Prev. Nutr. Food Sci. 2018;23(1):35-45
https://doi.org/10.3746/pnf.2018.23.1.35
pISSN 2287-1098 · eISSN 2287-8602

Antioxidant Activities of Selected Berries and Their Free, Esterified, and Insoluble-Bound Phenolic Acid Contents

Ji-Sang Kim

Department of Food, Nutrition and Biotechnology, Kyungnam University, Gyeongnam 51767, Korea

ABSTRACT: To explore the potential of berries as natural sources of bioactive compounds, the quantities of free, esterified, and insoluble-bound phenolic acids in a number of berries were determined. In addition, the antioxidant activities of the berries were determined using 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity, ferric reducing antioxidant power, and Trolox equivalent antioxidant capacity assays, in addition to determination of their metal ion chelating activities. Furthermore, several phenolic compounds were detected using high-performance liquid chromatography. Of the 6 tested berries, black chokeberry and blackberry exhibited the strongest antioxidant activities, and the various berry samples were found to contain catechin, caffeic acid, *p*-coumaric acid, epicatechin, vanillic acid, quercitrin, resveratrol, morin, naringenin, and apigenin. Moreover, the antioxidant activities and total phenolic contents of the fractions containing insoluble-bound phenolic acids were higher than those containing the free and esterified phenolic acids. The results imply that the insoluble-bound fractions of these berries are important natural sources of antioxidants for the preparation of functional food ingredients and preventing diseases associated with oxidative stress.

Keywords: antioxidant activity, alkaline hydrolysis, phenolic profile, berries

INTRODUCTION

Berries, which are particularly important in the Finnish diet, contain many essential functional components, including flavonoids and phenolic acids, which constitute two large and heterogeneous groups of biologically active non-nutrients. Although the contents of flavonoids and phenolic acids vary widely within berries, the contents of phenolic compounds within a single species also vary due to differences in the berry varieties (1) and growth conditions (2).

In a structural context, phenolic compounds are composed of aromatic rings that bear one or more hydroxyl groups, and are generally categorized as phenolic acids, flavonoids, anthocyanins, or tannins (3). Indeed, such phenolic compounds constitute one of the most numerous and ubiquitously distributed groups of plant secondary metabolites, and have been demonstrated to exhibit various beneficial effects in the treatment of a multitude of diseases (4). In the case of the phenolic acids, these compounds exist in three main forms, namely soluble free acids, esterified acids, which are esterified with sugars and low-molecular mass components, and insoluble-bound acids, which are covalently bound to the structural components of cell walls (5,6). In terms of their biological activities, phenolic compounds are considered to contribute to the antioxidant, anti-carcinogenic, anti-inflammatory, and anti-angiogenic properties of berries (7-9), while also improving the nutritional value of processed foods by preventing the oxidation of lipids and proteins in such products (10). Although a number of studies have examined the nutritional and chemical components of berries in addition to their bioactivities (11,12), few reports on the free, esterified, and insoluble-bound phenolic acid contents of berries and their resulting antioxidant capacities are available. Thus, we herein aim to investigate the contents and antioxidant capacities of these three classes of phenolics following their isolation from selected berries. In addition, the polyphenols present in the free, esterified, and insoluble-bound components were identified and quantified by high-performance liquid chromatography (HPLC).

Received 14 November 2017; Accepted 19 December 2017; Published online 31 March 2018

Correspondence to Ji-Sang Kim, Tel: +82-55-249-2185, E-mail: jisangkim@kyungnam.ac.kr

MATERIALS AND METHODS

Chemicals and reagents

2,2-Diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), gallic acid, *p*-hydroxybenzoic acid, chlorogenic acid, catechin, caffeic acid, epicatechin, epigallocatechin gallate, *p*-coumaric acid, ferulic acid, *m*-coumaric acid, *o*-coumaric acid, quercitrin, myricetin, resveratrol, morin, quercetin, naringenin, apigenin, vanillic acid, kaempferol, and formic acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Aluminum chloride, anhydrous sodium sulfate, Folin-Ciocalteu reagent, methanol, *n*-hexane, sodium carbonate, and sodium nitrite were obtained from Merck (Darmstadt, Germany). All other chemicals used in the experiments were of analytical grade, and deionized water was used throughout.

Sample preparation

Six berries were studied, namely, raspberry (*Rubus idaeus* L.), blackcurrant (*Ribes nigrum*), blackberry (*Rubus croceacanthus*), cranberry (*Vaccinium macrocarpon*), black chokeberry (*Aronia melanocarpa*), and blueberry (*Vaccinium* spp). The berries were collected from local markets in Gimhae, Korea. After washing with deionized water and immediately freeze-drying, the lyophilized berries were ground into powders using a grinder and stored at −80°C prior to analysis.

Extraction and separation of the phenolic fractions

***Free phenolic acid fractions*:** Extraction of the free phenolic acid fraction was carried out following the method described by Neo et al. (13) with slight modifications. More specifically, a sample of the desired berry powder (1 g) was homogenized using a mixture of aqueous methanol (MeOH) and acetone (both 70%, 1:1, v/v) at room temperature. Each homogenized mixture was then subjected to centrifugation and the supernatants were combined prior to reducing their volumes by evaporation under reduced pressure. The pH of the resulting aqueous suspension was adjusted to pH 2, then extracted with *n*-hexane to remove lipid contaminants. Subsequently, the free phenolics present in the aqueous phase were extracted using a mixture of diethyl ether/ethyl acetate (EtOAc) (1:1, v/v), and the combined organic extracts were dried over anhydrous Na_2SO_4, filtered (Whatman No.1 filter paper), and evaporated to dryness under reduced pressure at 45°C, prior to re-dissolving the residue in MeOH (5 mL). The recovered aqueous extract was then combined with the precipitate obtained following the initial centrifugation step for extraction of the esterified phenolic acid components.

***Esterified phenolic acid fractions*:** The esterified phenolic acid fraction was extracted according to the method described by Neo et al. (13) using the aqueous phase obtained following extraction of the free phenolic acid-containing fraction. Initially, this aqueous phase was directly hydrolyzed using 4 N NaOH at room temperature, after which the pH of the hydrolyzed solution was adjusted to pH 2, and *n*-hexane was added to remove any residual oils. The pH-adjusted extract was then washed using the above described diethyl ether/EtOAc mixture, and the resulting extracts were combined and evaporated to dryness under vacuum at 45°C to give the esterified phenolic compounds, which were re-dissolved in MeOH (5 mL).

***Insoluble-bound phenolic acid fractions*:** The insoluble-bound phenolic acids were extracted from the above residue according to the method described by Neo et al. (13). In this case, the berry samples obtained following extraction with methanol/acetone were hydrolyzed using 4 N NaOH at room temperature. The pH of the solution was then adjusted to pH 2, and the resulting solution subjected to centrifugation. The obtained supernatant was extracted using *n*-hexane, followed by diethyl ether/EtOAc, prior to evaporation to dryness and re-dissolving in MeOH (5 mL).

