Food Sci. Biotechnol. 25(1): 55-61 (2016)
DOI 10.1007/s10068-016-0008-8

Food Science
and
Biotechnology

Effects of Drying Methods on Contents of Bioactive Compounds and Antioxidant Activities of Black Chokeberries (*Aronia melanocarpa*)

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Received April 28, 2015
Revised October 22, 2015
Accepted October 26, 2015
Published online February 29, 2016

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pISSN 1226-7708
eISSN 2092-6456

Abstract Optimal drying techniques for maintaining high levels of bioactive compounds and antioxidant activities in black chokeberries were investigated. Effects of 3 drying methods on total bioactive compound contents and *in vitro* antioxidant activities in 80% ethanol extracts were evaluated. Fresh black chokeberries were dried using sun-drying, freeze-drying, and oven-drying. Highest amounts of total polyphenols, flavonoids, and anthocyanins were detected in freeze-dried black chokeberry extracts after sun and oven-drying. 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS), and superoxide anion scavenging activities in black chokeberry extracts were also evaluated. Freeze-dried berries produced strongest antioxidant activities. Freeze-drying was the optimal drying method for maintaining high levels of bioactive compounds in 80% ethanol extracts of dried black chokeberries.

Keywords: *Aronia melanocarpa*, black chokeberry, polyphenol, flavonoid, anthocyanin

Introduction

Black chokeberry, *Aronia melanocarpa*, is a perennial, deciduous shrub belonging to the Rosacea family (1). Previous studies confirmed that black chokeberry has higher quantities of phenolic compounds than apples, red raspberries, blackberries, sweet rowanberries, sweet cherries, and sour cherries (2). The total phenolic content of black chokeberry fruit has been reported to exceed 20 mg/g of gallic acid equivalents (GAE) (3). In addition, anthocyanin levels of black chokeberries were reported to be as high as 1% on a dry weight (DW) basis (1). The high contents of phenolics and anthocyanins in black chokeberry fruit may contribute to potential health benefits (4). Black chokeberries have been reported to have anticancer and antimutagenic activities, as well as beneficial properties related to blood pressure and cardiovascular disease (5,6).

Fresh black chokeberries are not available all year. Therefore, it is important to identify viable substitutes for fresh berries during periods of no availability. Chokeberries can be eaten raw off the bush, but are usually processed before consumption. In recent years, black chokeberries have been used as a major ingredient in food processing for juice, syrup, jam, and wine, in addition to being used as a food colorant (7). Identification of an optimal method for processing black chokeberries that ensures maximum preservation of antioxidative compounds is important.

Drying is an important method for processing raw foods because the drying process inhibits enzymatic degradation and limits microbial growth (8). However, drying can also have negative effects on attributes of final food products. For instance, water removal leads to loss of both nutritive and sensory properties of food products (9).

In this study, effects of sun-drying, freeze-drying, and oven-drying methods on total bioactive compound levels and antioxidant activities in black chokeberries were evaluated using spectrophotometric methods.

Materials and Methods

Materials Folin-Ciocalteu 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), and nicotinamide adenine dinucleotide (NADH) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Anthocyanin standards for HPLC analysis were purchased from Extrasynthese (Genay, France). HPLC-grade water, methanol, acetonitrile, and trifluoroacetic acid (TFA) were purchased from Fisher Scientific Inc. (Fair Lawn, NJ, USA). All chemicals used were of analytical grade.

Berry preparation Berries of the Aronia Nero cultivar of black

chokeberry (*Aronia melanocarpa*) was harvested from a local farm in Yeongcheon, Korea in June of 2012 at optimal ripeness. Berries were stored immediately at 4°C in refrigerator for 2 days before drying. Effects of drying methods on bioactive compounds and antioxidant activities in black chokeberries were evaluated using the 3 controlled drying methods of sun-drying, freeze-drying, and oven-drying. For sun-drying, black chokeberries were arranged in a single layer in a basket and dried in direct sunlight for 7 days. For freeze-drying, black chokeberries were frozen at −80°C freezer overnight and freeze-dried (PVTFD20R; Ilshin Lab Co., Yangju, Korea) at 35°C and 0.010~0.015 torr for 2 days. For oven-drying, black chokeberries were arranged in a single layer on a food dehydrator tray (L'equip, Seoul, Korea) and heated at 60°C for 12 h. All dried berry samples were ground in a food grinder (HMF-3260S; Hanil, Seoul, Korea), sieved through 30 mesh and 100 mesh screens twice to produce a fine powder, then stored at −80°C freezer prior to extraction.

Powdered berry samples were subjected to extraction with 80% ethanol at 85°C for 2 h. In brief, powdered berry samples were mixed with 80% ethanol at a ratio of 1:25 (g/mL), then the mixture was filtered through Whatman #2 filter paper (Whatman International Ltd., Maidstone, UK) under vacuum. Extracted filtrates were evaporated using a rotary evaporator (N-1200A; Eyela Co., Tokyo, Japan) under reduced pressure at 40°C, then the resulting residue was dissolved in an additional 50 mL of distilled water. The solution was then freeze-dried (PVTFD20R; Ilshin Lab) and stored at −20°C freezer prior to analysis. Moisture contents were measured by AOAC methods (10) and dried berry samples contained 14.7-15.1% moisture.

