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Effects of storage temperature on the bioactive compound content and antioxidant activity of aronia (*Aronia melanocarpa*) fruit

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아로니아의 저장온도가 생리활성물질 및 항산화에 미치는 영향

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Abstract

The fruit of aronia (*Aronia melanocarpa*, also called the "black chokeberry), which is rich in anthocyanin, polyphenol, and flavonoid content and possesses antioxidant, anticancerous, and anti-inflammatory properties. In this study, the influence of storage temperature and storage period on the phytochemical content and antioxidant activity of aronia was determined. The total polyphenol and flavonoid contents of aronia extract were found to be 308.48 μg gallic acid equivalent/g dry weight and 5.33 μg quercetin equivalent/g dry weight, respectively. HPLC analysis of aronia reveled four anthocyanin peaks corresponding to cyanidin-3-*O*-galactoside, cyanidin-3-*O*-glucoside, cyanidin-3-*O*-arabinose, and cyanidin-3-*O*-xylose and three polyphenol peaks corresponding to chlorogenic acid, vanillic acid, and rutin hydrate. Long-term storage at a refrigerated temperature (4℃) reduced the levels the levels of health promoting compounds. We found that the highest amounts of total polyphenols, flavonoids, and anthocyanins were retained in aronia samples stored at -80℃ followed by those stored at -20℃ and 4℃ samples. Furthermore, the samples stored at -80℃ showed the stronger antioxidant activities than those stored at other temperatures. Based on these findings, we concluded that freezing aronia at -80℃ can help preserve its antioxidant activity by maintaining high levels of anthocyanins and other bioactive compounds.

Key words : aronia, freezing, polyphenols, anthocyanins, antioxidant activity

Introduction

Aronia (*Aronia melanocarpa*, commonly known as black chokeberry) belongs to the Rosaceae family. Aronia is native to North America and has been grown in Europe since the early 20th century (1). The berries have high anthocyanin, polyphenol, and flavonoid contents and exhibit excellent antioxidant, anti-cancerous, and anti-inflammatory activities (2-4). The contents of physiologically active substances in plants, including aronia, varies depending on the cultivation environment, soil, climate, fertilizer, variety, season, storage, and cooking methods (6,7). Aronia contains large amounts of polyphenols, including tannins, and is used as a raw material in a variety of processed foods and cosmetics. However, the fresh fruit is typically not consumed owing to its distinctive bitter taste (8-10). Therefore, harvested aronia is frozen or stored at refrigerated temperatures for a certain period of time and is often processed as needed. Anthocyanins, which are major physiologically active substances in aronia, are highly unstable and have been reported to be influenced by freezing temperature, freezing rate, light, pH, oxygen, and the presence of coexisting compounds such as enzymes, proteins, and metal ions (11-14). According to Zhang et al. (15), storage at low temperatures

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in a high-oxygen atmosphere increases anthocyanin stability. On the contrary, the stability of berry products is often enhanced by the removal of oxygen, inactivation of enzymes, and naturally existing compounds such as flavanols and phenolic acids (11,16).

Freezing is the most common storage method for storing large quantities of seasonal fruits. Aronia and other berries are frozen for long-term storage, and frozen berries are used as ingredients in various processed foods. The levels of bioactive compounds can differ between fresh and stored vegetables/fruits depending on the storage time and temperature. In addition, storage conditions can modify nutrient content. There have been several studies (13,14) on the stability of anthocyanins in berries at drying temperatures; however, few studies have examined the stability of physiologically active compounds in berries and their antioxidant activities at refrigeration and freezing temperatures. Therefore, it is imperative to understand the effects of storage conditions, such as time and temperature, on the bioactive compound content of aronia. This study investigated the effects of storage period (0-20 weeks) and temperature (4℃, -20℃, and -80℃) on the levels of bioactive compounds, such as total phenols, flavonoids, and anthocyanins in aronia. In addition, the antioxidant activity of aronia stored under various conditions was examined.

Materials and methods

Materials and reagents

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

The aronia (*Aronia melanocarpa*, *Nero* cultivar) fruit was harvested when optimally ripe at a local fram in Sangju, Korea, The fruit was free from insect and mechanical damage. The aronia samples were subdivided into 100 g portions, packed in plastic zipper bags (200×149×47 mm, Ziploc, SC Johnson & Son, Inc. Bay City, MI, USA), and stored for fixed period of time.

Sample preparation and storage

The effect of storage on the content of bioactive compounds and antioxidant activity was evaluated by storing aronia for up to 20 weeks (0, 4, 8, 12, 16, and 20 weeks) at three different temperatures: 4℃ (refrigerated), -20℃ (freezing), and -80℃(deep-freezing). After the designated storage period, the samples were freeze-dried at -80℃. The freeze-dried samples were ground with a food grinder (Hanil, Wonju, Korea). Lyophilized powdered samples were stored at -80℃ until analysis.

Determination of total polyphenols and flavonoids

Lyophilized aronia powder was extracted with 25 volumes of ethanol for 24 h at room temperature. The ethanol extract was filtered through Whatman No.1 filter paper and concentrated using a rotary vacuum evaporator (EYELA 400 series, Tokyo, Japan). The dried extracts was then used in the analyses of total polyphenol and flavonoid contents. The total polyphenol content of aronia was measured using Folin-Ciocalteu's phenol reagent according to the method of Thi and Hwang (17). The total flavonoid content of aronia was measured using the method of Thi and Hwang (17).