Qualitative and quantitative analysis of the phenolic acid fractions

***Determination of the total phenolic content (TPC)*:** The TPC of each extract was colorimetrically estimated using the Folin-Ciocalteu method (14). More specifically, a portion of the Folin-Ciocalteu reagent (0.5 mL) was added to each extract (0.1 mL), after which distilled water was added (7 mL). The resulting mixtures were allowed to stand at room temperature for 5 min prior to the addition of an aqueous 7.5% Na_2CO_3 solution (1.5 mL). The obtained solutions were then allowed to stand at room temperature for a further 2 h, after which time their absorbances at 765 nm were measured using gallic acid and methanol as the reference standard and blank solutions, respectively. All values were expressed as milligrams of gallic acid equivalents (GAE) per 100 g of sample dry matter.

***HPLC analysis of the phenolic fractions*:** HPLC was used to separate and identify the individual polyphenolic compounds present in the berry samples according to a previously reported method (15) with slight modifications. Each extract was filtered through a 0.45 μm filter prior to injection into the HPLC system (Agilent 1260 Infinity Quaternary liquid chromatograph, Hewlett Packard, Wilmington, NC, USA), which was equipped with a multiple wavelength detector operating at 280 nm. Chromatographic separations were achieved using an Agilent Zorbax RRHD SB-C18 column (2.1 mm i.d.×100 mm, 1.8 μm particle size; Agilent Technologies, Santa Clara, CA, USA). The column temperature and flow rate were set at 30°C and 0.3 mL/min, respectively. Two solvents

(solutions A and B) were used to achieve a gradient elution. Solution A was composed of water containing 0.1% formic acid, while solution B was composed of acetonitrile containing 0.1% formic acid, and the following gradient was employed: 0% B (0 min), 5% B (0~3.5 min), 15% B (3.5~7.1 min), 40% B (7.1~25 min), 40% B (25~26 min), 100% B (26~27 min), 100% B (27~29 min), and 0% B (29~35 min). The standards employed for analysis were caffeic acid, chlorogenic acid, ferulic acid, gallic acid, *m*-coumaric acid, *o*-coumaric acid, *p*-coumaric acid, *p*-hydroxybenzoic acid, vanillic acid, catechin, epicatechin, epigallocatechin gallate, quercitrin, myricetin, resveratrol, morin, quercetin, naringenin, apigenin, and kaempferol.

Evaluation of the antioxidant activities of the phenolic acid fractions

***DPPH radical scavenging activity*:** The radical scavenging activities of the extracts were estimated according to the procedure described by Delgado-Andrade et al. (16). An aliquot of the desired extract (200 μL) was added to a solution of the DPPH radical in MeOH (1 mL, 74 mg/L). It should be noted that a freshly prepared solution of the DPPH radical gave a final absorption of 1.8 AU at 520 nm. The above mixture (i.e., containing the DPPH radical and the sample extract) was then shaken for 30 min, after which time its absorption at 520 nm was measured. Trolox solutions of various concentrations were used for calibration (0.15~1.15 mM), and the results were expressed as mM equivalents of Trolox (TE)/g of sample.

***Ferric reducing antioxidant power (FRAP) assay*:** The ferric reducing antioxidant power of each extract was evaluated according to the Benzie and Strain method (17). A portion of the freshly prepared FRAP reagent warmed to 37°C (900 μL) was mixed with distilled water (90 μL), and either the desired extract or water (as a blank) was added (30 μL). The final dilution of each test sample was 1:34. The FRAP reagent employed herein contains a 10 mM 2,4,6-tripyridyl-*S*-triazine solution (2.5 mL) prepared in 40 mM aqueous HCl, in addition to 20 mM $FeCl_3 \cdot 6H_2O$ (2.5 mL), and 0.3 M acetate buffer (25 mL) at pH 3.6. The absorption of each solution was recorded at 595 nm every 15 s using a Synergy HTX spectrophotometer (Biotech Instruments, Winooski, VT, USA), and the reaction was monitored for 30 min at 37°C. Trolox solutions of various concentrations were used for calibration, and the results were expressed as mM TE/g of sample.

***Determination of the total antioxidant capacity using the Trolox equivalent antioxidant capacity (TEAC) assay*:** The antioxidant capacity of each extract was estimated according to the procedure reported by Re et al. (18). Thus, the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) radical cation ($ABTS^{+\cdot}$) was prepared by reacting a 7 mM ABTS stock solution with a 2.45 mM aqueous solution of $K_2S_2O_8$. The resulting $ABTS^{+\cdot}$ solution was diluted using 5 mM phosphate-buffered saline buffer (pH 7.4) to obtain an absorbance of 0.70±0.02 at 730 nm. Following addition of the desired extract (10 μL), the Trolox standard was added to a portion of the diluted $ABTS^{+\cdot}$ solution (4 mL), and the absorbance was recorded over 20 min (Synergy HTX spectrophotometer, Biotech Instruments). Trolox solutions of various concentrations were used for calibration, and the results were expressed as mM TE/g of sample.

***Metal ion chelating activity*:** The metal ion chelating abilities of the extracts were investigated according to the procedures reported by Wang and Xiong (19) and Dinis et al. (20). To determine the Cu^{2+} chelating ability, a 2 mM solution of $CuSO_4$ (1 mL) was mixed with pyridine (1 mL) at pH 7.0, and a 0.1% solution of pyrocatechol violet (20 μL) was added. Following the addition of the desired extract (1 mL), the blue color of the $CuSO_4$ solution disappeared, due to dissociation of the Cu^{2+} ions. After allowing the reaction to proceed for 5 min, the absorbance was recorded at 632 nm (Synergy HTX spectrophotometer). To determine the Fe^{2+} chelating ability, a portion of each extract (100 μL) was added to a mixture of distilled water (600 μL) and 0.2 mM $FeCl_2 \cdot 4H_2O$ (100 μL), and the resulting mixture allowed to stand at room temperature for 30 s. After this time the reaction mixture was added to a 1 mM solution of ferrozine (200 μL), and the absorbance was recorded at 562 nm (Synergy HTX spectrophotometer, Biotech Instruments) after allowing to stand for 10 min at room temperature. The Cu^{2+} and Fe^{2+} chelating activities were calculated using the following equation:

$$\text{Chelating activity (\%)} = \frac{A_0 - A_s}{A_0} \times 100$$

where A_0 and A_s are the absorbance values of the control and the extract samples, respectively.

Statistical analysis

The experimental data were analyzed by analysis of variance (ANOVA), and the significant differences between the mean values determined from measurements carried out in quintuples (i.e., $P<0.05$) were obtained by Duncan's multiple range test using statistical analysis software (SPSS 17.0, IBM Inc., Armonk, NY, USA).