Determination of total phenolic contents Total polyphenol contents were measured using Folin-Ciocalteu's phenol reagent following the method of Zhou *et al.* (11) with minor modification. In brief, 200 μL of a diluted berry sample was mixed with 400 μL of 2% 2N-Folin-Ciocalteu phenol reagent. After 3 min of standing at room temperature, 800 μL of 10% Na_2CO_3 was added and mixed, then the mixture was kept in the dark by covering with aluminum foil at room temperature for 1 h. After vortexing, the absorbance was measured at 750 nm with a microplate reader (Infinite M200 Pro; Tecan Group Ltd., San Jose, CA, USA). Gallic acid was used as a calibration standard, and results were expressed as gallic acid equivalents (GAE) per g of DW (mg of GAE/g).

Determination of total flavonoid contents Total flavonoid contents of black chokeberry ethanol extracts were measured using the method of Woisky and Salatino (12) with minor modification. In brief, 500 μL of a berry extract sample was mixed with 30 μL of 5% $NaNO_2$ and allowed to react for 6 min at room temperature. Subsequently, 60 μL of 10% $AlCl_3 \cdot 6H_2O$ was added and mixed, and then the mixture was kept at room temperature for 6 min. Then, 200 μL of 1.0 M NaOH was added to the mixture. Finally, 110 μL of distilled water was added and mixed. The absorbance of the colored flavonoid-aluminum complex was immediately measured at 510 nm with a microplate reader. Catechin was used as a calibration standard and results were expressed as catechin equivalents (CE) per g of DW (mg of CE/g).

Determination of total anthocyanin contents Total anthocyanin contents were measured using the official AOAC method (10) with minor modification. In brief, 50 μL of a berry extract sample was mixed with 950 μL of distilled water to obtain analytical samples used for this analysis. Then, 950 μL of a buffer at a pH value of 1 containing potassium chloride and 0.2 M hydrochloric acid was added to 50 μL of a berry extract sample. Subsequently, 50 μL of the berry extract sample was mixed with a buffer at pH 4.5. The absorbance was measured at 520 and 700 nm using a microplate reader after vortexing. Total anthocyanin contents were calculated as:

$$\text{Total anthocyanin content (mg/100 g)} = (A \times Mw \times DF \times 1 \times 1000)/(\varepsilon \times 1)$$

where A is the difference in absorbance between pH 1.0 and pH 4.5, Mw=molecular weight of cyanidin-3-glucoside (449.2), DF=a dilution factor, ε=cyanidin-3-glucoside molar absorptivity (26,900), and 1=total volume (1 mL).

Extraction and quantification of anthocyanins and polyphenols using HPLC Powdered berry extract samples of approximately 100 mg were combined with 5 mL of methanol containing 0.1% formic acid, vortexed for 1 min, centrifuged (Mega 17R; Hanil Science Industryal, Incheon, Korea) for 5 min, then the upper fraction was transferred to a glass tube. Residue was extracted from this upper fraction with addition of another 5 mL of methanol containing 0.1% formic acid until extracts were colorless. Methanol fractions were combined and evaporated to dryness in a rotary evaporator. The resulting residue was re-dissolved in methanol at a concentration appropriate for HPLC analysis, and a 10 μL volume was injected into an HPLC apparatus (Ultimate 3000; Dionex, Sunnyvale, CA, USA). Anthocyanins were separated using a C_{18} Zorbox SB column (4.6×250 mm, 5 μm particle size) (Agilent Technologies Inc., Santa Clara, CA, USA).

The solvent system used was (A) water with 5% formic acid and (B) acetonitrile with 5% formic acid. Extract samples were separated using a gradient of: A/B=95/5 (0-5 min), 90/10 (8 min), 85/15 (13-18 min), 80/20 (25 min), 70/30 (28-32 min), and 95/5 (35-40 min) at a flow rate of 0.8 mL/min. Absorbance peaks at 520 nm were detected using a UV detector (Waters 2487; Waters, Milford, MA, USA). Anthocyanin standard stock solutions were prepared in methanol containing 0.1% formic acid and calibration curves were constructed at concentrations from 6.25 to 100 μg/mL. High linearity (r^2>0.999) was obtained for each standard curve.

Polyphenol levels were analyzed via HPLC (Ultimate 3000; Dionex) on an Agilent XDB C_{18} column (4.6x150 mm, 5 μm). The solvent system used was (A) water with 0.3% TFA and (B) acetonitrile. Extract samples were separated using a gradient of: A/B = 95/5 (0-39 min), 40/60 (40 min), 0/100 (45-50 min), and 95/5 (55-60 min) at a flow rate

of 0.8 mL/min. Absorbance peaks at 280 nm were detected using a UV detector (190-800 DAD scanning) (Waters). Polyphenol standard stock solutions were prepared in methanol and calibration curves were constructed at concentrations of 6.25 to 100 μg/mL. High linearity (r^2>0.999) was obtained for each standard curve.

