Comparison of Bioactive Compounds Contents in Different Fruit Tissues of June-bearing Strawberry Cultivars

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Abstract. We evaluated the bioactive compounds including carbohydrates (CH), organic acids (OA), ascorbic acid (AA), anthocyanin (AC), and ellagic acid (EA) in the achenes, epidermis, and flesh of fully ripe strawberry fruits of the 'Maehyang', 'Seolhyang', 'Keumhyang', 'Akiheme', and 'Red pearl' cultivars. The total contents of CH, OA, AA, and AC were significantly greater in the epidermis than in other tissues, while the EA content was significantly greater in the achenes than in other tissues. The AA content of the epidermis ranged from 49.1-69.2 mg·100 g⁻¹ FW and was significantly greater than the content of the flesh, while AA was not detected in the achenes of any of the tested strawberry cultivars. The AA contents of the epidermis and flesh of the 'Maehyang' were 69.2 and 42.2 mg·100 g⁻¹ FW, respectively, and were greater than those of the other cultivars. The AC contents in the epidermis and achenes of the 'Keumhyang' were 74.0 mg·100 g⁻¹ FW and 36.7 mg·100 g⁻¹ DW, respectively, greater than those of the other cultivars. The EA content of the achenes of the 'Seolhyang' was 215.5 mg·100 g⁻¹ DW, significantly greater than those of the other cultivars. Results indicate that the antioxidant levels and other chemical compounds of strawberry fruits vary significantly among different fruit tissues. Our results also suggest that the recently introduced Korean 'Maehyang', 'Seolhyang', and 'Keumhyang' contain higher levels of antioxidants than other major June-bearing strawberry cultivars. These cultivars are feasible selections for both growers and consumers.

Additional key words: achene, antioxidant, epidermis, flesh, Fragaria × ananassa

Introduction

Strawberries (Fragaria × ananassa Duch.) are a good source of natural antioxidants, which play an important role in maintaining health. Antioxidant sources in strawberries include ascorbic acids and phenolic compounds, such as anthocyanins and ellagic acids (Hannum, 2004). Ascorbic acid plays a pivotal role in plant cells by preventing oxidative stress from photosynthesis, oxidative metabolism, or exposure to pollutants (Nascimento et al., 2005). Anthocyanins comprise of a large subclass of flavonoid plant pigments found in most of the red, blue, and purple flowers and fruits. Anthocyanins are the most abundant flavonoids in strawberries. Ellagic acid comprises 51% of the phenolic compounds in strawberries (Häkkien et al., 2000) and is found both in free form and esterified to glucose in water soluble hydrolysable ellagitannins (Clifford and Scalbert, 2000). Strawberries, together with

raspberries and blackberries, are the main sources of ellagic acid regularly eaten by humans (Clifford and Scalbert, 2000; Daniel at al., 1989). Additionally, carbohydrate sources in food are of major interest with regard to chronic diseases. Different types of carbohydrates give rise to different glycemic responses; the glycemic potential of glucose is, for example, higher than that of sucrose, which in turn is higher than that of fructose. Interestingly, different types of indigestible carbohydrates give rise to different profiles of short-chain fatty acids. Thus, the carbohydrate pattern is of great importance from a nutritional point of view.

Strawberry fruits consist of seeds, called achenes, which are the actual fruits of the berries, and flesh, which is modified receptacle tissue. Strawberry fruit originates from a single inflorescence and is actually an aggregate composed of many ovaries, each with a single ovule (Perkins-Veazie, 1995). The achenes, embedded in the epidermis of the swollen receptacle,

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are the true fruit of the species. The receptacle is composed of an internal pith, a cortex layer, and an epidermal layer (Suutarinen et al., 1998). Fibrovascular strands connect the achenes to the interior of the receptacle and supply nutrients to the achenes and the surrounding parenchymal cells. Receptacle growth follows either a single or a double sigmoid curve, depending on the cultivar. It is mainly the result of cell enlargement; however, cell division accounts for 15% to 20% of total growth (Cheng and Breen, 1992; Havis, 1943). Previous studies have shown that the flesh and achenes possess high antioxidant activity and are high in phenolic compounds, especially ellagic acid derivatives (Aaby et al., 2005; Mass et al., 1991; Williner et al., 2003). Most fruit peel and seed fractions exhibit higher antioxidant activity than pulp fractions based on the ferric reducing/antioxidant power values (Guo et al., 2003). However, little work has been conducted regarding the polyphenolic composition and antioxidant properties of the pulp and achenes of strawberry fruits. In addition, no comparisons have been performed regarding the contributions of the achenes, epidermis, and flesh to the bioactive compound content between Korean strawberry cultivars and other major foreign cultivars.

