

Development of low-sugar antioxidant jam by a combination of anthocyanin-rich berries

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Abstract A major challenge for the food industry is to satisfy the consumer demand for health benefits as well as taste. In this study, we investigated the antioxidant activities of extracts from four anthocyanin-rich jams, as well as their low-sugar versions, which used white grape juice concentrate as a white refined sugar substitute. It was determined that blueberry + Korean black raspberry jam dramatically scavenged 1,1-diphenyl-2-picrylhydrazyl, 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid), peroxy radicals, and reduced ferric ions in the tested jams and contained high amounts of phenols, flavonoids, and anthocyanins. In addition, the antioxidant capacity and content of phenolics, flavonoids, and anthocyanins were retained during the processing of berry jams. Berry jams and their low-sugar jams exhibited inhibitory effects on reactive oxygen species production in HepG2 cells and lipid peroxidation in the liver and brain homogenates. In conclusion, blueberry + Korean black raspberry and raspberry + strawberry jams have strong antioxidant capacities, which are retained in jams containing sugar substitutes. These results suggest that anthocyanin-rich berry jams with natural sugar replacements could be developed as high functional foods and also decrease the risk for metabolic diseases.

Keywords Anthocyanin · Antioxidant · Berry · Jam · Low sugar

Introduction

Blueberries (*Vaccinium corymbosum*), raspberries (*Rubus strigosus*), Korean black raspberries (*Rubus* sp.), and strawberries (*Fragaria x ananassa* D.) are rich in phenols and flavonoids and possess high radical-scavenging capacity (Wang and Lin 2000; Garzon et al. 2009; Nurmi et al. 2009). These berries are known to have high anthocyanin content. The anthocyanins in *V. corymbosum* are petunidin, cyanidins, and malvidin glycosides, including petunidin 3-glucoside, cyanidin 3-glucoside, and malvidin 3-galactoside (Ichiyanagi et al. 2000). *Rubus strigosus* also contain various kinds of anthocyanins, mainly pelargonidin and cyanidin (Kassim et al. 2009). The anthocyanins present in black raspberries are cyanidin-3-glucoside, cyanidin-3-rutinoside, cyanidin-3-sambubioside, and cyanidin-3-xylosylrutinoside (Bowen-Forbes et al. 2010). The anthocyanin present in strawberries is mainly pelargonidin; low levels of cyanidin are also present (Silva et al. 2007). These anthocyanins have been found to exert cytotoxic, cytostatic, anticarcinogenic, anti-inflammatory, and antioxidant effects (Wang and Lin 2000).

Jam is one of the shelf-stable products made from fruit. Fruits and sugar are generally combined in similar ratios and heated to produce fruit jams that are high in sugar content (Garcia-Martinez et al. 2002). However, high sugar intake can pose greater risks for metabolic disease such as obesity and diabetes, which is a current health concern especially in children (Birch and Fisher 1997; Weaver and Finke 2003; Albertson et al. 2011). It has been reported that sugar substitutes induce changes in gel formation and

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changes in the property of matter during the production of sugar-free sweets (Gajar and Badrie 2002). It is, therefore, important to find a natural alternative to artificial sugar in order to improve health functions in the population.

The objective of this study was to evaluate the antioxidant capacity of newly developed berry jams by measuring their contents of phenolics, flavonoids, and anthocyanins, and reactive oxygen species (ROS)-scavenging activity in the cells, and the inhibitory activity against lipid peroxidation in animal tissues.

Materials and methods

Reagents and chemicals

All chemical reagents used in this study were of ACS grade. 2,4,6-tripyridyl-s-triazine (TPTZ), ferric chloride, 7'-dichlorofluorescein diacetate (H₂DCFDA), cyanidin-3-glucoside, and 2,6-di-*tert*-butyl-4-methylphenol (BHT) were purchased from Sigma (USA). 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) was obtained from Invitrogen (Invitrogen-Life Technologies, USA). Trichloroacetic acid was purchased from Kanto Chemical Co. (Japan).