DPPH assay DPPH radical scavenging activities of 80% ethanol extracts of black chokeberries were determined following the method of Cheung *et al*. (13), with minor modification. First, 192 μL of a solution of 50 μM DPPH was mixed with 48 μL of a diluted extract sample at a ratio of 4:1 (v/v). The mixture was left covered with aluminum foil in the dark at room temperature for 30 min, then used as a control consisting of either 48 μL of distilled water in192 μL of 50 μM DPPH for the ascorbic acid standard, or 48 μL of 94% ethanol in 192 μL of 50 μM DPPH for berry extract samples. The extent to which DPPH was de-colored was read at 517 nm using a microplate reader. Ascorbic acid was used as a control. The DPPH radical-scavenging activity was calculated as:

$$\text{Inhibition (\%)}=[(A_{control}-A_{sample})/A_{control}]x100$$

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 activity of chokeberry extracts was determined following the method of Re *et al*. (14), with minor modification. First, ABTS was dissolved in distilled water to obtain a 7 mM concentration. The ABTS radical cation was produced by reaction of an ABTS stock solution with 2.45 mM $K_2S_2O_8$ at a ratio of 2:1 in the dark for 24 h before use. The ABTS reagent was diluted with 94% ethanol to an absorbance of 0.17±0.03, which was measured with a microplate reader at 734 nm. Then, 950 μL of this ABTS reagent was mixed with 50 μL of different concentrations of extract samples. After leaving the mixture covered with aluminum foil in the dark at room temperature for 10 min, the absorbance was recorded at 734 nm using a microplate reader. Ascorbic acid was used as a control. Each extract sample was measured in triplicate, and percentage inhibition (%) was calculated as:

$$\text{Inhibition (\%)}=[(A_{control}-A_{sample})/A_{control}]x100$$

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.

Superoxide anion scavenging activity The superoxide radical generated in a xanthine-xanthine oxidase system was analyzed spectrophotometrically with microplate reader using the NBT product as an end product (15). The reaction mixture was prepared with 50 μL of an extract sample, 0.5 mL of 1:1 ratio mixture of 0.4 mM xanthine and 0.24 mM NBT, 0.5 mL of 0.049 U/mL xanthine oxidase, and distilled water, to obtain a final volume of 2.0 mL. After incubation in water bath at 37°C for 40 min, 2 mL of 69 mM SDS was added to stop the reaction. The absorbance was measured with a microplate reader at 560 nm and compared with controls that had been run without xanthine oxidase. Ascorbic acid was used as a positive control. Each extract sample was measured in triplicate and percentage inhibition (%) was calculated as:

$$\text{Inhibition (\%)}=[1-(A_{sample})/(A_{control})]x100$$

where $A_{control}$ is the absorbance of a control blank without the test compound, and A_{sample} is the absorbance of the test compound.

Reducing power activity The Fe^{3+} reducing power of extracts was determined following the method of Oyaizu (16) with minor modification. Different concentrations (12.5~200 mg/mL) of 0.25 mL of extract samples were mixed with 0.25 mL of a 0.2 M phosphate buffer (pH 6.6) and 0.25 mL of potassium hexacyanoferrate ($K_3Fe(CN)_6$) (w/v, 1%). After incubation in water bath at 50°C in a water bath for 20 min, the reaction was stopped by addition of 0.25 mL of a trichloroacetic acid solution (w/v, 10%). Then, the mixture was centrifuged (Mega 17R; Hanil Science Industryal) at 15,000x*g* for 10 min. Subsequently, 0.5 mL of the supernatant was mixed with 0.5 mL of distilled water and 0.1 mL of a ferric chloride ($FeCl_3$) solution (0.1%, w/v) for 10 min. The absorbance was immediately measured at 700 nm using a microplate reader to determine the reducing power. Ascorbic acid was used as a standard with the final concentration ranging from 0 to 200 μg/mL.

Statistical analysis All values are presented as mean±standard deviation (SD). Statistical analysis was performed using the statistical analysis system (SPSS software package, Version 17.0). Data were compared using one-way analysis of variance (ANOVA) at p<0.05.

Results and Discussion

Determination of total phenolic, flavonoid, and anthocyanin contents Concentrations of total polyphenols, flavonoids, and anthocyanins were determined for black chokeberries that were dried using all 3 methods (Table 1). The highest phenolic content of 919.7 mg of GAE/g of DW was observed in 80% ethanol extracts of black chokeberries dried using the freeze-drying method. In contrast, the lowest phenolic content of 792.3 mg of GAE/g of DW was observed for oven-dried berries.

Total flavonoid contents of 80% ethanol extracts ranged from 52.0 to 66.1 mg of catechin equivalents (CE)/g. Extracts of freeze-dried black chokeberries contained the highest quantities of flavonoids at 66.1 mg of CE/g of DW, while extracts of sun-dried back chokeberries contained the lowest value at 52.0 mg of CE/g of DW. The 80% ethanol extract of oven-dried black chokeberries contained 58.5 mg of CE/g of DW.

