

Changes of Antioxidant Capacity, Total Phenolics, and Vitamin C Contents During *Rubus coreanus* Fruit Ripening

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Abstract Changes in antioxidant activity of *Rubus coreanus* fruit of 3 clones (S13, S14, and S16), which were selected from different sites, were studied at different ripening stages. Antioxidant activities (free radical scavenging activity and reducing power) were determined and their relationships to total phenolic contents and ascorbic acid were analyzed. The highest free radical scavenging activities of 3 clones (S13, S14, and S16) were 79.39, 75.80, and 81.16% at 125 µg/mL, respectively. In general, the antioxidant activity and the related parameters, including total phenolic content and vitamin C content decreased during fruit ripening. Total phenolic contents of the *R. coreanus* fruits (S13, S14, and S16) were correlated with free radical scavenging activity ($R^2=0.8114$, 0.9186, and 0.9714). These results improve knowledge of the effect of ripening on the antioxidant activity and related compounds contents that could help to establish the optimum *R. coreanus* fruit harvest data for various usages.

Key words: *Rubus coreanus*, antioxidant capacity, free radical scavenging activity, total phenolics, vitamin C content

Introduction

Rubus coreanus distributed in the southern part of the Korea is a perennial shrub. The fruit of this plant can be consumed at different ripening stages. The ripen fruit of *R. coreanus* has been used for food, juice, and functional food. The unripe fruit of *R. coreanus* called *bogunja* (Korean bramble) has been used in traditional herbal medicine for the treatment of diabetes mellitus and sexual disinclination (1). It is also used for the management for asthma and allergic disease (2). Since the uses of ripen and unripe fruit of *R. coreanus* are available, it is necessary to study the chemical composition and biological activity of *R. coreanus* fruits during ripening. From the fruit of *R. coreanus*, 4-hydroxybenzoic acid, 4-hydroxy-3-methoxy benzoic acid, and 3,4-dihydroxybenzoic acid were identified by gas chromatography-mass spectrometry (GC-MS) (3). These phenolic compounds inhibit lipid autoxidation by acting as radical scavengers (4). Since the fruit of *R. coreanus* are known to possess antioxidant activity at various extents, extracts from the fruit of this plant acts as free radical inhibitors (5).

Free radicals which have 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 cell chemical species as free radicals and reactive oxygen species (6). 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 (e.g., catechins, flavonols) and vitamins (C, E, and A) (7-9).

While there are some data on the constituents and biological activities of *R. coreanus* fruits (10,11), there are no studies of the chemical composition and antioxidant activity of *R. coreanus* fruits during ripening. Thus, in this study, we report, for the first time, changes in the overall antioxidant properties of *R. coreanus* fruit during ripening. Antioxidant activities and their contribution (phenolic compound and vitamin C) to the total antioxidant activity have been established. Further, we also evaluated the correlations between antioxidant activity, total phenolic content, and vitamin C content.

Materials and Methods

Materials *R. coreanus* fruits of 3 clones (S13, S14, and S16), grown in the Korea Forest Institute (Suwon) were utilized. A voucher specimen was deposited at the Korea Forest Research Institute, Suwon, Korea. Seven samples were collected according to their maturity. Among 7 samples, 6 samples were collected at 5-day intervals and the last sample was collected when the fruit were commercially matured. To characterize the ripening stages of the fruit utilized here, weight and diameter of fruits were determined (Table 1).

Extraction Dried *R. coreanus* 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 1,1-diphenyl-2-picrylhydrazyl (DPPH) method according to the procedure of Park *et al.* (12). Ethyl alcohol soluble fraction (0.5 mL) of samples at various concentrations (50, 100, and 125 ppm) was added to a solution of DPPH in EtOH (100 µM, 3 mL) 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 (8453

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Table 1. Characterization of *R. coreanus* fruit ripening stages by fruit weight and diameter

Days after fruit set (date)	Weight (g)			Diameter (cm)			W/D ¹⁾		
	S13	S14	S16	S13	S14	S16	S13	S14	S16
5 (June-12)	0.14	0.12	0.13	5.78	5.63	5.95	0.02	0.02	0.02
10 (June-17)	0.16	0.13	0.16	6.32	6.22	6.94	0.02	0.02	0.02
15 (June-22)	0.19	0.19	0.21	7.20	7.42	7.93	0.03	0.03	0.03
20 (June-27)	0.24	0.22	0.27	8.01	8.04	8.26	0.03	0.03	0.03
25 (July-2)	0.32	0.31	0.40	9.09	9.25	9.77	0.03	0.03	0.04
30 (July-7)	0.36	0.37	0.53	9.27	9.41	10.78	0.04	0.04	0.05
37 (July-14)	1.28	1.33	1.72	15.46	15.08	16.21	0.08	0.09	0.11

¹⁾Ratio of weight and diameter.