RESULTS AND DISCUSSION

Total phenolic contents of the berry samples

The TPCs of the berry samples and their isolated fractions are presented in Table 1. As indicated, the TPCs of the free, esterified, and insoluble-bound phenolic acids

Table 1. The total phenolic contents of the berry samples and their isolated fractions (mg gallic acid equivalents/100 g of sample)

	Free	Esterified	Insoluble-bound	Total
Raspberry	86.67±1.53^{fC}	130.00±1.00^{fB}	272.67±0.58^{fA}	489.67±2.52^{f}
Blackcurrant	189.67±0.58^{eC}	227.67±1.53^{eB}	292.33±1.53^{eA}	710.33±2.52^{e}
Blackberry	272.00±1.73^{cC}	476.67±2.08^{bB}	559.00±5.20^{bA}	1,307.33±9.24^{b}
Cranberry	285.00±1.73^{bC}	355.67±2.08^{cB}	492.33±2.31^{cA}	1,133.00±3.00^{c}
Black chokeberry	372.33±2.08^{aC}	735.00±2.65^{aB}	801.67±1.53^{aA}	1,909.33±5.51^{a}
Blueberry	214.67±3.06^{dC}	349.33±2.52^{dB}	372.67±2.52^{dA}	936.67±2.08^{d}

Data represent the mean value for each sample±standard deviation (n=5).
Different letters within the same row (A-C) and column (a-f) indicate significant differences at $P<0.05$.

from the various berry samples were within the range of 86.67～372.33, 130.00～735.00, and 272.67～801.67 mg GAE/100 g of dry weight (DW) samples, respectively. More specifically, the free phenolic acid fraction of black chokeberry had the highest TPC, followed by cranberry, blackberry, blueberry, and blackcurrant, while raspberry had the lowest TPC. For the esterified phenolic acid fraction, the highest TPC was observed for black chokeberry, followed by blackberry, cranberry, blueberry, blackcurrant, and raspberry. In the insoluble-bound phenolic acid fraction, black chokeberry again exhibited the highest TPC, followed by blackberry, cranberry, blueberry, blackcurrant, and raspberry. Upon combination of the obtained values for the three fractions of the different berry samples, the total TPCs ranged from 489.67 to 1,909.33 mg GAE/100 g DW, with black chokeberry, blackberry, and cranberry giving the highest values. In addition, we found that for all samples, the TPCs in the insoluble-bound phenolic acid fractions were significantly ($P<0.05$) higher than those of the free and esterified phenolic acid fractions, with a contribution of 39.78～55.68%.

More specifically, the TPCs of the insoluble-bound fractions obtained from the raspberry, blackcurrant, blackberry, cranberry, black chokeberry, and blueberry samples were 55.68, 41.15, 42.76, 43.45, 41.99, and 39.79%, respectively. Interestingly, Acosta-Estrada et al. (5) reported that insoluble-bound phenolic acid constituents were covalently bound to structural components of the cell walls, and that they play important roles in providing chemical and physical barriers, in determining the antioxidant properties of the berries, and in protecting against pathogen invasion by animals, insects, and microorganisms (21). In addition, release of the phenolic compounds was previously reported to be more efficient by alkaline hydrolysis than by acid hydrolysis (22), as alkaline hydrolysis resulted in cleavage of the ester bonds linking the phenolic acids to the polysaccharides present in the cell walls (5).

Identification and quantification of the phenolic acid compounds

The phenolic acid compounds present in the various berry samples were then identified and quantified as outlined in Table 2, with catechin, caffeic acid, *p*-coumaric acid, epicatechin, vanillic acid, quercitrin, resveratrol, morin, naringenin, and apigenin being present in all berry samples. In the free phenolic acid fraction, the TPC ranged from 2,960.71 to 18,936.78 μg/100 g DW, with the blackberry sample containing the highest content, followed by the blueberry, black chokeberry, cranberry, blackcurrant, and raspberry fractions. In addition, the esterified fraction contained a TPC ranging from 6,910.83 to 32,644.08 μg/100 g DW. In this case, black chokeberry contained the highest TPC, followed by blueberry, raspberry, blackberry, cranberry, and blackcurrant. Furthermore, the TPC of the insoluble-bound phenolic acid fraction ranged from 16,152.67 to 35,497.91 μg/100 g DW, with the raspberry extract containing the highest TPC, followed by the black chokeberry, blueberry, blackberry, blackcurrant, and cranberry extracts. Although the TPC of the esterified fraction obtained from the blueberries was significantly ($P<0.05$) higher than those of the free and insoluble-bound fractions, a different trend was observed for the other berries, with higher TPCs being detected in their insoluble-bound fractions. More specifically, high catechin contents were found in the free phenolic acid fractions of blueberry (12,072.87 μg/100 g DW), black chokeberry (10,985.48 μg/100 g DW), blackberry (4,150.90 μg/100 g DW), cranberry (1,982.41 μg/100 g DW), and blackcurrant (626.05 μg/100 g DW), while high contents of *p*-coumaric acid were detected in the free phenolic acid fractions of blackberry (10,491.54 μg/100 g DW), blueberry (2,170.81 μg/100 g DW), and black chokeberry (1,576.64 μg/100 g DW). In addition, high contents of morin were found in the cranberry (1,047.81 μg/100 g DW) and raspberry (407.38 μg/100 g DW) extracts, while in the case of the blackcurrant and cranberry samples, high contents of vanillic acid were detected in the free phenolic fractions (i.e., 1,102.24 and 584.78 μg/100 g DW, respectively). In the case of the esterified fraction, high catechin contents were found in the blueberry (10,615.92 μg/100 g DW), black chokeberry (5,288.70 μg/100 g DW), and blackcurrant (1,565.89 μg/100 g DW) samples, while high caffeic acid contents were detected for the blackberry, blueberry, blackcurrant, and raspberry extracts (i.e., 2,526.80, 1,804.70, 1,546.96,

Table 2. The main phenolic compounds and their contents of selected berries (unit: μg/100 g dry weight)

Phenolics	Free	Esterified	Insoluble-bound
Raspberry			
p-Hydroxybenzoic acid	ND	ND	ND
Gallic acid	ND	10.64±0.58^{B}	51.95±3.43^{A}
Chlorogenic acid	ND	18.90±0.25^{B}	701.07±37.55^{A}
Catechin	107.86±4.32^{C}	657.72±4.68^{A}	189.66±7.09^{B}
Caffeic acid	139.95±1.05^{C}	766.47±10.05^{A}	636.23±24.01^{B}
p-Coumaric acid	66.11±1.51^{C}	5,518.41±168.49^{B}	10,597.95±101.55^{A}
Epicatechin	168.97±2.63^{C}	10,838.60±145.40^{A}	9,561.10±123.49^{B}
Epigallocatechin gallate	ND	250.09±3.64^{A}	186.61±9.27^{B}
Ferulic acid	467.26±7.87^{A}	5.18±0.43^{C}	66.15±4.29^{B}
m-Coumaric acid	339.71±3.90^{C}	6,281.16±31.04^{B}	9,369.64±180.92^{A}
o-Coumaric acid	ND	ND	48.02±6.01
Vanillic acid	18.66±0.83^{C}	703.40±31.31^{A}	376.55±26.26^{B}
Quercitrin	939.21±8.28^{A}	30.93±0.22^{C}	146.99±5.03^{B}
Myricetin	11.89±0.33^{B}	ND	39.15±1.32^{A}
Resveratrol	237.19±1.63^{A}	53.89±0.06^{C}	72.25±1.34^{B}
Morin	407.38±2.26^{B}	1,006.23±3.74^{A}	173.46±2.28^{C}
Quercetin	1.86±0.08^{C}	7.79±0.26^{B}	17.43±1.08^{A}
Naringenin	14.06±0.30^{B}	9.34±0.28^{C}	38.24±2.46^{A}
Apigenin	40.59±0.39^{B}	12.65±0.17^{C}	103.88±1.95^{A}
Kaempferol	ND	ND	3,121.59±31.77
Total	2,960.71±21.97^{C}	26,171.39±80.97^{B}	35,497.91±227.42^{A}
Blackcurrant			
p-Hydroxybenzoic acid	575.37±2.17^{A}	495.47±6.69^{B}	ND
Gallic acid	51.13±1.83^{B}	27.74±4.36^{C}	66.98±2.39^{A}
Chlorogenic acid	382.59±40.76^{A}	37.13±4.04^{C}	173.18±1.04^{B}
Catechin	626.05±15.14^{C}	1,565.89±10.51^{A}	1,216.04±4.90^{B}
Caffeic acid	167.01±14.08^{C}	1,546.96±48.97^{A}	774.27±12.39^{B}
p-Coumaric acid	474.76±21.44^{A}	299.85±14.93^{B}	168.97±15.28^{C}
Epicatechin	194.24±2.66^{B}	152.97±2.43^{C}	236.56±6.52^{A}
Epigallocatechin gallate	16.17±1.03^{C}	2,216.32±43.98^{A}	1,269.71±29.88^{B}
Ferulic acid	ND	222.47±2.54^{B}	283.20±13.66^{A}
m-Coumaric acid	ND	ND	ND
o-Coumaric acid	29.88±1.48	ND	ND
Vanillic acid	1,102.24±21.19^{A}	14.95±1.50^{C}	696.85±9.63^{B}
Quercitrin	155.97±2.00^{A}	164.02±8.93^{A}	91.07±7.34^{B}
Myricetin	20.44±0.95^{A}	3.68±0.48^{B}	ND
Resveratrol	55.59±2.15^{C}	88.22±3.78^{B}	151.87±6.04^{A}
Morin	11.50±0.95^{C}	45.00±2.76^{A}	24.74±1.47^{B}
Quercetin	ND	2.28±0.21^{B}	3.91±0.16^{A}
Naringenin	27.66±0.84^{A}	9.31±0.36^{C}	22.98±0.66^{B}
Apigenin	28.88±1.75^{B}	18.59±1.04^{C}	80.89±2.57^{A}
Kaempferol	ND	ND	11,466.97±149.81
Total	3,919.47±38.51^{C}	6,910.83±31.69^{B}	16,728.16±140.56^{A}