Total anthocyanin contents in 80% ethanol extracts ranged from

Table 1. Total polyphenol, flavonoid, and anthocyanin contents obtained after drying black chokeberries using sun, freeze, and oven-drying

Drying method	Total polyphenol (mg of GAE[1])/g)	Total flavonoids (mg of CE[2])/g)	Total anthocyanin (mg of C3G[3])/g)
Sun-dried	876.4±23.6[b]	52.0±2.9[a4)]	1,146±20.3[a]
Freeze-dried	919.7±16.9[c]	66.1±4.5[b]	3,715±34.2[c]
Oven-dried	792.3±6.8[a]	58.5±4.3[ab]	2,231±11.4[b]

[1)]GAE=gallic acid equivalents
[2)]CE=catechin equivalents
[3)]C3G=cyanidin-3-glucoside
[4)a-c]Values with different superscript letters within the same column are significantly different at $p<0.05$.

Table 2. Summary of total polyphenol, flavonoid, and anthocyanin contents obtained after drying black chokeberries using sun, freeze, and oven-drying

Drying method	Total polyphenol (mg of GAE[1])/g)	Total flavonoids (mg of CE[2])/g)	Total anthocyanin (mg of C3G[3])/g)
Sun-dried	++[4)]	+	+
Freeze-dried	+++	++	+++
Oven-dried	+	+	++

[1)]GAE=gallic acid equivalents
[2)]CE=catechin equivalents
[3)]C3G=cyanidin-3-glucoside
[4)]The higher number of + means the higher total polyphenol, flavonoids, and anthocyanin contents.

1,146 to 3,715 mg/100 g. Anthocyanin contents differed between the 3 drying methods. Freeze-drying was the most effective method for preservation of anthocyanin levels with a value of 3,715 mg/100 g of DW, followed by oven-drying at 2,231 mg/100 g of DW and sun-drying at 1,146 mg/100 g of DW.

Total polyphenol, flavonoid, and anthocyanin contents obtained after drying black chokeberries using 3 different methods are shown in Table 2. Amounts of total phenolics, flavonoids, and anthocyanin differed among the drying methods. Fresh black chokeberries contain high amounts of polyphenols, particularly tannin (17,18). A previous study reported the black chokeberry phenolic content to be 690.2 mg/100 g of fresh weight (19). However, it is possible that a high temperature was used during the extraction process, leading to breakdown of tannin to simple phenols, thus increasing the number of compounds with free hydroxyl groups (20). In this study, the highest flavonoid contents were observed in extracts of black chokeberries using the freeze-drying method, while the lowest flavonoid contents were observed for oven-dried chokeberries. Amounts of phenolic compounds, flavonoids, and anthocyanins in black chokeberries reportedly vary according to the cultivar and growth year (18). Wild chokeberries and the cultivars Viking and Nero had similar total phenolic contents of 9,012-10,804 mg/kg in the first year, and 9,361-12,055 mg/kg of fresh weight in the second year.

Extraction and quantification of anthocyanins and polyphenols using HPLC Four types of anthocyanins were detected in black chokeberry extracts, including cyanidin-3-*O*-galactoside, cyanidin-3-*O*-glucoside, cyanidin-3-*O*-arabinoside, and cyanidin-3-*O*-xylose, with retention times of 16.6, 17.4, 18.4, and 22.9 min, respectively. An HPLC chromatogram of anthocyanin standard compounds is shown in Fig. 1. Cyanidin-3-*O*-galactoside was the dominant anthocyanin, followed by cyanidin-3-*O*-arabinoside, cyanidin-3-*O*-xylose, and cyanidin-3-*O*-glucoside.

Anthocyanin concentrations in black chokeberries dried using the 3 drying methods are shown in Table 3. Anthocyanin contents were affected differently by the 3 drying methods. The highest anthocyanin contents were observed in freeze-dried black chokeberries, followed by sun- and oven-dried black chokeberries. The cyanidin-3-*O*-galactoside content of freeze-dried black chokeberries was 798.1 mg/100 g of DW. With sun and oven-drying, the cyanidin-3-*O*-galactoside contents decreased dramatically to 388.4 and 119.6 mg/100 g of DW, decreases of 51.3% and 85.0% relative to freeze-dried berries, respectively. The cyanidin-3-*O*-arabinoside content of freeze-dried black chokeberries was 370.9 mg/100 g of compared with controls.