Preparation of fruit jams

To improve marketability according to the color clearness, jams were made of blueberry plus black raspberry and raspberry plus strawberry which have similar color tone, navy, and red, respectively. A standard commercial procedure was followed for the manufacturing of jams, as outlined previously (Downing 1996). Briefly, 3–5 kg of each type of fruit was sorted and washed. Fruit was coarsely grounded with a Robot Coupe commercial food processor model R302 V at 1750 rpm for approximately 30 s (Robot Coupe U.S.A. Inc., USA). As shown in Table 1, the jam formulation was 50 % fruit, 48 % sugar, and 2 % pectin mix (composed of dextrose, pectin, and fumaric acid; Pacific Pectin Inc., USA). The ground fruit was brought to a boil in a steam kettle (Model TDB6, Groen/A Dover Industries Co., USA) for enzyme inactivation. Pectin mix was added under manual agitation. The pH value was determined, and adjusted by addition of 50 % citric acid solution to reach a target pH range of 3.0–3.2. White refined sugar was subsequently added, and the mixture was evaporated to a final concentration of 65–68°Bx. The jam was hot-packed at 85 °C in 120 mL glass jars, immediately sealed with plastisol-lined metal lids, and inverted for 5 min to sterilize the lids. The jars were then returned to the normal position for air cooling. In the case of low-sugar jams, white refined sugar was replaced by similar volume

of white grape juice concentrate which is 68°Bx sugar concentration (Atacama Juice Co. Ltd., Chile).

Preparation of berry jam extracts

Berry jams were extracted with two volumes of distilled water for 2 h with agitation in dark, and jam extracts were diluted in distilled water at concentration of 200 mg/mL. Diluted jam extracts were stored at –20 °C.

Determination of pH, sugar content, and acidity

The pH of the samples was measured using an Accumet Basic pH meter (Fisher Scientific, USA). Sweetness was measured using a hand refractometer (ATAGO, Japan). Acidity was measured by the titration method with 0.1 N NaOH (Lee and Kim 2006).

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay

DPPH radical-scavenging activity was assayed as previous stated with minor modifications (Ozgen et al. 2006). Briefly, 50 µL of each sample was added to 200 µL of 200 µM DPPH methanolic solution in a 96-well microplate. The plate was slightly shaken, and incubated at 37 °C for 30 min, after which the absorbance was measured at 515 nm. Dimethyl sulfoxide and methanol were used as negative controls, whereas α -tocopherol was used as the positive control.

2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation-decolorization assay

Decolorization of ABTS^{•+} was determined according to a previously modified method (Katalinic et al. 2005). ABTS^{•+} was generated through a stoichiometric reaction that involved 5 mL of 7 mM ABTS^{•+} and 80 µL of 2.45 mM potassium persulfate and was performed in the dark at room temperature for 16 h. Dilution was carried out with ethanol in order to obtain the proper absorbance of 0.7 ± 0.02 at a wavelength of 734 nm. This was followed by addition of 90 µL of ABTS^{•+} solution to 10 µL of sample in a 96-well plate. Absorbance was detected at 734 nm following a 5-min incubation period at room temperature.

Ferric reducing antioxidant power (FRAP) assay

FRAP assays were conducted according to the previously published spectrophotometric methods (Braugher et al. 1986), where the ferric-2,4,6-tripyridyl-s-triazine (Fe(III)-TPTZ) complex is reduced to the blue-colored ferrous ion.

