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Variation of bioactive compounds content of 14 oriental strawberry cultivars

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ABSTRACT

Variation in bioactive compounds content was assessed in antioxidant rich June-bearing strawberry cultivars. Ascorbic acid, anthocyanin, and ellagic acid content were analyzed in ripe fruits of 14 cultivars. The bioactive content in strawberry fruit was found to vary significantly among cultivars and from year to year. The highest ascorbic acid content was found in 'Sugyeong'. The 'Red Pearl' and 'Sachinoka' had three to fourfold higher amounts of pelargonidin 3-glucoside than other cultivars. For cyanidin 3-glucoide and pelargonidin 3-rutinoside, two other characterized anthocyanins, 'Dahong' and 'Keumhyang' had the highest contents among all the tested cultivars. The ellagic acid content of 'Dahong' was generally all within the upper ranges. These results can be used for the validation of fruit antioxidant capacity and in addition, provide useful information for breeding programs looking to enhance the antioxidant capacity in strawberry fruit.

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1. Introduction

Strawberry (*Fragaria* × *ananassa* Dutch.) is one of the most commonly consumed small fruits, being utilized both in fresh and processed forms in jam, juice, yogurt, and even in dietary supplements. The significant economic and commercial impacts of strawberries have led to them being among the most heavily researched from the agronomic, genomic, and nutritional point of view. The nutritional quality of strawberry fruits has been correlated to the high content of bioactive compounds such as ascorbic acid (Giné Bordonaba & Terry, 2011), anthocyanin (Hannum, 2004), and ellagic acid (Mass, Galleta, & Stoner, 1991), most of which express relevant antioxidant capacities *in vitro* and *in vivo* (Olsson et al., 2004). Increasing evidence has highlighted that consuming strawberry fruits is beneficial for maintaining good health and reducing the risk of certain chronic diseases such as cancers (Seeram et al., 2006), cardiovascular disease (Azzini et al., 2010), and memory loss (Giampieri et al., 2012). Several studies have also suggested that polyphenols in strawberry fruits play a primary role in preventing cell damage due to the exposure to reactive oxygen species (Olsson et al., 2004; Rekika et al., 2005; Wang & Lewers, 2007).

Given these important findings, consumers, growers, breeders, and processors have become more interested in health-beneficial compounds in strawberry fruits for their nutraceutical properties. The breeding programs for rich-nutritious and better-tasting cultivars might be successful if the variability and diversity of the bioactive compounds (Mezzetti, 2013). Diamanti et al. (2012, 2014), revealed that increasing nutritional quality and health-beneficial compounds of strawberry fruits using wild and cultivated germplasm. Furthermore, the valuable for breeding programs have considered regarding flavor, studies of biochemical pathways and signaling compounds as well as marker development (Ulrich & Olbricht, 2014).

In the present study, contents of ascorbic acid, individual anthocyanins such as pelargonidin 3-glucoside, cyanidin 3-glucoside, and pelargonidin 3-rutinoside, and ellagic acid were assessed in 14 oriental June-bearing strawberry cultivars recently bred in Korea and Japan. In addition, in this investigation the bioactive compounds content were compared, in order to individuate compound for strawberry discrimination and individuation of strawberry cultivars with high content of those secondary metabolites. Thus, these results will be providing useful information for selective strawberry breeding.

2. Materials and methods

2.1. Strawberry samples

Fruit samples were analyzed from 14 strawberry genotypes. These were: 'Dahong', 'Keumhyang', 'Maehynag', 'Seolhyang', 'Ssanta', 'Sugyeong', 'Sunhong' (Korean cultivars), and 'Akihime',