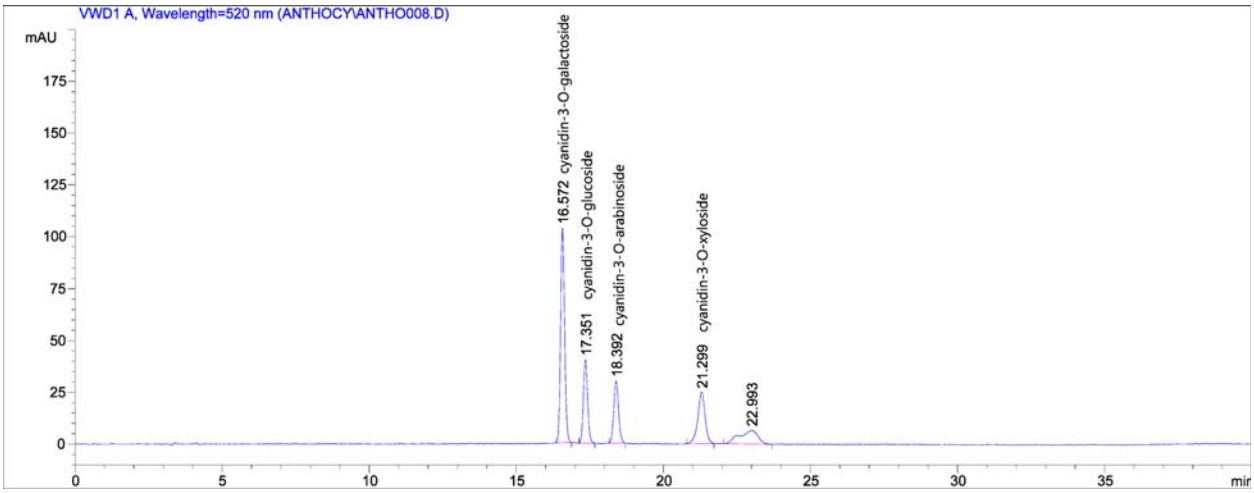

Fig. 1. HPLC chromatogram of anthocyanin standard compounds.

Table 3. Anthocyanin contents (mg/100 g) after drying black chokeberries using sun-, freeze-, and oven-drying

	Cyanidin-3-*O*-galactoside	Cyanidin-3-*O*-glucoside	Cyanidin-3-*O*-arabinose	Cyanidin-3-*O*-xylose
Sun-dried	388.4±23.1[b]	20.8±1.5[b]	159.2±0.10[b]	25.7±1.7[b]
Freeze-dried	798.1±32.7[c]	49.1±3.2[c]	370.9±0.18[c]	71.2±3.7[c]
Oven-dried	119.6±2.2[a1)]	6.1±0.7[a]	51.6±0.01[a]	5.2±0.7[a]

[1)a-c]Values with different superscript letters within the same column are significantly different at $p<0.05$.

Table 4. Polyphenol contents (mg/g) after drying black chokeberries using sun-, freeze-, and oven-drying

	Chlorogenic acid	Vanillic acid	Rutin hydrate
Sun-dried	3.61±0.13[a1)]	0.08±6.7[b]	0.09±0.01[ab]
Freeze-dried	4.60±0.26[b]	0.09±5.8[b]	0.10±0.01[b]
Oven-dried	3.60±0.10[a]	0.07±4.1[a]	0.08±0.01[a]

[1)a-c]Values with different superscript letters within the same column are significantly different at $p<0.05$.

The 3 phenolic compounds chlorogenic acid, vanillic acid, and rutin hydrate were identified in berry extracts with retention times of 14.0, 15.5, and 21.7 min, respectively. Chlorogenic acid was the major phenolic compound, while vanillic acid and rutin hydrate were present in lower, but similar, amounts.

Polyphenol concentrations in black chokeberries dried using 3 methods are shown in Table 4. Polyphenol contents were affected differently by the 3 drying methods. The highest amount of polyphenols was observed in freeze-dried black chokeberry extracts, while similar, but lower, amounts of phenolic compounds were detected in sun and oven-dried berry extracts. The chlorogenic acid content in freeze-dried black chokeberry extracts was 4.60 mg/g of DW. After sun- and oven-drying, chlorogenic acid contents decreased to 3.61 and 3.60 mg/g of DW, decreases of 21.6% and 21.8% relative to freeze-dried berry extracts, respectively. The vanillic acid content of freeze-dried black chokeberry extracts was 0.09 mg/g of DW. After sun- and oven-drying, vanillic acid contents decreased by 11.1 and 22.2%, respectively, relative to freeze-dried berry extracts.

Antioxidant activities of black chokeberry extracts DPPH radical scavenging activities of black chokeberry extracts dried using 3 different methods are shown in Fig. 2. The DPPH radical scavenging activity of 80% ethanol extracts for berries of all 3 drying methods increased in a concentration-dependent manner from 12.5-200 mg/mL. The average inhibition of DPPH radical formation in freeze-dried black chokeberry extracts at 200 mg/mL in 80% ethanol was 74.3%, whereas it was 71.2 and 73.8% in sun-dried and oven-dried extracts, respectively. The freeze-drying method produced the strongest DPPH scavenging activity. Sun-dried berry extracts exhibited the lowest DPPH radical scavenging activity in 80% ethanol extracts.

