Original Article

Journal of Apiculture 32(2) : 133~138 (2017)
DOI: 10.17519/apiculture.2017.06.32.2.133

Antioxidant Activity, Total Phenolics and Vitamin C Contents of the Unripe and Ripe Fruit of Hardy Kiwi (*Actinidia arguta*) 'Saehan' as Honey Plant

Youngki Park*

Department of Forest Genetic Resources, National Institute of Forest Science, Suwon 16631, Korea

(Received 27 June 2017; Revised 29 June 2017; Accepted 29 June 2017)

Abstract

The aim of this investigation was to find the knowledge of the changes of physiochemicals associated with fruit quality, vitamin C, total phenolics and antioxidant properties during fruit ripening. Changes in antioxidant activity of *Actinidia arguta* fruit of Saehan cultivar was studied at different ripening stages. Antioxidant activities (free-radical scavenging activity and reducing power) were determined and the total phenolic contents, and vitamin C contents were also analyzed. The highest free-radical scavenging activity (at 100ug/ml) and reducing power (at 100ug/ml) in *A. arguta* fruit were 45.43% and 0.14, respectively. Total phenolic content and vitamin C content in fruit of 40 days after fruit set were 540.2ug/g and 1238.0ug/g, respectively. In general, the antioxidant activity and the related parameters, including total phenolic content and vitamin C content decreased during fruit ripening. These results improve knowledge of the effect of ripening on the antioxidant activity and related compounds contents that could help to establish the optimum *A. arguta* fruit harvest data for various usages.

Key words: *Actinidia arguta*, Antioxidant capacity, Free radical scavenging activity, Total phenolics, Vitamin C content

INTRODUCTION

Free radicals in the form of superoxide radical, hydroxyl radical and singlet oxygen are molecules having an unpaired electron in the outer orbit and are unstable and reactive. They appear to be an important factor in cellular degeneration included aging. Antioxidant compounds are produced by the plant to protect the cell against the attack from other cellular chemical species as free radicals and reactive oxygen species (Ferreyra *et al*., 2007). Antioxidants act by neutralizing free radical activity. The capacity to neutralize free radical activity is based on the properties of a group of enzymes and phenolic compounds of various chemical structures (eg. catechins, flavonols) and vitamins (C, E, and A) (Fang *et al*., 2002). While there are some data on the constituents and biological activities of *A. arguta* fruits, there are no studies of the changing chemical composition and antioxidant activity of *A. arguta* fruits during ripening.

Actinidia arguta (Sieb. & Zucc.) called hardy kiwi, can be used for fruit tree and honey plants (Ryu, 2003). The fruit has peel without hair and an edible smooth skin. The fruit of hardy kiwi can be eaten whole and contains high amounts of sugar, vitamin C (ascorbic acid) and distinctive flavor (Kim *et al*., 2006). From the flower of *A. arguta,* lilac alcohol epoxide was isolated and identified (Matich *et*

*Corresponding author. E-mail: woodpark@korea.kr

al., 2003). Additionally, a hardy kiwifruit is called a healthy fruit because it has a lot of vitamin C, lutein, phenolics, and some minerals, such as P, Ca, and Zn (Nishiyama *et al*., 2004). It is native to north China, Korea, and Japan. Because of its high frost resistance, it may be commercially cultivated in countries whose climate is colder, not suitable for *A. deliciosa* cultivation. To make new cultivar with larger fruit and high yield, we have selected new *A. arguta* cultivars, Seahan, Daesung, and Chilbo and registered as a new variety denomination and certificated variety production and merchandising in 2013 (Park *et al*., 2007).

In this study, we report the changes in the overall antioxidant properties of *A. arguta* fruit during ripening, for the first time. We also evaluated the changes of the total phenolic contents and vitamin C content during fruit ripening.