UV-Vis Spectrophotometer; Agilent Technologies, Palo Alto, CA, USA) at 517 nm. The mixture of 0.5 mL EtOH with a solution of 3 mL 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 (13). Each extracts (100 and 50 µg/mL) in EtOH (2.5 mL) was mixed with 2.5 mL of 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 mL potassium ferricyanide (10 mg/mL), the mixture then incubated at 50°C for 20 min. After 2.5 mL trichloroacetic acid (100 mg/mL) was added, the mixture was centrifuged at 1,220×g for 10 min. The upper layer (5 mL) was mixed with 5 mL distilled water and 1 mL ferric chloride (1 mg/mL). The absorbance was measured at 700 nm. 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.* (14). Each sample (1 mL) was mixed with Folin and Ciocalteu's phenol reagent (1 mL, Sigma-Aldrich, St. Louis, MO, USA). After 3 min, 1 mL of saturated Na₂CO₃ was added to the mixture and it was made up to 10 mL by adding distilled water. After the reaction was kept in the dark for 90 min, absorbance was taken at 725 nm. A calibration curve was constructed with different concentrations of gallic acid (Wako Pure Chemicals, Osaka, Japan) (0.01-0.1 mM) 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 Jagota and Dani (15). A 0.5 g sample of dried fruits was weight and extracted with distilled water then filtered. Two-tenth mL extracts was mixed with 0.8 mL 10%(w/v) trichloroacetic acid (TCA) at 4°C. After centrifugation at 3,000×g for 5 min, 0.5 mL of supernatant was made up to 2 mL volume with distilled water. 0.2 mL 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 760 nm. The absorption maximum of the color developed by the interaction of ascorbic acid with Folin reagent is 760 nm.

Results and Discussion

Morphological characteristics Visually, the fruits changed colour during fruit ripening from an initial green to a red, and finally to a dark red at harvest. Table 1 shows the weight and diameter along the various ripening stages considered here. The weight and diameter of *R. coreanus* fruit were increased with the progress of ripening and the weight/diameter ratio of each maturity stage was also increased.

Antioxidant activity Because the antioxidant activity of fruits is important for assessing their nutritional value (16), the free radical scavenging activity of fruit was measured. Free radicals are chemical fragments that cause oxidation and antioxidants act as free radical scavengers. Free radical scavenging activity of extracts from the fruits was evaluated by the colorimetric decrease in the absorbance of DPPH due to the chemical trapping of unpaired electron. The free radical scavenging activities of *R. coreanus* fruit during ripening are shown in Fig. 1.

The activities decreased during the ripening of fruit, and the highest free radical scavenging activities of 3 clones (S13, S14, and S16) were 79.39, 75.80, and 81.16% at 125 µg/mL, respectively. The antioxidant activity of the fruit at each maturity stage appeared to be concentration dependent.

According to Beltran *et al.* (17), the antioxidants and the related parameters decreased as olive fruit ripened. Similar results were also reported by Ferreyra *et al.* (6) in strawberry fruits.

Reducing power Reducing power has been found to be associated with the antioxidant activity (18) and thus may serve as a significant indicator of the potential antioxidant activity (19). Reducing powers of the fruit at different maturity stages were measured using the potassium ferricyanide method (Fig. 2). 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 *R. coreanus* fruit from 3 clones (S13, S14, and S16) were 1.04, 1.12, and 0.92 at 100 µg/mL, respectively. As the reducing power are generally associated with the presence of reductones and antioxidant activity, antioxidant activity expressed as free radical scavenging capacity may be associated with its reducing power.

