Fruit Quality and Chemical Contents of Hybrid Boysenberry (*Rubus ursinus*) Lines Developed by Hybridization and Gamma Irradiation

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ABSTRACT The *Rubus* fruit is an economically important berry crop that contains various functional compounds. The objective of this study was to analyze fruit qualities (i.e., pH, soluble solids content, titratable acidity, and mineral content) as well as fatty acid and phenolic compounds (i.e., ellagic acid and anthocyanins) among hybrid boysenberry lines developed by hybridization and gamma irradiation. There were no significant differences in the hybrid boysenberry fruit pH and titratable acidity (%) among the tested genotypes. However, the soluble solids content was higher in the BSA119 and BSA144 mutants than in the original genotype (BS_Hybrid). Meanwhile, linoleic acid was the most abundant fatty acid in the analyzed hybrid boysenberry fruits. The fatty acid composition did not differ significantly among the genotypes. The ellagic acid content of all genotypes ranged from 8.72 mg/100 g to 46.10 mg/100 g, with the highest concentration observed for the BSB127 genotype. Additionally, cyanidin-3-*O*-sophoroside (M-H⁺, 611 m/z) and cyanidin-3-*O*-glucoside (M-H⁺, 449 m/z) were the two major anthocyanins detected in the boysenberry and mutant genotypes, while cyanidin-3-*O*-glucoside was the predominant blackberry anthocyanin. The total anthocyanin concentrations of four mutant genotypes (i.e., BSA036, BSA078, BSA101, and BSB127) were significantly higher than that of the original genotype (382.0 mg/100 g). The highest total anthocyanin concentration was observed for the BSA078 genotype (467.9 mg/100 g). These results may be useful for identifying the optimal genotypes for breeding new cultivars with enhanced qualities and potential health benefits.

Keywords Edible oils, GC-MS, LC-MS, Mutation breeding, Phenolic compounds, Phytochemicals, Thornless mutant

INTRODUCTION

Boysenberry (*Rubus ursinus* Chamisso & Schlenhtendal) is a hybrid *Rubus* berry derived from a cross between loganberry (*Rubus loganobaccus* Bailey) and trailing blackberry (*Rubus fruticosus* L.) (Zafra-Stone *et al.* 2007). *Rubus* fruits are recognized for the health benefits of their natural phytochemicals, such as phenolics, vitamin C, minerals, and fatty acids (Kaume *et al.* 2012; Lee *et al.* 2012; Lee *et al.* 2014). These fruits are rich in phenolics,

and the contribution of these compounds to antioxidant activities will be of interest to breeders whose goals include developing new varieties that produce higher quality and healthier fruits than what is currently available (Wada and Ou 2002; Parry *et al.* 2005; Guedes *et al.* 2013). Anthocyanins and ellagic acid are abundant phenolic compounds in *Rubus* fruits (Wada and Ou 2002; Cooney *et al.* 2004; Ryu *et al.* 2016). The potential health benefits of anthocyanins have led to a renewed interest in the consumption of foods rich in anthocyanins (e.g., berry fruits) and the bio-

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absorption of these phenolic compounds. Previous studies revealed that the possible positive health effects resulting from the intake of *Rubus* fruit anthocyanins include decreased oxidative stress, lowered serum cholesterol levels, and protection from liver ischemia-reperfusion injury (Cooney *et al.* 2004; Ghosh *et al.* 2006; Cho *et al.* 2015). Among the *Rubus* species, boysenberry has the most ellagic acid, which is a potential anticarcinogenic compound (Wada and Ou 2002). Additionally, specialty high-value *Rubus* fruits are gaining attention owing to their health benefits, which are linked to their abundance of polyunsaturated fatty acids (Parry *et al.* 2005; Van Hoed *et al.* 2009; Ferreira de Araujo *et al.* 2011).

