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Kiwifruit cultivar 'Halla gold' functional component changes during preharvest fruit maturation and postharvest storage

You Jin Lim, Seok Hyun Eom*

Department of Horticultural Biotechnology, College of Life Sciences, Kyung Hee University, Yongin 17104, Republic of Korea

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ABSTRACT

There is an increasing interest in the use of immature fruit for its health benefits; however, little is known about changes in the molecular composition of functional compounds during the stages of fruit maturation and over their postharvest ripening process. This study investigates the changing content levels of key functional molecules in the golden kiwifruit cultivar harvested between 70 and 160 days after full blooming (DAFB) and stored at 4 °C for 9 weeks. We found that mature fruit contained higher levels of total phenolics, total flavonoids, and vitamin C, while having lower levels of pigments such as lutein, β-carotene, 9'-*cis*-neoxanthin, and pheophytins. Lutein and β-carotene content was maintained during postharvest storage regardless maturity at harvest, with the notable exception of β-carotene which increased dramatically between 3 and 6 weeks of storage in fruit harvested 160 DAFB. Other pigments tended to decrease during ripening and maturation. Overall these results suggest that less mature kiwifruits are likely to be a better source health-beneficial pigment molecules like lutein and β-carotene.

1. Introduction

The main functional molecules in fruits can change significantly during maturation and storage; this is particularly true of climacteric fruit, such as kiwifruit. Kiwifruit has been shown to change in both the accumulation and degradation of bioactive molecules during pre-harvest maturation as well as post-harvest storage. Soluble solids content (SSC) and dry matter of kiwifruits have previously been used as important indicators for assessing harvest time, edible texture, and postharvest management (Scott et al., 1986; Burdon et al., 2004; Famiani et al., 2012). It has commonly known that the optimal harvesting time of kiwifruit for fresh consume is when the fruit has 6.2–6.5°brix soluble solids content and 15–20% dry matter (Burdon et al., 2016), and the optimal storage conditions for commercial distribution are 95% relative humidity and 1 °C storage (Crisosto and Kader, 1999). The storage condition can prolonged shelf life of kiwifruits.

Previous studies demonstrated that many bioactive components found in kiwifruits, such as vitamins, carotenoids, phenolic compounds, and flavonoids, undergo varying degrees of accumulation and/or degradation during fruit maturation and storage (Ampomah-Dwamena et al., 2009; Burda et al., 1990; Kulkarni and Aradhya, 2005). Vitamin C, a major antioxidant molecule found in fruits, provides a valuable benchmark for a more general estimate of a fruit's nutritional value. Vitamin C concentrations in kiwifruits range from 20 mg to over 1000 mg per 100 g fresh weight, depending on the species. It was reported that the content of 'Hayward' kiwifruit is about 85 mg per 100 g fresh weight (Ferguson and MacRae, 1991). The vitamin C content of climacteric fruits, such as kiwifruits, has been found to either increase or decrease during the maturation process, depending on harvest point and on storage conditions (Lee and Kader, 2000). Additionally, other major carotenoid pigments, including lutein (non-provitamin A) and β-carotene (provitamin A), are found in significant quantities in kiwifruit (Cano, 1991; Ampomah-Dwamena et al., 2009). Golden kiwifruit cultivars bred from *Actinidia chinensis* have been used in several studies of carotenoid changes, since their color change is evident during fruit maturation (Cano, 1991; Ampomah-Dwamena et al., 2009). Lutein and β-carotene act as antioxidants and have been shown to protect cells from reactive oxygen species, particularly in ameliorating eye-related diseases (Sies and Stahl, 1995; Granado et al., 2003). Carotenoids have been found to vary between pre- and post-harvest in various horticultural crops (Bergquist et al., 2006; Lee, 1986; Lima et al., 2005; Montefiori et al., 2009; Rodrigo and Zacarias, 2007). For example, preharvest kiwifruits have been found to contain lower lutein levels compared to mature fruit, reaching a maximum level around 90 DAFB (Ampomah-Dwamena et al., 2009). β-carotene levels in *A. chinensis* have been shown to increase until peaking 150 DAFB (Ampomah-Dwamena et al., 2009).