Table 1 Formulation of berry jams and low-sugar berry jams

	Jam		Low-sugar jam	
	Blueberry + Korean black raspberry jam	Raspberry + strawberry jam	Blueberry + Korean black raspberry jam	Raspberry + strawberry jam
Blueberry	33.0	–	35.0	–
Korean black raspberry	15.0	–	15.0	–
Mulberry	5.0	–	5.0	–
Raspberry	–	18.0	–	18.0
Strawberry	–	47.0	–	37.0
MBA grape	–	–	10	–
White grape juice concentrate	–	–	44.0	34.6
Organic sugar	43.4	31.0	–	–
Pectin	0.7	0.2	0.6	0.2
Citric acid	0.4	0.2	0.4	0.2
Oligosaccharide	2.5	1.6	–	–
Total percentage	100	100	100	100

Briefly, 30 μL of FRAP working reagent consisting of 300 mM acetate buffer (pH 3.6), 10 mM Fe(III)-TPTZ in 40 mM HCl, and 20 mM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ at a ratio of 10:1:1 was mixed with 7 μL of sample or standard. Subsequently, 170 μL of distilled water was added to the sample mixture in a 96-well microplate. The microplate was incubated for 10 min at room temperature, and absorbance was measured at 593 nm via an ELISA reader (Tecan Sunrise microplate reader, Tecan Group Ltd., Switzerland) with α -tocopherol as the positive control.

Oxygen radical absorbance capacity (ORAC) assay

Antioxidant activities of the sample extracts were investigated for their peroxy radical-scavenging capacity using the ORAC assay system. The ORAC assay was carried out using a fluorescence microplate reader (Infinite 200; Tecan Group Ltd.) with fluorescent filters (excitation wavelength, 485 nm; emission filter, 535 nm). In the final assay mixture, fluorescein (91.4 nM) was used as a target of free radical attack with AAPH (final conc. 11.4 mM) as a peroxy radical generator in the peroxy radical-scavenging capacity ($\text{ORAC}_{\text{ROO}\cdot}$) assay (Nakao et al. 1998; Sato et al. 1999). Trolox (1 μM) was used as a control standard and was freshly prepared on a daily basis. The analyzer was programmed to record the fluorescence of fluorescein every 2 min after AAPH was added. All fluorescence measurements were expressed relative to the initial reading. Final results were calculated based on the difference in the area under the fluorescence decay curve between the blank and the sample. All data have been expressed as μmol of trolox

equivalents. One ORAC unit is equivalent to the net protection area provided by 1 μM of trolox.

Determination of total phenolic compounds

Total phenolic content was determined using the Folin-Ciocalteu reagent (Kuhnen et al. 2014). Briefly, 100 μL of extract was mixed with 50 μL of sodium carbonate (10 %, w/v) solution, followed by addition of 15 μL of the Folin-Ciocalteu reagent (previously diluted 5-fold with distilled water). Following 5-min incubation at room temperature, the sample mixture was transferred to a 96-well microplate, and absorbance at 655 nm was measured using a microplate reader. Results are expressed as gallic acid equivalents (GAEs).

Determination of total flavonoids

Total flavonoid content was determined by aluminum chloride using a colorimetric method previously described with slight modifications (Chung et al. 1999). Briefly, 25 μL of the sample was mixed with 75 μL of 95 % methanol in a 96-well microplate. Then, 5 μL of 10 % $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$, 1 M potassium acetate, and 14 μL of distilled water were added, and the mixture was incubated for 40 min at room temperature. Absorbance readings were obtained at 415 nm with a microplate reader (Sunrise: Tecan Group Ltd.). The total flavonoid content of the samples was extrapolated from standard curves established with quercetin at 0–50 $\mu\text{g}/\text{mL}$.

Colorimetric analysis of total anthocyanins

Total anthocyanin content was evaluated by the pH differential method as described by Giusti and Wrolstad (2001). Water-soluble extracts of fruits and their jams in 0.025 M potassium chloride buffer (pH 1.0) and 0.4 M sodium acetate buffer (pH 4.5) were measured at 510 nm after 15 min of incubation at room temperature. Total anthocyanin content was expressed in mg cyanidin 3-glucoside equivalents (mg CGE)/100 g of jam.