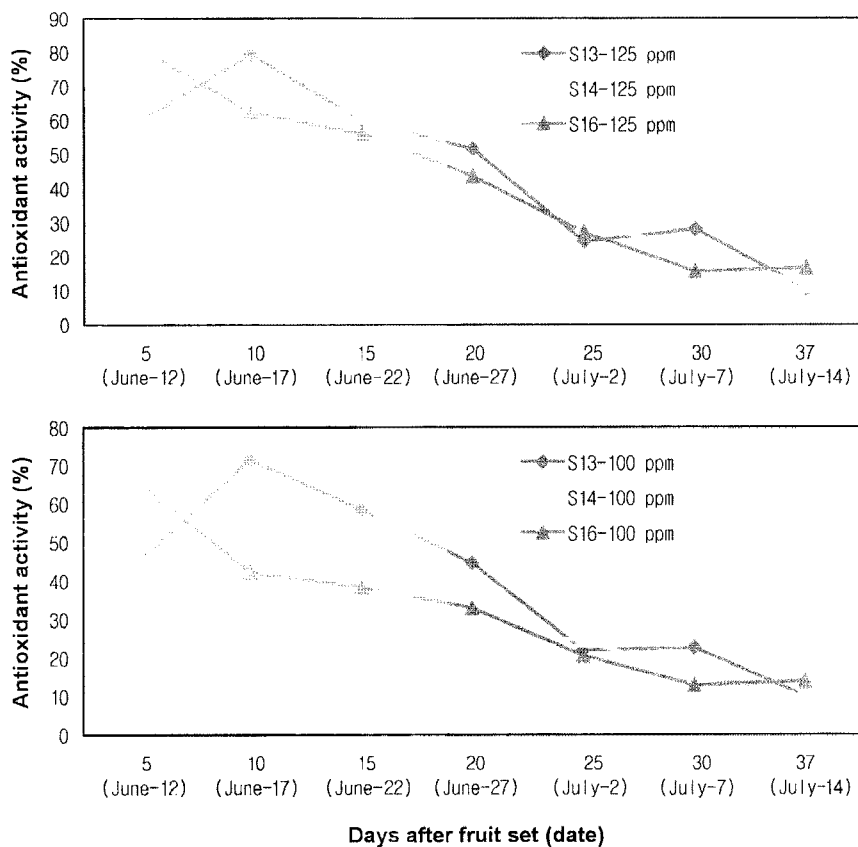


Fig. 1. Free radical scavenging activity of the *R. coreanus* fruit during ripening in different concentration.

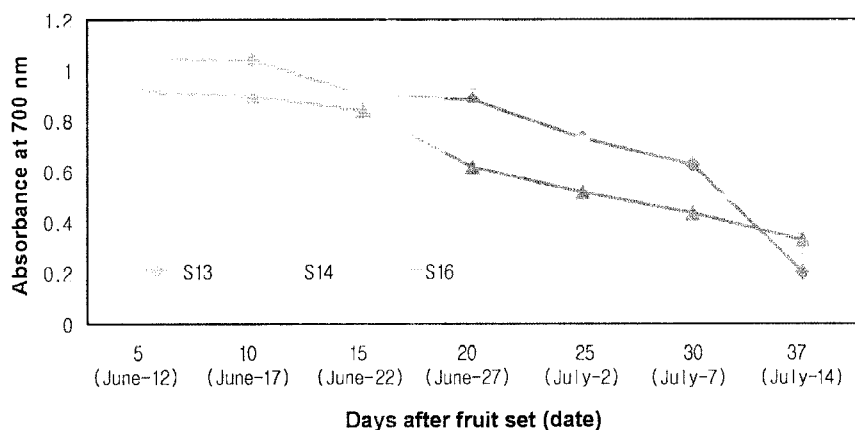


Fig. 2. Reducing power of the *R. coreanus* fruit during ripening at 100 µg/mg. The values are mean±SD (n=3).

Total phenolic contents It is reported that total phenolic contents played an important role in the DPPH radical scavenging activity (20). Generally, the antioxidant activity increased with the increase in the total phenolic contents. The total phenolic contents of *R. coreanus* fruit during ripening are presented in Fig. 3.

Total phenolic content in fruit of 5 days after fruit set of 3 clones (S13, S14, and S16) were 260.50, 254.34, and 235.51 µg/g, respectively. From the results obtained from Fig. 3, a graduate decrease in total phenolic content was observed during fruit ripening. The results revealed that total phenolic contents were present in high quantities in the first stage, and that when fruits attained completely matured only traces of total phenolic contents were contained.