Determination of anthocyanins and polyphenols by HPLC

Freeze dried samples (-100 mg) were mixed with 5 mL methanol containing 0.1% formic acid and vortexed for 1 min. Then, the mixture was centrifuged for 5 min and the upper layer was transferred to another glass tube. The extraction process was performed twice. The methanolic fractions were combined and evaporated to dryness using a rotary evaporator (EYELA, Tokyo, Japan). The residue was redissolved in the extraction solvent with an appropriate dilution for HPLC analysis (Ultimate 3,000, Dionex, Sunnyvale, CA, USA), and an injecition volume of 10 μL was used.

Anthocyanins were separated by C_{18} Zorbox SB column (4.6×250 mm, 5 mm particle size, Agilent Technologies Inc., Santa Clara, CA, USA) and detected at 520 nm using a UV detector (Waters, Manchester, UK). The mobile phase was a mixture of (A) water with 5% formic acid and (B) acetonitrile with 5% formic acid. The samples were separated

according to the following gradient: A/B (v/v) = 95/5 (0-5 min), 90/10(8 min), 85/15(13-18 min), 80/20(25 min), 70/30(28-32 min), 95/5(35-40 min) at a flow rate of 0.8 mL/min.

Polyphenols were separated using Agilent XDB C_{18} column (4.6×150 mm, 5 μm) and detected at 280 nm using a a UV detector (190-800 DAD scanning, Waters). The mobile phase was a mixture of (A) water with 0.3% TFA and (B) acetonitrile. The samples were separated according to the following gradient: A/B (v/v) = 95/5 (0-39 min), 40/60 (40 min), 0/100 (45-50 min), 95/5 (55-60 min) at a flow rate of 0.8 mL/min.

Determination of antioxidant activity

The DPPH, ABTS, and superoxide radical scavenging activities of 80% ethanolic extract of aronia were determined using the method described by Thi and Hwang (18). The reducing power of the aronia extracts were determined according to the method of Thi and Hwang (17). Briefly, 0.25 mL of 0.2 M phosphate buffer (pH 6.6) and 0.25 mL of 1% potassium hexacyanoferrate ($K_3Fe(CN)_6$) were mixed and incubated at 50℃ for 20 min. To terminate the reaction, 0.25 mL of 10% trichloroacetic acid solution was added, and then the mixture was centrifuged at 3,000 rpm for 10 min. Subsequently, a mixture of 0.5 mL of the supernatant, 0.5 mL of distilled water and 0.1 mL of 0.1% ferric chloride ($FeCl_3$) solution was allowed to react for 10 min. The absorbance of this mixture was then measured at 700 nm using a microplate reader (Infinite M200 Pro, Tecan Group Ltd. San Jose, CA, USA) to determine the reducing power.

Statistical analysis

All results are presented as mean±SD. Statistical analysis was performed using the SPSS software package (version 17.0). Data were compared using one-way analysis of variance; $p<0.05$ was considered significant.

Results and discussion

Total polyphenol and flavonoid contents

The concentration of total polyphenols in aronia samples during storage at three different temperatures over a 20 week period are presented in Table 1. The total polyphenol content in fresh aronia before storage was 905.4 mg. However, the total polyphenol content decreased during storage. Over the 20 week storage period, we observed that a lower storage temperature resulted in a higher retention of the total polyphenol content. Notably, deep freezing of aronia at -80℃ was the best mode for preserving the total polyphenol content over 20 weeks. By contrast, refrigeration at 4℃ resulted in the highest loss of total polyphenols, with a total polyphenol content of 658.0 mg after 20 weeks. The total polyphenol retention rate after storage for 20 weeks were 89.6%, 77.1%, and 72.7% at -80℃, -20℃, and 4℃, respectively. According to Misiak and Irzyniec (19), storage of aronia at 3℃ for 6 months resulted in a 30% reduction in polyphenol compounds. Aronia has much hiher contents of polyphenol compounds than any other berries such as blueberries, raspberries, and strawberries, and the stability of polyphenol compounds is cloasely related to the storage temperature. The polyphenol content varies depending on the variety of aronia, and it is generally reported that 25-45% of the phenolic compounds are anthocyanins (20,21).

The concentrations of total flavonoids in aronia samples during storage at three different temperatures over a 20 week period are presented in Table 2. The total flavonoid content in fresh aronia before storage was 423.5 mg. However, the total flavonoid content decreases during storage. Over the 20 week storage period, we observed that a lower the storage temperature resulted in greater retention of the total flavonoid content. Notably, deep freezing at -80℃ allowed that greatest amount of total flavonoids to be retained during storage of aronia for 20 weeks. By contrast, refrigeration at 4℃ resulted

Table 1. The total polyphenol content of aronia stored at different storage temperatures

(mg GAE/g dry weight)

Temp. (℃)	Storage Period (week)					
	0	4	8	12	16	20
4	905.4±23.7^{aE}	782.8±31.6^{aD}	776.5±26.2^{aCD}	761.3±19.1^{aC}	743.3±23.1^{aB}	658.0±20.1^{aA}
-20	905.4±23.7^{aF}	854.5±21.1^{bE}	839.0±27.2^{bD}	813.5±23.2^{bC}	793.8±26.7^{bB}	698.4±23.5^{bA}
-80	905.4±23.7^{aE}	891.0±29.6^{cD}	859.5±24.2^{cC}	840.0±24.6^{cB}	833.3±24.6^{cB}	811.5±20.8^{cA}

Data were the mean±SD of three separate experiments.
$^{a-c}$Values with the different superscript within the same column are significantly different at $p<0.05$.
$^{A-E}$Means with different superscripts in the same row are significantly different at $p<0.05$.
GAE, gallic acid equivalent.

