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Effects of Different Growing Regions on Quality Characteristics, Bioactive Compound Contents, and Antioxidant Activity of Aronia (*Aronia melanocarpa*) in Korea

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ABSTRACT: The objective of this study was to determine the effects of different growing regions on quality characteristics, total bioactive compound contents, and *in vitro* antioxidant activity in aronia. Aronia grown in 3 different regions (Sangjoo, Ulju, and Youngcheon) in Korea was obtained and used fresh or as a freeze-dried powder. No statistically significant differences were observed for moisture, ash, crude lipid, and crude protein contents in aronia sampled from the 3 different regions. Aronia grown in Sangjoo had the highest total acid content and the lowest sugar content and pH value. Conversely, aronia grown in Youngcheon possessed the lowest total acid content and the highest sugar content and pH value. Aronia grown in Sangjoo possessed relatively high levels of polyphenols, flavonoids, and anthocyanins, as well as high antioxidant activity in comparison with aronia produced in other regions. Aronia grown in Youngcheon scored the highest for taste and overall acceptability in sensory evaluations, which may be related to the high sugar content and pH, and the low total acidity of the fruits. It is possible that higher sugar contents and pH, and lower total acidity in the aronia grown in Youngcheon result in more preferable sensory characteristics. However, they also contain relatively low levels of total polyphenols, flavonoids, and anthocyanins, and have low antioxidant activity as measured by 2,2-diphenyl-1-picrylhydrazyl and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging assays.

Keywords: aronia, growing region, polyphenols, anthocyanins, antioxidant activity

INTRODUCTION

Aronia (*Aronia melanocarpa*) belongs to the Rosacea family, and it is primarily cultivated in Poland, European countries, and North America (1). Aronia is known as a "super fruit" because it is a good source of bioactive phytochemicals such as polyphenols, flavonoids, and anthocyanins (2,3). It has various health benefits such as anticancer and antimutagenic activities, as well as blood pressure lowering properties (2-5). Aronia was introduced in Korea 5~6 years ago and the growing areas are increasing in several regions. Aronia is being evaluated as a potential new fruit for use in natural colorants, juices, and as a source of ingredients with good antioxidant activities in several processed foods (6-8).

Internal and external factors affect fruit characteristics, as well as the quantity and composition of bioactive compounds in aronia. Ecological factors such as geology, soil characteristics, and climatic conditions (temperature, sunlight, water status, and humidity) have been known to influence the quality of fruits and vegetables (9-11). The biosynthesis and accumulation of phenolic compounds can be controlled endogenously during developmental differentiation (12). Furthermore, differences in the levels of phenolic compounds in the fruit depend on a number of factors, such as genotype, environmental conditions in growing regions, and the degree of maturity at harvest (13). The increased application of fertilizers to aronia has been shown to increase vegetative growth and yield, but to decrease the anthocyanin content and total acidity (14). Mphahlele et al. (15) noted that the chemical content of pomegranate and other types of fresh produce was associated with the elevation of the growing location, and reported that fruit grown at high altitude locations and under high light intensity had significantly higher vitamin C contents.

The purpose of our research was to determine the effects of different geographic growing regions on quality characteristics, bioactive compound contents, and antioxidant activities of aronia.

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MATERIALS AND METHODS

Chemicals

Folin-Ciocalteu's phenol reagent, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), catechin, gallic acid, nitro blue tetrazolium chloride (NBT), nicotinamide adenine dinucleotide (NADH), Tris-HCl, and *p*-methyl styrene (PMS) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Anthocyanin and polyphenol standards for high performance liquid chromatography (HPLC) analysis were purchased from Extrasynthese (Genay, France) and Sigma-Aldrich Co., respectively. HPLC grade water, methanol, acetonitrile, and trifluoroacetic acid (TFA) were purchased from Fisher Scientific Inc. (Fair Lawn, NJ, USA). All chemicals used were of analytical grade.

