Prev. Nutr. Food Sci. 2016;21(4):330-337
https://doi.org/10.3746/pnf.2016.21.4.330
pISSN 2287-1098 · eISSN 2287-8602

Quality Characteristics and Antioxidant Activity of Yogurt Supplemented with Aronia (*Aronia melanocarpa*) Juice

Linh Nguyen and Eun-Sun Hwang

Department of Nutrition and Culinary Science, Hankyong National University, Gyeonggi 17579, Korea

ABSTRACT: We investigated the quality characteristics and antioxidant activities of yogurt supplemented with 1%, 2%, and 3% aronia juice and fermented for 24 h at 37°C. The total acidity increased with increasing levels of aronia juice and incubation time. Lightness and yellowness of the yogurt decreased, but redness increased, with increasing aronia juice content and incubation time. The number of lactic acid bacteria (LAB) increased with increased incubation time, and yogurt containing 2% and 3% aronia juice showed higher LAB counts than 1% aroinia juice-supplemented yogurt. The total polyphenol and flavonoid contents increased proportionally with increasing levels of aronia juice. Antioxidant activity of aronia-containing yogurt was significantly higher than that of the control and increased proportionally with aronia juice concentration. Yogurt with 2% aronia juice had the best taste ($P<0.05$). Aronia juice may be a useful additive for improving the taste and antioxidant potential of yogurt.

Keywords: aronia, yogurt, lactic acid bacteria, sensory evaluation, antioxidant activity

INTRODUCTION

Yogurt is a coagulated milk product obtained from fermentation by lactic acid bacteria (LAB), *Streptococcus thermophilus* and *Lactobacillus delbrueckii* spp. *bulgaricus* (1). Yogurt is traditionally consumed as a healthy food due to its high nutritional value and health benefits as well as its sensory properties (2,3). Yogurt is a rich source of bioactive peptides with antioxidant activity that are produced during fermentation (4). Compared with milk, yogurt is more nutritious and is an excellent source of protein, calcium, phosphorus, riboflavin, thiamin, vitamin B_{12}, folate, niacin, magnesium, and zinc (3). Probiotic bacteria can tolerate acid and bile and can survive in the intestinal tract. Thus, they have a beneficial effect on intestinal function and offer health benefits such as decreasing cholesterol absorption, reducing blood pressure, and ameliorating intestinal disorders and chronic diseases (5). Since lactose in milk is converted to lactic acid during fermentation and yogurt contains lactose-fermenting bacteria, lactose intolerant people can consume yogurt without adverse effects (6). Moreover, consumption of fermented milk products causes a slight reduction in stomach pH, which reduces the risk of pathogen transit and ameliorates the effects of low gastric juice secretion (7).

Aronia (*Aronia melanocarpa*) berries, also known as black chokeberries, contain many bioactive compounds, including anthocyanins, carotenoids, fatty acids, flavonoids, phenolic compounds, and vitamins (8,9). Originating from the eastern parts of North America, aronia berries have been cultivated and medicinally used in the former Soviet Union since the middle of the 20th century. The health-promoting potential of this berry is widely recognized, and increasing numbers of preclinical and clinical studies support the health benefits of aronia (10, 11). Aronia berries have received significant attention in recent years due to their antioxidant, anti-inflammatory, and anti-cancer activities, which are associated with a reduced risk of high blood pressure, cardiovascular diseases, and cancer (12,13).

Usually, yogurt is flavored by adding natural ingredients or synthetic flavor compounds (14). Yogurt is mainly pigmented and flavored by adding fruit juices or pulp from berry fruits such as strawberries, blueberries, and raspberries, which provide natural color and flavor as well as bioactive compounds. Fruit juices, powders, and extracts have potential as functional ingredients in the food industry, including the dairy sector (15). The effects of different types of plant materials added as functional ingredients on the quality and antioxidant properties of yogurt have been studied (16,17), but aronia has

Received 29 June 2016; Accepted 31 August 2016; Published online 31 December 2016

Correspondence to Eun-Sun Hwang, Tel: +82-31-670-5182, E-mail: ehwang@hknu.ac.kr

not been studied in this context.

The objective this study was to determine the quality and sensory characteristics, bioactive compound contents, and antioxidant activities of yogurt supplemented with aronia juice.