2,7-dichlorofluorescein assay (H₂DCFDA)

Intracellular ROS production was evaluated according to a previously reported method (Poljak-Blazi et al. 2011). Briefly, human hepatocarcinoma HepG2 cells which maintained in Dulbecco's modified Eagle's medium supplemented with 10 % fetal bovine serum (FBS), 100 units/mL of penicillin, and 100 µg/mL of streptomycin at 37 °C in 5 % CO₂, were seeded onto 0.1 % gelatin-coated cover glasses in a 24-well plate. Cells were allowed to adhere to the cover glass for 24 h, followed by incubating in the presence of 200 µg/mL of the sample for another 24 h. The cells treated with the sample were then incubated with 30 µM dichlorofluorescein diacetate (H₂DCFDA) for 1 h, followed by incubation with 500 µM hydrogen peroxide diluted in phosphate-buffered saline (PBS) containing 0.5 % FBS for 30 min. Fluorescence was visualized with a fluorescent microscope (Eclipse TE2000-U, Nikon, Japan).

Thiobarbituric acid reactive substance (TBARS) assay

The lipid peroxidation assay was carried out as previously described (Braugher et al. 1986). Mice were anesthetized with diethyl ether, and sacrificed by exsanguination. The liver and brain were isolated and homogenized with isotonic PBS (twenty parts) at 4 °C. The homogenate was then centrifuged at 10,000×g for 5 min, after which the supernatant was used in an ex vivo lipid peroxidation assay. Briefly, various concentrations of the sample were mixed with 0.5 mL of 0.15 M potassium chloride and 0.5 mL of mouse liver or brain homogenates. Peroxidation was initiated by the addition of 100 µL of 0.4 mM ferric chloride. After incubation at 37 °C for 30 min, the reaction was terminated by the addition of 0.5 mL of ice-cold 0.25 M HCl containing 15 % trichloroacetic acid, 0.38 % thiobarbituric acid, and 0.5 % BHT. The mixture was then heated at 90 °C for 1 h, cooled, and centrifuged at 10,500×g for 2 min, followed by measuring absorbance at 532 nm. BHT was used as a positive control. The inhibition ratio (%) was calculated with the following equation: inhibition ratio (%) = (A–Al)/A × 100, where A is the

absorbance of the control and Al is the absorbance of the test sample.

Statistical analysis

All data are expressed as mean ± standard deviation (SD). Statistical analysis was performed by analysis of variance (ANOVA), followed by Duncan's multiple range test, using the SPSS statistics 22 software (SPSS Inc., USA). Statistical significance was reached at $p < 0.05$.

Results and discussion

Sugar content, pH, and acidity of fresh berries

As shown in Table 2, the sugar content of raspberry was the highest among the fresh berries tested with 10.9 Bx. The sugar contents of Korean black raspberry, blueberry, and strawberry were 9.4, 9.0, and 8.6 Bx, respectively. The pH values of raspberry, Korean black raspberry, blueberry, and strawberry were 2.80, 1.60, 0.83, and 0.82, respectively. In addition, the pH values of blueberry, raspberry, Korean black raspberry, and strawberry which contained less sugar than other fruit candidates to produce jams such as Campbell grape (15.8 Bx), Muscat Bailey A (MBA) grape (20.5 Bx), mulberry (14.6 Bx), mini apple (13.5 Bx), aronia (18.8 Bx) (data not shown) were 3.2, 3.1, 3.7, and 3.7, respectively. In particular, the combinations of blueberry + Korean black raspberry and raspberry + strawberry were the best choice for jam production when considered color of each berry, color tone of these jams, and consumer acceptability for jam color.