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'Benihoppe', 'Red Pearl', 'Sachinoka', 'Sagahonoka', 'Tochinomine', and 'Tochiotome' (Japanese cultivars) (Table 1). Samples were collected from an experimental field established in the National Institute of Horticultural & Herbal Science experimental farm, located at Suwon, Korea (36° 43′N, 128° 07′E, and elevation 30 m). The experimental design was completely randomized with three replications. The cultivation type was substrate culture using drip irrigation system in a greenhouse. Strawberry plants (crown diameter: approximately 10 mm) were transplanted into plastic containers (740 mm × 250 mm × 200 mm, L × W × H) filled with a mixture of peatmoss (BM-4, Berger Peat Moss Ltd., Quebec, Canada) and perlite (Parat No 3, Kyung Dong Ceratech Co., Ltd., Seoul, Korea) (1:1, v/v). The plant density was 24 plants/m^2. Mean daily average air temperature and integral radiation from flowering to harvest for fruit production years 2011 and 2012 were −0.6 and 0.7 °C, and 303.9 and 286.9 MJ m^{-2}, respectively. The night temperature inside the greenhouse was maintained above 10 °C. The secondary or tertiary strawberry fruits in the first cluster were harvested at the optimal fruit maturity when ca. 90% of the fruit surface had reached full red color.

2.2. Extraction and analysis of ascorbic acid

Strawberry fruit sample (10 g) was homogenized with 50 mL of buffer solution (4% metaphosphoric acid) and filtered using an 8 μm cellulose filter paper (Whatman International Ltd., Kent, UK). The mixture was filtered through a 0.45 μm polyvinylidene fluoride membrane syringe filter (Agilent Co., Palo Alto, CA, USA) and injected into an HPLC system (Ultimate 3000, Dionex, Sunnyvale, CA, USA) under the following conditions. The mobile phase was acetonitrile and 50 mM $NH_4H_2PO_4$ (70:30, v/v), and the flow rate was 1.0 mL/min. The ascorbic acid components were detected at 254 nm. A C18 reverse phase column (4.6 × 250 mm, 0.5 μm; Supelcosil TM C-18, Supelco, Bellefonte, PA, USA) was used for analysis (Kim, Kim, & Park, 2006). Each measurement had three replications, three separate extractions from three different fruit samples.

2.3. Extraction and analysis of anthocyanin

Anthocyanin was extracted from the fruit skin (2 g), less than 2 mm thick, by homogenizing with 5 mL HCl (1%)-methanol solution. The extract was filtered through an 8 μm cellulose filter paper (Whatman International Ltd., Kent, UK), and then the solutions were filtered through a 0.45 μm polyvinylidene fluoride membrane syringe filter (Agilent Co., Palo Alto, CA, USA) prior to injection into an HPLC system (Ultimate 3000, Dionex, Sunnyvale, CA, USA). Solvents used were: (A) 0.1% trifluoroacetic acid in water, and (B) HPLC-grade acetonitrile, establishing the following gradient: isocratic 10% of B for 5 min, 10–15% of B over 15 min, isocratic 15% of B for 5 min, 15–18% of B over 5 min, and 18–35% of B over 20 min, using a flow rate of 0.5 mL min^{-1}. Separation was achieved using a C18 column (4.6 × 150 mm, 5 μm; Zorbax SB-C18, Agilent Co., New York, USA) and peaks were identified with a UV/Vis detector at 520 nm (da Silva, Escribano-Bailon, Alonso, Rivas-Gonzalo, & Santos-Buelga, 2007). Individual anthocyanin standards (Pelargonidin 3-glucoside, pelargonidin 3-rutioside, and cyaniding 3-glucoside) were purchase from Extrasynthese SA. (Lyon, Nord Genay, France). Each measurement had three replications, three separate extractions from three different fruit samples.

Table 1
Parentage and origin of 14 June-bearing strawberry cultivars.