MATERIALS AND METHODS

Materials

Folin-Ciocalteu's reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), gallic acid, and catechin were purchased from Sigma Chemical Co. (St. Louis, MO, USA). de Man, Rogosa, Sharpe (MRS) broth was purchased from BD Biosciences (San Jose, CA, USA). All chemicals were of analytical grade. Skim milk powder and commercial milk were purchased from Seoul Dairy Co. (Seoul, Korea). Kefir starter powder (TSI Inc., Surrey, BC, Canada) and isomalto oligosaccharide (Daesang, Osan, Korea) were used to prepare the yogurt.

Preparation of aronia juice

Aronia (*Aronia melanocarpa* cv Nero) berries were harvested at optimal ripeness from a local farm in Yeongcheon, Korea in August of 2015. The berries were washed with tap water, ground with a food grinder (HMF-3260S, Hanil, Seoul, Korea), and filtered with cheese cloth 2 times to obtain juice.

Preparation of yogurt

To prepare aronia yogurt, a mixture of market milk (450 mL) and skim milk (50 mL) was heated until boiling, then was cooled until it reached 30°C. To the mixture, aronia juice and oligosaccharides were added at 0%, 1%, 2%, and 3%. The mixture was well-mixed and inoculated with 0.4% Kefir starter powder which consisted of *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Lactobacillus lactis*, and yeast as starters, and the mixture was incubated at 37°C for 24 h. Samples were collected after 0, 3, 6, 12, and 24 h for analysis of several parameters. For the sensory evaluation, yogurts stored at 4°C after 24 h incubation were used. The mixing ratios for yogurt fortified with different amounts of aronia juice are presented in Table 1.

Table 1. Mixing ratios of yogurt fortified with various amounts of aronia juice

	Aronia juice (%)			
	0	1	2	3
Aronia juice (mL)	0	5	10	15
Milk (mL)	450	445	440	435
Skim milk powder (g)	50	50	50	50
Starter (g)	2	2	2	2
Oligosaccharide (g)	5	5	5	5

Changes in pH, titratable acidity, and sugar contents of yogurt during incubation

The pH values of yogurt were measured during fermentation using a calibrated glass electrode pH meter (420 Benchtop, Orion Research Inc., Beverly, MA, USA). For determining titratable acidity, 10 mL of yogurt was titrated with 0.1 M sodium hydroxide solution. The titratable acidity was expressed as gram of lactic acid/100 g of yogurt and was calculated using the following equation:

$$\text{Total acidity}=\frac{\text{V}\times\text{NaOH factor}\times\text{A}\times\text{D}}{\text{volume of sample}}\times 100$$

where V is volume of NaOH added (mL), A is conversion factor (0.009 for lactic acid), D is dilution factor, and F is factor of 0.1 N NaOH.

Sugar content was measured using a refractometer (PR-201α, ATAGO Co., Ltd., Tokyo, Japan) calibrated with distilled water. Sugar content was expressed as °Brix.

Color determination

Changes in yogurt color during fermentation were measured using a colorimeter (Chrome meter CR-400, Minolta, Osaka, Japan) calibrated with a standard calibration slide. L, a, and b values for standard tile were 97.10, +0.24, and +1.75, respectively. Results were expressed as L^* (lightness/darkness), a^* (redness/greenness), and b^* (yellowness/blueness).

Determination of LAB count

MRS agar was used to quantify the viable LAB in the yogurt. Yogurt (10 g) was diluted 10 times with 0.85% saline solution and homogenized with a Stomacher (Stomacher R400, INTERSCIENCE, Saint-Nom-la-Brètèche, France) for 2 min at speed 4. After further serial dilution, 1 mL of sample was added to MRS agar in petri dishes and then incubated for 48 h at 37°C. Colonies appearing on the plates were counted, multiplied by the dilution factor, and expressed as the log colony forming units per milliliter (log CFU/mL).

Determination of total phenolic contents

Total polyphenol content was measured with Folin-Ciocalteu's phenol reagent using the method described by Zhou et al. (18) with slight modifications. Briefly, 200 μL of the appropriately diluted sample was mixed with 400 μL of 2% 2 N Folin-Ciocalteu's phenol reagent. After incubation for 3 min at room temperature, 800 μL of 10% Na_2CO_3 was added to the mixture. The mixture was covered with aluminum foil and kept in the dark at room temperature for 1 h. The mixture was vortexed, and

the absorbance was measured at 750 nm with a microplate reader (Infinite M200 Pro, Tecan Systems Inc., San Jose, CA, USA). A standard curve was created using gallic acid, and results were expressed as gallic acid equivalents (GAE) per gram of dry weight (mg/g GAE).