MATERIALS AND METHODS

Sample collection and preparation

A. arguta fruits of Saehan cultivar, grown in the National Institute of Forest Science (Suwon) were utilized. A voucher specimen was deposited at the National Institute of Forest Science, Suwon, Korea. Five samples were collected according to their maturity in this study. Among five samples, four samples were collected at 30-day intervals and the last sample was used when the fruits were matured at room temperature after 5 days. Following five stages were chosen at various stages of fruit ripening (Fig. 1): Stage 1. collected 10 days after fruit set, Stage 2. collected 40 days after fruit set, Stage 3. collected 70 days after fruit set, Stage 4. collected 100 days after fruit set, Stage 5. stored for 5 days at room temperature for mature. To characterize the ripening stages of the fruit utilized here, weight and diameter of fruits were determined (Table 1). Freeze-dried *A. arguta* fruits were finely ground and extracted with ethanol (EtOH) at 60°C for 30 min and then evaporated to give the crude extract.

Free radical scavenging activity

The antioxidant activity was measured by the DPPH (1, 1-diphenyl-2-picrylhydrazyl) method according to the procedure of Park *et al*. (2006). Free radical scavenging activity of extracts from the fruits was evaluated by the colorimetrical decrease in the absorbance of DPPH due to the chemical trapping of unpaired electron. Ethyl alcohol soluble fraction (0.5mL) of samples at various concentrations (50, 100 and 125 ppm) was added to a solution of DPPH in EtOH (100μM, 3mL) and the reaction mixture were shaken vigorously. After incubating the mixtures for 10 min at room temperature, the remaining amounts of DPPH were determined by colorimetry (852A Diode Array Spectrophotometer, Hewlett Packard Co.) at 517nm. The mixture of 0.5mL EtOH with a solution of 3mL DPPH was used as control. The mean values were obtained from triplicate experiments.

Reducing power

The reducing power was determined according to the method of Oyaizu (1986). Each extracts (100μg/mL and 50μg/mL) in EtOH (2.5mL) was mixed with 2.5mL of 0.2 M sodium phosphate buffer (pH 6.6) and 2.5mL potassium ferricyanide (10mg/mL), the mixture then incubated at 50°C for 20 min. After 2.5mL trichloroacetic acid (100mg/mL) was added, the mixture was centrifuged at 4,000 rpm for 10 min. The upper layer (5mL) was mixed with 5mL distilled water and 1mL ferric chloride (1mg/mL). The absorbance was measured at 700nm. Increased absorbance of the reaction mixture indicated increased reducing power.

Total phenolic contents

Total phenolic contents were measured according to the method of Cheung *et al*. (2003). Each sample (1mL) was mixed with Folin and Ciocalteu's phenol reagent (1mL, Sigma). After 3 min, 1mL of saturated Na_2CO_3 was added

Table 1. Characterization of *A. arguta* fruit ripening stages by fruit weight and diameter (The values are mean±SD, n=3)

Stage	Days after fruit set (Dates)	Weight (g)	Diameter (cm)	W/D*
Stage 1	10 (June-17)	5.07±0.51	2.23±0.24	2.27±0.07
Stage 2	40 (July-17)	9.39±0.96	3.15±0.14	2.98±0.32
Stage 3	70 (Aug.-16)	17.17±1.19	3.67±0.13	4.67±0.17
Stage 4	100 (Sep.-15)	17.50±2.50	3.68±0.46	4.76±0.24
Stage 5	Mature	17.30±1.19	3.66±0.26	4.73±0.55

*W/D means the ratio of weight and diameter.

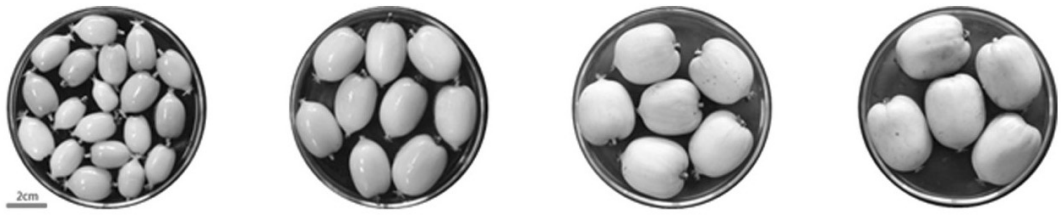

Fig. 1. Morphological characteristics of collected *A. arguta* fruit during ripening.

to the mixture and it was made up to 10mL by adding distilled water. After the reaction was kept in the dark for 90 min, absorbance was taken at 725nm. A calibration curve was constructed with different concentrations of gallic acid (Wako pure chemical Industries) (0.01-0.1mM) as a standard. Total phenolic contents were expressed as gallic acid equivalents (mg GAE/g extract).