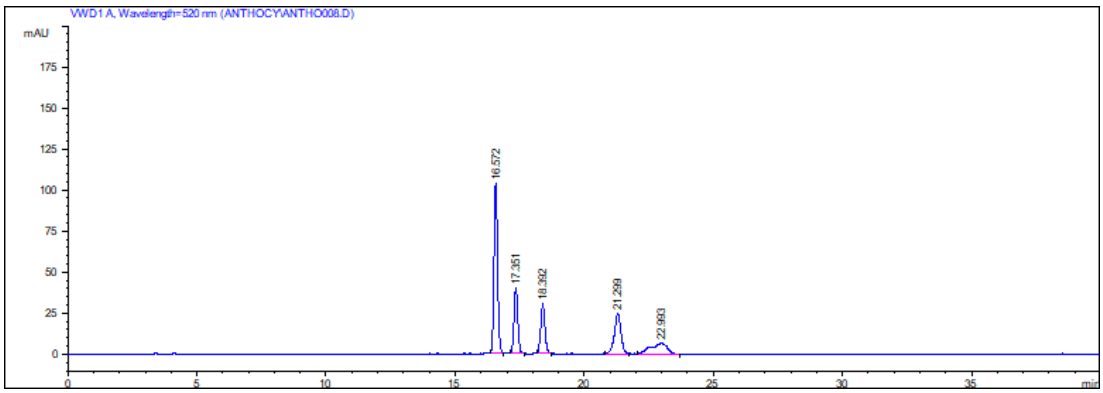

Fig. 1. Representative HPLC chromatogram of anthocyanins in standards. RT: 16.8 min, cyanidin-3-O-galactoside; 17.5 min, cyanidin-3-*O*-glucoside; 18.6 min, cyanidin-3-*O*-arabinoside; 22.8 min, cyanidin-3-*O*-xylose.

Table 2. The total flavonoid content of aronia stored at different storage temperatures

(mg CE/g dry weight)

Temp. (℃)	Storage period (week)					
	0	4	8	12	16	20
4	423.5±20.4^{aF}	344.4±18.9^{aE}	322.2±13.0^{aD}	307.1±16.2^{aC}	285.8±11.5^{aB}	217.1±17.4^{aA}
-20	423.5±20.4^{aD}	380.3±13.4^{bCD}	374.7±15.3^{bC}	347.6±13.8^{bB}	326.8±18.2^{bA}	323.1±15.7^{bA}
-80	423.5±20.4^{aE}	401.4±14.7^{cD}	390.1±14.2^{cC}	383.6±17.4^{cBC}	376.7±12.9^{cB}	351.3±16.3^{cA}

Data were the mean±SD of three separate experiments.
$^{a-c}$Values with the different superscript within the same column are significantly different at $p<0.05$.
$^{A-E}$Means with different superscripts in the same row are significantly different at $p<0.05$.
CE=catechin equivalent

the highest loss of total flavonoids, with the total flavonoid content decreasing to 217.1 mg after 20 weeks. The total flavonoid retention rate after storage for 20 weeks were 83.0%, 76.3%, and 51.3% at -80℃, -20℃, and 4℃, respectively.

Anthocyanins content by HPLC

Fig. 1 shows a typical chromatogram of anthocyanins in aronia extract. We detected four anthocyanins, namely, cyanidin-3-*O*-galactoside, cyanidin-3-*O*-glucoside, cyanidin-3-*O*-arabinoside, and cyanidin-3-*O*-xylose, at 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*-arabinoside, cyanidin-3-*O*-xylose, and cyanidin-3-*O*-glucoside. Table 3 shows the anthocyanin contents of aronia at different storage temperatures over 20 weeks. The storage temperatures affected the anthocyanin content, and the highest anthocyanin contents were detected at the lowest storage temperature of -80℃ followed by -20℃ and 4℃. The initial cyanidin-3-*O*-galactoside content of aronia was 9462.6 mg/kg dry weight. After storage for 4, 12, and 20 weeks at 4℃, it decreased 88.6%, 67.0% and 28.7%, respectively, of the initial value. After storage for 4, 12, and 20 weeks at -20℃, the cyanidin-3-*O*-galactoside contents were found to be 97.4%, 90.5% and 68.5%, respectively, of the initial value. After 4, 12, and 20 weeks of storage at -80℃, the cyanidin-3-*O*-galactoside contents were 98.7%, 86.7%, and 85.2%, respectively, of the initial value. At each temperature, the loss of anthocyanins increased as the storage period became longer. The content of cyanidin-3-*O*-arabinose was 4376.8 mg/kg before storage, but it decreased after storage depending on the temperature and duration. In the case of storage for 20 weeks at 4℃, -20℃, and -80℃, the cyanidin-3-*O*-arabinose contents were 39.7%, 68.5%, and 84.5%, respectively, of the initial value. Similarly, higher losses of cyanidin-3-*O*-glucoside and cyanidin-3-*O*- xylose were observed as the storage temperatures increased.