and 766.47 μg/100 g DW). For the insoluble-bound fractions, high catechin contents were found in the blueberry (6,970.62 μg/100 g DW), black chokeberry (4,391.78 μg/100 g DW), blackberry (1,708.61 μg/100 g DW), and blackcurrant (1,216.04 μg/100 g DW) samples, whereas high *p*-coumaric acid contents were detected in the insoluble-bound fractions of raspberry, blueberry, and blackberry (i.e., 10,597.95, 3,311.30, and 3,254.54 μg/100 g DW, respectively). To date, a number of studies have reported that the polyphenol components present in plants correlated with their respective antioxidant activities (23, 24). Indeed, Intra and Kuo (25) reported that the antioxidant activity of catechin was related to its free radical scavenging and metal chelating activities, which rendered catechin a more potent lipid antioxidant than vitamins C and E. In addition, it should be noted that catechin does not exhibit pro-oxidant activity in physiological achievable concentrations. In terms of its structure, catechin contains two aromatic rings and a dihydropyran heterocyclic moiety bearing a hydroxyl group at the C-3 position (26), which can chelate to metal ions (27). As such, catechin could be considered a powerful antioxidant for the neutralization of free radicals (28). In addition, vanillic acid is a phenolic derivative present in numerous edi-

Table 2. Continued

Phenolics	Free	Esterified	Insoluble-bound
Blackberry			
p-Hydroxybenzoic acid	685.01±2.16	ND	ND
Gallic acid	163.39±3.27^{A}	21.11±2.80^{B}	ND
Chlorogenic acid	ND	ND	63.24±17.81
Catechin	4,150.90±183.57^{A}	2,364.52±28.53^{B}	1,708.61±43.24^{C}
Caffeic acid	1,296.79±54.11^{C}	2,526.80±10.2^{A}	1,587.30±15.06^{B}
p-Coumaric acid	10,491.54±152.65^{A}	1,560.84±101.78^{C}	3,254.54±102.45^{B}
Epicatechin	176.51±6.80^{C}	2,418.42±33.17^{B}	5,023.41±55.78^{A}
Epigallocatechin gallate	226.39±13.44^{C}	2,114.32±12.47^{A}	1,155.83±18.02^{B}
Ferulic acid	179.18±5.16^{A}	92.94±7.98^{C}	127.75±4.09^{B}
m-Coumaric acid	363.10±32.54^{C}	7,046.05±334.46^{A}	5,686.20±213.23^{B}
o-Coumaric acid	191.08±2.22	ND	ND
Vanillic acid	432.90±19.20^{C}	519.66±47.92^{B}	693.35±21.43^{A}
Quercitrin	129.25±13.30^{C}	868.90±15.50^{A}	307.57±41.50^{B}
Myricetin	6.62±0.59^{B}	4.33±0.44^{B}	25.07±5.84^{A}
Resveratrol	228.89±8.16^{A}	104.14±0.41^{C}	181.59±2.68^{B}
Morin	98.40±0.33^{B}	257.49±2.61^{A}	86.27±7.29^{C}
Quercetin	8.12±0.94^{B}	3.81±0.26^{C}	24.42±0.50^{A}
Naringenin	20.63±0.68^{A}	13.05±0.38^{B}	13.59±0.60^{B}
Apigenin	88.09±1.54^{A}	9.72±0.88^{C}	49.94±5.21^{B}
Kaempferol	ND	ND	249.48±14.83
Total	18,936.78±101.81^{C}	19,926.11±273.06^{B}	20,238.14±272.13^{A}
Cranberry			
p-Hydroxybenzoic acid	435.41±0.06^{A}	415.26±0.21^{B}	413.64±5.47^{B}
Gallic acid	ND	27.03±1.10	ND
Chlorogenic acid	ND	41.95±1.12^{B}	1,134.36±25.22^{A}
Catechin	1,982.41±5.05^{A}	294.40±14.87^{C}	676.34±20.34^{B}
Caffeic acid	651.92±9.54^{B}	37.71±20.65^{B}	1,523.64±56.92^{A}
p-Coumaric acid	94.01±2.99^{C}	228.38±6.42^{B}	1,051.06±54.95^{A}
Epicatechin	1,343.81±9.28^{A}	26.32±1.90^{C}	1,140.79±9.24^{B}
Epigallocatechin gallate	373.38±11.62^{C}	4,001.21±54.26^{B}	4,870.57±82.50^{A}
Ferulic acid	129.23±5.43^{C}	350.46±9.39^{B}	444.71±30.91^{A}
m-Coumaric acid	110.14±8.30^{A}	24.70±0.96^{B}	ND
o-Coumaric acid	39.66±1.01^{C}	57.96±1.04^{B}	368.49±14.95^{A}
Vanillic acid	584.78±13.69^{B}	119.20±1.93^{C}	1,626.01±55.84^{A}
Quercitrin	97.87±0.91^{B}	75.58±1.41^{C}	366.36±11.65^{A}
Myricetin	113.93±5.39^{A}	7.27±0.84^{B}	ND
Resveratrol	233.78±1.58^{A}	79.29±1.96^{C}	87.10±3.22^{B}
Morin	1,047.81±15.70^{A}	912.02±9.24^{B}	434.03±10.79^{C}
Quercetin	5.06±0.17^{C}	24.57±0.15^{A}	22.88±0.50^{B}
Naringenin	17.25±0.28^{B}	23.01±3.39^{A}	10.11±0.38^{C}
Apigenin	29.60±0.38^{A}	7.18±0.08^{C}	19.73±1.22^{B}
Kaempferol	ND	ND	1,962.86±93.33
Total	7,290.03±25.27^{B}	7,353.50±112.99^{B}	16,152.67±133.48^{A}

ble plants and fruits, and is also an intermediate in the production of vanillin from ferulic acid (29). Furthermore, morin is a type of flavonoid belonging to the flavonol group, while *p*-coumaric acid is a hydroxyl derivative of cinnamic acid. Interestingly, this compound exhibits a range of medicinal properties, including antifilarial, anticancer, and antimicrobial activities (30), which can be attributed to its free-radical scavenging ability (31).