The ABTS radical scavenging activities of black chokeberry extracts dried using the 3 different methods are shown in Fig. 3. The ABTS radical scavenging activity increased in a concentration-dependent manner from 12.5-200 mg/mL. The average inhibition of ABTS radical formation in freeze-dried black chokeberry extracts at 100 mg/mL was 43.4%, whereas it was 38.2% for oven-dried berry extracts. Sun-drying exhibited the lowest ABTS radical scavenging activity, with 34.7 and 61.4% activities for 100 and 200 mg/mL 80% ethanol extracts, respectively. The freeze-drying method was more effective at retaining the ABTS radical scavenging activity than the other 2 drying methods.

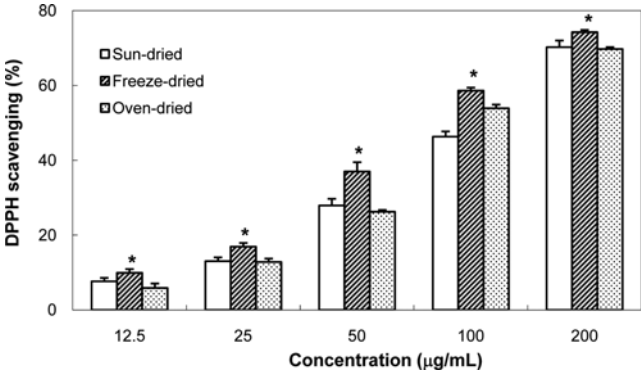

Fig. 2. DPPH radical scavenging activities of black chokeberries dried using sun, freeze, and oven-drying. Each value is expressed as a mean±SD of 3 separate experiments. The asterisk symbol indicates that mean values are significantly different from controls at $p<0.05$.

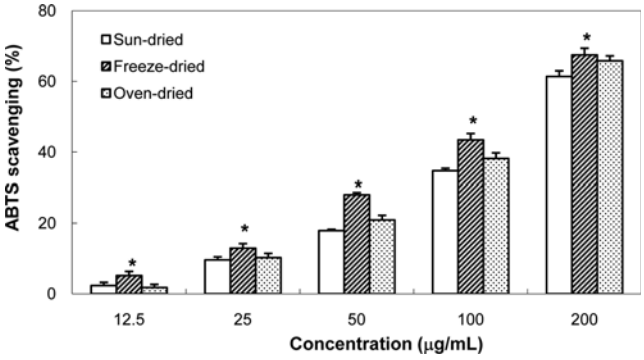

Fig. 3. The ABTS radical scavenging activities of black chokeberries dried using sun, freeze, and oven-drying. Each value is expressed as a mean±SD of 3 separate experiments. The asterisk symbol indicates that mean values are significantly different from controls at $p<0.05$.

Scavenging activities of the superoxide anion in black chokeberry extracts are shown in Fig. 4. The highest inhibition of superoxide anion radical formation was obtained in freeze-dried black chokeberry extracts with 31.6% inhibition. In comparison, inhibition levels were 28.3 and 25.3%, respectively, in sun-dried and oven-dried berry extracts at 200 mg/mL, respectively. Therefore, the freeze-drying method was the most effective technique for the superoxide anion scavenging activity.

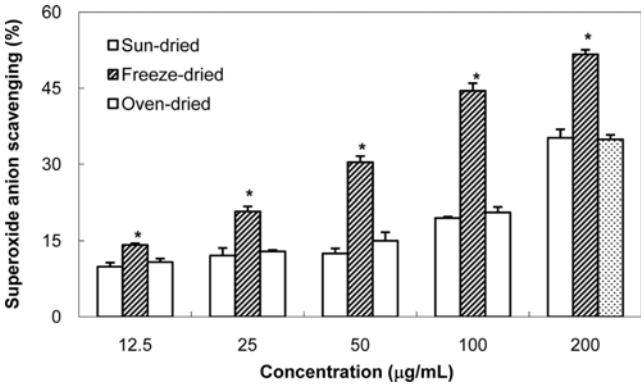

Fig. 4. The superoxide anion radical scavenging activities of black chokeberries dried using sun, freeze, and oven-drying. Each value is expressed as a mean±SD of 3 separate experiments. The asterisk symbol indicates that mean values are significantly different from controls at $p<0.05$.

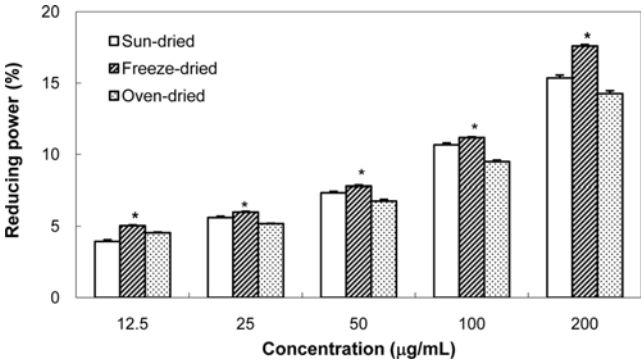

Fig. 5. Reducing powers of black chokeberries dried using sun, freeze, and oven-drying. Each value is expressed as a mean±SD of 3 separate experiments. The asterisk symbol indicates that mean values are significantly different from controls at $p<0.05$.