When stored for a long time, the physiologically active substances in foods change and decompose, even at freezing temperature, which has an important influence on antioxidant activity (22). The stability of bioactive compound content during freezing is influenced by various factors such as freezing rate, freezing temperature, plant variety, and pH (23). Ancos et al. (24) showed that the total anthocyanin content

Table 3. The anthocyanin content of aronia stored at different storage temperatures

(mg/kg dry weight)

Storage temp. (℃)	Storage time (week)	cyanidin-3-O-galactoside	cyanidin-3-O-glucoside	cyanidin-3-O-arabinose	cyanidin-3-O-xylose
Fresh (Initial)		9462.6±314.2^{f}	588.1±48.1^{f}	4376.8±147.0^{f}	850.6±10.9^{f}
4	4	8386.2±529.2^{d}	555.4±39.9^{e}	3930.7±249.8^{e}	777.8±45.6^{e}
	8	6598.9±504.9^{c}	414.3±28.6^{c}	3075.4±201.6^{c}	585.0±24.9^{c}
	12	6336.6±192.6^{c}	407.6±18.8^{c}	3000.6±190.1^{c}	576.8±32.2^{c}
	16	5955.5±671.1^{b}	379.3±47.2^{b}	2799.0±321.8^{b}	535.0±71.8^{b}
	20	3659.4±231.1^{a}	226.7±24.7^{a}	1714.0±123.3^{a}	324.9±14.3^{a}
-20	4	9217.3±128.3^{f}	574.5±20.5^{a}	4218.4±97.0^{e}	837.3±40.1^{ef}
	8	8710.5±111.0^{e}	560.3±38.6^{a}	4065.0±88.1^{e}	817.7±55.7^{ef}
	12	8564.5±136.5^{de}	541.0±23.0^{a}	3943.8±70.8^{e}	796.3±40.2^{e}
	16	6502.7±176.4^{c}	398.7±12.8^{c}	3012.2±68.5^{c}	572.7±15.8^{c}
	20	6477.4±129.8^{c}	393.1±12.7^{c}	2999.4±46.3^{c}	560.4±17.9^{c}
-80	4	9341.1±336.7^{f}	583.7±39.3^{f}	4305.1±117.6^{f}	837.0±49.9^{f}
	8	8831.8±395.4^{e}	548.9±32.4^{e}	4041.0±171.3^{e}	792.4±45.8^{e}
	12	8207.6±301.9^{d}	498.9±17.7^{d}	3942.6±142.8^{e}	774.1±33.7^{e}
	16	8179.4±353.2^{d}	485.1±26.2^{d}	3750.8±183.5^{d}	709.4±40.8^{d}
	20	8059.0±330.1^{d}	483.7±23.2^{d}	3697.1±179.9^{d}	707.2±41.3^{d}

Data were the mean±SD of three separate experiments.
$^{a-f}$Values with the different superscript within the same column are significantly different at $p<0.05$.

Table 4. The polyphenol content of aronia stored at different storage temperatures

(mg/kg dry weight)

Storage temp.(℃)	Storage time (week)	Chlorogenic acid	Vanillic acid	Rutin hydrate
Fresh (Initial)		5160.1±126.9^{f}	92.8±9.7^{f}	112.3±8.9^{f}
4	4	4351.3±98.6^{d}	76.8±8.6^{e}	83.2±7.6^{d}
	8	3696.7±87.3^{c}	68.2±7.3^{d}	73.6±6.8^{c}
	12	3603.1±78.9^{c}	41.7±5.1^{b}	65.2±5.4^{b}
	16	2970.7±65.4^{b}	39.0±4.2^{b}	64.4±5.7^{b}
	20	1493.5±35.8^{a}	24.4±2.7^{a}	52.4±6.2^{a}
-20	4	4790.4±87.5^{e}	89.3±9.1^{f}	101.0±8.7^{e}
	8	4751.9±82.4^{e}	80.6±8.5^{e}	95.8±8.4^{e}
	12	4701.3±76.3^{e}	72.7±4.3^{e}	85.2±7.6^{d}
	16	3451.2±70.8^{c}	68.9±4.6^{de}	69.8±6.3^{c}
	20	3442.2±71.2^{c}	56.4±3.8^{c}	58.4±4.9^{b}
-80	4	5160.1±103.2^{f}	90.4±9.2^{f}	105.9±9.4^{e}
	8	5048.6±116.3^{f}	88.4±8.7^{f}	96.7±7.8^{e}
	12	4846.6±93.1^{e}	78.3±5.9^{e}	91.4±8.4^{de}
	16	4829.2±90.6^{e}	75.2±6.2^{e}	82.8±6.3^{d}
	20	4702.5±75.7^{e}	68.2±6.5^{de}	77.2±6.1^{c}

Data were the mean±SD of three separate experiments.
$^{a-f}$Values with the different superscript within the same column are significantly different at $p<0.05$.

in raspberry significantly decreased by 15-27%, cyanidin-3-glucoside decreased by 21-34% in Rubi cultivar and 15-40% in Zeva cultivar compared with those in the fresh fruit after storage for 1 year at -20℃. Syamaldaevi et al. (25) confirmed that the anthocyanin content decreased by 21%, 29%, and 16% when red raspberry was stored for 378 days at -20℃, -35℃, and -80℃, respectively. Jacques et al. (26) reported that the anthocyanin content was reduced by 57%, 50%, and 31% when blackberry was stored at -10℃, -18℃, and -80℃, respectively for 6 months. Thus, the anthocyanin content is closely related to the freezing temperature and lower freezing temperature reduces the reduction rate. As a result, deep-freezing at -80℃ has been suggested to be superior to refrigeration or normal freezing at -20℃ for preserving antioxidants during the storage of food products.