Determination of total flavonoid content

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

DPPH assay

The DPPH radical scavenging activities of yogurt samples were determined using the method of Cheung et al. (20) with slight modifications. First, 192 μL of 50 μM DPPH was mixed with 48 μL of diluted sample. The mixture was covered with aluminum foil and kept in the dark at room temperature for 30 min. The control consisted of 48 μL distilled water in 192 μL of 50 μM DPPH for the ascorbic acid standard or 48 μL of 94% ethanol in 192 μL of 50 μM DPPH for the test samples. Decolorization of DPPH was measured at 517 nm with a microplate reader. 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 the test compound) and A_{sample} is the absorbance of the test compound.

ABTS assay

The ABTS radical scavenging activities of yogurt samples were determined using the method of Re et al. (21) with slight modifications. First, ABTS was dissolved in distilled water to a concentration of 7 mM. ABTS radical cations were produced by reacting the ABTS stock solution with 2.45 mM $K_2S_2O_8$ (in a 2:1 ratio) in the dark, covered with aluminum foil, for 24 h before use. The ABTS reagent was diluted with 94% ethanol to the appropriate absorbance (0.17±0.03), which was measured at 734 nm. The ABTS reagent (950 μL) was mixed with 50 μL of the indicated concentrations of test samples. The mixture was covered with aluminum foil and kept in the dark at room temperature for 10 min. The absorbance at 734 nm was recorded with a microplate reader. Each sample was measured in triplicate, and percent 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 the test compound) and A_{sample} is the absorbance of the test compound.

Reducing power assay

The Fe^{3+} reducing power of yogurt samples was determined using the method of Oyaizu (22) with slight modifications. Various concentrations of samples (0.25 mL) were mixed with 0.25 mL of 0.2 M phosphate buffer (pH 6.6) and 0.25 mL of 1% (w/v) $K_3Fe(CN)_6$. The mixture was incubated in a 50°C water bath for 20 min, and the reaction was stopped by adding 0.25 mL of 10% (w/v) trichloroacetic acid solution. The solution was then centrifuged at 3,000 rpm for 10 min. The supernatant (0.5 mL) was mixed with 0.5 mL of distilled water and 0.1 mL of 0.1% (w/v) $FeCl_3$ for 10 min. The reducing power was determined by measuring the absorbance at 700 nm with a microplate reader. A standard curve of ascorbic acid was generated with concentrations ranging from 0 to 200 μg/mL.

Sensory evaluation

Yogurt samples were subjected to sensory evaluation by 20 panelists after 24 h of fermentation and storage at 4°C. Each panelist was asked to rank the 4 samples for color, flavor, taste, mouth feel, thickness, and overall acceptance using a 9-point hedonic scale, ranging from 1 (dislike extremely) to 9 (like extremely). All samples were placed into 50 mL white plastic cups with random 3 digit codes and were served to the panelists in a random order. Water was provided for panelists to rinse their mouth between samples.

Statistical analysis

The results are presented as mean±standard deviation. Statistical analysis was performed with the statistical analysis system (version 22.0, SPSS Statistics software package, IBM, Armonk, NY, USA). Data were compared using one-way analysis of variance; $P<0.05$ was considered significant.

RESULTS AND DISCUSSION

Changes in pH, titratable acidity, and sugar contents of yogurt during incubation

The pH values of yogurt during incubation for 24 h at 37°C are presented in Table 2. An overall decline in yogurt pH occurred during the 24 h period. The initial pH values for all samples were 6.33~6.46, with control showing higher pH values than samples with aronia juice added. The pH began to drop significantly after 6 h of fermentation. The pH gradually decreased during fermentation and reached 4.25~4.29 in yogurt containing 1~3% aronia juice after 24 h incubation. In the yogurt samples containing aronia juice, pH values were lower than in the plain yogurt with no aronia juice. Reductions in pH were essentially proportional to the aronia juice concentration. Aronia-containing yogurt had faster rates of pH reduction than control yogurt. Aronia juice contains several acidic compounds such as acetic, malic, succinic, citric, tartaric, and phosphoric acids (23,24), and these acids may contribute to the lower pH values in yogurts containing aronia juice.