DPPH radical scavenging activity

The scavenging activities of the phenolic acids extracted from the various berry samples were then investigated using the DPPH radical scavenging assay. This assay was selected due to the greater stability of the DPPH radical compared to the superoxide and hydroxyl radicals (32). Thus, Table 3 shows the DPPH radical scavenging capacities of the free, esterified, and insoluble-bound phenolic acid fractions obtained from the berry samples, which were in the range of 86.67～156.95, 78.11～183.11, and 97.90～272.75 μmol TE/g DW, respectively. Upon combination of the results obtained for these three fractions, the total radical scavenging activities were calculated to range from 265.58 to 568.80 μmol TE/g DW. Interestingly, for all samples the DPPH radical scavenging activ-

Table 2. Continued

Phenolics	Free	Esterified	Insoluble-bound
Black chokeberry			
p-Hydroxybenzoic acid	424.40±7.33	ND	ND
Gallic acid	289.27±17.13^{A}	211.19±16.94^{B}	ND
Chlorogenic acid	1,707.39±21.75^{C}	4,896.15±131.98^{B}	13,253.03±579.46^{A}
Catechin	10,985.48±80.44^{A}	5,288.70±497.19^{B}	4,391.78±339.28^{C}
Caffeic acid	235.20±4.32^{C}	2,603.96±401.22^{B}	11,818.82±177.50^{A}
p-Coumaric acid	1,576.64±85.08^{B}	1,718.38±19.09^{A}	796.57±94.40^{C}
Epicatechin	30.28±6.07^{C}	1,760.75±77.11^{A}	518.93±10.51^{B}
Epigallocatechin gallate	ND	2,348.95±23.43^{A}	1,426.52±23.86^{B}
Ferulic acid	ND	1,212.88±23.81^{A}	539.83±12.67^{B}
m-Coumaric acid	73.17±4.83	ND	ND
o-Coumaric acid	12.99±0.72^{B}	29.56±4.65^{A}	ND
Vanillic acid	325.76±33.36^{B}	1,152.81±91.05^{A}	21.62±3.09^{C}
Quercitrin	603.76±15.18^{B}	9,754.80±176.79^{A}	215.68±12.90^{C}
Myricetin	38.79±0.15^{B}	151.35±3.72^{A}	28.36±1.33^{C}
Resveratrol	246.32±1.88^{A}	92.23±1.22^{B}	95.13±5.38^{B}
Morin	177.90±9.91^{C}	1,386.48±5.57^{A}	530.75±27.82^{B}
Quercetin	5.80±0.55^{B}	1.77±0.10^{C}	14.94±1.69^{A}
Naringenin	18.28±1.23^{A}	18.82±0.88^{A}	19.51±1.67^{A}
Apigenin	13.82±0.35^{B}	15.30±0.59^{B}	912.63±17.98^{A}
Kaempferol	ND	ND	501.35±29.62
Total	16,765.25±197.45^{C}	32,644.08±716.22^{B}	34,952.25±477.52^{A}
Blueberry			
p-Hydroxybenzoic acid	454.35±5.38^{A}	ND	421.76±0.79^{B}
Gallic acid	ND	ND	ND
Chlorogenic acid	ND	29.56±1.97^{B}	156.06±1.92^{A}
Catechin	12,072.87±189.74^{A}	10,615.92±436.88^{B}	6,970.62±109.02^{C}
Caffeic acid	286.52±19.74^{C}	1,804.70±90.11^{B}	6,680.09±115.81^{A}
p-Coumaric acid	2,170.81±320.36^{C}	10,598.66±145.29^{A}	3,311.30±78.62^{B}
Epicatechin	334.77±28.30^{B}	71.19±5.62^{C}	366.69±19.32^{A}
Epigallocatechin gallate	ND	709.56±21.56^{B}	759.16±22.42^{A}
Ferulic acid	4.42±0.52^{C}	302.70±3.74^{A}	248.16±11.52^{B}
m-Coumaric acid	ND	102.42±12.89	ND
o-Coumaric acid	458.18±18.26^{A}	122.62±8.67^{B}	ND
Vanillic acid	571.09±31.64^{C}	3,261.67±9.81^{A}	975.37±9.15^{B}
Quercitrin	344.30±11.18^{A}	111.68±4.28^{B}	112.45±2.28^{B}
Myricetin	69.87±2.43^{A}	34.62±0.80^{A}	54.48±52.93^{A}
Resveratrol	147.73±4.35^{A}	98.74±2.99^{B}	148.60±17.82^{A}
Morin	45.26±1.13^{C}	403.05±40.44^{B}	841.43±2.61^{A}
Quercetin	50.03±0.31^{A}	28.29±1.95^{C}	31.71±0.83^{B}
Naringenin	24.62±0.70^{A}	17.02±1.52^{B}	15.93±0.75^{B}
Apigenin	8.45±0.15^{C}	10.02±0.25^{B}	14.64±1.42^{A}
Kaempferol	ND	ND	2,995.57±263.63
Total	17,043.27±565.13^{C}	28,322.43±489.79^{A}	24,104.01±372.49^{B}

Data represent the mean value for each sample±standard deviation (n=5).
Different letters (A-C) within a row indicate significant differences at $P<0.05$ level.
ND, not detected.

ities of the insoluble-bound fractions were significantly ($P<0.05$) higher than those of the free and the esterified fractions. In addition, black chokeberry exhibited the highest DPPH scavenging activity (i.e., 568.80 μmol TE/g DW), followed by the raspberry, blackberry, cranberry, blueberry, and blackcurrant extracts. Interestingly, Madhujith and Shahidi (33) reported that the DPPH radical scavenging activities of insoluble-bound phenolic acids were higher than of free and esterified phenolic acids. Other studies have also indicated that insoluble-bound phenolics acids exhibit higher antioxidant activities than free phenolics (34,35). It therefore appears that the antioxidant activities of plant extracts are related to the presence of certain individual phenolic compounds and their corresponding structures (36), where the positions and quantities of the hydroxyl groups are likely of particular importance (37). The results obtained herein therefore indicate that the insoluble-bound phenolic acids present

Table 3. The DPPH radical scavenging capacities of the free, esterified, and insoluble-bound phenolic acid fractions obtained from the berry samples (unit: μmol Trolox equivalent/g dry weight)

	Free	Esterified	Insoluble-bound	Total
Raspberry	86.67±6.92^{cC}	130.37±1.60^{bB}	272.75±5.22^{aA}	489.79±13.75^{b}
Blackcurrant	67.63±7.28^{dB}	100.04±6.02^{cA}	97.90±4.04^{dA}	265.58±17.34^{e}
Blackberry	156.95±6.68^{aB}	98.33±4.01^{cC}	199.58±4.47^{bA}	454.87±15.16^{c}
Cranberry	93.30±6.13^{cB}	99.67±4.42^{cB}	123.58±5.22^{cA}	316.55±15.77^{d}
Black chokeberry	114.59±6.92^{bC}	183.11±2.02^{aB}	271.10±3.62^{aA}	568.80±12.56^{a}
Blueberry	109.99±6.57^{bA}	78.11±4.84^{dB}	116.14±5.26^{cA}	304.25±16.67^{d}

Data represent the mean value for each sample±standard deviation (n=5).
Different letters within the same row (A-C) and column (a-e) indicate significant differences at $P<0.05$.