Polyphenols content by HPLC

Fig. 2 shows a typical chromatogram of polyphenols in aronia extract. We detected three polyphenols, namely, chlorogenic acid, vanillic acid, and rutin hydrate, at retention times of 14.0, 15.5, and 21.7 min, respectively. Chlorogenic acid was the major polyphenol followed by rutin hydrate and vanillic acid. Table 4 shows the polyphenol content of aronia at different storage temperatures over 20 weeks. The storage temperatures affected the polyphenol content, and the highest amount of polyphenol contents were detected in the lowest storage temperature of -80℃ followed by -20℃ and 4℃. The initial chlorogenic acid content in aronia was 5,187.1 mg/kg dry weight. After storage for 4, 12, and 20 weeks at 4℃, the chlorogenic acid content decreased to 83.9%, 69.5%, and 28.8%, respectively, of the initial value. After storage for 4, 12, and 20 weeks at -20℃, the chlorogenic acid contents were 92.4%, 90.6% and 66.4%, respectively, of the inithial value. After 4, 12, and 20 weeks of storage at -80℃, the chlorogenic acid contents were 99.5%, 93.4%, and 90.7%, respectively, of the initial value. The content of vanillic acid was 92.8 mg/kg before storage, but decreased during storage depending on the storage temperature and duration. When stored for 20 weeks at 4℃, -20℃, and -80℃, the vanillic acid contents were 26.3%, 60.8%, and 73.5%, respectively of the initial value. Similar to the anthocyanin content, the polyphenol content decreased with increasing storage period and the loss of polyphenol content decreased at lower storage temperatures.

Antioxidant activity

The DPPH radical scavenging activities of aronia stored at various temperatures over 20 weeks are shown in Table 5. The DPPH radical scavenging activity showed a strong relationship with the storage period and tended to decrease as the storage period became longer. Additionally, the DPPH radical scavenging activity was significantly higher for the samples stored at -80℃ than for those refrigerated at 4℃ or frozen at -20℃. For all the samples, the DPPH radical

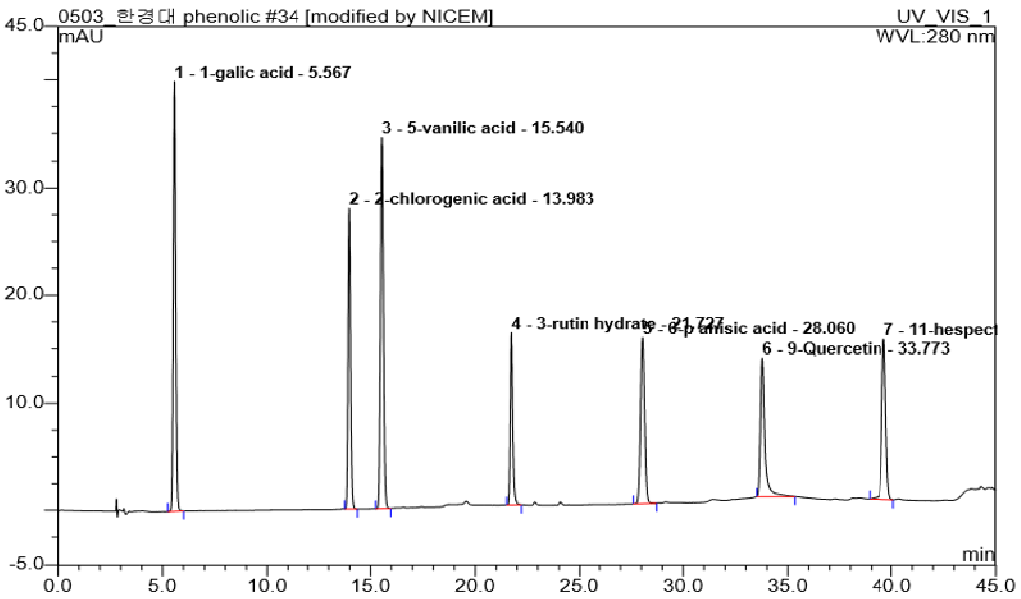

Fig. 2. Representative HPLC chromatogram of polyphenols in standards. RT: 14.0 min, chlorogenic acid; 15.5 min, vanillic acid; 21.7 min rutin hydrate.