Total phenolics, flavonoid, and anthocyanin content in berry jams and low-sugar berry jams

Total phenolic contents of berry jams and low-sugar berry jams are presented as GAEs in Table 3. It was found that blueberry + Korean black raspberry jam contained relatively high level of phenolics (153.1 ± 5.2 mg GAE per 100 g jams), whereas raspberry + strawberry had relatively low level of phenolics. A slight increase in total phenolic content was found in low-sugar berry jams,

Table 2 Acid level, pH, and sweetness of fresh berries

	pH	Sugar content (°Brix)	Acidity
Blueberry	3.2	9	0.83
Korean black raspberry	3.7	9.4	1.60
Raspberry	3.1	10.9	2.80
Strawberry	3.7	8.6	0.82

Table 3 Total phenols, flavonoids, and anthocyanins of berry jam and low-sugar berry jam extracts

	Total phenolics (mg GAE/100 g w.b.)	Total flavonoids (mg QE/100 g w.b.)	Total anthocyanins (mg CGE/100 g w.b.)
Jams			
Blueberry + Korean black raspberry jam	153.1 ^a ± 5.2	62.9 ^a ± 4.7	23.1 ^a ± 0.7
Raspberry + strawberry jam	72.2 ^c ± 1.7	9.5 ^c ± 1.5	6.1 ^c ± 1.2
Low-sugar jams			
Blueberry + Korean black raspberry jam	158.4 ^a ± 7.6	58.0 ^a ± 2.8	24.2 ^a ± 1.8
Raspberry + strawberry jam	109.5 ^b ± 2.8	16.3 ^b ± 1.0	8.4 ^b ± 0.3

Values with different superscript represent statistically significant difference at $P < 0.05$

compared with berry jams in which refined white sugar replaced white grape juice concentrate. The slight increase of total phenolic content in low-sugar berry jams compared to other berry jams was most likely due to the phenolic compounds present in white grape juice concentrate. In fact, white grape juice concentrate used in this study contained 20.6 mg total phenolics per 100 g solid content (data not shown). It has been reported that the levels of total phenolics, total flavonoids, and antioxidant capacity were decreased up to 10 %, and particularly the anthocyanin content was reduced by the maximum of 40 % during jam preparation. In addition, weakly acidic condition of berry jams helps the phytochemicals such as flavonoids and anthocyanins maintain intact and minimize the destruction during jam processing (Lee et al. 2013).

Total amounts of flavonoids in berry jams and low-sugar berry jams are shown in Table 3. The changes of total flavonoids during jam processing exhibited a similar fashion to those of phenolics, where the replacement of refined white sugar with fruit juices in jam processing increased the overall flavonoid content.

Total anthocyanin contents in berry jams and low-sugar berry jams are presented in Table 3, which were analyzed by using the colorimetric analysis. Blueberry + Korean black raspberry jam had 3.8 times higher total anthocyanin content than raspberry + strawberry jam. In cases that sugar in jams was replaced with white grape juice concentrate, the total phenolic content was slightly increased, compared to normal berry jams.

Scavenging activities of DPPH, ABTS cation, peroxy radicals, and FRAP in berry jams and low-sugar jams

Most samples used in the study showed a strong DPPH radical-scavenging activity. As presented in Table 4, blueberry + Korean black raspberry jam and its low-sugar counterpart effectively scavenged DPPH radical. The ABTS⁺ radical-scavenging activities of the above jam products showed similar tendency to DPPH radical-scavenging activity. As shown in Table 4, low-sugar jam

extracts had relatively stronger ABTS⁺ radical-scavenging activity than normal berry jam extracts. Trolox equivalent antioxidant capacity (TEAC) in berry jams and low-sugar jams was evaluated on the basis of a chemical reaction using peroxy radicals which were generated by AAPH, a thermolabile radical initiator. Peroxy radicals were found to be partially scavenged by the extracts of berry jams and low-sugar jams. TEACs of berry jams and low-sugar jams are shown in Table 4. In particular, the extracts from blueberry + Korean black raspberry low-sugar jam were found to efficiently scavenge peroxy radicals.

FRAP which measures the ability of sample to reduce the Fe³⁺-TPTZ complex into Fe²⁺ form under acidic conditions was employed to evaluate the antioxidant potential of jam samples. The extract of blueberry-Korean black raspberry jam showed higher FRAP value than that of raspberry + strawberry jam.