Mutation breeding involves the use of a mutagen to create plants exhibiting a few new mutant characteristics that do not disturb other varietal traits. The fruit industry relies on a limited number of clonally propagated cultivars established based on specific fruit quality parameters and consumer familiarity with the product, and is very resistant to changes (Predieri *et al.* 2001; Kim *et al.* 2015; Jo *et al.* 2016; FAO/IAEA Mutant Variety Database [<https://mvd.iaea.org/>]). This limits the application of cross-breeding in fruit-bearing plant species, as fruit cultivars are generally highly heterozygous, and progenies resulting from cross-breeding express many traits that are different from those of the parents (Predieri *et al.* 2001; Jo *et al.* 2016; FAO/IAEA Mutant Variety Database). In *Rubus* species, mutagenesis has been used to manipulate various traits, including the presence of spines, fruit size and color, disease resistance, timing of maturity, yield, and cold-induced resistance to

pathogens (Donini 1982; Ryu *et al.* 2016; FAO/IAEA Mutant Variety Database). Improved thornless cultivars have been produced by breeders to meet the demands of growers who wish to maintain an economic advantage in the marketplace. Additionally, evaluating boysenberry fruit resources regarding phytochemical contents is important and may be considerably beneficial for the food and pharmaceutical industries (Predieri *et al.* 2001; Clark *et al.* 2007; Ryu *et al.* 2016).

The objective of this study was to investigate the nutritional characteristics [i.e., mineral content, soluble solids content (SSC), and soluble sugar content] and functional compounds (i.e., anthocyanins, ellagic acid, and fatty acid) of *Rubus* genotypes.

MATERIALS AND METHODS

Plant materials and harvest

Eleven genotypes were analyzed in this study (Table 1). Among these genotypes, the BS_PI cultivars was introduced from Japan and produced spines, while the others were stabilized lines from advanced generations and were thornless (Fig. 1). The BS_Hybrid line was obtained from crosses between the thornless blackberry (*Rubus fruticosus* L. cv. V3) and boysenberry (Shin *et al.* 2008). V3 line was developed from spiny blackberry explants exposed to somaclonal variation. Six mutant genotypes (BSA036 to BSA144) were developed from hybrid boysenberry explants exposed to 20 Gy gamma radiation.

Table 1. Origin of hybrid boysenberry genotypes used in this study.

No.	Line	Origin	Treatment	Stem spiny
1	BS_PI	Boysenberry from Japan		Spiny
2	Blackberry (V3)	Hybrid Blackberry	Somaclonal variation	Thornless
3	BS_Hybrid	Cross breeding	Blackberry(V3) Boysenberry	Thornless
4	BSA-036	BS_Hybrid	Gamma-ray 20 Gy	Thornless
5	BSA-065	BS_Hybrid	Gamma-ray 20 Gy	Thornless
6	BSA-078	BS_Hybrid	Gamma-ray 20 Gy	Thornless
7	BSA-101	BS_Hybrid	Gamma-ray 20 Gy	Thornless
8	BSA-119	BS_Hybrid	Gamma-ray 20 Gy	Thornless
9	BSA-144	BS_Hybrid	Gamma-ray 20 Gy	Thornless
10	BSB-032	BS_Hybrid	Gamma-ray 40 Gy	Thornless
11	BSB-127	BS_Hybrid	Gamma-ray 40 Gy	Thornless

Fig. 1. Stem profile of boysenberry genotype used in this study. (A) Spiny, (B) thornless.

Another two mutant genotypes (BSB032 and BSB127) were produced from hybrid boysenberry explants treated with 40 Gy gamma radiation. The eight mutant lines exhibited improved agronomic characteristics, including higher fruit yields and sugar contents than the original parent. Fully ripened fruits were collected from a $3 \times 3 \text{ m}^2$ quadrant. Three replicates were used for each sample.

Estimation of pH, soluble solids content, and titratable acidity

The pH was measured using a Docu-pH meter (Sartorius Inc., Göttingen, Germany). The SSC was determined using the PR-101 hand-held refractometer (Atago USA Inc., Bellevue, WA, U.S.A.). The titratable acidity (%) was calculated according to the AOAC (1995) method.

Mineral content

We determined the mineral contents using the AOAC (1995) methods. Samples (1.0 g) were weighed and subjected to dry ashing in a clean porcelain crucible at 550°C in a muffle furnace. The resultant ash was dissolved in 5.0 mL nitric acid: hydrogen chloride: purified water (1:2:3) and gently heated on a heating mantle until the brown fumes disappeared. Then, 5.0 mL distilled water was added to each sample, which was then heated until a

colorless solution was obtained. The mineral solution was filtered into a 100 mL volumetric flask. The elemental composition of the solution was analyzed in triplicate using a Model 403 atomic absorption spectrophotometer (Perkin Elmer, Waltham, MA, U.S.A.).