Sample preparation

A 'Nero' cultivar of aronia of similar ripeness was harvested at the end of August, 2014 from growing farms in 3 different regions (Sangjoo, Ulju, and Youngcheon) of Korea. Immediately after harvest, aronia was manually removed from stems, washed in cold water, surface dried for 2 h at ambient temperature, and immediately stored at different temperatures for use in subsequent experiments. For the determination of proximate contents, total sugars, total acidity, and pH were measured, and for sensory evaluation, the samples were stored and measurements were conducted at 4°C. For the determination of total polyphenols, flavonoids, anthocyanins, amino acid contents, and antioxidant activities, the black chokeberries were frozen at −80°C overnight and freeze-dried for 2 days. The freeze-dried samples were finely ground in a food grinder. Samples of lyophilized powder were stored at −80°C until analysis.

Proximate analysis

Moisture, ash, crude lipid, and crude protein levels of aronia were determined according to the Association of Official Analytical Chemists (AOAC) standard methods (16).

Total sugar content, total acid content, and pH

Aronia samples were ground in a food grinder and filtered through cheesecloth to obtain the juice, which was subsequently used to determine the total sugar content, total acidity, and pH. Total sugar content of aronia was determined using a digital refractometer (PR-100, Atago Co., Ltd., Tokyo, Japan) and expressed as °Brix. The total acid content was determined by titrating the juice with a standardized base, using citric acid equivalents based on the AOAC standard method (17). Sample pH was determined using a pH meter (420 Benchtop, Orion Research Inc., Beverly, MA, USA).

Determination of total polyphenols, total flavonoids, and total anthocyanins

The total polyphenol content was measured using the Folin-Ciocalteu's phenol reagent according to the method described by Zhou et al. (18) with slight modifications. Briefly, 200 μL of the appropriately diluted sample was mixed with 400 μL 2% 2 N Folin-Ciocalteu's phenol reagent. After 3 min at room temperature, 800 μL 10% Na_2CO_3 was added and mixed. Next, the mixture was kept in the dark by covering with aluminum foil at room temperature for 1 h. After vortexing, the absorbance was measured at 750 nm using a microplate reader (Infinite M200 Pro, Tecan Group Ltd., San Jose, CA, USA). Gallic acid was used as a calibration standard, and the results were expressed as gallic acid equivalents (GAE) per gram dry weight (mg GAE/g).

The total flavonoid content of the ethanolic extract of aronia was measured using the method described by Woisky and Salatino (19) with slight modifications. Briefly, 500 μL of sample was mixed with 30 μL 5% $NaNO_2$ and allowed to react for 6 min at room temperature. Next, 60 μL 10% $AlCl_3 \cdot 6H_2O$ was added, and the mixture was continuously mixed at room temperature for 6 min before 200 μL of 1.0 M NaOH was added. Finally, 110 μL distilled water was added and mixed. The absorbance of the colored flavonoid-aluminum complex was measured immediately at 510 nm using a microplate reader. Catechin was used as a calibration standard, and the results were expressed as catechin equivalents (CE) per gram dry weight (mg CE/g).

The total anthocyanin content was measured by Giusti and Wrolstad (20) with slight modifications. Briefly, 50 μL samples were mixed well with 950 μL distilled water to obtain the sample for this experiment. Next, 950 μL buffer (pH 1), which contained potassium chloride and 0.2 M hydrochloric acid, was added to 50 μL of the sample, and 50 μL of the resultant sample was mixed with buffer pH 4.5. The absorbance was measured at 520 and 700 nm using a microplate reader after vortexing. Total anthocyanin content was calculated using the following equation:

$$\text{Total anthocyanin content (mg/100 g)} = \frac{A \times MW \times DF \times 1 \times 1{,}000}{\varepsilon \times 1}$$

$$A = (OD_{520nm} - OD_{700nm})_{pH\ 1.0} - (OD_{520nm} - OD_{700nm})_{pH\ 4.5}$$

where MW is molecular weight of cyanidin-3-glucoside (C3G) (449.2), DF is dilution factor, ε is C3G molar absorptivity (26,900), and 1 is total volume (1 mL).