The increase in total acidity, which represents the amount of lactic acid present in the yogurt during fermentation, is shown in Table 2. In general, the total acidity increased during incubation, and this upward trend was almost linear compared to the pH change. From 12 h of incubation onward, the difference between control and aronia yogurt became more pronounced. The yogurt fortified with 2% and 3% aronia juice showed the greater increases in total acidity from 6~24 h of fermentation. Total acidity of the control sample was 1.01%, while yogurt containing 2% and 3% aronia juice had 1.13% and 1.16% total acidity after 24 h incubation, respectively.

The sugar content of yogurt samples during incubation at 37°C is presented in Table 2. The initial sugar content in yogurt samples was 23.45~23.50°Brix and there were no significant differences between control and aronia juice yogurt samples. Sugar content was reduced during fermentation in each yogurt sample. Until 6 h of fermentation, sugar contents were not changed much in any treatment, but after 12 h of fermentation, sugar content dropped significantly. The sugar content in control yogurt was 15.40°Brix, and both yogurt samples containing 2% and 3% aronia juice had 16.40°Brix after 24 h of fermentation. Aronia juice contains several sugars such as glucose, mannitol, and sorbitol (25), and aronia itself shows 10.5~14.3°Brix in the 'Viking' cultivar and 13~19°Brix in the 'Nero' cultivar (26). This is the reason why yogurt containing 2~3% aronia juice had greater sugar content than control yogurt or yogurt containing 1% aronia juice.

Color evaluation

Color properties of the yogurt samples are shown in Table 3. L^* values, indicating brightness, ranged from 44.21 to 48.71 for all yogurt samples at 0 h of incubation. The control sample showed the highest L^* values, and L^* value was decreased with increasing amounts of aronia juice; yogurt with 3% aronia juice showed the lowest L^*

Table 2. Changes in pH, total acidity, and sugar content during incubation of yogurt containing various amounts of aronia juice

Incubation time (h)	Aronia juice (%)			
	0	1	2	3
pH				
0	6.46±0.01^{dE}	6.43±0.00^{cE}	6.38±0.01^{bE}	6.33±0.00^{aE}
3	6.39±0.00^{cD}	6.39±0.01^{cD}	6.35±0.01^{bD}	6.31±0.00^{aD}
6	6.04±0.00^{bC}	5.88±0.00^{aC}	5.89±0.00^{aC}	5.89±0.00^{aC}
12	5.20±0.00^{cB}	5.04±0.00^{bB}	4.99±0.00^{aB}	4.97±0.00^{aB}
24	4.39±0.01^{cA}	4.29±0.00^{bA}	4.29±0.00^{bA}	4.25±0.00^{aA}
Total acidity (%)				
0	0.14±0.01^{nsA}	0.14±0.01^{A}	0.14±0.01^{A}	0.13±0.01^{A}
3	0.14±0.01^{nsA}	0.14±0.00^{A}	0.14±0.01^{A}	0.15±0.00^{A}
6	0.30±0.01^{aB}	0.32±0.01^{aB}	0.33±0.01^{bB}	0.34±0.01^{bB}
12	0.58±0.00^{aC}	0.65±0.01^{bC}	0.67±0.03^{bC}	0.67±0.03^{bC}
24	1.01±0.10^{aD}	1.08±0.00^{abD}	1.13±0.01^{bD}	1.16±0.03^{bD}
Sugar content (°Brix)				
0	23.50±0.00^{nsC}	23.45±0.07^{C}	23.50±0.14^{C}	23.45±0.07^{C}
3	23.45±0.00^{nsC}	23.37±0.00^{C}	23.25±0.35^{C}	23.45±0.07^{C}
6	23.15±0.21^{nsC}	23.05±0.35^{C}	23.05±0.07^{C}	23.00±0.42^{C}
12	16.80±1.41^{nsB}	16.40±0.00^{B}	17.00±0.28^{B}	17.10±0.01^{B}
24	15.40±0.28^{nsA}	15.80±0.28^{A}	16.40±0.00^{A}	16.40±0.00^{A}

Data are presented as the mean±SD of three separate experiments.
Values with different letters within the same row (a-d) and the same column (A-E) are significantly different at $P<0.05$.
^{ns}Not significant.