Fatty acid composition

Fatty acid composition was analyzed by gas chromatography-mass spectrometry (GC-MS). According to the Soxhlet extraction procedure, 5 g crushed dried fruit (80 mashed) was packed into a thimble and the oils were extracted with diethyl ether for 6 h. The fruit lipid extracts were mixed with a 20% boron trifluoride-methanol reagent, and the fatty acids were converted into methyl ester derivatives. The methyl esters of the fatty acids were dissolved in chloroform and analyzed by GC-MS (Plus-2010; Shimadzu, Kyoto, Japan). The fruit fatty acid composition was analyzed using a GC-MS instrument equipped with an HP-88 capillary column ($60 \text{ m} \times 0.25 \text{ mm}$; J & W Scientific, CA, U.S.A.) under the following conditions: ionization voltage, 70 eV; mass scan range, 35–450 mass units; injector temperature, 230°C ; detector temperature, 230°C ; injection volume, 1 μL ; split ratio, 1:20; carrier gas, helium; and flow rate, 1.7 mL/minute. The column temperature program specified an isothermal temperature of 40°C for 5 minutes followed by an increase to 180°C at a rate of $5^\circ\text{C}/\text{minute}$ and a subsequent increase to 230°C at a rate of $1^\circ\text{C}/\text{minute}$. We identified the substances present in extracts by comparing their mass spectra against a database of GC-MS spectra (Nist. 62 Library) and based on their retention indices.

Ellagic acid analysis

The lyophilized, powdered fruits were diluted with purified water and methanol (37.5:63.5). We then added 10 mL 1.2 mol hydrogen chloride to the diluted mixture, which was refluxed for 16 hours at $80 \pm 5^\circ\text{C}$. The extract was cooled and filtered through a $0.45 \mu\text{m}$ membrane. The following gradients were used: 0–3 minutes, 10–20% B; 3–6 minutes, 20–30% B; 6–9 minutes, 30–50% B; 9–15 minutes, 50–60% B; 15–18 minutes, 60–70% B; 18–21 minutes, 70–50% B; 21–24 minutes, 50–30% B; and 24–27 minutes, 30–0% B. Ellagic acid was detected at 254 nm and

identified using commercial standards (Sigma, St. Louis, MO, USA).

Anthocyanin analysis

Anthocyanins were extracted from samples as described by Ryu *et al.* (2016). Briefly, 0.5 g freeze-dried fruits were treated with 5 mL methanol:water:hydrochloric acid (70:29:1, v/v) at 4°C in darkness for 24 hours, with periodic vortexing. The material was filtered through a 0.45 µm membrane filter. Three extract replicates were analyzed for each sample. The extracted anthocyanins were analyzed using a 1260 series high-performance liquid chromatography system, a 380 evaporative light scattering detector, and a 6130 quadrupole mass spectrometry system (Agilent Technologies, USA) equipped with a Poroshell 120 SB-C18 column (150 mm × 4.6 mm internal diameter, 2.7 µm particle size; Agilent Technologies, CA, U.S.A.) with a compatible C18 guard column (4 mm × 3 mm internal diameter, 3 µm particle size; Phenomenex, CA, USA). The mobile phase was composed of water (A, 0.05% formic acid) in acetonitrile (B, 0.05% formic acid). The following gradients were used: 0-3 minutes, 95% A and 5% B; 3-28 minutes, 100% B; and 28-35 minutes, 100% B. The flow rate of the mobile phase was adjusted to 0.5 mL/minute, and the column temperature was set to 24°C. The injection volume was 10 µL. The optimum parameters were as follows: drying gas pressure, 12.0 L/minute; positive and negative capillary voltage, 3000 Volt; drying gas temperature, 350°C; and nebulizer gas pressure, 35 psig

(Lb/in²). The MSD signal settings in the positive/negative mode were as follows: mass range (m/z), 100-1000; fragmentor, 70; and scan mode. The evaporative light scattering detector settings were as follows: gas flow rate, 1.60 SLM; LED intensity, 100%; evaporative temperature, 30°C; nebulizer temperature, 30°C.

Data analysis

Data were subjected to analysis of variance using the multiple comparisons method of SPSS version 12 (SPSS Inc., Chicago, IL, USA). Correlation analyses were based on Pearson's correlation coefficient. A *P*-value<0.05 indicated statistical significance. If a treatment effect was determined to be significant, the means were differentiated using Duncan's multiple range tests.