Table 4. The ferric reducing antioxidant power of free, esterified, and insoluble-bound phenolic fractions obtained from the berry samples (unit: μmol Trolox equivalent/g dry weight)

	Free	Esterified	Insoluble-bound	Total
Raspberry	28.11±5.91^{bcC}	133.97±2.47^{bB}	218.36±2.90^{bA}	380.44±11.29^{b}
Blackcurrant	17.81±1.46^{dC}	27.46±1.50^{dA}	23.55±1.33^{dB}	68.83±4.29^{d}
Blackberry	111.63±7.26^{aB}	63.51±8.13^{cC}	135.35±6.70^{cA}	310.49±22.09^{c}
Cranberry	22.41±3.26^{cdB}	22.87±0.77^{deB}	29.38±1.15^{dA}	74.65±5.17^{d}
Black chokeberry	33.16±4.75^{bC}	177.90±4.67^{aB}	238.80±6.90^{aA}	449.86±16.31^{a}
Blueberry	17.27±2.33^{dB}	18.27±0.97^{eB}	30.30±2.18^{dA}	65.84±5.48^{d}

Data represent the mean value for each sample±standard deviation (n=5).
Different letters within the same row (A-C) and column (a-e) indicate significant differences at $P<0.05$.

in the various berry samples could effectively react with DPPH radicals to convert them into stable products and terminate the radical chain reaction.

FRAP assay

Two main mechanisms exist for the scavenging of free radicals by antioxidants, namely hydrogen atom transfer and single electron transfer (38,39). The FRAP assay is a typical electron transfer-based method that measures the reduction of ferric ions (Fe^{3+}) to intensely blue colored ferrous ions (Fe^{2+}) by antioxidants in acidic media. Thus, we determined the FRAP values of the free, esterified, and insoluble-bound phenolic acid fractions from the various berry samples, and the results are outlined in Table 4. In general, the samples exhibiting higher ferric-reducing antioxidant powers also contained the highest TPCs. As shown in Table 4, for the fraction containing the free phenolic acids, the FRAP values varied from 17.27 to 111.63 μmol TE/g DW, with blackberry giving the highest value, followed by the black chokeberry, raspberry, cranberry, blackcurrant, and blueberry samples. In the fraction containing the esterified phenolic compounds, the FRAP values ranged 18.27 to 177.90 μmol TE/g DW. In this case, black chokeberry gave the highest FRAP value, followed by raspberry, blackberry, blackcurrant, cranberry, and blueberry. Furthermore, for the insoluble-bound phenolic acid fraction, the FRAP values ranged from 23.55 to 238.80 μmol TE/g DW, with black chokeberry again exhibiting the highest FRAP value, in this case followed by raspberry, blackberry, blueberry, cranberry, and blackcurrant. With the exception of the blackcurrant sample, the FRAP values of the insoluble-bound phenolic acid fractions were significantly ($P<0.05$) higher than those of the free and esterified phenolics. Upon combination of the results obtained for the three fractions, the total FRAP values ranged from 65.84 to 449.86 μmol TE/g DW. Similarly, Liyana-Pathirana and Shahidi (40) reported that the contribution of insoluble-bound phenolic acids to the TPC in wheat was significantly higher than those of the free and esterified fractions, with the insoluble-bound phenolics demonstrating a significantly higher antioxidant capacity. The berry samples examined herein that contain significant quantities of insoluble-bound phenolic acids should therefore be considered a good source of phenolics that exhibit numerous potential health benefits.

Determination of the total antioxidant capacity using the TEAC assay

The total antioxidant activities of the free, esterified, and insoluble-bound phenolic acid components isolated from the various berry samples were determined using the TEAC assay, and the results are given in Table 5, where values ranging from 47.10～178.35, 58.15～231.69, and 80.44～243.35 μmol TE/g DW are indicated, respectively. In this case, the black chokeberry samples in the insoluble-bound fraction exhibited the highest TEAC value (243.35 μmol TE/g DW), followed by blackberry, cranberry, raspberry, blueberry, and blackcurrant. In addition, the TEAC values of the raspberry, blackberry, cranberry, and black chokeberry extracts containing the insoluble-bound phenolic acids were significantly ($P<0.05$) higher

Table 5. The ABTS radical cation scavenging activity of the free, esterified, and insoluble-bound phenolic acid components isolated from the various berry samples (unit: μmol Trolox equivalent/g dry weight)

	Free	Esterified	Insoluble-bound	Total
Raspberry	51.48±1.57^{eC}	111.06±1.88^{cB}	158.56±3.13^{cA}	321.10±6.58^{d}
Blackcurrant	47.10±0.96^{fC}	102.73±2.53^{dA}	80.44±1.88^{eB}	230.27±5.36^{f}
Blackberry	178.35±2.20^{aB}	99.19±1.86^{dC}	186.90±2.53^{bA}	464.44±6.60^{b}
Cranberry	88.77±1.91^{cC}	125.23±2.82^{bB}	162.31±1.66^{cA}	376.31±6.38^{c}
Black chokeberry	77.10±1.58^{dC}	231.69±3.75^{aB}	243.35±1.58^{aA}	552.15±6.90^{a}
Blueberry	102.31±2.26^{bA}	58.15±2.20^{eB}	100.44±1.88^{dA}	260.90±6.33^{e}

Data represent the mean value for each sample±standard deviation (n=5).
Different letters within the same row (A-C) and column (a-f) indicate significant differences at $P<0.05$.

than those containing the free and esterified phenolics. However, a different trend was observed for the blackcurrant and blueberry samples, with the fractions containing the esterified and free phenolic acids giving higher TEAC values than that containing the insoluble-bound phenolics. Combination of the results for the three fractions gave total TEAC values ranging from 230.27 to 552.15 μmol TE/g DW, where the black chokeberry sample exhibited the highest total TEAC value, followed by the blackberry, cranberry, raspberry, blueberry, and blackcurrant samples.

However, the total TEAC values of the tested samples did not appear to show any clear relationship with the TPCs. In this case, the TEAC value of a compound represents the concentration of Trolox that exhibits the same antioxidant capacity as the compound or compounds of interest (41). Thus, the TEAC value can be considered to be a stoichiometric number related to a Trolox value of 1. Indeed, Alshikh et al. (39) suggested that the TEAC value was dependent not only on the phenolic concentration but also on the identity of the phenolic compounds and the reaction mechanisms taking place. This suggests that the TPCs may not sufficiently explain the observed antioxidant activities of fruit and plant phenolic extracts, which are mixtures of different compounds exhibiting variable activities.