Table 3. Changes in Hunter's color values during incubation of yogurt containing various amounts of aronia juice

	Incubation time (h)	Aronia juice (%)			
		0	1	2	3
L*	0	48.71±0.22^{dA}	46.02±0.11^{cA}	44.81±0.15^{bA}	44.21±0.10^{aA}
	3	50.35±0.26^{cB}	46.71±0.12^{aB}	47.28±0.21^{bB}	46.39±0.48^{aB}
	6	51.31±0.66^{dB}	49.58±0.17^{cC}	47.23±0.30^{bB}	46.52±0.18^{aB}
	12	52.65±0.06^{cC}	51.10±0.15^{bD}	51.23±0.68^{bC}	49.06±0.70^{aC}
	24	54.33±0.93^{cD}	52.61±1.34^{bD}	52.21±0.36^{abD}	50.79±0.07^{aD}
a*	0	−1.59±0.04^{aC}	−0.27±0.02^{bA}	0.62±0.04^{cA}	1.30±0.03^{dA}
	3	−1.59±0.02^{aC}	−0.25±0.03^{bA}	0.76±0.02^{cB}	1.61±0.02^{dB}
	6	−1.97±0.01^{aB}	−0.19±0.02^{bB}	0.86±0.03^{cC}	1.95±0.04^{dC}
	12	−2.10±0.05^{aA}	0.17±0.02^{bC}	1.43±0.03^{cD}	2.32±0.09^{dD}
	24	−1.48±0.06^{aD}	0.54±0.02^{bD}	1.86±0.05^{cE}	2.64±0.03^{dE}
b*	0	2.97±0.09^{dA}	1.63±0.03^{cA}	1.30±0.88^{bA}	0.88±0.02^{aA}
	3	2.89±0.09^{dA}	1.99±0.03^{cB}	1.69±0.03^{bB}	1.06±0.02^{aB}
	6	4.00±0.01^{dB}	2.70±0.03^{cC}	1.87±0.04^{bC}	1.24±0.01^{aC}
	12	4.25±0.18^{dC}	3.05±0.03^{cD}	2.46±0.04^{bD}	1.73±0.05^{aD}
	24	4.79±0.15^{dD}	3.24±0.02^{cE}	2.69±0.05^{bE}	2.00±0.01^{aE}

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

value among the samples. After fermentation, L* values increased. The L* value in control yogurt increased from 48.71 to 54.33 after 24 h of fermentation. The L* value in yogurt with 3% aronia juice increased from 44.21 to 50.79 after 24 h fermentation. a* values, indicating redness, ranged from −1.59 to 1.30 in all yogurt samples, with control yogurt and yogurt containing 1% aronia juice showed negative values at 0 h of incubation. The control yogurt showed the lowest a* value and a* value increased with increasing amounts of aronia juice. Upon fermentation, a* values increased. The a* value in yogurt with 1% aronia juice increased from −0.27 to 0.54 after 24 h of fermentation. The a* value in yogurt with 3% aronia juice increased from 1.30 to 2.64 after 24 h of fermentation. The b* values, indicating yellowness, ranged from 0.88 to 2.97 in yogurt samples at 0 h of incubation. Control yogurt showed the highest b* values and the value decreased with increasing amounts of aronia juice. Upon fermentation, b* values increased. The b* value in control yogurt increased from 2.97 to 4.79 after 24 h of fermentation. The b* value in yogurt with 3% aronia juice also increased from 0.88 to 2.00 after 24 h of fermentation. Overall, the results showed that the yogurts supplemented with aronia juice were redder, darker, and less yellow than control yogurt. Aronia is a dark purplish fruit (8) and its color undoubtedly contributed to the color of yogurt by decreasing the L* and b* values and increasing the a* value.

LAB count

Table 4 shows the LAB counts in yogurts fortified with various amounts of aronia juice. The LAB counts were 6.45~6.46 CFU/mL at 0~3 h in yogurt samples, and the initial number of LAB was not different among samples. After 3 h of incubation, the number of LAB increased rapidly in both control and aronia juice containing samples. The LAB count after 3 h of incubation was 9.00~9.59 log CFU/mL, and yogurt containing 2% and 3% ar-

Table 4. Changes in lactic acid bacteria number during incubation of yogurt containing various amounts of aronia juice (log CFU/mL)

Incubation time (h)	Aronia juice (%)			
	0	1	2	3
0	6.45±0.01^{nsA}	6.46±0.01^{A}	6.45±0.02^{A}	6.46±0.01^{A}
3	9.00±0.00^{aB}	9.00±0.00^{aB}	9.39±0.12^{bB}	9.59±0.16^{bB}
6	9.39±0.12^{aC}	9.54±0.34^{abC}	9.60±0.43^{bC}	10.34±0.19^{cC}
12	10.20±0.00^{aD}	10.32±0.20^{abD}	10.51±0.01^{bD}	10.67±0.14^{cD}
24	10.60±0.07^{aD}	10.64±0.05^{abE}	10.69±0.05^{abE}	10.82±0.10^{bE}