Table 5. DPPH radical scavenging ability of aronia stored at different storage temperatures

(%)

Storage temp (℃)	Storage time (week)	Concentration (μg aronia extract/mL)			
		12.5	25	50	100
Fresh (initial)		25.6±2.2^{fA}	37.9±3.6^{fA}	58.9±5.1^{fA}	65.1±5.6^{fA}
4	4	18.1±1.4^{eA}	29.8±2.9^{eB}	44.3±4.2^{dC}	54.9±4.5^{dD}
	8	12.2±1.5^{bcA}	26.3±2.5^{dB}	30.9±3.4^{eC}	43.9±4.3^{eD}
	12	8.1±0.6^{bA}	15.1±1.2^{bB}	20.4±2.9^{bC}	32.9±3.2^{bD}
	16	9.5±0.3^{bA}	12.9±1.2^{aB}	15.1±1.4^{aB}	29.4±1.6^{bC}
	20	5.3±0.5^{aA}	9.7±1.4^{aAB}	12.9±1.7^{aB}	20.1±1.2^{aC}
-20	4	22.9±2.8^{eA}	35.7±2.7^{eB}	54.8±4.5^{eC}	62.8±5.5^{eD}
	8	20.2±1.4^{dA}	33.2±2.3^{dB}	49.9±3.7^{dC}	62.1±4.3^{eD}
	12	19.3±1.2^{cA}	30.5±2.3^{cB}	47.9±2.4^{dC}	61.4±1.4^{eD}
	16	15.2±3.5^{cA}	29.9±1.7^{cB}	37.2±2.9^{cdC}	58.2±3.3^{deD}
	20	12.2±3.5^{bcA}	19.7±1.4^{bA}	32.9±2.7^{cB}	49.1±2.2^{dC}
-80	4	23.3±2.6^{eA}	35.8±1.9^{eB}	56.2±4.1^{eC}	62.8±1.6^{eD}
	8	21.1±2.7^{dA}	33.5±2.5^{dB}	52.9±3.4^{dC}	60.7±4.9^{eD}
	12	20.1±2.9^{dA}	31.3±1.2^{cB}	51.4±2.8^{dC}	61.5±4.4^{eD}
	16	18.1±2.3^{cA}	29.0±2.3^{cB}	50.7±5.1^{dC}	59.1±3.4^{deD}
	20	16.3±1.2^{cA}	30.7±2.2^{cB}	47.7±3.5^{dC}	58.0±3.4^{deD}

Data were the mean±SD of triplicate experiment.
$^{a-f}$Means with different superscripts in the same column are significantly different at $p<0.05$.
$^{A-D}$Means with different superscripts in the same row are significantly different at $p<0.05$.

scavenging activity of the aronia extracts increased in a concentration-dependent manner (12.5-100 μg/mL). Before storage, the DPPH radical scavenging activity of the 25 μg/mL aronia extract was 37.9%, whereas higher extract concentration of 50 μg/mL and 100 μg/mL exhibited increased DPPH radical scavenging activities of 58.9% and 65.1%, respectively. The average inhibition of DPPH radical formation in 100 μg/mL arnoia extracts stored at -80℃, -20℃, and 4℃ for 20 weeks was 58.0%, 49.1%, and 20.1%, respectively. Although the DPPH radical scavenging activity decreased when aronia was stored at -80℃, the reduction was less than at 4℃ or -20℃. Thus, storing aronia at cryogenic temperatures rather than at normal refrigeration or freezing temperatures is effective for maintaining the DPPH radical scavenging activity.

The ABTS radical scavenging activities of aronia stored at different temperatures are shown in Table 6. The ABTS radical scavenging activities increased in a concentration dependent manner (12.5-100 μg/mL). The ABTS radical scavenging activities of the 100 μg/mL aronia extract before storage was 71.2%. However, the ABTS radical scavenging activity of the 100 μg/mL aronia extract stored at 4℃ for 4, 12, and 20 weeks decreased to 58.4%, 36.3% and 17.8%, respectively. By contrast, the ABTS radical scavenging activities of 100 μg/mL aronia extract stored at -80℃ for 4, 12, and 20 weeks were 68.4%, 65.9%, and 65.6%, respectively, indicating that the antioxidant activity was not lost in the storage period of 20 weeks.

The superoxide anions scavenging activities of aronia extract are shown in Table 7. The superoxide anion scavenging activity of 100 μg/mL aronia extract before storage was 45.8%. However, after storage at 4℃ for 4, 12, and 20 weeks, the superoxide anion scavenging activity of the 100 μg/mL aronia extract decreased to 35.1%, 31.2%, and 11.0%, respectively. By contrast, when stored at -80℃ for 4, 12, and 20 weeks, the superoxide anion scavenging activities of 100 μg/mL aronia extract was 43.1%, 42.1%, and 37.0%, respectively, and the antioxidant activity was not lost in the storage period of 20 weeks. Thus, deep-freezing at -80℃ was more effective than storage at both 4℃ or -20℃ in terms of retaining the superoxide anion scavenging activity of aronia extract.

Table 6. ABTS radical scavenging ability of aronia stored at different storage temperatures

(%)