Cultivar	Parentage	Origin[a]
Dahong	Sachinoka × Maehyang	NIHHS, South Korea, 2007
Keumhyang	Akihime × Red Pearl	NSES, South Korea, 2005
Maehyang	Tochinomine × Akihime	NSES, South Korea, 2001
Seolhyang	Akihime × Red Pearl	NSES, South Korea, 2005
Ssanta	Maehyang × Seolhyang	SFVES, South Korea, 2009
Sugyeong	Johong × Maehyang	NIHHS, South Korea, 2008
Sunhong	Johong × Maehyang	NIHHS, South Korea, 2005
Akihime	Kunowase × Nyoho	Ogiwara, A. in Shizuoka, Japan, 1992
Benihoppe	Akihime × Sachinoka	SAES, Japan, 2002
Red Pearl	Aiberry × Toyonoka	Nishida, A. in Ahime, Japan, 1993
Sachinoka	Toyonoka × Aiberry	KBNRIVOT, Japan, 1996
Sagahonoka	Oonish × Toyonoka	SGAES, Japan, 2001
Tochinomine	(Florida 69–226 × Reiko) × Nyoho	TAES, Japan, 1993
Tochiotome	Kurume 49 × Tochinomine	TAES, Japan, 2006

[a] NIHHS, National Institute of Horticultural & Herbal Science, RDA, South Korea; NSES, Nonsan Strawberry Experiment Station, South Korea; SFVES, Seongju Fruit Vegetable Experiment Station, South Korea; SAES, Shizuoka Agricultural Experiment Station, Japan; KBNRIVOT, Krume Branch of National Research Institute Vegetable, Ornamental, and Tea, Japan; SGAES, Saga Agricultural Experiment Station, Japan; TAES, Tochigi Agricultural Experiment Station, Japan.

2.4. Extraction and analysis of ellagic acid

Strawberry fruit samples (2.5 g) were homogenized in 7.5 mL of purified water, and 12.5 mL of methanol was added. In addition, 5.0 mL of 6.0 mol L^{-1} HCl was added. The mixtures were refluxed for 2 h at 85 °C and then filtered through a 0.45 μm polyvinylidene fluoride membrane syringe filter (Agilent Co., Palo Alto, CA, USA) prior to injection into an HPLC system (Ultimate 3000, Dionex, Sunnyvale, CA, USA). Solvent A was 1% formic acid and solvent B was acetonitrile with 0.5 mL/min flow rate. The gradient was: 0–15 min, 10–55% of B in A; 15–20 min, 55–100% of B in A; 20–25 min, 100–10% of B in A; 25–35 min, 10–10% of B in A. Separation was achieved using a C18 column (4.6 × 150 mm, 5 μm; Zorbax SB-C18, Agilent Co., Palo Alto, CA, USA) and peaks were identified using UV/Vis detector at 260 nm (Kim, Bea, Na, Ko, & Chun, 2013). Each measurement had three replications, three separate extractions from three different fruit samples.

2.5. Statistical analysis

Influence of strawberry cultivars and years on health-related compounds in strawberry fruit was assessed using Analysis of Variance (ANOVA) in SAS 9.2 (SAS Institute Inc., Cary, NC, USA) to identify least significant differences ($P < 0.001$).

3. Results and discussion

3.1. Ascorbic acid content

Ascorbic acid content strongly differed among cultivars and fruit production years (Table 2). Across fruit production year, ascorbic acid content ranged from 56.8 to 108.1 mg/100 g fresh weight (Fig. 1). The highest ascorbic acid content was found for 'Sugyeong' harvested in 2011, while low content was analyzed for 'Sagahonoka' and 'Seolhyang' harvested in 2012. The 'Sugyeong' showed higher contents of dietary antioxidant such as ascorbic acid. This 'Sugyeong' could thus be useful for developing cultivars with high antioxidant capacity. Significant variation in different quality traits and antioxidants by strawberry cultivars were also reported by Capocasa, Scalzo, Mezzetti, and Battino (2008), Singh et al. (2011), Tsao et al. (2005) and Wang and Jiao (2000). The ascorbic acid content of 'Sagahonoka' and 'Seolhyang' harvested in 2011 was 23.7% and 34.4% lower than that of fruits harvested in 2012. Ascorbic acid content of 'Sagahonoka' and 'Seolhyang' was most easily influenced by changes in environmental conditions. Ascorbic acid content can be modified by several pre-harvest factors (Lee & Kader, 2000). Among them, light

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