The reducing power of black chokeberry extracts using the 3 different drying methods is shown in Fig. 5. The overall reducing power was weak, compared to results obtained from other anti-oxidant assays. The reducing power in freeze-dried black chokeberry extracts at 200 mg/mL was 17.6%, whereas it was 15.4 and 14.3% in sun-dried and oven-dried berry extracts, respectively. However, the freeze-drying method showed the strongest reducing powder.

A summary of DPPH, ABTS, and superoxide anion radical scavenging activities and reducing powers after drying black chokeberries using 3 different methods is shown in Table 5. Antioxidant activities differed among the drying methods. Amounts of flavonoids and phenolic acids correlated with levels of antioxidant activities based on scavenging of free radicals (21). Antioxidant activities of black chokeberry extracts were evaluated using different *in vitro* assays. Use of at least 2 methods is usually recommended for determination of antioxidant activities due to differences between testing systems (22). High levels of polyphenols, flavonoids, and anthocyanins caused significant ($p<0.05$) increases in DPPH, ABTS, and superoxide anion radical scavenging activities and reducing-powers, compared with controls.

Table 5. Summary of DPPH, ABTS, and superoxide anion radical scavenging activities and reducing power after drying black chokeberries using sun-, freeze-, and oven-drying

Drying method	DPPH	ABTS	Super oxide anion	Reducing power
Sun-dried	++[1)]	++	++	++
Freeze-dried	+++	+++	+++	+++
Oven-dried	++	++	+	+

[1)]The higher number of + means the more strong DPPH, ABTS, and super oxide anion radial scavenging activities and reducing power.

In general, the drying process causes loss of naturally occurring antioxidants present in raw plant materials, and longer drying times cause a greater decline in product quality (23,24). Thus, intense and prolonged thermal treatment may be responsible for loss of natural antioxidants. Sun-drying is the most commonly used commercial technique for drying vegetables and fruits. However, this technique has the major disadvantage of a long drying time, which degrades food quality (25). For sun-drying, fresh black chokeberries were kept at an ambient temperature for 7 days to achieve complete drying. During drying, metabolically active plants lose moisture slowly and may sense moisture loss as stress (26).

Oven-drying, a commonly used method, destroys undesirable enzymes and preserves foodstuff quality for a long time. However, the high temperature of oven-drying is likely to cause declines in levels of heat-sensitive nutrients and phytochemicals. Freeze-drying is an existing technology that can be used to retain product quality and generate benefits of dried foods for shelf-life and reduction in transport and storage costs. However, the major disadvantage of freeze-drying is a relatively high cost (27). Yousif *et al*. (28) reported that freeze-drying showed a less pronounced damaging effect on tissue structure than other drying methods. The freeze-drying method reportedly can be used for food products to produce anti-oxidants (29-31). Results reported herein also supported advantages of freeze-drying to retain anti-oxidant compounds in black chokeberries. Black chokeberry extracts can be used in a functional strategy to protect against free radical-associated diseases.

Bioactive compounds in black chokeberry extracts were quantified for determination of whether freeze, sun, or oven-drying was most effective for preservation of bioactive compound contents. Black chokeberry extracts were effective scavengers of active oxygen species, including DPPH and ABTS radicals, and superoxide anions. Moreover, freeze-drying preserved the highest levels of bioactive and anti-oxidant compounds in black chokeberries, in addition to inhibition of the activities of several harmful free radicals.

Acknowledgments This research was supported by the High Value-added Food Technology Development Program (112078-3), Ministry of Agriculture, Food and Rural Affairs, Republic of Korea.

Disclosure The authors declare no conflict of interest.