Multiple papers have reported the correlation between antioxidant capacities and total phenolic, flavonoid, and anthocyanin contents (Ebrahimzadeh et al. 2014; Hosu et al. 2014; Yuri et al. 2014; Mitrovic et al. 2015). These findings suggest that polyphenols can scavenge free radicals by upregulation of certain metal chelation reactions (Bors et al. 1990). Our results indicated that jams that contain higher amounts of phenols, flavonoids, and anthocyanins had relatively high scavenging potential for DPPH, ABTS⁺, and peroxy radicals. For instance, extracts from blueberry to Korean black raspberry jam and its low-sugar counterpart contain high levels of phenols, flavonoids, and anthocyanins, and exhibited high antioxidant activities as compared with other berry jams and low-sugar berry jams.

Inhibition of ROS generation in berry jams and low-sugar jams in mouse hepatoma HepG2 cells

As berry jams and their low-sugar jams were shown to be effective antioxidants in the radical-scavenging assays, they were further evaluated for the ability to suppress H₂O₂-induced ROS generation in human hepatocarcinoma HepG2 cells which are sensitive and quickly responsive to

Table 4 Antioxidant activities of jam extracts against radical scavenging and ferric ion reduction

	DPPH* (% inhibition)	ABTS ⁺ (% inhibition)	ORAC _{ROO-} (TE, mM)	FRAP** (FeSO ₄ , mM)
Jams				
Blueberry + Korean black raspberry jam	81.7 ^b ± 1.04	52.9 ^c ± 7.68	18.7 ^{ab} ± 0.70	0.6 ^a ± 0.06
Raspberry + strawberry jam	66.1 ^c ± 1.16	68.0 ^{bc} ± 9.24	11.4 ^{cd} ± 4.65	0.2 ^b ± 0.02
Low-sugar jams				
Blueberry + Korean black raspberry jam	86.7 ^a ± 1.34	79.3 ^{ab} ± 4.94	23.3 ^a ± 3.74	0.5 ^a ± 0.04
Raspberry + strawberry jam	69.2 ^c ± 1.09	70.7 ^b ± 6.69	14.2 ^{bc} ± 4.86	0.3 ^b ± 0.02

Values with different superscript represent statistically significant difference at $P < 0.05$

* Berry jams and low-sugar berry jam extracts were diluted 1:4 ratio with distilled water to reduce color intervention

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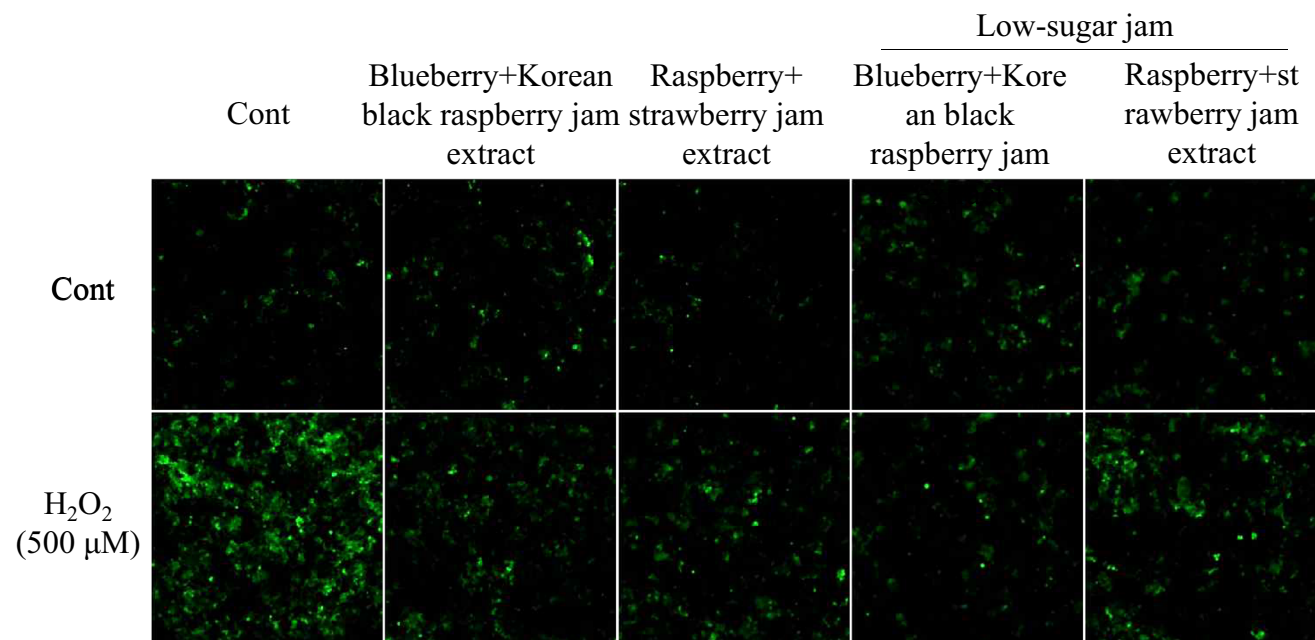