Storage temp (℃)	Storage time (week)	Concentration (μg aronia extract/mL)			
		12.5	25	50	100
Fresh (initial)		32.8±1.2^{fA}	51.3±3.8^{fB}	60.2±5.2^{fC}	71.2±5.5^{fD}
4	4	8.0±3.3^{bA}	24.4±2.7^{aB}	40.4±2.3^{aC}	58.4±4.4^{eD}
	8	7.0±2.3^{bA}	23.8±2.9^{cB}	32.6±1.7^{cC}	46.9±3.5^{dD}
	12	6.5±1.3^{bA}	19.2±1.8^{bB}	26.9±1.1^{bC}	36.3±2.8^{cD}
	16	4.4±1.2^{abA}	18.6±1.3^{bB}	20.7±1.0^{abC}	24.4±2.4^{bD}
	20	2.2±0.5^{aA}	7.6±1.1^{aB}	17.1±1.8^{aC}	17.8±2.3^{aC}
-20	4	21.9±2.5^{eA}	35.0±3.6^{deB}	54.8±4.9^{efC}	62.7±4.7^{efD}
	8	17.4±1.7^{dA}	29.7±2.9^{dB}	50.0±5.2^{eC}	55.2±4.8^{eD}
	12	11.1±1.5^{cA}	25.6±2.6^{cB}	47.3±4.9^{eC}	47.1±3.8^{dC}
	16	7.8±3.1^{bA}	20.8±2.1^{bB}	37.1±1.2^{cdC}	41.2±2.2^{cdD}
	20	3.4±1.6^{aA}	17.1±1.2^{bB}	33.6±1.8^{cC}	40.2±2.9^{cdD}
-80	4	31.4±3.6^{fA}	49.1±3.8^{fB}	58.8±5.2^{fC}	68.4±5.4^{fD}
	8	30.1±2.5^{fA}	47.8±3.9^{eB}	57.6±3.9^{efC}	67.2±5.6^{efD}
	12	28.2±2.6^{eA}	43.9±2.7^{eB}	55.7±3.7^{efC}	65.9±2.7^{efD}
	16	27.8±3.7^{eA}	43.1±3.2^{eB}	55.6±2.3^{efC}	65.7±2.8^{efD}
	20	25.7±3.3^{eA}	41.0±2.5^{eB}	53.5±3.6^{efC}	65.6±2.2^{efD}

Data were the mean±SD of triplicate experiment.
$^{a-f}$Means with different superscripts in the same column are significantly different at $p<0.05$.
$^{A-D}$Means with different superscripts in the same row are significantly different at $p<0.05$.

Table 7. Superoxide anion radical scavenging ability of aronia stored at different storage temperatures

(%)

Storage temp (℃)	Storage time (week)	Concentration (μg aronia extract/mL)			
		12.5	25	50	100
Fresh (initial)		10.3±0.3^{fA}	18.1±0.5^{fB}	27.4±1.2^{fC}	45.8±1.1^{fD}
4	4	4.9±1.2^{dA}	8.3±0.9^{eB}	15.7±1.3^{cC}	35.1±0.3^{cD}
	8	3.6±1.0^{cA}	7.2±1.4^{dB}	14.4±1.3^{cC}	34.4±0.8^{cD}
	12	3.1±0.6^{cA}	5.9 ±0.9^{cB}	12.6±0.9^{bC}	31.2±1.5^{bD}
	16	2.7±0.5^{bA}	4.6±1.2^{bB}	11.6±2.0^{bC}	29.4±0.4^{bD}
	20	0.8±1.4^{aA}	1.9±2.5^{aB}	6.5±2.6^{aC}	11.0±2.0^{aD}
-20	4	8.0±2.4^{eA}	10.6±0.4^{eB}	17.8±0.6^{dC}	41.9±0.2^{dD}
	8	4.8±0.8^{dA}	9.4±2.8^{eB}	16.6±2.6^{dC}	41.3±0.5^{dD}
	12	4.2±0.2^{cA}	7.5±0.1^{dB}	16.5±0.7^{dC}	39.2±1.4^{dD}
	16	2.8±0.6^{bA}	5.3±0.3^{bcB}	14.8±0.4^{cC}	33.3±0.6^{cD}
	20	1.7±0.6^{aA}	4.2±0.4^{bB}	12.5±0.4^{bC}	32.6±0.2^{cD}
-80	4	9.6±0.5^{fA}	16.2±0.7^{aB}	25.5±1.1^{eC}	43.1±0.2^{eD}
	8	6.4±0.6^{eA}	12.9±0.3^{aB}	23.2±0.9^{deC}	42.7±0.9^{eD}
	12	5.2±0.8^{dA}	10.7±0.7^{aB}	21.2±0.6^{deC}	42.1±0.4^{eD}
	16	4.3±0.6^{cdA}	8.2±0.4^{aB}	19.0±0.9^{dC}	40.6±0.2^{dD}
	20	2.9±0.5^{cA}	6.9±1.1^{aB}	15.9±1.2^{cC}	37.0±0.8^{cD}

Data were the mean±SD of triplicate experiment.
$^{a-f}$Means with different superscripts in the same column are significantly different at $p<0.05$.
$^{A-D}$Means with different superscripts in the same row are significantly different at $p<0.05$.

Table 8. Reducing power (absorbance measured at 700 nm) of aronia stored at different storage temperatures

(O.D)