Extraction and quantification of anthocyanins and polyphenols by HPLC

Powdered samples (~100 mg) were weighed; 5 mL methanol containing 0.1% formic acid was added to the mixture, followed by vortex mixing for 1 min. The mixture was centrifuged for 5 min, and the top layer was removed to another glass tube. The aqueous layer was extracted with another 5 mL of methanol containing 0.1% formic acid. The extraction was performed 3~4 times until aronia extracts were colorless. The methanolic fractions were combined and evaporated to dryness in a rotary evaporator (EYELA, Tokyo, Japan). The residue was dissolved again in extraction solvent obtaining an appropriate concentration for HPLC analysis, and a 10 μL volume was injected. The anthocyanins were separated using a C_{18} Zorbox SB column (4.6×250 mm, 5 μm particle size, Agilent Technologies Inc., Santa Clara, CA, USA).

The solvent system employed water with 5% formic acid (A) and acetonitrile with 5% formic acid (B). The samples were separated according to the following gradient: A/B=95/5 (0~5 min), 90/10 (8 min), 85/15 (13~18 min), 80/20 (25 min), 70/30 (28~32 min), and 95/5 (35~40 min) at a flow rate of 0.8 mL/min. The peaks were detected using an UV detector at 520 nm (Waters Corporation, Milford, MA, USA).

The polyphenol content was analyzed by HPLC (Ultimate 3000, Dionex, Sunnyvale, CA, USA) on an Agilent XDB C_{18} column (4.6×150 mm, 5 μm, Agilent Technologies Inc.). The solvent system employed was water with 0.3% TFA (A) and acetonitrile (B). The samples were separated according to the following gradient: A/B=95/5 (0~39 min), 40/60 (40 min), 0/100 (45~50 min), and 95/5 (55~60 min) at a flow rate of 0.8 mL/min. The peaks were detected using an UV detector (190−800 DAD scanning) at 280 nm (Waters Corporation).

Extraction and quantification of amino acids by HPLC

Powdered samples (~100 mg) were weighed; 5 mL 80% ethanol was added to the mixture, followed by vortex mixing for 1 min. The mixture was centrifuged for 5 min, and the upper layer was removed to another glass tube. The aqueous layer was extracted with another 5 mL 80% ethanol and the extraction was performed 3 times. The ethanolic fractions were combined and evaporated to dryness in a rotary evaporator. The residue was dissolved again in extraction solvent to obtain an appropriate concentration for HPLC analysis, and a 0.5-μL volume was injected. The free amino acids were separated using a VDSpher 100 C18-E column (4.6 mm×150 mm, 3.5 μm, VDS optilab Chromatographie Technik GmbH, Würzburg, Germany) and a fluorescence detector (FLD, excitation: 340 nm, emission: 450 nm).

For analysis of amino acids, an Ultimate 3000 chromatograph (Dionex) using an amino acid analyzer (Dionex) was used. Standard solutions of amino acids (Sigma-Aldrich Co.) and DL-methionine-*S*-methylsulfonium chloride (Sigma-Aldrich Co.) were used as standards. The concentration of the standards was 2.5 μM/mL. The solvent system employed was 40 mM sodium phosphate dibasic, pH 7.8 (A) and water/acetonitrile/methanol (10:45:45 v/v%) (B). The samples were separated according to the following gradient: A/B=100/0 (0~23 min), 45/55 (24 min), 0/80 (24.5~26 min), and 100/0 (26.5~30 min) at a flow rate of 0.8 mL/min. Temperatures used were 20°C for the samples and 40°C for the columns.

DPPH radical scavenging assay

The DPPH radical scavenging activity of 80% ethanolic extract of aronia was determined using the method described by Cheung et al. (21) with slight modifications. First, 192 μL solution of 50 μM DPPH was mixed with 48 μL diluted sample at a 4:1 (v/v) ratio. After leaving the mixture in the dark covered with aluminum foil at room temperature for 30 min, a control consisting of 48 μL distilled water in 192 μL 50 μM DPPH was used for the ascorbic acid standard, or 48 μL of 94% ethanol in 192 μL of 50 μM DPPH was used for the samples. The decolorization of DPPH was read at 517 nm using a microplate reader. Ascorbic acid was used as a control. The DPPH radical scavenging activity was calculated according to the following equation:

$$\text{Inhibition (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

where $A_{control}$ is the absorbance of the control reaction (containing all reagents except for the test compound) and A_{sample} is the absorbance of the test compound.