Vitamin C content

Vitamin C (L-ascorbic acid) was determined by a colorimetric method defined by Jagot and Dani (1982). A 0.5g sample of dried fruits was weight and extracted with distilled water then filtered. 0.2mL extracts was mixed with 0.8mL 10% (w/v) trichloroacetic acid (TCA) at 4°C After centrifugation at 3000 rpm for 5 min, 0.5mL of supernatant was made up to 2mL volume with distilled water. 0.2mL 10% (v/v) Folin phenol reagent was then added to the mixture, and vigorously shaken. After 10 min reaction time, maximum absorbance was measured at 760nm. The absorption maximum of the color developed by the interaction of ascorbic acid with Folin reagent is 760nm.

RESULTS AND DISCUSSION

Morphological characteristics

The weight and diameter along the various ripening stages considered here was showed in Table 1. The weight and diameter of *A. arguta* Saehan cultivar fruit were increased with the progress of ripening and the weight/diameter ratio (W/D) of each maturity stage was also increased. Because of the dryness of fruit, the end of stage, there was an decrease in fruit weight and diameter. The morphological characteristics of *A. arguta* fruit during ripening was showed in Fig. 1.

Antioxidant activity

The free radical scavenging activities of *A. arguta* fruit during ripening are shown in Fig. 2. The activities decreased during the ripening of fruit, and the highest free-radical scavenging activities of Saehan cultivar was 45.43% at 100μg/mL. The antioxidant activity of the fruit at each maturity stage appeared to be concentration dependent.

Because the antioxidant activity of fruits is important for assessing their nutritional value (Rice-Evans *et al*., 1996),

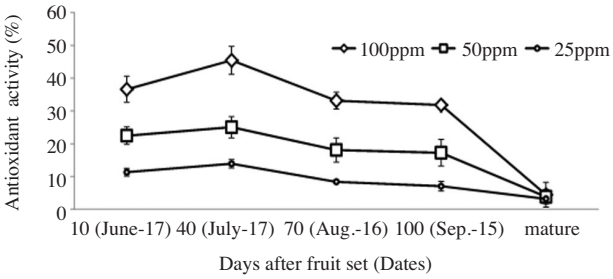

Fig. 2. Free radical scavenging activity of the *A. arguta* fruit during ripening in different concentration. The values are mean±SD (n=3).

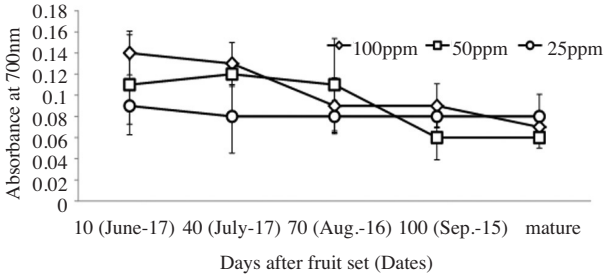

Fig. 3. Reducing power of the *A. arguta* fruit during ripening at 100μg/mg. The values are mean±SD (n=3).

the free radical scavenging activity of fruit was measured. Free radicals are chemical fragments that cause oxidation and antioxidants act as free radical scavengers. According to Beltran *et al*. (2005), the antioxidants and the related parameters decreased as olive fruit ripened. Similar results were also reported by Ferreyra *et al*. (2007) in strawberry fruits which supports our result. Some results (Yoryyon and Supapvanich, 2017) reported that the decrease of antioxidant activity as fruit ripening may be attributed to the total phenolic contents.