RESULTS

Estimation of pH, soluble solids content, and titratable acidity

The pH, SSC, and titratable acidity of the analyzed hybrid boysenberry fruits are listed in Table 2. There were no significant differences in the pH and titratable acidity (%) of *Rubus* fruits among genotypes. In contrast, the SSC value of the BS_Hybrid genotype was lower than that of the mother cultivars (i.e., boysenberry and blackberry). The SSC was higher in the BSA119 and BSA144 genotypes than in the original genotype (BS_Hybrid). The BSA101

Table 2. The hydrogen exponent, sugars content and titratable acidity of fruit for hybrid boysenberry genotypes.

Line	Hydrogen exponent	SSC (°Brix)	Titratable acidity (%)
BS_PI	3.7±0.1a ²⁾	7.6±0.1b ²⁾	1.8±0.2a ²⁾
Blackberry (V3)	3.6±0.0a	7.4±0.2bc	1.7±0.0a
BS_Hybrid	3.8±0.2a	6.0±0.3d	1.5±0.3a
BSA-036	3.6±0.1a	6.1±0.2d	1.5±0.3a
BSA-065	3.8±0.0a	6.3±0.2d	1.6±0.3a
BSA-078	3.7±0.1a	6.2±0.2d	1.5±0.3a
BSA-101	3.6±0.1a	5.9±0.1d	1.8±0.2a
BSA-119	3.6±0.0a	8.8±0.1a	1.5±0.3a
BSA-144	3.6±0.0a	7.1±0.3c	1.6±0.3a
BSB-032	3.6±0.1a	6.1±0.2d	1.7±0.2a
BSB-127	3.5±0.1a	6.2±0.2d	1.8±0.2a

²⁾Mean separation within columns by Duncan's multiple range tests (*P*≤0.05, n=3).

genotype had the lowest SSC, although it was not significantly different than the SSC of the original genotype.

Mineral content

The mineral contents of the hybrid boysenberry genotypes are provided in Table 3. Potassium, calcium, and magnesium were the most abundant minerals in the fruits. Among the minerals, potassium was detected at the highest concentrations for all genotypes, with BSB127 having the highest potassium content. The mutant genotypes contained less calcium and magnesium than the boysenberry and blackberry cultivars. The boysenberry cultivar (BS_PI) had the highest fruit sodium content. Meanwhile,

there were no significant differences in the iron and zinc contents among the genotypes.

Fatty acid composition

The fruit fatty acid compositions of the hybrid boysenberry genotypes are presented in Table 4 and Supplementary Fig. S1. The GC-MS chromatograms revealed the presence of five fatty acids (i.e., palmitic acid, stearic acid, oleic acid, linoleic acid, and linolenic acid). Linoleic acid was the major fatty acid present in fruits, ranging from 65.00% (BSA036) to 67.61% (BS_PI). The saturated fatty acid (SFA) content ranged from 7.92% (BSA119) to 9.33% (BSA036), while the unsaturated fatty

Table 3. Mineral content of fruit in hybrid boysenberry genotypes. (mg/100 g)

Line	Ca	Mg	K	Na	Fe	Zn
BS_PI	22.88a ²⁾	18.14a ²⁾	181.70b ²⁾	3.03a ²⁾	0.57a ²⁾	0.39a ²⁾
Blackberry (V3)	23.03a	16.97a	123.60g	1.45b	0.34a	0.17a
BS_Hybrid	19.47b	8.91c	111.80h	0.99b	0.27a	0.21a
BSA-036	17.45b	14.20b	144.80e	0.65b	0.37a	0.31a
BSA-065	19.68b	15.30b	145.62e	1.24b	0.29a	0.33a
BSA-078	18.69b	13.98b	145.96e	0.94b	0.33a	0.31a
BSA-101	18.66b	14.52b	136.94f	0.97b	0.60a	0.31a
BSA-119	19.42b	15.25b	148.55e	1.10b	0.25a	0.19a
BSA-144	19.74b	14.72b	164.58d	1.26b	0.29a	0.20a
BSB-032	18.47b	13.97b	174.82c	1.53b	0.29a	0.18a
BSB-127	19.60b	13.97b	193.40a	1.63b	0.32a	0.28a

²⁾Mean separation within columns by Duncan's multiple range tests ($P \leq 0.05$, $n=3$).