ABTS radical scavenging assay

The ABTS radical scavenging activity of aronia extract was determined using the method described by Re et al. (22) with slight modifications. First, ABTS was dissolved in distilled water to obtain a 7 mM concentration. The ABTS radical cation was produced by reacting the ABTS stock solution with 2.45 mM $K_2S_2O_8$ (at a ratio of 2:1) in the dark covered with aluminum foil for 24 h before use. The ABTS reagent was diluted with 94% ethanol to obtain an appropriate absorbance (0.17±0.03), which was measured at 734 nm. Then, 950 μL of the ABTS reagent was mixed with 50 μL of several concentrations of the tested samples. After leaving the mixture in the dark covered with aluminum foil at room temperature for 10 min, the absorbance at 734 nm was measured using a microplate reader. Ascorbic acid was used as a control. Each sample was measured in triplicate, and the percentage (%) inhibition was calculated using the following equation:

$$\text{Inhibition (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

where $A_{control}$ is the absorbance of the control reaction (containing all reagents except for the test compound) and A_{sample} is the absorbance of the test compound.

Sensory evaluation

For the sensory evaluation, 50 potential consumers between the ages of 20 and 29 years were questioned about their preferences for aronia. The berries were allowed to reach room temperature, and 20-g samples were presented in random order with a glass of warm water, a spit cup for expectoration, and a paper napkin. The panel individually evaluated color, taste, texture, flavor, and overall acceptance of the berries, and scored the berries from 1 to 9 for each character. The judges wrote their evaluation on paper, which was collected for data analysis after the end of the evaluation.

Statistical analysis

All the results are presented as means±standard deviation (SD). Statistical analyses were performed using the statistical analysis system Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL, USA). Data were compared using the one-way analysis of variance. $P<0.05$ was considered significant.

RESULTS AND DISCUSSION

Proximate analysis

The moisture, ash, crude lipid, and crude protein contents of aronia grown in different regions in Korea are presented in Table 1. The moisture contents in aronia grown in Sangju, Ulju, and Youngcheon were 31.6, 29.5, and 31.2%, respectively, and there were no statistically significant differences among the samples from the 3 growing regions. Ash contents in aronia grown in Sangju, Ulju, and Youngcheon were 0.34, 0.38, and 0.35%, respectively, and there were no statistically significant difference among the growing regions. Crude lipid and protein contents of the 3 samples were 0.93～1.04% and 1.43～1.54%, respectively, and there were no statistically significant differences among the growing regions.

Total sugar content, total acid content, and pH

The sugar and acid contents, and pH of aronia grown in different regions are presented in Table 2. The sugar contents of aronia grown in Sangju, Ulju, and Youngcheon were 12.20, 12.63, and 14.10°Brix, respectively, and aronia grown in Youngcheon showed higher sugar contents than those from the other regions. The aronia grown in Sangju showed the highest total acid content (7.33%) and the lowest pH (3.70), while aronia grown in Youngcheon showed the lowest total acid content of (5.21%) and the highest pH (4.02).

The sugar and acid contents of aronia vary according to the variety, season, and growth stage. Aronia has been shown to have higher sugar contents, especially sorbitol, than other berries such as blueberries, strawberries, and raspberries (23). Previous studies reported a range of 13～19°Brix in 'Nero' aronia over 3 seasons, and a range of 10.5～14.3°Brix depending on the harvest date in 'Viking' aronia (24).