The objective of the present investigation was to compare five June-bearing strawberry cultivars, the recently introduced Korean cultivars 'Maehyang', 'Seolhyang', and 'Keumhyang', and the current major Japanese cultivars in Korea, 'Akihime' and 'Red pearl', with regard to estimated carbohydrate, organic acid, ascorbic acid, anthocyanin, and ellagic acid contents in different fruit tissues.

Materials and Methods

Preparation of Strawberry Fruit Samples

Ripe secondary or tertiary strawberry fruits in the third fruit cluster were harvested from 'Maehyang', 'Seolhyang', 'Keumhyang', 'Akihime', and 'Red pearl' cultivars grown at the Experimental Farm of Seoul National University located in Suwon, Korea. Strawberries were selected to assess uniformity of color development and freedom from defects. The achenes, epidermis (without achenes), and flesh were separated and analyzed for their bioactive compound contents. The achenes were separated by blending in a food processor (HR 2870, Philips Electro., Netherlands). The achenes were then separated from the flesh by pressing them through a strainer. The collected achenes were rinsed several times with cold running water and left to dry at room temperature.

Extraction and Analysis of Carbohydrate and Organic acid

The achenes (0.125 g), epidermis (1.250 g), and flesh (5.000

g) of all samples were diluted with purified water to 12.5, 25.0, and 50.0 mL, respectively. The samples were then homogenized and filtered using Whatman No. 2 filter paper. The mixtures were filtered through a 0.45 µm syringe filter and each diluted sample was injected into a chromatography system (Dionex 2500, Dionex Co., New York, NY, USA). The carbohydrate analysis was performed using an 18 mM sodium hydroxide solvent with a CarboPac PA10 (4 × 250 mm) column and a pulsed amperonmetry detector with an Au electrode. The organic acids were analyzed with an eluent of 0.4 mM heptafluorobutyric acid. Five mN of tetrabutylammonium hydroxide was used as a regenerate solution, and an Anion-ICE micromembrane suppressor and an IonPac ICE-AS6 (9 × 250 mm) column were also used. The flow rate was 1.0 mL·min⁻¹ and suppressed conductivity was used as a detector for the organic acid analysis. Data are reported as means \pm standard deviation of at least three replicates.

Extraction and analysis of ascorbic acid

The achenes (0.25 g), epidermis (2.50 g), and flesh (10.00 g) of the strawberry fruit samples were homogenized with 12.5, 25.0, and 50.0 mL of buffer solution (4% metaphosphoric acid) and filtered using Whatman No. 2 filter paper. The mixtures were filtered through a 0.45 µm syringe filter and injected into an HPLC system (Ultimate 3000, Dionex, Sunnyvale, CA, USA) for L-ascorbic acid analysis. The mobile phase used acetonitrile and 50 mM NH4H2PO4 (70:30, v/v) with a flow rate of 1.0 mL·min⁻¹. The 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 et al., 2006). Data are reported as means \pm standard deviation of at least three replicates.

Extraction and analysis of anthocyanin

The achenes (0.05 g), epidermis (0.20 g), and flesh (2.00 g) of the strawberry fruit samples were homogenized with 7.5, 12.5, and 30.0 mL of buffer solution (1% HCl-methanol solution) and filtered using Whatman No. 2 filter paper. The mixtures were analyzed with a spectrophotometer at 520 nm (UV-2550, Shimadzu, Kyoto, Japan). The results were expressed as mg of pelargonidin 3-glucoside ($\varepsilon = 36,000 \text{ L}$ · mol⁻¹·cm⁻¹) 100 g⁻¹ FW (Ferreyra et al., 2007). Data are reported as means ± standard deviation of at least three replicates.

Extraction and analysis of ellagic acid

The achenes (0.5 g), epidermis (2.0 g), and flesh (2.5 g) of the strawberry fruit samples were diluted with purified water to 7.5 mL and homogenized. Then 12.5 mL of methanol and 5 mL of 6 mol·L⁻¹ HCl were added. The mixtures were refluxed for 2 hrs at $85 \pm 5^{\circ}$ °C. The refluxed mixtures were filtered through a 0.45 µm syringe filter prior to injection into the HPLC system (Ultimate 3000, Dionex, Sunnyvale, CA, USA). Solvent A was 1% formic acid and solvent B was acetonitrile. These solvents (flow rate 0.5 mL·min⁻¹) were used to achieve the best purity and separation for the ellagic acid peak. UV detection (260 nm) was used to quantify the ellagic acid content, a column (4.6 \times 150 mm, 5 μ m; Zorbax SB-C18, Agilent Co., New York, NY, USA) was also used. Data are reported as means \pm standard deviation of at least three replicates.