References

1. Slimestad R, Torskangerpoll K, Nateland HS, Johannessen T, Giske NH. Flavonoids from black chokeberries, *Aronia melanocarpa*. J. Food Compos. Anal. 18: 61-68 (2005)
2. Jakobek L, Šeruga M, Novak I, Medvidović-Kosanović M. Flavonols, phenolic acids and antioxidant activity of some red fruits. Deut. Lebensm.-rundsch. 103: 369-378 (2007)
3. Kähkönen MP, Hopia AI, Vuorela HJ, Rauha J, Pihlaja K, Kujala TS, Heinonen M. Antioxidant activity of plant extracts containing phenolic compounds. J. Agr. Food Chem. 47: 3954-3962 (1999)
4. Carvalho IS, Cavaco T, Brodelius M. Phenolic composition and antioxidant capacity of six *Artemisia* species. Ind. Crop. Prod. 33: 382-388 (2011)
5. Hellström JK, Shikov AN, Makarova MN, Pihlanto AM, Pozharitskaya ON, Ryhänen E, Kivijärvi P, Makarov VG. Blood pressure-lowering properties of chokeberry (*Aronia mitchurinii*, var. *Viking*). J. Funct. Food. 2: 163-169 (2010)
6. Kedzierska M, Olas B, Wachowicz B, Glowacki R, Bald E, Czernek U, Szydlowska-Pazera K, Potemsk P. Effect of the commercial extract of aronia on oxidative stress in blood platelets isolated from breast cancer patients after the surgery and various phases of the chemotherapy. Fitoterapia 83: 310-317 (2012)
7. Oszmiañski J, Wojdylo A. Aronia melanocarpa phenolics and their antioxidant activity. Eur. Food Res. Technol. 221: 809-813 (2005)
8. Ahrné LM, Pereira NR, Staack N, Floberg P. Microwave convective drying of plant foods at constant and variable microwave power. Dry. Technol. 25: 1149-1153 (2007)
9. Stojanovic J, Silva JL. Influence of osmotic concentration, continuous high frequency ultrasound and dehydration on antioxidants, colour and chemical properties of rabbiteye blueberries. Food Chem. 101: 898-906 (2007)
10. AOAC. Official Method of Analysis of AOAC Intl. 16th ed. pp. 1-26. Association of official analytical chemists, Washington, DC, USA (1995)
11. Zhou K, Su L, Yu L. Phytochemicals and antioxidant properties in wheat bran. J. Agr. Food Chem. 52: 6108-6114 (2004)
12. Woisky R, Salatino A. Analysis of propolis: Some parameters and procedures for chemical quality control. J. Apic. Sci. 37: 99-105 (1998)
13. Cheung LM, Cheung PC, Ooi VE. Antioxidant activity and total phenolics of edible mushroom extracts. Food Chem. 81: 249-255 (2003)
14. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Bio. Med. 26: 1231-1237 (1999)
15. Wang J, Yuan X, Jin Z, Tian Y, Song H. Free radical and reactive oxygen species scavenigng activities of peanut skins extract. Food Chem. 104: 242-250 (2007)
16. Oyaizu M. Studies on product of browning reaction prepared from glucose amine. Jap. J. Nutr. 44: 307-315 (1986)
17. Drewnowski A, Gomez-Carneros C. Bitter taste, phytonutrients, and the consumer: A review. Am. J. Clin. Nutr. 72: 1424-1435 (2000)
18. Jakobek L, Drenjanèeviæ M, Jukiæ V, Šeruga M. Phenolic acids, flavonols, anthocyanins and antiradical activity of "Nero", "Viking", "Galicianka" and wild chokeberries. Sci. Hortic.-Amsterdam 147: 56-63 (2012)
19. Benvenuti S, Pellati F, Melegari M, Bertelli D. Polyphenols, anthocyanins, ascorbic acid, and radical scavenging activity of *Rubus*, *Ribes*, and *Aronia*. J. Food Sci. 69: 164-169 (2004)
20. Oboh G. Effect of blanching on the antioxidant properties of some tropical green leafy vegetables. LWT-Food Sci. Technol. 38: 513-517 (2005)
21. Valcheva-Kuzmanova S, Borisova P. Hepatoprotective effect of the natural fruit juice from *Aronia melanocarpa* on carbon tetrachloride-induced acute liver damage in rats. Exp. Toxicol. Pathol. 56: 195-201 (2004)
22. Pantelidis GE, Vasilakakis M, Manganaris GA, Diamantidis Gr. Antioxidant capacity, phenol, anthocyanin and ascorbic acid contents in raspberries, black berries, red currants, gooseberries and Cornelian cherries. Food Chem. 102: 777-783 (2007)
23. Hsu C-L, Chen W, Weng Y-M, Tseng C-Y. Chemical composition, physical properties and antioxidant activities of yam flours as affected by different drying methods. Food Chem. 83: 85-92 (2003)
24. Lim YY, Murtijaya J. Antioxidant properties of *Phyllanthus amarus* extracts as affected by different drying methods. LWT-Food Sci. Technol. 40: 1664-1669 (2007)
25. Laguerre JC, Tauzin V, Grenier E. Hot air and microwave drying of onions: A comparative study. Dry. Technol. 17: 1471-1480 (1999)
26. Hossain MB, Barry-Ryan C, Martin-Diana AB, Brunton NP. Effect of drying method on the antioxidant capacity of six *Lamiceae* herbs. Food Chem. 123: 85-91 (2010)
27. Chan EWC, Lim YY, Wong SK, Lim KK, Tan SP, Lianto FS, Yong MY. Effects of different drying methods on the antioxidant properties of leaves and tea of ginger species. Food Chem. 113: 166-172 (2009)
28. Yousif AL, Scaman CH, Durance TD, Girard B. Flavor volatiles and physical properties of vacuum-microwave and airdried sweet basil (*Ocimum basilicum* L.). J. Agr. Food Chem. 47: 4777-4781 (1999)
29. Ratti C. Hot air and freeze-drying of high-value foods: A review. J. Food Eng. 49: 311-319 (2001)
30. Borchani C, Besbes S, Masmoudi M, Blecker C, Paquot M, Attia H. Effect of drying methods on physico-chemical and antioxidant properties of date fibre concentrates. Food Chem. 125: 194-1201 (2011)
31. Ma L, Chen H, Zhu W, Wang Z. Effect of different drying methods on physicochemical properties and antioxidant activities of polysaccharides extracted from mushroom Inonotus obliquus. Food Res. Int. 50: 633-640 (2013)