Determination of total polyphenols, flavonoids, and anthocyanins

The total phenolic, flavonoid, and anthocyanin contents of aronia grown in different regions are presented in Table 3. The total polyphenol, flavonoid, and anthocyanin contents in different regions occurred in the following decreasing order: Sangju> Ulju> Youngcheon. The highest phenolic content was obtained in samples from Sangju (412.11 mg GAE/g dry weight), and the lowest phenolic content was found in samples from Youngcheon (276.73 mg GAE/g dry weight). The total flavonoid contents of aronia grown in different regions were in the range of 234.87～171.75 mg CE/g dry weight. The aronia extract obtained from Sangju contained the highest level of flavonoids (234.87 mg CE/g dry weight) and aronia from Youngcheon contained the lowest level of flavonoids (171.75 mg CE/g dry weight). The aronia grown in Sangju contained the highest levels of anthocyanins compared to those from the other regions. The highest anthocyanin content was obtained in samples from Sang-

Table 1. Proximate analysis of aronia grown in different regions

Component	Growing region		
	Sangju	Ulju	Youngcheon
Moisture (%)	31.6±0.7[ns1)]	29.5±0.7	31.2±0.8
Ash (%)	0.34±0.02[ns]	0.38±0.05	0.35±0.03
Crude lipid (%)	1.04±0.0[ns]	0.93±0.06	1.01±0.07
Crude protein (%)	1.54±0.16[ns]	1.47±0.19	1.43±0.15

Data are presented as means±SD of triplicate experiments. [1)]Not significant.

Table 2. The pH, total acidity, and sugar contents of aronia grown in different regions

Growing region	pH	Total acidity (%)	Sugar contents (°Brix)
Sangju	3.70±0.02[a]	7.33±0.26[b]	12.20±0.01[a]
Ulju	3.88±0.02[a]	5.78±0.13[ab]	12.63±0.06[a]
Youngcheon	4.02±0.02[b]	5.21±0.13[a]	14.10±0.17[b]

Data are presented as means±SD of triplicate experiments. Values with different letters (a,b) within the same column are significantly different at $P<0.05$.

Table 3. The total polyphenol, flavonoid, and anthocyanin contents of aronia grown in different regions

Growing region	Total polyphenol (mg GAE/g)	Total flavonoid (mg CE/g)	Total anthocyanin (mg C3G/100 g)
Sangju	412.11±4.75^{c}	234.87±6.33^{c}	16.48±0.57^{c}
Ulju	330.93±2.74^{b}	180.71±9.02^{b}	15.11±3.17^{b}
Youngcheon	276.73±5.31^{a}	171.75±6.44^{a}	13.75±2.01^{a}

Data are presented as means±SD of triplicate experiments. Values with different letters (a-c) within the same column are significantly different at $P<0.05$.
GAE, gallic acid equivalent; CE, catechin equivalent; C3G, cyanidin-3-glucoside.

ju (16.48 mg C3G/100 g dry weight), and the lowest phenolic content was obtained in those from Youngcheon (13.75 mg C3G/ 100 g dry weight).

Extraction and quantification of anthocyanins and polyphenols by HPLC

Table 4 shows the concentration of anthocyanins in aronia grown in different regions. We detected 4 types of anthocyanins, namely cyanidin-3-*O*-galactoside, cyanidin-3-*O*-glucoside, cyanidin-3-*O*-arabinose, and cyanidin-3-*O*-xylose, with retention times of 16.8, 17.5, 18.6, and 22.8 min, respectively. Cyanidin-3-*O*-galactoside was the major anthocyanin followed by cyanidin-3-*O*-arabinose, cyanidin-3-*O*-xylose, and cyanidin-3-*O*-glucoside. Different growth regions affected the anthocyanin contents, and the highest anthocyanin levels were detected in the samples from Sangju followed by those from Ulju and Youngcheon. The level of cyanidin-3-*O*-galactoside in aronia grown in Sangju was 9,179.13 mg/kg dry weight. The levels of cyanidin-3-*O*-galactoside in samples from Ulju and Youngcheon were statistically lower than those in the samples from Sangju, with 7,135.6 and 6,541.87 mg/kg dry weight, respectively. The level of cyanidin-3-*O*-arabinose in aronia grown in Sangju was 3,924.2 mg/kg dry weight, and the levels in the samples from Ulju and Youngcheon were statistically lower than those from Sangju, with 2,963.57 and 3,088.33 mg/kg dry weight, respectively. There was no statistical difference in the individual anthocyanin contents in the samples from Ulju and Youngcheon. The samples were found to contain lower levels of cyanidin-3-*O*-xylose and cyanidin-3-*O*-glucoside than cyanidin-3-*O*-galactoside and cyanidin-3-*O*-arabinose.