Statistical analysis

Appropriate statistical techniques were applied to evaluate the existence of significant differences among the strawberry cultivars and fruit tissues and to discriminate between different samples. ANOVA were carried out using Statistical Analysis Systems software (SAS 9.2, SAS Institute Inc., Cary, NC, USA). Means were separated using Duncan's multiple range tests at the 0.05 significance level.

Results and discussion

Carbohydrate and organic acid content in different tissues of strawberry fruits

We identified differences in carbohydrate and organic acid contents among fruit tissues (Table 1). The highest level of total carbohydrate was found in the epidermis, followed by the flesh. The carbohydrate content of the achenes was low compared to those of other tissues. The mean glucose content in the epidermis of the 'Maehyang' was 31.9 mg·g⁻¹ FW, and this was the highest value among all tested cultivars. The mean value of fructose in the epidermis of the 'Seolhyang' was the highest compared to the other cultivars. The carbohydrates were higher than those of the previous reports (Crespo et al., 2010). This difference may reflect a cultural preference for sweetness in Korea and Japan, where strawberries are

Table 1. Carbohydrate and organic acid content of different tissues in ripe strawberry fruits.

Cultivar	Tissues	Carbohydrate content (mg·g ⁻¹)			Organic acid content (mg·g ⁻¹)			TO/TOZ
		Glucose	Fructose	Total	Citric acid	Malic acid	Total	- TC/TO ^z
Maehyang	Achene	6.9 e ^y	5.3 f	12.2 d	0.5 hi	ND ^x	0.5 c	NC ^w
	Epidermis	31.9 a	29.4 ab	61.3 a	6.5 cd	1.9 a	8.4 b	7.5 c
	Flesh	23.6 bcd	21.7 cde	45.3 bc	1.3 gh	1.9 a	3.2 e	14.2 ab
Seolhyang	Achene	5.8 e	4.4 f	10.2 d	0.5 hi	ND	0.5 f	NC
	Epidermis	29.3 abc	31.1 a	60.4 a	8.8 a	1.8 ab	10.6 a	5.7 c
	Flesh	30.6 ab	27.4 abcd	58.0 ab	2.7 f	1.3 bc	4.0 de	15.9 a
Keumhyang	Achene	8.2 e	6.2 f	14.4 d	0.5 hi	ND	0.5 c	NC
	Epidermis	26.0 abcd	27.7 abc	53.6 abc	7.6 bc	1.2 c	8.7 b	6.2 c
	Flesh	22.2 cd	18.9 e	41.1 c	3.0 ef	1.5 abc	4.5 de	9.2 bc
Akihime	Achene	5.1 e	3.4 f	8.5 d	ND	ND	ND	NC
	Epidermis	21.4 d	23.7 bcde	45.1 bc	6.3 d	0.6 d	6.9 c	6.6 c
	Flesh	23.5 bcd	18.9 e	42.4 c	1.9 fg	1.2 c	3.1 e	9.2 bc
Red pearl	Achene	6.8 e	4.7 f	11.5 d	ND	ND	ND	NC
	Epidermis	25.3 abcd	26.6 abcd	51.9 bc	7.8 ab	1.2 bc	9.1 b	5.8 c
	Flesh	28.7 abcd	20.2 de	49.0 bc	4.0 e	1.3 bc	5.3 d	8.7 bc
Significance								
Cultivar (A)		ns	ns	ns	***	**	**	ns
Tissue (B)		***	***	***	***	***	***	***
АВ		ns	ns	ns	**	*	*	ns

^zTC/TO; Total carbohydrate/organic acid ratio.

^yMean separation within columns by Duncan's multiple range test at a 5% significance level.

^{*}ND; non-detectable.