Table 4. Fatty acid composition of fruit in hybrid boysenberry genotypes. (%)

Line	C16:0	C18:0	C18:1	C18:2	C18:3	SFA	UFA
BS_PI	6.70a ²⁾	2.32c ²⁾	10.55a ²⁾	67.61a ²⁾	12.83b ²⁾	9.02a ²⁾	90.98a ²⁾
Blackberry (V3)	6.74a	1.48f	10.66a	67.19a	13.94a	8.22a	91.79a
BS_Hybrid	6.75a	2.06d	10.42a	66.51a	14.27a	8.81a	91.20a
BSA-036	6.32a	3.01a	10.93a	65.00b	14.76a	9.33a	90.68a
BSA-065	6.39a	2.52a	10.79a	66.87a	13.44a	8.91a	91.10a
BSA-078	6.28a	2.85a	10.43a	66.57a	13.89a	9.12a	90.88a
BSA-101	6.56a	1.92e	11.02a	66.98a	13.54a	8.47a	91.53a
BSA-119	6.06a	1.86e	10.12a	67.52a	14.45a	7.92a	92.08a
BSA-144	6.56a	2.44b	10.99a	65.99a	14.03a	9.00a	91.01a
BSB-032	6.47a	2.60a	10.97a	66.49a	13.48a	9.07a	90.94a
BSB-127	6.67a	2.23c	10.44a	66.68a	13.99a	8.90a	91.11a

C16:0 = palmitic acid, C18:0 = stearic acid, C18:1 = oleic acid, C18:2 = linoleic acid, C18:3 = α -linolenic acid.

²⁾Mean separation within columns by Duncan's multiple range tests ($P \leq 0.05$, $n=3$).

SFA: saturated fatty acid, UFA: unsaturated fatty acid.

acid (UFA) content ranged from 90.68% (BSA036) to 92.08% (BSA119). There were no significant differences in the contents of palmitic acid, oleic acid, SFA, and UFA among all genotypes.

Ellagic acid content

The ellagic acid contents are presented in Fig. 2. We observed significant differences among the analyzed hybrid boysenberry genotypes. The BSB127 genotype had the highest ellagic acid concentration (46.40 ± 1.33 mg/100 g), followed by blackberry (39.31 ± 0.05 mg/100 g), BSA036 (36.05 ± 0.61 mg/100 g), BSA101 (20.04 ± 0.43 mg/100 g), boysenberry and BSA144 (13.37 ± 0.19 mg/100 g),

BSA032 (11.89 ± 1.33 mg/100 g), BSA078 (12.30 ± 0.18 mg/100 g), BSA119 (11.13 ± 0.33 mg/100 g), and BSA065 (8.72 ± 0.36 mg/100 g).

Anthocyanin content

The anthocyanin contents of the hybrid boysenberry genotypes are provided in Table 5 and Supplementary Fig. S2. Cyanidin-3-*O*-glucoside ($M-H^+$, 449 m/z) was the major blackberry anthocyanin. In contrast, cyanidin-3-*O*-sophoroside ($M-H^+$, 611 m/z) and cyanidin-3-*O*-glucoside were the major anthocyanins in boysenberry and BS_Hybrid lines.

The BSA078 genotype had the highest cyanidin-3-*O*-

Fig. 2. Ellagic acid content in hybrid boysenberry genotypes. The letters above each point indicate a significant difference at the 5% level (Duncan's multiple range tests, $n=3$).

Table 5. Anthocyanin content of fruit for hybrid boysenberry genotypes. (mg/100 g)

Line	Cy-3- <i>O</i> -Sop	Cy-3- <i>O</i> -Glu	Total anthocyanin
Boysenberry	48.3 ± 08.9 de ²⁾	177.4 ± 8.7 g ²⁾	225.7 ± 0.2 g ²⁾
Blackberry	NDf	184.0 ± 2.0 g	184.0 ± 2.0 h
BS_Hybrid	72.6 ± 12.4 bc	309.4 ± 6.6 d	382.0 ± 5.8 d
BSA-036	82.3 ± 14.0 b	344.6 ± 7.8 bc	426.9 ± 6.3 c
BSA-065	56.5 ± 09.5 c	205.4 ± 8.9 f	261.9 ± 0.7 f
BSA-078	107.5 ± 19.2 a	360.5 ± 16.2 ab	467.9 ± 3.0 a
BSA-101	91.2 ± 15.8 ab	373.7 ± 09.1 a	464.9 ± 6.7 a
BSA-119	71.2 ± 12.3 bc	273.3 ± 08.6 e	344.4 ± 3.7 e
BSA-144	33.6 ± 05.7 e	118.3 ± 03.4 h	152.0 ± 2.3 i
BSB-032	40.4 ± 06.9 de	95.2 ± 03.5 i	135.5 ± 3.4 j
BSB-127	85.9 ± 14.4 b	353.4 ± 08.2 b	439.3 ± 6.2 b

²⁾Mean separation within columns by Duncan's multiple range tests ($P \leq 0.05$, $n=3$).