The concentrations of polyphenols in aronia grown in different regions are presented in Table 4. We detected 3 phenolic compounds; chlorogenic acid, vanillic acid, and rutin hydrate, with retention times of 14.0, 15.5, and 21.7 min, respectively. Chlorogenic acid was found to be the major phenolic compound with similar levels of vanillic acid and rutin hydrate observed. Different growth regions affected the polyphenol contents, and the highest polyphenols were detected in Sangju followed by Ulju and Youngcheon. Chlorogenic acid in the aronia grown in Sangju was 225.1 mg/kg dry weight. The levels of chlorogenic acid in the samples from Ulju and Youngcheon were statistically lower than in the samples from Sangju, with 170.1 and 191.5 mg/kg dry weight, respectively. The vanillic acid content in aronia grown in Sangju was 4.3 mg/kg dry weight. The vanillic acid content in the samples from Youngcheon was statistically lower than in the samples from Sangju and Ulju.

Phenolic compounds may be present in different amounts and quantities in fruits and vegetables depending on a number of factors such as genotype, environmental conditions in growing regions, as well as the degree of maturity at harvest (13). Bolling et al. (24) found that aronia juice contained lower levels of undetermined polyphenols, such as jaboticabin, pruning, hyperin, or isorhamnetin 3-*O*-rutinoside. It has been reported that aronia contains 4 anthocyanins: 3-*O*-galactoside, 3-*O*-glucoside, 3-*O*-arabinose, and 3-*O*-xyloside of cyanidin. The same compounds were detected in the present study, which are the compounds responsible for the dark red color of aronia (25,26).

Table 4. Levels of individual anthocyanin and polyphenols in aronia grown in different regions (unit: mg/kg)

	Growing region		
	Sangju	Ulju	Youngcheon
Anthocyanins			
Cyanidin-3-*O*-galactoside	9,179.13±421.21^{b}	7,135.6±618.35^{a}	6,541.87±918.28^{a}
Cyanidin-3-*O*-arabinose	3,924.2±179.99^{b}	2,963.57±260.39^{a}	3,088.33±434.86^{a}
Cyanidin-3-*O*-xylose	812.33±43.11^{b}	561.77±55.91^{a}	617.37±93.49^{a}
Cyanidin-3-*O*-glucoside	513.1±28.11^{b}	371.83±37.8^{a}	418.13±56.61^{a}
Polyphenols			
Chlorogenic acid	225.1±618.4^{c}	170.1±918.3^{a}	191.5±997.3^{b}
Vanillic acid	4.3±0.4^{b}	4.6±0.6^{b}	2.5±0.6^{a}
Rutin hydrate	3.1±0.3^{a}	4.5±0.4^{b}	3.3±0.4^{a}

Data are presented as means±SD of triplicate experiments.
Values with different letters (a-c) within the same row are significantly different at $P<0.05$.

Table 5. The amino acid contents of aronia grown in different regions (unit: mg/kg)

Amino acids	Growing region		
	Sangju	Ulju	Youngcheon
Aspartic acid	0.16±0.27$^{ns1)}$	0.35±0.30	0.10±0.18
Glutamic acid	0.29±0.01^{a}	0.28±0.02^{a}	0.40±0.01^{b}
Serine	0.39±0.02^{ns}	0.23±0.20	0.31±0.01
Histidine	0.08±0.01^{b}	0.07±0.00^{ab}	0.07±0.00^{a}
Glycine	0.18±0.07^{ns}	0.11±0.01	0.09±0.00
Threonine	0.39±0.03^{b}	0.37±0.01^{b}	0.33±0.00^{a}
Arginine	0.13±0.00^{b}	0.11±0.00^{a}	0.10±0.01^{a}
Alanine	0.19±0.00^{b}	0.22±0.01^{c}	0.15±0.00^{a}
Tyrosine	0.04±0.01^{a}	0.03±0.00^{a}	0.06±0.00^{b}
Valine	0.25±0.00^{b}	0.24±0.01^{b}	0.21±0.00^{a}
Methionine	ND$^{2)}$	0.01±0.02	ND
Phenylalanine	0.06±0.01^{ab}	0.04±0.01^{a}	0.06±0.00^{b}
Isoleucine	0.12±0.01^{c}	0.11±0.01^{b}	0.08±0.00^{a}
Leucine	0.08±0.03^{ns}	0.10±0.01	0.07±0.00