Metal ion chelating activity

In their higher valence states, metals such as iron, copper, manganese, nickel, and cobalt are known to participate in the direct initiation of lipid oxidation via electron transfer and lipid alkyl radical formation, while lower valence metals can directly initiate lipid oxidation via the formation of reactive oxygen species (ROS) (42). In addition, the chelation of iron can prevent the formation of free radicals in addition to preventing the impairment of vital organ functions *in vivo*. More specifically, the formation of a complex between the antioxidant and the metal renders the metal ions inactive and so they cannot act as initiators of lipid oxidation (43). Thus, the metal ion chelating activities of the free, esterified, and insoluble-bound phenolic acid fractions from the various berry samples are given in Table 6. In the case of the cupric ion (Cu^{2+}) chelating ability, values of 33.58～54.34, 41.60～54.95, and 42.61～59.23% were obtained for the berry extracts containing the free, esterified, and insoluble-bound phenolics, respectively. Combination of the results for the three different fractions gave total Cu^{2+} chelating abilities ranging from 135.35 to 160.18%, where the black chokeberry sample exhibited the highest Cu^{2+} chelating ability. In addition, in the case of ferrous ion (Fe^{2+}) chelating ability, the values obtained for the berry extracts containing free phenolic acids and those released from their esterified and insoluble-bound forms were in the range of 33.25～47.60, 44.21～53.56, and 44.21～

Table 6. The metal ion chelating activity of the free, esterified, and insoluble-bound phenolic acid fractions from the various berry samples (unit: %)

	Cu^{2+} chelating ability				Fe^{2+} chelating ability			
	Free	Esterified	Insoluble-bound	Total	Free	Esterified	Insoluble-bound	Total
Raspberry	54.34±2.19^{aA}	43.40±0.11^{eB}	42.61±0.19^{fB}	140.36±2.48^{b}	37.27±0.41^{dC}	53.56±0.30^{aA}	47.60±0.39^{bB}	138.43±1.10^{b}
Blackcurrant	44.32±1.67^{cB}	46.32±0.10^{dA}	46.64±0.09^{dA}	137.28±1.86^{c}	45.57±0.39^{bA}	44.21±0.40^{dB}	44.89±0.40^{dAB}	134.66±1.18^{c}
Blackberry	49.51±1.21^{bA}	47.68±0.82^{cB}	44.64±0.10^{eC}	141.83±2.13^{b}	45.57±0.39^{bB}	47.35±0.08^{cA}	44.21±0.40^{dC}	137.12±0.86^{b}
Cranberry	33.58±0.50^{dC}	53.36±0.87^{bA}	48.50±0.09^{bB}	135.44±1.45^{c}	47.60±0.39^{aA}	45.11±0.40^{dB}	45.79±0.40^{cB}	138.50±1.18^{b}
Black chokeberry	45.99±0.25^{cC}	54.95±0.14^{aB}	59.23±0.10^{aA}	160.18±0.48^{a}	42.84±0.40^{cC}	47.15±1.06^{cB}	55.53±0.38^{aA}	145.51±1.80^{a}
Blueberry	46.37±0.25^{cB}	41.60±0.11^{fC}	47.38±0.16^{cA}	135.35±0.52^{c}	33.25±0.42^{eC}	52.47±0.75^{bA}	46.47±0.39^{cB}	132.19±1.56^{d}

Data represent the mean value for each sample±standard deviation (n=5).
Different letters within the same row (A-C) and column (a-f) indicate significant differences at $P<0.05$.

55.53%, respectively, with combination of these results giving total Fe^{2+} chelating abilities of 132.19～145.51%. However, the Cu^{2+} and Fe^{2+} chelating abilities of the tested samples showed no clear trend between the free, esterified, and insoluble-bound phenolic acid fractions. Although the phenolic compounds present in these berry samples are the main components responsible for chelation to metal ions, it is also possible that nonphenolic constituents present in the extracts may also participate in this process (44).

Thus, in conclusion, we herein investigated the antioxidant capacities and total phenolic acid contents (free, esterified, and insoluble-bound fractions) of 6 selected berries, in addition to identifying and quantifying a number of phenolic compounds present in the berry samples using HPLC. Interestingly, we found that the contents and antioxidant capacities of the free, esterified, and insoluble-bound phenolic acid components varied considerably. More specifically, the black chokeberry and blackberry samples exhibited superior antioxidant activities to the other berry samples, as determined by a combination of the results obtained from DPPH, FRAP, and TEAC assays, in addition to determination of their metal ion chelating activities.

Furthermore, catechin, caffeic acid, *p*-coumaric acid, epicatechin, vanillic acid, quercitrin, resveratrol, morin, naringenin, and apigenin were found to be widely abundant in the selected berry samples. Moreover, the antioxidant activities and TPCs of the fractions containing the insoluble-bound phenolics were higher than those of the fractions containing the free and esterified phenolic acids. Our results therefore suggest that the insoluble-bound fractions of the various berries examined herein could be regarded as good sources of natural antioxidants, and so may be suitable for application in the preparation of functional food ingredients and preventing diseases associated with oxidative stress.

ACKNOWLEDGEMENTS

This work was supported by Kyungnam University Foundation Grant, 2013.

AUTHOR DISCLOSURE STATEMENT

The author declares no conflict of interest.

REFERENCES

1. Prior RL, Cao G, Martin A, Sofic E, McEwen J, O'Brien C, Lischner N, Ehlenfeldt M, Kalt W, Krewer G, Mainland CM. 1998. Antioxidant capacity as influenced by total phenolic and anthocyanin content, maturity, and variety of *Vaccinium* species. *J Agric Food Chem* 46: 2686-2693.
2. Dixon RA, Paiva NL. 1995. Stress-induced phenylpropanoid metabolism. *Plant Cell* 7: 1085-1097.
3. Masisi K, Beta T, Moghadasian MH. 2016. Antioxidant properties of diverse cereal grains: a review on *in vitro* and *in vivo* studies. *Food Chem* 196: 90-97.
4. Soobrattee MA, Neergheen VS, Luximon-Ramma A, Aruoma OI, Bahorun T. 2005. Phenolics as potential antioxidant therapeutic agents: mechanism and actions. *Mutat Res* 579: 200-213.
5. Acosta-Estrada BA, Gutiérrez-Uribe JA, Serna-Saldívar SO. 2014. Bound phenolics in foods, a review. *Food Chem* 152: 46-55.
6. Ayoub M, de Camargo AC, Shahidi F. 2016. Antioxidants and bioactivities of free, esterified and insoluble-bound phenolics from berry seed meals. *Food Chem* 197: 221-232.
7. Clifford MN. 2000. Anthocyanins-nature, occurrence and dietary burden. *J Sci Food Agric* 80: 1063-1072.
8. Kong JM, Chia LS, Goh NK, Chia TF, Brouillard R. 2003. Analysis and biological activities of anthocyanins. *Phytochemistry* 64: 923-933.
9. Rossi A, Serraino I, Dugo P, Di Paola R, Mondello L, Genovese T, Morabito D, Dugo G, Sautebin L, Caputi AP, Cuzzocrea S. 2003. Protective effects of anthocyanins from blackberry in a rat model of acute lung inflammation. *Free Radic Res* 37: 891-900.
10. Viljanen K, Kivikari R, Heinonen M. 2004. Protein-lipid interactions during liposome oxidation with added anthocyanin and other phenolic compounds. *J Agric Food Chem* 52: 1104-1111.
11. de Souza VR, Pereira PA, da Silva TL, de Oliveira Lima LC, Pio R, Queiroz F. 2014. Determination of the bioactive compounds, antioxidant activity and chemical composition of Brazilian blackberry, red raspberry, strawberry, blueberry and sweet cherry fruits. *Food Chem* 156: 362-368.
12. Namiesnik J, Vearasilp K, Nemirovski A, Leontowicz H, Leontowicz M, Pasko P, Martinez-Ayala AL, González-Aguilar GA, Suhaj M, Gorinstein S. 2014. *In vitro* studies on the relationship between the antioxidant activities of some berry extracts and their binding properties to serum albumin. *Appl Biochem Biotechnol* 172: 2849-2865.
13. Neo YP, Ariffin A, Tan CP, Tan YA. 2008. Determination of oil palm fruit phenolic compounds and their antioxidant activities using spectrophotometric methods. *Int J Food Sci Technol* 43: 1832-1837.
14. Singleton VL, Rossi JA. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am J Enol Vitic* 16: 144-158.
15. Mattila P, Kumpulainen J. 2002. Determination of free and total phenolic acids in plant-derived foods by HPLC with diode-array detection. *J Agric Food Chem* 50: 3660-3667.
16. Delgado-Andrade C, Seiquer I, Navarro MP. 2004. Bioavailability of iron from a heat treated glucose-lysine model food system: assays in rats and in Caco-2 cells. *J Sci Food Agric* 84: 1507-1513.
17. Benzie IF, Strain JJ. 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Anal Biochem* 239: 70-76.
18. 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.
19. Wang LL, Xiong YL. 2005. Inhibition of lipid oxidation in cooked beef patties by hydrolyzed potato protein is related to its reducing and radical scavenging ability. *J Agric Food Chem* 53: 9186-9192.