WNC; non-calculable.

vns,*,****Nonsignificant or significant at p = 0.05, 0.01, or 0.001% level, respectively.

consumed fresh and intact, whereas in Europe berries are frequently processed and consumed in a salad. The dominant carbohydrates in the strawberry fruits were fructose and glucose, but a considerable amount of sucrose was also present (Olsson et al., 2004). The highest levels of total organic acids were found in the epidermis, followed by the flesh. The organic acid content in the achenes was low or non-detectable. The mean value of the citric acid content in the epidermis of the 'Seolhyang' was 8.8 mg·g⁻¹ FW, and this was highest among all the tested cultivars. The primary organic acid in strawberry fruits is citric acid, the amount of which ranges from 4 to 12 mg·g⁻¹ FW and comprises 88% of the total acid content (Perkins-Veazie, 1995). The mean value of the malic acid content in the epidermis of the 'Maehyang' was the highest among all the tested cultivars. Organic acids are minor components of the strawberry fruit; however, they are important attributes of flavor that, in combination with carbohydrates, impact on the sensory quality of the fruit (Wang et al., 2002). The carbohydrate/organic acid ratio is a major determinant of the taste of a strawberry (Perkins-Veazie, 1995); a higher ratio indicates better flavor. The total carbohydrate/ organic acid ratio in the flesh of the 'Seolhyang' was 15.9, this was the highest among the tested cultivars. In addition, that ratio in the epidermis of the 'Maehyang' was 7.5, this was the highest among the tested cultivars. Therefore, the 'Seolhyang' and 'Maehyang' cultivar had the most flavors among the tested cultivars and is therefore appropriate for sale in luxury departments or markets.

Ascorbic acid content of different tissues in strawberry fruits

We detected differences in the ascorbic acid contents of

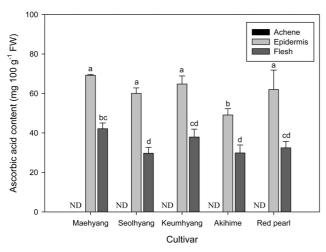


Fig. 1. The ascorbic acid contents of different tissues in ripe strawberry fruits. ND: non-detectable. Vertical bars show standard deviation (n = 3). Small letters inside the figure indicate mean separation by Duncan's multiple range test at p = 0.05.

different fruit tissues and cultivars (Fig. 1). The highest level of ascorbic acid was found in the epidermis, followed by the flesh. The mature achene contains a hard and relatively thick pericarp, a thin testa, a single layer endosperm, and a small embryo. Embryo formation is completed 10 days after anthesis; the newly formed embryo stores protein and fat (Nitsch, 1950). As such, ascorbic acid was not detected in any achenes of the tested strawberry cultivars. The ascorbic acid contents of the epidermis and flesh of the 'Maehyang' were 69.2 and 42.2 mg·100 g⁻¹ FW, respectively, and these values were the highest among all tested cultivars. Strawberry fruits are known to contain a large amount of ascorbic acid; the average amount is 60 mg·100 g⁻¹ FW, but it varied by cultivars (Lundergan and Moore, 1975; Slate and Robinson, 1945; Sturm et al., 2003). Interestingly, the peel fractions of some fruits possess higher antioxidant activity than the pulp fractions. As such, the peel fraction of fruits may potentially contain more antioxidants quantitatively or qualitatively than the pulp fraction (Guo et al., 2003).

Anthocyanin content of different tissues in strawberry fruits

We found differences in the anthocyanin content of different fruit tissues and cultivars (Fig. 2). The highest level of anthocyanin was found in the epidermis, followed by the achenes and then the flesh (except in the 'Red Pearl'). In the epidermis, the anthocyanin content of the 'Keumhyang' was the highest at 74.0 mg·100 g⁻¹ FW, while the lowest content was found in the 'Seolhyang' at 29.8 mg·100 g⁻¹ FW. An up to 2.5-fold difference in anthocyanin content was found between cultivars. The skin color of the fruit is usually redder than the inner flesh (Yoshida and Tamura, 2005). Holcroft

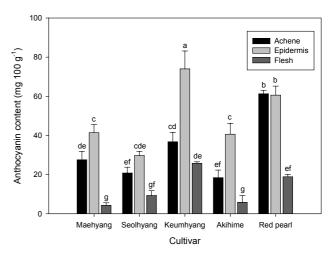


Fig. 2. The anthocyanin contents of different tissues in ripe strawberry fruits. Vertical bars show standard deviation (n = 3). Small letters inside the figure indicate mean separation by Duncan's multiple range test at p = 0.05.

and Kader (1999) reported that not only does the concentration of pigments differ between the outer and inner tissues of the 'Selva' cultivar, but so does the composition of pigments. Red color, which is a result of anthocyanin accumulation, usually occurs in the achenes, followed by the epidermis and finally in the inner receptacular tissue. Anthocyanin concentration in the cortex and pith was significantly lower in Korean and Japanese cultivars compared to European cultivars (Yoshida and Tamura, 2005). This difference may reflect cultural preferences, as in Korea and Japan strawberries are consumed intact and the color of the inner flesh may be less important than in Europe, where the berries are frequently cut and displayed or processed.