Data are presented as means±SD of triplicate experiments. Values with different letters (a-c) within the same row are significantly different at $P<0.05$.
[1)]Not significant.
[2)]Not detected.

Table 6. DPPH radical scavenging activity of aronia grown in different regions (unit: %)

Concentration (μg/mL)	Growing region		
	Sangju	Ulju	Youngcheon
12.5	9.93±1.01$^{ns1)A}$	9.13±1.06^{A}	9.67±0.88^{A}
25	16.92±1.02^{nsB}	16.68±1.09^{B}	16.05±0.98^{B}
50	36.99±2.51^{bC}	33.13±1.46^{bC}	27.92±1.79^{aC}
100	58.63±1.88^{cD}	54.57±1.34^{bD}	46.33±1.43^{aD}
200	74.26±1.58^{bE}	72.19±1.67^{abE}	71.21±1.84^{aE}

Data are presented as means±SD of triplicate experiments. Values with different letters within the same row (a-c) and column (A-E) are significantly different at $P<0.05$.
[1)]Not significant.

Table 7. ABTS radical scavenging activity of aronia grown in different regions (unit: %)

Concentration (μg/mL)	Growing region		
	Sangju	Ulju	Youngcheon
12.5	5.48±0.63^{bA}	5.35±0.69^{bA}	2.11±0.74^{aA}
25	12.58±1.86^{bB}	9.93±0.48^{aB}	9.23±0.51^{aB}
50	21.04±0.58^{bC}	20.54±0.82^{bC}	17.87±0.37^{aC}
100	43.44±1.80^{cD}	40.47±0.56^{bD}	34.69±0.65^{aD}
200	67.51±1.94^{bE}	64.59±2.67^{abE}	61.38±1.60^{aE}

Data are presented as means±SD of triplicate experiments. Values with different letters within the same row (a-c) and column (A-E) are significantly different at $P<0.05$.

Extraction and quantification of amino acids by HPLC

The amino acid composition of aronia is presented in Table 5. We detected 14 amino acids and, overall, the samples contained relatively high levels of glutamic acid (0.28～0.40 mg), serine (0.23～0.39 mg), and threonine (0.33～0.39 mg) per kilogram of dry weight. No relationship was found between the growing region of black chokeberries and the levels of amino acids. The aronia grown in Sangju contained relatively high levels of serine, threonine, glutamic acid, and valine. The aronia grown in Ulju contained relatively high levels of threonine, aspartic acid, glutamic acid, and alanine. The aronia grown in Youngcheon contained relatively high levels of glutamic acid, threonine, and serine. Relatively low amounts of tyrosine, glycine, methionine, and phenylalanine were detected in aronia.

DPPH and ABTS radical scavenging assays

The DPPH radical-scavenging activity of aronia dried by 3 different methods is shown in Table 6. The DPPH radical scavenging activity of all of the samples increased in a concentration-dependent manner (12.5～200 μg/mL). The average inhibition of the DPPH radical scavenging activity of 200 μg/mL aronia extract from Sangju was 74.26%, whereas the average inhibitions of 72.19% and 71.21% were obtained for the samples from Ulju and Youngcheon, respectively. Aronia grown in Sangju exhibited stronger DPPH scavenging ability than aronia grown in Ulju or Youngcheon, especially at high concentrations of the extract.