Most fruit quality research to date has focused on kiwifruit

* Corresponding author.
E-mail address: se43@khu.ac.kr (S.H. Eom).

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harvested at late maturity and after the postharvest ripening process, as this is the type most commonly consumed. On the other hand, the focus of research must be broadened to examine other stages of maturity in order to optimize functional molecule levels for industrial extraction, like the use of polyphenol extracts from by-products of green kiwifruit juicing (Sun-Waterhouse et al., 2009). Kiwifruit, which has antioxidant and anti-inflammatory activity (Farr et al., 2007; Nishiyama et al., 2004), has been used industrially as a raw material for cosmetics and health functional food products. Many industrially processed products utilize kiwifruits harvested at many different stages of maturity and storage.

This study thus seeks to characterize the changes in fruit content of major functional molecules during preharvest maturation and postharvest storage in an *A. chinensis* cultivar, in order to determine the optimal harvest timing to maximize the content of bioactive compounds for industrially processed products. Although there are certainly many material changes that occur during fruit maturation, studies that follow their levels during multiple stages across pre- and post-harvest maturation and storage remain rare.

2. Materials and methods

2.1. Harvest and postharvest of kiwifruits

Korean-bred *A. chinensis* 'Halla gold' were produced at the Research Institute of Agriculture for Climate Change in Rural Development Administration of Korea in 2015 (Jeju, Korea), which is located at 33° 28′ 7.32″ N 126° 31′ 4.58″ E. Fruits were harvested from the seven-year-old kiwifruit trees. Five fruits per plant were harvested every 30 days from 70 to 160 DAFB from 5 plants. Harvested fruits were kept in a 4 °C chamber controlled at 50–60% relative humidity to rapidly identify phytochemical changes in the fruit harvested in both immature and mature states by stimulating post-harvest ripening although the storage condition has not been applied in the industry of actual kiwifruit storage as we mentioned above. Five stored fruit samples were collected every 3 weeks and analyzed for fruit quality and functional molecule composition.

2.2. Measurement of fruit quality

The flesh of each sample fruit was obtained by peeling and removing the skin within 1mm thickness for quality assessment. Five peeled fruit was ground using a commercial grinder for measurement of SSC. The ground fruit samples were centrifuged at 12,000 rpm for 10 min and the SSC was measured with the supernatant using a handled refractometer (Atago Co., Tokyo, Japan) and is described as degree of brix (°Bx). SSC was measured in 5 replicates. Five fruits were used for firmness measurement and flesh firmness was measured at three points in the central part of fruit per fruit using a handheld fruit penetrometer with 5 mm probe (Takemura Denki Seisakusho Ltd., Tokyo, Japan) and expressed in kilograms. Dry matter was investigated in five fruits and was examined after flesh slices that were cut the middle portion of fruits to 2 mm of thickness horizontally and dried at 65 °C for 24 h. Kiwifruit horizontal cross-sections are shown to illustrate the major morphological changes (Fig. 1).

2.3. Crude extraction procedure

Peeled fruits were homogenized in bulk using a commercial grinder and 50 g of ground samples was dissolved into 250 mL of 80% ethanol (v/v). Extraction was performed in three replicates. After 24 h, solid debris was removed by filtration with whatman No.1 filter paper (Whatman International Ltd., Maidstone, UK). This extraction was performed three times per sample over 24 h at room temperature. Excess solvent was removed using a vacuum rotary evaporator (Eyela Co., Tokyo, Japan) at temperatures above 40 °C. Final soluble extracts were lyophilized and stored at 4 °C until experiments were performed.