20. Dinis TC, Maderia VM, Almeida LM. 1994. Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. *Arch Biochem Biophys* 315: 161-169.
21. Sancho AI, Bartolomé B, Gómez-Cordovés C, Williamson G, Faulds CB. 2001. Release of ferulic acid from cereal residues by barley enzymatic extracts. *J Cereal Sci* 34: 173-179.
22. Kim KH, Tsao R, Yang R, Cui SW. 2006. Phenolic acid profiles and antioxidant activities of wheat bran extracts and the effect of hydrolysis conditions. *Food Chem* 95: 466-473.
23. Lizcano LJ, Bakkali F, Ruiz-Larrea MB, Ruiz-Sanz JI. 2010. Antioxidant activity and polyphenol content of aqueous extracts from Colombian Amazonian plants with medicinal use. *Food Chem* 119: 1566-1570.
24. Wu CR, Lin WH, Hseu YC, Lien JC, Lin YT, Kuo TP, Ching H. 2011. Evaluation of the antioxidant activity of five endemic *Ligustrum* species leaves from Taiwan flora *in vitro*. *Food Chem* 127: 564-571.
25. Intra J, Kuo SM. 2007. Physiological levels of tea catechins increase cellular lipid antioxidant activity of vitamin C and vitamin E in human intestinal Caco-2 cells. *Chem Biol Interact* 169: 91-99.
26. Braicu C, Ladomery MR, Chedea VS, Irimie A, Berindan-Neagoe I. 2013. The relationship between the structure and biological actions of green tea catechins. *Food Chem* 141: 3282-3289.
27. Zhang HY, Ge N, Zhang ZY. 1999. Theoretical elucidation of activity differences of five phenolic antioxidants. *Zhongguo Yao Li Xue Bao* 20: 363-366.
28. Zheng LT, Ryu GM, Kwon BM, Lee WH, Suk K. 2008. Anti-inflammatory effects of catechols in lipopolysaccharide-stimulated microglia cells: inhibition of microglial neurotoxicity. *Eur J Pharmacol* 588: 106-113.
29. Civolani C, Barghini P, Roncetti AR, Ruzzi M, Schiesser A. 2000. Bioconversion of ferulic acid into vanillic acid by means of a vanillate-negative mutant of *Pseudomonas fluorescens* strain BF13. *Appl Environ Microbiol* 66: 2311-2317.
30. Delaquis P, Stanich K, Toivonen P. 2005. Effect of pH on the inhibition of *Listeria* spp. by vanillin and vanillic acid. *J Food Prot* 68: 1472-1476.
31. Prince PSM, Dhanasekar K, Rajakumar S. 2011. Preventive effects of vanillic acid on lipids, bax, bcl-2 and myocardial infarct size on isoproterenol-induced myocardial infarcted rats: a biochemical and *in vitro* study. *Cardiovasc Toxicol* 11: 58-66.
32. Siriwardhana SSKW, Shahidi F. 2002. Antiradical activity of extracts of almond and its by-products. *J Am Oil Chem Soc* 79: 903-908.
33. Madhujith T, Shahidi F. 2009. Antioxidant potential of barley as affected by alkaline hydrolysis and release of insoluble-bound phenolics. *Food Chem* 117: 615-620.
34. Chandrasekara A, Shahidi F. 2011. Determination of antioxidant activity in free and hydrolyzed fractions of millet grains and characterization of their phenolic profiles by HPLC-DAD-ESI-MS^n. *J Funct Foods* 3: 144-158.
35. Govardhan Singh RS, Negi PS, Radha C. 2013. Phenolic composition, antioxidant and antimicrobial activities of free and bound phenolic extracts of *Moringa oleifera* seed flour. *J Funct Foods* 5: 1883-1891.
36. Djeridane A, Yousfi M, Nadjemi B, Boutassouna D, Stocker P, Vidal N. 2006. Antioxidant activity of some algerian medicinal plants extracts containing phenolic compounds. *Food Chem* 97: 654-660.
37. Silva FA, Borges F, Guimarães C, Lima JL, Matos C, Reis S. 2000. Phenolic acids and derivatives: studies on the relationship among structure, radical scavenging activity, and physicochemical parameters. *J Agric Food Chem* 48: 2122-2126.
38. Albishi T, John JA, Al-Khalifa AS, Shahidi F. 2013. Phenolic content and antioxidant activities of selected potato varieties and their processing by-products. *J Funct Foods* 5: 590-600.
39. Alshikh N, Costa de Camargo A, Shahidi F. 2015. Phenolics of selected lentil cultivars: antioxidant activities and inhibition of low-density lipoprotein and DNA damage. *J Funct Foods* 18: 1022-1038.
40. Liyana-Pathirana CM, Shahidi F. 2006. Importance of insoluble-bound phenolics to antioxidant properties of wheat. *J Agric Food Chem* 54: 1256-1264.
41. van den Berg R, Haenen GRMM, van den Berg H, Bast A. 1999. Applicability of an improved Trolox equivalent antioxidant capacity (TEAC) assay for evaluation of antioxidant capacity measurements of mixtures. *Food Chem* 66: 511-517.
42. Kanner J, Harel S, Hazan B. 1986. Muscle membranal lipid peroxidation by an "iron redox cycle" system: initiation by oxy radicals and site-specific mechanism. *J Agric Food Chem* 34: 506-510.
43. Shahidi F, Zhong Y. 2007. Measurement of antioxidant activity in food and biological systems. In *Antioxidant Measurement and Applications*. Shahidi F, Ho CT, eds. American Chemical Society, Washington, DC, USA. p 36-66.
44. Wettasinghe M, Shahidi F. 2002. Iron (II) chelation activity of extracts of borage and evening primrose meals. *Food Res Int* 35: 65-71.