Ellagic acid content of different tissues in strawberry fruits

We found large differences in the ellagic acid content among various strawberry fruit tissues. The highest level of ellagic acid was found in the achenes, followed by the epidermis and then the flesh (Fig. 3). The ellagic acid contents of the epidermis and flesh tissues of all cultivars were below 3.2 mg·100 g⁻¹ FW. However, the levels in the achenes ranged from 70.8 to 215.5 mg·100 g⁻¹ DW. In the achenes, the ellagic acid content was highest in the 'Seolhyang' at 215.5 mg·100 g⁻¹ DW. These levels are in agreement with previously reported values for strawberries (Mass et al., 1991; Williner et al., 2003). In raspberries, 87.8% of the ellagic acid resides in the seeds, whereas in strawberries, 95.7% of the ellagic acid can be found in the pulp (Daniel et al., 1989). As such, it is likely that the ellagic acid in strawberries is more bioavailable than in raspberries.

The bioactive compound characteristics of the strawberries

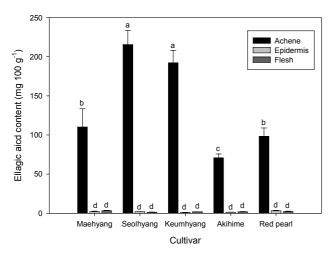


Fig. 3. The ellagic acid contents of different tissues in ripe strawberry fruits. Vertical bars show standard deviation (n = 3). Small letters inside the figure indicate mean separation by Duncan's multiple range test at p = 0.05.

in this study varied across different fruit tissues and cultivars. That information should make it possible to select strawberries with an optimal content of bioactive compounds for consumers. In addition, breeders might use those profiles to identify high levels of antioxidants in parents for future strawberry breeding programs. Moreover those foundational data could be used by the fruit processing industry for production of health nutrition supplements or research fields of food nutrition. Our results also suggest that the recently introduced Korean 'Maehyang', 'Seolhyang', and 'Keumhyang' contain higher levels of bioactive compound than other major June-bearing strawberry cultivars. Thus recently introduced Korean strawberry cultivars are comparable to other major cultivars in East Asian countries.

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일계성 딸기 과실의 부위에 따른 바이오 활성 화합물 함량 비교

김성 $\mathbf{a}^{1,2} \cdot \mathbf{m}$ 로나 $^3 \cdot \mathbf{v}$ 현 $\mathbf{c}^1 \cdot \mathbf{a}$ 무정 $^2 \cdot \mathbf{d}$ 혜령 $^1 \cdot \mathbf{d}$ 장후 1,2*

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조 록. '매향', '설향', '금향', '아키히메'와 '레드펄' 딸기 과실 부위에서 환원당, 유기산, 아스코르빈산, 안토시아닌 및 엘라직산의 함량을 비교하였다. 환원당, 유기산, 아스코르빈산 및 안토시아닌 함량은 딸기 표피에서 다른 부위의 함량 보다 유의하게 높았으나 엘라직산의 함량은 딸기 수과에서 높았다. '매향' 딸기의 표피 및 과육에서 아스코르빈산 함량은 각각 69.2와 42.2mg·100g¹ FW로 다른 품종에 비해 높았다. 그리고 '금향' 딸기의 표피 및 수과에서 안토시아닌 함량은 각각 74.0mg·100g¹ FW과 36.7mg·100g¹ DW으로 타 품종에 비해 유의하게 높았으며 '설향' 딸기 수과에서 엘라직산의 함량은 215.5mg·100g¹ DW로 타 품종에 비해 유의하게 높았다. 딸기 과실의 부위에 따라 바이오 활성 화합물들의 함량은 크게 차이가 있었으며 '매향', '설향' 및 '금향' 딸기 품종은 바이오 활성 화합물들의 함량이 높아 소비자들에게는 고품질 딸기로 인식될 수 있어 국내 육성 품종 딸기의 보급이 확산될 것으로 기대된다. 그리고 본 기초적 결과들은 딸기 가공 분야 및 영양학 연구 분야에도 유용하게 활용될 것으로 판단된다.

추가 주요어 : 수과, 항산화, 표피, 과육, Fragaria × ananassa