R D and Viola R 2005 Biosynthesis and catabolism of L-ascorbic acid in plants *Critical Reviews in Plant Sciences* vol 24 p 167-88

[7] Linster C L, Gomez T A, Christensen K C and et al 2007 *Arabidopsis* VTC2 encodes a GDP-L-galactose phosphorylase, the last unknown enzyme in the Smirnoff-Wheeler pathway to ascorbic acid in plants *Journal of Biology Chemistry* vol 26 p 18879-85

[8] Li M J, Chen X S, Wang P P and Ma F W 2011 Ascorbic acid accumulation and expression of genes involved in its biosynthesis and recycling in developing apple fruit *Soc. Hort. Sci.* vol 4 p 231-8

[9] Li M J, Ma F W, Liang D and et al 2010 Ascorbate biosynthesis during early fruit development is the main reason for its accumulation in kiwi *PLoS ONE* vol 5 p 14281

[10] Huang M, Xu Q and Deng X X 2014 L-Ascorbic acid metabolism during fruit development in an ascorbate-rich fruit crop chestnut rose (*Rosa roxburghii* Tratt) *J. Plant Physiol.* vol 171 p 1205-16

[11] Zhang C M, Huang J and Li X G 2016 Transcriptomic analysis reveals the metabolic mechanism of L-ascorbic acid in *Ziziphus jujuba* Mill *Front. Plant Sci.* vol 7 p 122

[12] Davey M W and Keulemans J 2004 Determining the potential to breed for enhanced antioxidant status in *Malus*: mean inter- and intravarietal fruit vitamin C and glutathione contents at harvest and their evolution during storage *J. Agric. Food Chem.* vol 52 p 8031-8

[13] Li M J, Liang D, Pu F and et al 2009 Ascorbate levels and the activity of key enzymes in ascorbate biosynthesis and recycling in the leaves of 22 chinese persimmon cultivars *Sci. Hort.* vol 120 p 250-6