According to Spayd and Morris (21), similar results were obtained in strawberry fruits. Zhang *et al.* (22) also reported that total phenolic contents of fresh and core of pears decreased gradually as the fruit ripened. According to Kim (23), 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.

Vitamin C contents Vitamin C (ascorbic acid) is usually selected as an index of the nutrient quality because of its liable nature as compared to the other nutrients in food (24). It is also reported that chemical contributors to antioxidant activity in fruit are numerous and included

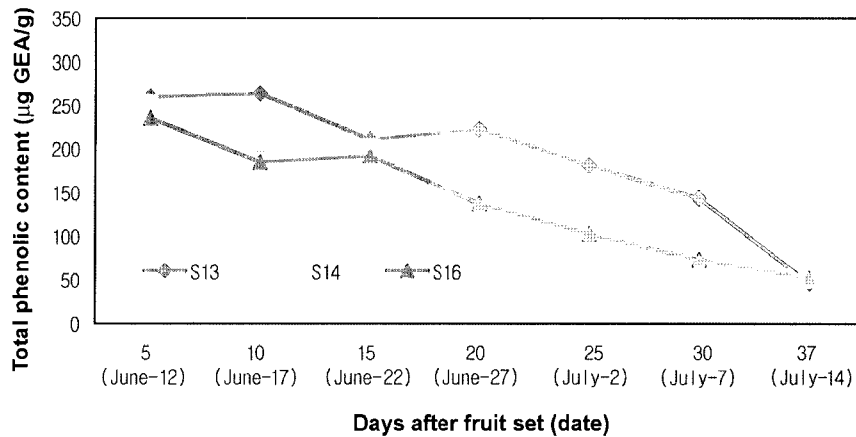


Fig. 3. The total phenolic contents of *R. coreanus* fruit during ripening. The values are mean±SD (n=3).

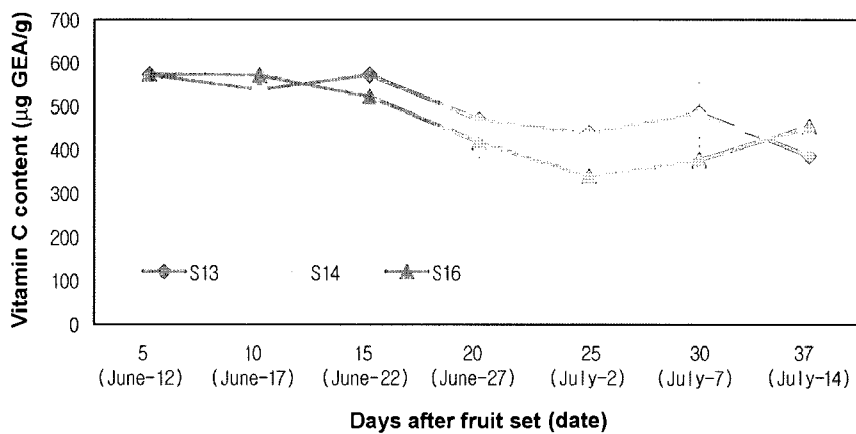


Fig. 4. Vitamin C contents of *R. coreanus* fruit during ripening. The values are mean±SD (n=3).

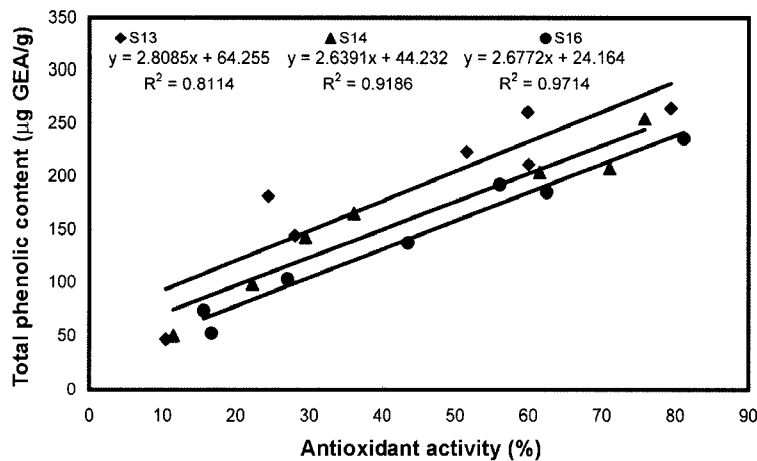


Fig. 5. Correlation between free radical scavenging activity with total phenolic content of *R. coreanus* fruit (S13, S14, and S16 clones).

vitamin C (25). Therefore, vitamin C contents of *R. coreanus* fruit during ripening are presented in Fig. 4.