Fig. 1 Inhibitory action of jam and low-sugar jam extracts against intracellular ROS production induced by hydrogen peroxide in HepG2 cells. Human hepatocarcinoma HepG2 cells were treated

with 200 μg/mL of anthocyanin-rich berry jam extracts for 24 h. The ROS scavenging activity of samples was assessed in the presence of hydrogen peroxide (500 μM)

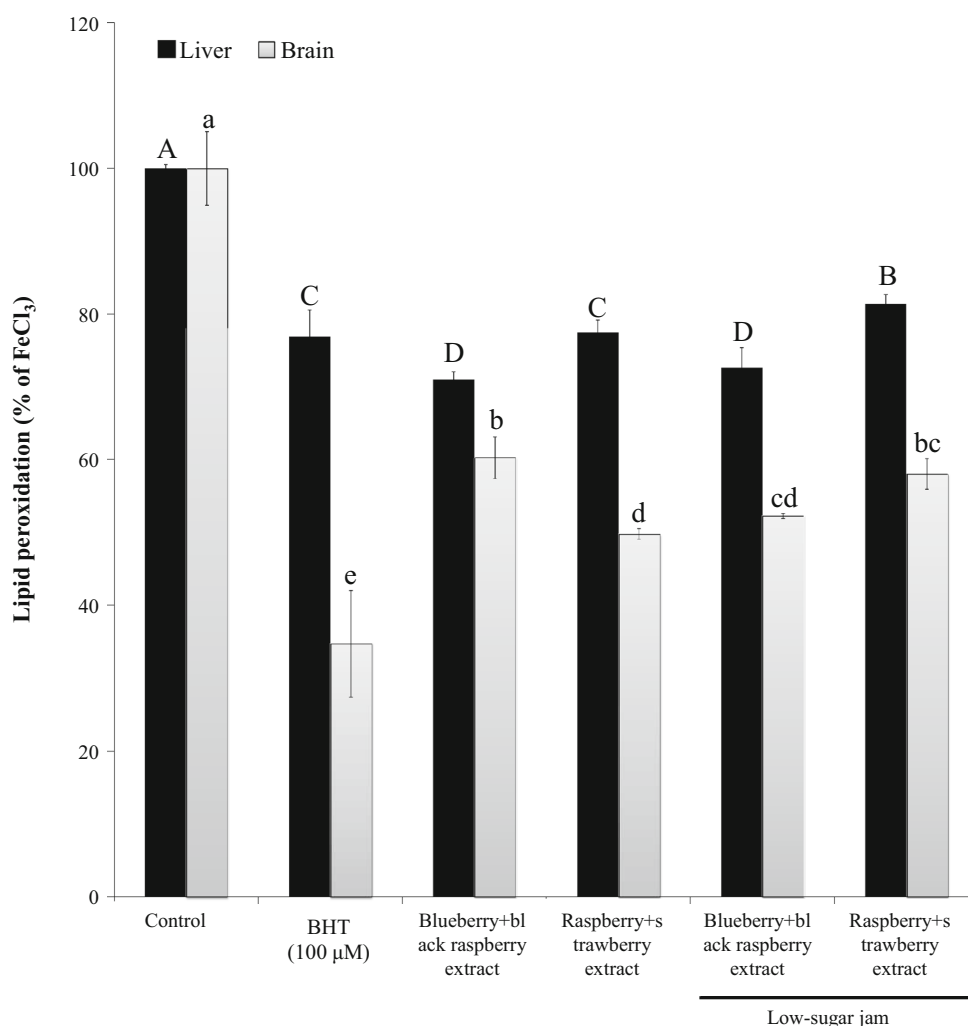
external oxidative stress by modulating expression of inducible antioxidant enzymes. As shown in Fig. 1, the extracts from blueberry + Korean black raspberry and raspberry + strawberry jam effectively inhibited ROS production, which was indicated by the bright green fluorescence, in the cells treated with H₂O₂. These results suggest that berry jams possess powerful antioxidant capacity in cells as well.