Reducing power

Reducing powers of the *A. arguta* fruit during ripening were showed in Fig. 3. Like the antioxidant activity of fruit, as the fruit ripening, reducing power of fruit of each clone was gradually decreased. The highest reducing power of *A. arguta* fruit from Saehan cultivar were 0.14 at 100μg/mL in the first step.

As the reducing power are generally associated with the presence of reductones and antioxidant activity, antioxidant

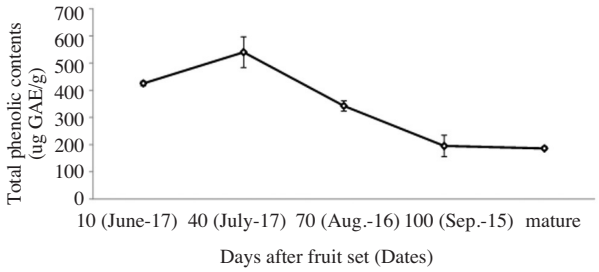

Fig. 4. The total phenolic contents of *A. arguta* fruit during ripening. The values are mean±SD (n=3).

activity expressed as free radical scavenging capacity may be associated with its reducing power (Siddhuraju *et al*., 2002). According to the study of Burda *et al*. (1990), with the enlargement of the fruit, the reduction of the antioxidant activities during the growth of fruit may be associated with the relative decrease of the content of the polyphenol compounds. It was also studied that reducing power of grape seed was associated with its antioxidant (Jayaprakasha *et al*., 2001).

Total phenolic contents

The total phenolic contents of *A. arguta* fruit during ripening are presented in Fig. 4. The highest total phenolic content in fruit of Saehan cultivar was 540.23μg/g. From the results obtained from Fig. 4, a gradual decrease in total phenolic content was observed during fruit ripening.

Generally, the antioxidant activity increased with the increase in the total phenolic contents. According to Spayd and Morris (1981), similar results were obtained in strawberry fruits. The reasons of decreasing total phenolic contents as the fruit matures are that polyphenols in fruit react with other substances to form other compounds and accumulate in fruit (Kim, 1975). Zhang (2006) also reported that total phenolic contents of fresh and core of pears decreased gradually as the fruit ripened.

Vitamin C contents

The vitamin C contents of *A. arguta* fruit of Saehan cultivar during ripening are presented in Fig. 5. As observed in Fig. 5, changes in vitamin C content were

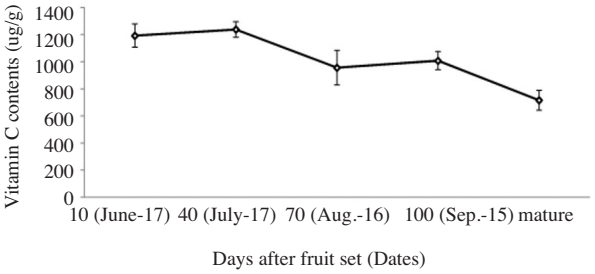

Fig. 5. Vitamin C contents of *A. arguta* Saehan cultivar fruit during ripening. The values are mean±SD (n=3).

found during fruit ripening, which ranged from 715.7 to 1238.0μg/g for Saehan cultivar. The content of vitamin C in *A. arguta* fruit decreased during ripening. The vitamin C content was affected by the ripening stage. These results are in agreement with those of Hong *et al*. (2006), who reported that vitamin C content of *Elaeagnus multiflora* fruit gradually decreased during ripening. Other researchers have also demonstrated a decrease in vitamin C content during fruit growth (Bashir and Abu-Goukh, 2003). The change of vitamin C content was in accordance with the results of total phenolic contents (Liu *et al*., 2015).

In this study, we report changes in the overall antioxidant activity, total phenolics and vitamin C content of *A. arguta* fruit during ripening. We aim to increase the understanding knowledge of fruit ripening process, which may be useful for optimal harvest timing and the processing and utilization of hardy kiwi fruit, especially unripe fruit.

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