Storage temp (℃)	Storage time (week)	Concentration (μg aronia extract/mL)			
		12.5	25	50	100
Fresh (initial)		0.201±0.002^{fA}	0.312±0.002^{fB}	0.458±0.003^{fC}	0.735±0.005^{eD}
4	4	0.146±0.000^{dA}	0.203±0.001^{dB}	0.306±0.002^{dC}	0.552±0.001^{dD}
	8	0.140±0.000^{dA}	0.193±0.003^{cB}	0.282±0.002^{cC}	0.456±0.002^{bD}
	12	0.137±0.001^{cA}	0.184±0.003^{cB}	0.266±0.002^{cC}	0.442±0.001^{bD}
	16	0.128±0.001^{bA}	0.143±0.001^{bB}	0.220±0.001^{bC}	0.434±0.001^{bD}
	20	0.117±0.005^{aA}	0.131±0.000^{aB}	0.199±0.004^{aC}	0.322±0.001^{aD}
-20	4	0.145±0.000^{dA}	0.207±0.001^{dB}	0.313±0.000^{dC}	0.523±0.001^{cD}
	8	0.142±0.001^{dA}	0.194±0.000^{cB}	0.299±0.001^{cC}	0.516±0.000^{cD}
	12	0.140±0.002^{dA}	0.193±0.002^{cB}	0.288±0.000^{cC}	0.513±0.001^{cD}
	16	0.135±0.000^{cA}	0.171±0.000^{cB}	0.260±0.001^{cC}	0.432±0.001^{bD}
	20	0.126±0.001^{bA}	0.157±0.001^{bB}	0.216±0.001^{bC}	0.341±0.000^{aD}
-80	4	0.193±0.003^{fA}	0.290±0.002^{eB}	0.429±0.002^{eC}	0.718±0.003^{dD}
	8	0.174±0.001^{eA}	0.196±0.001^{dB}	0.394±0.004^{eC}	0.526±0.002^{cD}
	12	0.163±0.001^{eA}	0.192±0.001^{cB}	0.352±0.002^{dC}	0.504±0.001^{cD}
	16	0.158±0.000^{deA}	0.190±0.001^{cB}	0.317±0.001^{dC}	0.502±0.004^{cD}
	20	0.147±0.000^{dA}	0.188±0.000^{cB}	0.284±0.001^{cC}	0.472±0.001^{bD}

Data were the mean±SD of triplicate experiment.
$^{a-f}$Means with different superscripts in the same column are significantly different at $p<0.05$.
$^{A-D}$Means with different superscripts in the same row are significantly different at $p<0.05$.

The reducing powers of aronia extract during storage for 20 weeks at different storage temperatures are presented in Table 8. The reducing power is expressed as the absorbance at 700 nm, where a higher the absorbance value corresponds to a higher reducing power. The reducing power of 25 μg/mL aronia extract before storage was 0.312. As the extract concentration increased to 50 μg/mL and 100 μg/mL, the reducing power increased to 0.458 and 0.735, respectively. However, after storage at 4℃, -20℃, and -80℃ for 4 weeks, the reducing powers of the 100 μg/mL aronia extract were 0.552, 0.523, and 0.718, respectively. Similar to the results of the other antioxidant tests, the reducing power of aronia stored at cryogenic temperatures was higher than those of aronia stored at normal refrigeration and freezing temperatures. Furthermore, the reducing power tended to decrease with increasing storage period.

Antioxidant activity measurements allow quantification of the protection ability against free radicals. Recently, the high antioxidant power of aronia has attracted much attention. The correlation between the total polyphenol, flavonoid, and anthocyanin contents in aronia have been correlated to the antioxidant activities using *in vitro* assays. Various *in vitro* and *in vivo* studies have shown that antioxidant and lipid peroxidation activities increase in proportion to the concentrations of polyphenol and anthocyanin compounds in aronia (27-29). Rugina et al. (30) showed that the total proanthocyanidin and anthocyanin content in aronia had a direct correlation with antioxidant activity, which is consistent with the results of this study. Furthermore, antioxidant activity have been shown to be directly proportional to the contents of chlorogenic acid, neochlorogenic acid, cyanidin-3-arabinoside, cyanidin-3-galactoside, and epicatechin in aronia (27).

In conclusion, the extracts of aronia are good scavengers of active oxygen species, including DPPH radicals, ABTS radicals, and superoxide anions. In general, it was found that deep freezing at -80℃ retained high amounts of bioactive compounds and effectively preserved the antioxidant compounds in aronia, which have been shown to inhibit several harmful free radicals. In particular, deep freezing at -80℃ was a more effective storage method than freezing at -20℃ or refrigeration at -4℃. The findings of this study also suggested that owing to its inhibitory effects, aronia extract

can potentially be used as a functional ingredient against free-radical-associated diseases.

요 약

본 연구에서는 아로니아의 저장온도에 따른 폴리페놀, 플라보노이드, 안토시아닌의 함량과 항산화 활성의 변화를 측정하였다. 저장하기 전의 신선한 아로니아 추출물의 총 폴리페놀과 총 플라보노이드 함량은 g 건조 중량 당 각각 gallic acid와 catechin을 기준으로 905.4 mg 및 423.5 mg으로 나타났다. 아로니아 추출물에서 4종류의 안토시아닌(cyanidin-3-O-galactoside, cyanidin-3-O-glucoside, cyanidin-3-O-arabinose 및 cyanidin-3-O-xylose)과 3종류의 폴리페놀(chlorogenic acid, vanillic acid및 rutin hydrate)을 HPLC 분석으로 확인하였다. 아로니아의 저장 온도에 따른 유효물질의 함량은 -80℃에서 가장 많은 양이 검출되었고, -20℃ 및 -4℃의 순으로 냉동온도가 높아짐에 따라 유효물질의 함량이 감소함을 확인하였다. 또한, -80℃에서 냉동 저장한 시료는 다른 온도에서 저장 한 시료와 비교하여 가장 강력한 항산화 활성을 나타냈다. 이상의 결과를 통해 아로니아를 냉동 저장할 경우에는 가급적 -80℃ 와 같은 초저온에서 보관하는 것이 생리활성 물질 및 항산화 활성의 손실을 최소화할 수 있을 것으로 사료된다.

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