Cy-3-*O*-Sop: Cyanidin-3-*O*-sophoroside ($M-H^+$, 611 m/z), Cy-3-*O*-Glu: Cyanidin-3-*O*-glucoside ($M-H^+$, 449 m/z), ND: Not detected.

sophoroside concentration (107.5 mg/100 g), followed by BSA101 (91.2 mg/100 g), BSB127 (85.9 mg/100 g), BSA036 (82.3 mg/100 g), BS_Hybrid (72.6 mg/100 g), BSA119 (71.2 mg/100 g), BSA065 (56.5 mg/100 g), boysenberry (48.3 mg/100 g), BSB032 (40.4 mg/100 g), and BSA114 (33.6 mg/100 g). Cyanidin-3-*O*-sophoroside pigments were undetectable in blackberry fruits. The highest cyanidin-3-*O*-glucoside concentration (373.7 mg/100 g) was observed for the BSA101 cultivar, followed by BSA078 (360.5 mg/100 g), BSB127 (353.4 mg/100 g), BSA36 (344.6 mg/100 g), BS_Hybrid (309.4 mg/100 g), BSA119 (273.3 mg/100 g), BSA065 (205.4 mg/100 g), blackberry (184.0 mg/100 g), boysenberry (177.4 mg/100 g), BSA144 (118.3 mg/100 g), and BSB032 (95.2 mg/100 g). The total anthocyanin contents differed significantly among the genotypes, ranging from 135.5 mg/100 g (BSB032) to 467.9 mg/100 g (BSA078) (i.e., a 3.5-fold difference among genotypes).

DISCUSSION

A limited number of *Rubus* genetic resources have been established based on fruit quality and functional compound contents. This has limited the application of cross-breeding to generate novel *Rubus* varieties. However, induced mutations change only one or a few specific traits of an elite cultivar, and may be useful for improving fruit production (Predieri 2001; Basaran and Kepenek 2011). In this study, the BS_Hybrid genotype, which was obtained from crosses between thornless blackberry (*R. fruticosus* L. cv. V3) and boysenberry, has unfavorable fruit characteristics such as a malformed fruit and relatively low sugar content. In contrast, the eight mutant genotypes produced by the gamma irradiation of BS_Hybrid explants, exhibited improved agronomic characteristics, such as higher fruit yields and sugar contents than the original parent.

The pH, SSC, and titratable acidity are very important determinants of fruit quality. Previous studies concluded that variations in these traits depend on the genotype, harvest time, and environmental conditions (Connor *et al.* 2005; Basaran and Kepenek 2011; Ryu *et al.* 2016). The SSC was higher for BSA119 and BSA144 than for the

original genotype. These genotypes may be useful as potential sources of fresh fruit materials. The analyzed *Rubus* fruits contained high levels of potassium, calcium and magnesium, which is consistent with the findings of Ryu *et al.* (2016). Fruit mineral contents are considerably influenced by soil conditions, fertilizer treatments, climatic conditions, and cultivars (Guedes *et al.* 2013). Because all of the genotypes were grown under the same conditions, any differences in mineral content were likely cultivar-dependent, which may have implications for the selection of *Rubus* breeding lines (Clark *et al.* 2007). All of the mutant genotypes contained less calcium and magnesium than the boysenberry and blackberry cultivars. In contrast, the BSB127 potassium content was approximately 1.7-fold higher than that of the original genotypes.