As observed in Fig. 4, decrease in vitamin C content were found during fruit ripening, which ranged from 572.64 to 386.70 µg/g for S13 clones. Similar results were obtained from the fruit of S14 and S16 clones. The vitamin C contents in the fruit of S14 and S16 clones at first maturity stage was 541.20 and 574.44 µg/g, respectively. The content of vitamin C in *R. coreanus* fruit decreased

during ripening. These results are in agreement with those of Hong *et al.* (26), who reported that vitamin C content of *Elaeagnus multiflora* fruit gradually decreased during ripening. However, it is also reported that the content of vitamin C in guava and strawberry increased slowly during ripening (6,27).

Correlation between antioxidant activity and total phenolic contents It is reported that total phenolic

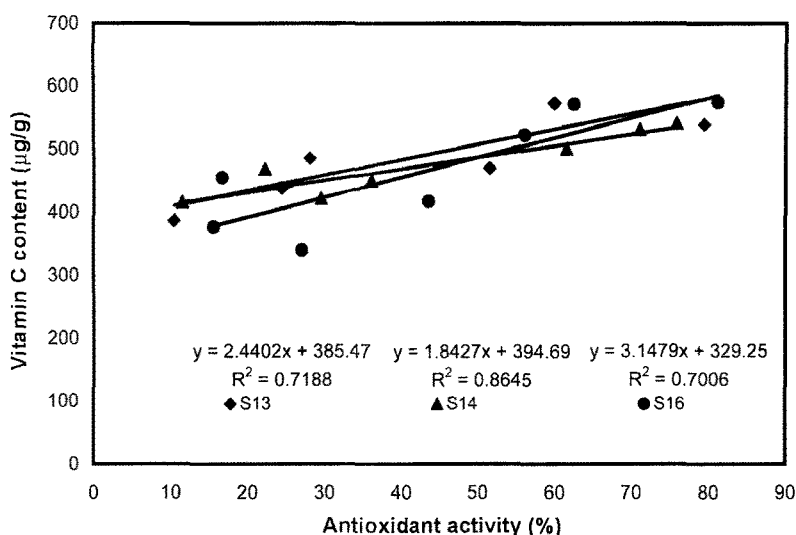


Fig. 6. Correlation between free radical scavenging activity with vitamin C content of *R. coreanus* fruit (S13, S14, and S16 clones).

contents played an important role in the DPPH radical scavenging activity (18). The scavenging activity of the *R. coreanus* fruit during ripening on DPPH radicals increased with increasing total phenolic contents. A linear correlation ($R^2=0.8114$, 0.9186 , and 0.9714) was shown between antioxidant activity (at $125 \mu\text{g/mL}$) and total phenolic contents of *R. coreanus* fruit (S13, S14, and S16 clones).

Using strawberry cultivar 'Selva', Ferreyra *et al.* (6) have found that the antioxidant capacity correlated linearly with the phenolic content. Yen and Chen (28) also discovered that polyphenols are the most abundant group of compound in tea leaf and seem to be the responsible for antioxidant activity. The increase in the antioxidant activity with the increase of total phenolics may be the results of reaction that polyphenols exert their antioxidant action by donating hydrogen atoms to free radicals (29).

Correlation between antioxidant activity and vitamin C content As observed in Fig. 6, a linear correlation ($R^2=0.7188$, 0.8645 , and 0.7006) was shown between antioxidant activity (at $125 \mu\text{g/mL}$) and vitamin C contents of *R. coreanus* fruit (S13, S14, and S16 clones). These results provided evidence that the antioxidant activity of *R. coreanus* fruit is also closely correlated with the vitamin C contents. It is reported that vitamin C prevents browning tissue, which is an oxidation reaction, directly and indirectly, it played an important role in the DPPH radical scavenging activity (30).

Based on the results in this study, we could suggest that during fruit ripening the antioxidant capacity and total phenolic content were decreased. Although the vitamin C contents were decreased during fruit ripening, the decrease rate was not significant. These results improve the knowledge of the effect of ripening on the antioxidant activity and related compounds contents that could help to establish the optimum *R. coreanus* fruit harvest data for various usages (for medicine or food).

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