Data are presented as the mean±SD of three separate experiments.
Values with different letters within the same row (a-c) and the same column (A-E) are significantly different at $P<0.05$.
^{ns}Not significant.

onia juice showed higher LAB counts than other samples. The number of LAB increased further at 12 h of incubation, but only a slight increase was observed at 24 h of incubation. The number of LAB in yogurt increased with increasing aronia juice concentration, and yogurts supplemented with 2% and 3% aronia juice had LAB counts of 10.51 and 10.67 log CFU/mL, respectively after 12 h incubation. Longer incubation times resulted in higher numbers of LAB and the number of LAB in aronia-containing yogurt met the required number of LAB (1.0×10^8 CFU/mL) for semi-liquid yogurt in Korea (17).

Aronia juice does not inhibit the growth of LAB and actually improves the growth of LAB during the incubation from 0~24 h, possibly by providing some sugars for LAB growth. Previous studies showed that adding flowering cherry into yogurt improves LAB growth (16), but adding acanthopanax powder to yogurt prevents LAB growth (17). Some plant materials contain antimicrobial compounds that may affect LAB growth (17).

Total phenolic and flavonoid contents

Table 5 shows the total polyphenol and flavonoid contents of yogurt fortified with different amounts of aronia juice after 24 h incubation. The highest total polyphenol content (54.05 mg GAE/g dry weight) was found in the yogurt fortified with 3% aronia juice, and the lowest polyphenol content (16.34 mg GAE/g dry weight) was found in the control yogurt. The total polyphenol content increased 2.5- and 3.3-fold in yogurt containing 2% and 3% aronia juice, respectively. The total flavonoid content in the control yogurt was 117.71 mg CE/g, while yogurt containing 1~3% aronia juice had higher flavonoid contents than the control, with values ranging from 122.40 to 152.10 mg CE/g. Compared to the control, the total flavonoid content increased 1.2- and 1.3-fold in yogurt with 2% and 3% aronia juice, respectively.

Incorporation of aronia juice into yogurt resulted in higher polyphenol and flavonoid contents. Aronia contains a relatively high amount of polyphenols, particularly tannins (27,28), and a previous study reported the phenolic content of aronia to be 690.2 mg/100 g fresh weight (29).

Table 5. Total polyphenol and flavonoid contents of yogurt containing various amounts of aronia juice

Aronia juice (%)	Total polyphenol (mg GAE/g)	Total flavonoids (mg CE/g)
0	16.34±1.04[a]	117.71±2.43[a]
1	28.27±1.20[b]	122.40±1.36[b]
2	41.30±1.43[c]	142.50±2.32[c]
3	54.05±1.43[d]	152.10±1.18[d]

GAE, gallic acid equivalent; CE, catechin equivalent.
Data are presented as the mean±SD of three separate experiments.
Values with different letters (a-d) within the same column are significantly different at $P<0.05$.

Antioxidant activity

The antioxidant activities of yogurt samples containing various amounts of aronia juice are shown in Table 6. Aronia-containing yogurt samples had higher antioxidant activity than plain yogurt. The DPPH radical scavenging activity of plain yogurt was 59.47%, and DPPH radical scavenging activity of yogurt increased in an aronia juice concentration-dependent manner. The average inhibition of DPPH radical formation in yogurt containing 2% and 3% aronia juice was 72.26% and 77.87%, respectively. The average inhibition of ABTS radical formation in plain yogurt was 45.96%, which increased to 59.98% in yogurt with 1% aronia juice added. The average ABTS radical scavenging activities of yogurt containing 2% and 3% aronia juice were 69.79% and 70.90%, respectively. The reducing power activity in control yogurt was 27.62%, which increased up to 29.55~29.86% in yogurt with aronia juice added, but there was no statistically significant difference between yogurt with aronia juice and control yogurt. The higher antioxidant activity in aronia-containing yogurt might result from the phyto-

Table 6. DPPH and ABTS radical scavenging activities and reducing power activity of yogurt containing various amount of aronia juice

Aronia juice (%)	DPPH assay (%)	ABTS assay (%)	Reducing power (%)
0	59.47±0.31[a]	45.96±0.55[a]	27.62±0.09[a]
1	68.50±0.40[b]	59.98±0.53[b]	29.55±0.28[b]
2	72.26±0.79[c]	69.79±1.48[c]	29.82±0.57[b]
3	77.87±0.44[d]	70.90±0.26[c]	29.86±0.31[b]

Data are presented as the mean±SD of three separate experiments.
DPPH, 2,2-diphenyl-1-picrylhydrazyl; ABTS, 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid.
Values with different letters (a-d) within the same column are significantly different at $P<0.05$.