Edible oils have been extracted and used as sources of food ingredients since ancient times (Parry *et al.* 2005; Van Hoed *et al.* 2009; Ferreira de Araujo *et al.* 2011). Linoleic acid was the most abundant fatty acid in all examined hybrid boysenberry genotypes. Additionally, the fruits contained very high levels of linoleic acids (65.00–67.61%) and UFA (90.68–92.08%). The observed linoleic acid levels in hybrid boysenberry genotypes were higher than those reported for different berries (i.e., red raspberry, marionberry, boysenberry, and blueberry) (Parry *et al.* 2005; Kaume *et al.* 2012). The UFA concentrations for all hybrid boysenberry genotypes were similar to those reported by Parry *et al.* (2005) (e.g., 91.3% for a boysenberry cultivar). Previous studies confirmed that *Rubus* fruit fatty acid compositions are strongly influenced by genotype, maturation stage, and climate (Parry *et al.* 2005; Van Hoed *et al.* 2009). However, in the present study, there were no significant differences in the SFA and UFA contents of all genotypes.

We observed significant differences in the fruit ellagic acid contents among hybrid boysenberry genotypes. The ellagic acid levels of the BS_PI, BS_Hybrid, BSA065, BSA078, BSA101, BSA119, BSA144, and BSA032 genotypes were lower than those of previously analyzed blackberry mutant lines (Ryu *et al.* 2016). Ellagic acid contents generally decrease as fruits mature (Ryu *et al.* 2016). The ellagic acid levels of the blackberry (V3), BSA036, and BSB127 genotypes were similar to those

reported by Ryu *et al.* (2016) (i.e., 35-47 mg/100 g for ripe blackberry fruits). In the current study, the highest ellagic acid concentration was observed for the BSB127 fruits.

Anthocyanins are important for responses to oxidative stress because of their ability to quickly scavenge reactive oxygen species (Lee *et al.* 2014; Ryu *et al.* 2016). The major anthocyanin in blackberries is cyanidin-3-*O*-glucoside, which can prevent cell damage, and may be beneficial in treatments for obesity and various cancers (Ghosh *et al.* 2006; Cho *et al.* 2015). The predominant anthocyanins in boysenberries are the disaccharides cyanidin-3-*O*-sophoroside and cyanidin-3-*O*-glucoside (Cooney *et al.* 2004; Fan-Chiang and Wrolstad 2005; Ghosh *et al.* 2006). Cyanidin-3-*O*-sophoroside is a natural colorant in cherries, raspberries, and boysenberries (Sugimoto *et al.* 2003; Cooney *et al.* 2004). This compound is important because it protects against oxidative stress and DNA damage, and may have beneficial health effects for diabetics (Sugimoto *et al.* 2003; Cooney *et al.* 2004; Ghosh *et al.* 2006). The anthocyanin concentrations of the hybrid boysenberry genotypes included in the current study ranged from 135.5 mg/100 g to 467.9 mg/100 g. These findings are similar to those described by Ryu *et al.* (2016) (i.e., 191.6-558.2 mg/100 g for five mutant blackberry cultivars). The highest anthocyanin content was detected in the BSB127 genotype. Lee *et al.* (2012) reported that anthocyanins (e.g., cyanidin-3-*O*-sophoroside, cyanidin-3-*O*-glucosylrutinoside, and cyanidin-3-*O*-glucoside), ellagic acid, and folate exhibit antioxidant activities. Ryu *et al.* (2016) concluded that the antioxidant activities of blackberries are correlated with total anthocyanin contents ($P \leq 0.05$). The application of *Rubus* species as a resource for functional compounds may result in an increase in *Rubus* fruit consumption (Cho *et al.* 2015; Ryu *et al.* 2016). Additionally, breeding novel *Rubus* cultivars with high anthocyanin and ellagic acid concentrations may be possible. Our results suggest that the BSA078, BSA101, and BSB127 genotypes are potentially the most appropriate materials for improving the health benefits of fruits.

In this study, we obtained abundant information about the nutritional characteristics and functional compounds of eleven hybrid boysenberry. Among the detected mineral, potassium was found to be the most abundant in all the

genotypes. As for the detected fatty acid, linoleic acid (C18:2) was found as a major one. The major anthocyanin in boysenberry, BS_hybrid and mutant lines were cyanidin-3-*O*-glucoside and cyanidin-3-*O*-sophoroside. Whereas, cyanidin-3-*O*-sophoroside compound was not detected in blackberry. Among the experimental genotypes even BSA119 seemed to have a high in soluble solids content. In addition, our results suggest that the BSA078, BSA101, and BSB127 genotypes are the most appropriate for the selection of functional boysenberry because they contained higher antioxidant components (anthocyanin and ellagic acid) contents.

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