2.4. Assessment of vitamin C content

Kiwifruit vitamin C content was assayed by reverse-phase HPLC (Waters 2695 Alliance HPLC, Waters, Milford, MA, USA) using an octadecylsilane column (Hypersil GOLD C18 column (5 µm, 150 × 4.6 mm), Thermo scientific, MA, USA) based on previously published methods (Nishiyama et al., 2004; Nováková et al., 2008). The mobile phases consisted of 2% (v/v) formic acid in HPLC grade water (solvent A) and 2% (v/v) formic acid in HPLC grade acetonitrile (solvent B). We next performed a gradient elution on the mobile phases at a flow rate of 1.0 mL/min, starting with 0–5% solvent B for 1 min, 5–9.4% solvent B for 4 min. We quantified serial sample concentration using an L-ascorbic acid standard (Daejung Chemicals & Metals Co., Siheung, Korea). After peeling, all fresh fruit was homogenized using a commercial blender. The homogenate (2 g) was then mixed in 40 mL of ice-cold 8% acetic acid. The mixture was shaken for 90 s and centrifuged at 10,000 rpm at 4 °C for 10 min. The supernatant was filtered through a 0.45 µm membrane filter (Syringe filter, Toyo Roshi Kaisha Ltd., Tokyo, Japan) and used for all subsequent analyses.

2.5. Total phenolics (TP) and flavonoid (TF) content

TP content was determined using previously published methods (Singleton and Rossi, 1965) with minor modifications. Briefly, 0.2 mL of kiwifruit extract was mixed with Folin- Ciocalteu's phenol reagent (Sigma-Aldrich Co., St. Louis, MO, USA) along with 2.6 mL of distilled water. After 6 min, 0.2 mL of 7% Na_2CO_3 was added. Samples were then incubated for 90 min at room temperature and 750 nm absorbance was measured using a spectrophotometer (S-4100; Scinco Co., Seoul, Korea). Total phenolic content is described in gallic acid equivalent (GAE) per 100 g fresh weight (F.W.).

TF content was determined using a previously described colorimetric method (Zhishen et al., 1999) with minor modifications. Briefly, 0.5 mL of kiwifruit extract was combined with 0.15 mL of 5% $NaNO_2$ and mixed in with 3.2 mL distilled water. After 5 min, 0.15 mL of 10% $AlCl_3$ and 1.0 mL of 1 M NaOH were also added. Absorbance was measured immediately at 510 nm using a spectrophotometer (S-4100, Scinco Co.). TF content is described as catechin equivalent (CE) per 100 g F.W.

2.6. Pigment analysis using reversed-phase HPLC

Lutein, β-carotene, 9'-*cis* -neoxanthin, pheophytin b, pheophytin a, chlorophyll *b*, and chlorophyll *a* were assayed using previously published methods (Nishiyama et al., 2005) with minor modifications. Fresh fruit was peeled and homogenized with Na_2CO_3 (2 g per 100 g fruit) using a commercial blender. The homogenate (5 g) was then submerged and mixed with 30 mL of 80% aqueous acetone (v/v). After actively mixing for 1.5 min, the supernatant was then filtered using sterilized cheese cloth. The extraction procedure was repeated three times until the solvent-containing mixture appeared colorless. Extracts were centrifuged at 12,000 rpm at 4 °C for 2 min and the supernatant was then filtered through a 0.45 µm membrane filter (Syringe filter, Toyo Roshi Kaisha Ltd.) before further analysis. Extracts were analyzed using reversed-phase HPLC (Waters 2695 Alliance HPLC; Waters Inc.) with an octadecylsilane column (Prontosil 120-5-C18-ace-EPS (5 µm, 250 × 4.6 mm), Bischoff, Leonberg, Germany). The mobile phase flow rate was 1.0 mL/min. The mobile phases were determined to be a combination of (A) water/acetonitrile (10:90 v/v) with 0.1% formic acid and (B) ethyl acetate with 0.1% formic acid. Subsequent gradient elution was performed as follows; 0 to 60% of solvent B for 10 min, and maintained at 60% solvent B for 15 min. Sample injection volume was 20 µL. Peaks were monitored at 445 nm with a Waters 996 photodiode array detector (PDA; Waters Inc.). HPLC grade solvents were used for