The ABTS radical-scavenging activity of the aronia dried by 3 different methods is shown in Table 7. The ABTS radical scavenging activity increased in a concentration-dependent manner (12.5～200 μg/mL). The average inhibition of the ABTS radical scavenging activity of 100 μg/mL aronia extract obtained from Sangju was 43.44%, whereas the average inhibitions of 40.47% and 34.69% were obtained for aronia grown in Ulju and Youngcheon, respectively. Aronia grown in Sangju and Ulju showed stronger ABTS scavenging capacity than aronia grown in Youngcheon. Aronia grown in Sangju contained the highest amount of polyphenols, flavonoids, and anthocyanins, and the antioxidant activity of aronia was correlated with the levels of these compounds.

Previous studies have shown that the high levels of phenolics, flavonoids, and anthocyanins are positively correlated with the antioxidant activity in aronia (27,28). This indicates that the high DPPH and ABTS radical scavenging activities of aronia grown in Sangju and Ulju could be attributed to the high levels of total polyphenols, flavonoids, and anthocyanins, which is consistent with the observations made by Hwang et al. (29) and Wang et al. (30).

Sensory evaluation

Table 8 shows the sensory scores obtained from the consumers' tests of aronia. There were no significant differences in color, texture, and flavor among the 3 samples. The aronia grown in Youngcheon scored the highest for

Table 8. Sensory evaluation of aronia grown in different regions

Evaluation item	Growing region		
	Sangju	Ulju	Youngcheon
Color	6.5±0.5[ns1)]	6.5±0.5	6.8±0.5
Taste	4.7±0.2[a]	5.6±0.2[b]	6.6±0.3[c]
Texture	5.8±0.3[ns]	5.7±0.3	5.4±0.4
Flavor	5.4±0.3[ns]	5.6±0.2	5.6±0.3
Overall acceptance	4.8±0.3[a]	5.7±0.3[b]	6.9±0.3[c]

Data are presented as means±SD of triplicate experiments. Values with different letters (a-c) within the same row are significantly different at $P<0.05$.
[1)]Not significant.

taste and overall acceptability. The taste score for aronia grown in Youngcheon was 6.6, while those for aronia from Sangju and Ulju were 4.7 and 5.6, respectively. Overall acceptability scores were 4.8～6.9, with aronia grown in Youngcheon presenting the highest value and aronia grown in Sangju presenting the lowest value. Anthocyanins contributed the most to the dark purple color of aronia and there was little difference in the color of aronia grown in the 3 different regions because they were harvested at similar stages of maturity. This may be associated with the sugar contents, total acidity, and pH of the fruits. It is possible that higher sugar contents and pH, and lower total acidity in aronia resulted in preferable sensory characteristics. Stavang et al. (31) reported that the sugar levels and sugar/acid ratios are closely correlated with the sensory properties in raspberry fruit. Aronia grown in Sangju contained higher levels of polyphenols and flavonoids compared to aronia grown in Youngcheon, which may be related to the astringency or bitter taste of those compounds. Based on the results of the sensory evaluation, we conclude that consumers prefer a less astringent or bitter taste and prefer a sweeter taste.

CONCLUSION

The quality characteristics, levels of bioactive compounds, and antioxidant activities of aronia grown in 3 different regions in Korea were investigated. Our results indicated no significant differences in the proximate composition of aronia grown in the 3 different regions. Furthermore, no relationship was found between the growing region of aronia and the levels of amino acids. However, aronia grown in Sangju had higher levels of polyphenols, flavonoids, and anthocyanins, as well as high antioxidant activity, in comparison to aronia from the other regions. The aronia grown in Sangju showed the highest total acid content, the lowest sugar content, and the lowest pH value compared to the other samples. Overall, samples from Youngcheon presented the best scores for taste and overall acceptability in the sensory evaluations, which may be related to the sugar contents, total acidity, and pH of the fruits. It is possible that the higher sugar contents and pH, and the lower total acidity in the aronia grown in Youngcheon result in more preferable sensory characteristics. Aronia grown in Sangju contained higher levels of polyphenols and flavonoids compared to aronia grown in Youngcheon, which may be related to the astringency or the bitter taste of those compounds.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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