Effects of berry jams and low-sugar jams on lipid peroxidation

The inhibitory effects of the berry jams on lipid peroxidation were evaluated by measuring TBARS levels in

homogenates of mouse tissues such as liver and brain (Fig. 2). Lipid peroxidation in liver and brain triggered by ferric chloride was significantly inhibited by all four berry jams. The levels of TBARS in the liver homogenates treated with 1 mM ferric chloride plus blueberry-Korean black raspberry, raspberry-strawberry jams, and their low-sugar jams were 71.0 ± 1.1, 77.4 ± 1.7, 72.6 ± 2.8, or 81.3 ± 1.4 % of the control, respectively. The inhibitory effects of berry jams and their low-sugar counterparts on lipid peroxidation were comparable with that of BHT, which was 76.9 ± 3.7 % of the control (treated with FeCl₃ alone) in TBARS level. We found that inhibition of lipid peroxidation in the liver by berry jams and their low-sugar counterparts was similar or slightly more potent than that observed in the brain.

Fig. 2 Inhibition of lipid peroxidation by jam and low-sugar jam extracts in liver (A), and brain (B) tissue extracts. Berry jam extracts were diluted at 1:10 (v/v) ratio with distilled water. BHT (100 μ M) was used as a positive control. Results are expressed as the mean \pm SD of three independent experiments



It was extensively reported that the total phenolic content in various kinds of berry jams is not changed during processing and manufacturing, differently from the raw fruits (Amakura et al. 2000). However, in some other studies, it has suggested that anthocyanins and procyanidins were degraded in processed products at room temperature due to instability, which resulted in loss of pigments (Howard et al. 2012). In this study, we found that blueberry + Korean black raspberry and raspberry-strawberry jams retained their antioxidant capacity well, as seen in antioxidant assays conducted using cells and tissue homogenates.

Blueberry, Korean black raspberry, raspberry, and strawberry contained relatively high levels of phenolics, flavonoids, and anthocyanins, and showed powerful antioxidant capacity. Further, the antioxidant capacity was not significantly reduced during jam manufacturing process which involves the disruption of the berry peel, heating with sugar under acidic conditions, although considerable loss of anthocyanins was observed. In conclusion, we developed low-sugar jams with high antioxidant capacity

using a combination of anthocyanin-rich berries. That is, blueberry + Korean black raspberry and raspberry + strawberry jams in which white grape juice was used instead of sugar retained high levels of total phenolics, flavonoids and anthocyanins, and antioxidant capacity.

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