Table 7. Sensory evaluation of yogurt containing various amount of aronia juice

Sensory property	Aronia juice (%)			
	0	1	2	3
Color	3.00±2.03[a]	4.45±1.99[b]	6.35±1.09[c]	7.35±0.99[c]
Flavor	5.00±1.84[ns]	4.74±1.73	5.10±1.33	5.60±1.50
Taste	4.55±1.85[a]	5.50±1.73[ab]	6.00±1.97[b]	5.74±1.91[ab]
Mouth feel	5.25±1.77[ns]	5.05±1.64	5.70±1.84	5.56±1.85
Thickness	5.95±1.51[ns]	5.84±1.74	5.55±1.96	5.79±1.93
Overall acceptance	5.25±1.86[ns]	5.40±2.39	5.85±2.35	5.80±2.19

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

chemical content of the juice and microbial metabolic activity.

Sensory evaluation

Table 7 shows the sensory scores of the yogurt samples fortified with various amount of aronia juice. Fortifying yogurt with aronia juice was associated with a statistically significant effect on sensory parameters such as color, taste, and overall acceptance. The color score of control showed the lowest value of 3.00 while scores increased with increasing amounts of aronia juice. The color scores were 4.45, 6.35, and 7.35 in yogurt containing 1%, 2%, and 3% aronia juice, respectively. Aronia contains a purple color from anthocyanins, which positively contributes to yogurt color. The taste score of yogurt with 2% aronia juice showed the highest score of 6.00, while taste scores for yogurt with 1% and 3% aronia juice were 5.50 and 5.74, respectively. However, there were no statistically differences in flavor, mouth feel, thickness, or overall acceptance between control yogurt and aronia-containing yogurt. Aronia itself usually has bitter or astringent taste, but even 3% aronia juice in the yogurt did not have adverse effects on flavor, mouthfeel, thickness, or overall acceptance of the yogurt. Moreover, aronia contained several bioactive compounds that may contribute to the antioxidative effects. The addition of 1∼3% aronia juice into yogurt is recommended for health benefits.

ACKNOWLEDGEMENTS

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF-2016R1A2B4014977).

AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

REFERENCES

1. Gahruie HH, Eskandari MH, Mesbahi G, Hanifpour MA. 2015. Scientific and technical aspects of yogurt fortification: a review. *Food Sci Hum Wellness* 4: 1-8.
2. Gilliland SE. 1989. Acidophilus milk products: a review of potential benefits to consumers. *J Dairy Sci* 72: 2483-2494.
3. Granato D, Branco GF, Cruz AG, Faria JAF, Shah NP. 2010. Probiotic dairy products as functional foods. *Compr Rev Food Sci Food Saf* 9: 455-470.
4. Muniandy P, Shori AB, Baba AS. 2016. Influence of green, white and black tea addition on the antioxidant activity of probiotic yogurt during refrigerated storage. *Food Packag Shelf Life* 8: 1-8.
5. Sanders ME. 1999. Probiotics. *Food Technol* 53: 67-77.
6. de Vrese M, Laue C, Offick B, Soeth E, Repenning F, Thoß A, Schrezenmeir J. 2015. A combination of acid lactase from *Aspergillus oryzae* and yogurt bacteria improves lactose digestion in lactose maldigesters synergistically: a randomized, controlled, double-blind cross-over trial. *Clin Nutr* 34: 394-399.
7. O'Connell JE, Fox PF. 2001. Significance and applications of phenolic compounds in the production and quality of milk and dairy products: a review. *Int Dairy J* 11: 103-120.
8. Kähkönen MP, Hopia AI, Heinonen M. 2001. Berry phenolics and their antioxidant activity. *J Agric Food Chem* 49: 4076-4082.
9. Kokotkiewicz A, Jaremicz Z, Luczkiewicz M. 2010. *Aronia* plants: a review of traditional use, biological activities, and perspectives for modern medicine. *J Med Food* 13: 255-269.
10. Valcheva-Kuzmanova S, Kuzmanov K, Tancheva S, Belcheva A. 2007. Hypoglycemic effects of *Aronia melanocarpa* fruit juice in streptozotocin-induced diabetic rats. *Methods Find Exp Clin Pharmacol* 29: 101-105.
11. Chrubasik C, Li G, Chrubasik S. 2010. The clinical effectiveness of chokeberry: a systematic review. *Phytother Res* 24: 1107-1114.
12. Bermúdez-Soto MJ, Tomás-Barberán FA. 2004. Evaluation of commercial red fruit juice concentrates as ingredients for antioxidant functional juices. *Eur Food Res Technol* 219: 133-141.
13. Carvalho IS, Cavaco T, Brodelius M. 2011. Phenolic composition and antioxidant capacity of six artemisia species. *Ind Crops Prod* 33: 382-388.
14. Coïsson JD, Travaglia F, Piana G, Capasso M, Arlorio M. 2005. *Euterpe oleracea* juice as a functional pigment for yogurt. *Food Res Int* 38: 893-897.
15. Wallace TC, Giusti MM. 2008. Determination of color, pigment, and phenolic stability in yogurt systems colored with nonacylated anthocyanins from *Berberis boliviana* L. as compared to other natural/synthetic colorants. *J Food Sci* 73: C241-C248.
16. Kim KH, Hwang HR, Jo JE, Lee SY, Kim NY, Yook HS. 2009. Quality characteristics of yogurt prepared with flowering cherry (*Prunus serrulata* L. var. *spontanea* Max. wils.) fruit powder during storage. *J Korean Soc Food Sci Nutr* 38: 1229-1236.
17. Oh HS, Kang ST. 2015. Quality characteristics and antioxidant activity of yogurt added with acanthopanax powder. *Korean J Food Sci Technol* 47: 765-771.
18. Zhou K, Su L, Yu LL. 2004. Phytochemicals and antioxidant properties in wheat bran. *J Agric Food Chem* 52: 6108-6114.
19. Woisky RG, Salatino A. 1998. Analysis of propolis: some parameters and procedures for chemical quality control. *J Apic Res* 37: 99-105.
20. Cheung LM, Cheung PCK, Ooi VEC. 2003. Antioxidant activity and total phenolics of edible mushroom extracts. *Food Chem* 81: 249-255.
21. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic Biol Med* 26: 1231-1237.
22. Oyaizu M. 1986. Studies on products of browning reaction-antioxidative activities of products of browning reaction prepared from glucosamine. *Jpn J Nutr Diet* 44: 307-315.
23. Wu X, Gu L, Prior RL, McKay S. 2004. Characterization of anthocyanins and proanthocyanidins in some cultivars of *Ribes*, *Aronia*, and *Sambucus* and their antioxidant capacity. *J Agric Food Chem* 52: 7846-7856.
24. Slimestad R, Torskangerpoll K, Nateland HS, Johannessen T, Giske NH. 2005. Flavonoids from black chokeberries, *Aronia melanocarpa*. *J Food Compos Anal* 18: 61-68.
25. Mikulic-Petkovsek M, Schmitzer V, Slatnar A, Stampar F, Veberic R. 2012. Composition of sugars, organic acids, and total phenolics in 25 wild or cultivated berry species. *J Food Sci* 77: C1064-C1070.
26. Bolling BW, Taheri R, Pei R, Kranz S, Yu M, Durocher SN, Brand MH. 2015. Harvest date affects aronia juice polyphe-

nols, sugars, and antioxidant activity, but not anthocyanin stability. *Food Chem* 187: 189-196.
27. Drewnowski A, Gomez-Carneros C. 2000. Bitter taste, phytonutrients, and the consumer: a review. *Am J Clin Nutr* 72: 1424-1435.
28. Jakobek L, Drenjančević M, Jukić V, Šeruga M. 2012. Phenolic acids, flavonols, anthocyanins and antiradical activity of "Nero", "Viking", "Galicianka" and wild chokeberries. *Sci Hortic* 147: 56-63.
29. Benvenuti S, Pellati F, Melegari M, Bertelli D. 2004. Polyphenols, anthocyanins, ascorbic acid, and radical scavenging activity of *Rubus*, *Ribes*, and *Aronia*. *J Food Sci* 69: FCT164-FCT169.