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Partial Characterization of Three Korean White Lotus Cultivars

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The nutritional and bioactive values of Korean white lotus cultivars (Muan, Chungyang, and Garam) extracted with methanol, water, and acetone solvents were evaluated by the contents of their bioactive compounds and antioxidant activity determined by ABTS, CUPRAC, FRAP, DPPH, and β-carotene−linoleic acid assays. HPLC, fluorometry, and FTIR spectroscopy were employed for evaluation of polyphenols and fatty acids. All lotus cultivar extracts possessed high amounts of total phenolics, ascorbic acid, proteins, and fatty acids and exhibited high levels of antioxidant activity, which were higher in water than in organic solvents. The significant highest content of the above-mentioned indices and biological activity were recorded in the Chungyang cultivar. Therefore, all studied Korean white lotus cultivars and in particular Chungyang could be recommended as effective nutritional and bioactive products, however, after investigation on humans.

KEYWORDS: White lotus cultivars; nutritional value; bioactive compounds; antioxidant activity

INTRODUCTION

The lotus plant (*Nelumbo nucifera* Gaertn.) is not known in Western countries for food consumption. However, for more than 3000 years, lotus has been cultivated as a crop in Far East Asia, for food and medicine. This plant also plays a significant role in religious and cultural activities (*1*). Therefore, it is not a surprise that most of the folk and scientific data, concerning lotus, are published by Far East scientists (*2*−*4*). According to the below-cited publications, lotus is rich in bioactive compounds and widely used in traditional Korean medicine (*5*−*9*).

In the past decade the interest of scientists in lotus health properties has grown (*10*−*12*). It was reported that lotus interrupted the propagation of Herpes simplex virus type 1 in HeLa cells (*10*). Experiments showed that extracts from lotus leaves display antiobesity effects on mice and rats (*11*) and decrease platelet aggregation in rabbits (*12*).

In recent years consumption of fresh lotus has increased rapidly because of its high nutritional value (*9*). According to some authors, lotus seeds contain high quantities of protein, crude fat, and carbohydrates (*9*).

However, the nutritional and bioactive properties of Korean white lotus cultivars have not been sufficiently investigated. Therefore, it was decided to determine the nutritional value and bioactive properties of Muan, Garam, and Chungyang Korean white lotus cultivars. Total phenols, phenolic and ascorbic acids, proteins, fatty acids, and antioxidant activity by ABTS CUPRAC, FRAP, DPPH, and β-carotene−linoleic acid assay complementary tests were determined (*13*−*17*).

As far as we know no results of such investigation have been published.

MATERIALS AND METHODS

Chemicals. 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), Folin−Ciocalteu reagent (FCR), lanthanum(III) chloride heptahydrate, $FeCl_3 \cdot 6H_2O$, $CuCl_2 \cdot 2H_2O$, 2,9-dimethyl-1,10-phenanthroline (neocuproine), and butylated hydroxyanisole (BHA) were purchased from Sigma Chemical Co., St. Louis, MO. 2,4,6-Tripyridyl-*s*-triazine (TPTZ) was purchased from Fluka Chemie, Buchs, Switzerland. Reference compounds of gallic acid (GA), protocatechuic acid (PCA), *p*-hydroxybenzoic acid (*p*-HBA), vanillic acid (VA), caffeic acid (CafA), ferulic acid (FA), *p*-coumaric acid (*p*-CoumA), cinnamic acid (CA), (+)-catechin (Cat), quercetin (Quer), and kaempferol (Kaempf) were obtained from Sigma-Aldrich, Steinheim, Germany. Individual fatty acids were as well purchased from Sigma-Aldrich. Acetonitrile (ACN) and tetrahydrofuran (THF) used as mobile phase were of HPLC grade and were purchased from Merck, Darmstadt, Germany. All solvents used as reaction media were of analytical grade and were obtained from POCh, Gliwice, Poland.

Lotus Samples. White lotus (*Nelumbo nucifera* Gaertn.) as a commercial mature plant was obtained from a local farm in January 2008, transported to the laboratory, and held overnight at 0 °C. Uniformly sized roots were selected and discarded off grade from local cultivars 'Muan', 'Chungyang', and 'Garam'.

Determination of the Contents of the Studied Bioactive Compounds. Most of the bioactive compounds were determined as previously described (*18*, *19*). The presence of polyphenols (flavonoids and phenolic acids) in the investigated lotus cultivars was studied by Fourier transform infrared (FT-IR) spectroscopy and fluorometry.

A Bruker Optic GMBH Vector FT-IR spectrometer (Bruker Optic GMBH, Attingen, Germany) was used to record IR spectra. A potassium bromide microdisk was prepared from 2 mg of finely ground lyophilized lotus powder with 100 mg of KBr (*20*−*22*). Fluorescence measurements were done using a model FP-6500, Jasco spectrofluorometer, serial N261332, Japan. Fluorescence emission spectra measurements for all lotus samples at a concentration of 0.02 mg/mL were taken at emission wavelengths of 330 and 740 nm and recorded from a wavelength of 265 to a wavelength of 310 nm and from 300 to 740 nm. Standards of 0.01 mM caffeic acid and quercetin in methanol were used.

The bioactive substances in lotus samples were extracted at room temperature for 3 h twice with different solvents of various polarities with a sample/solvent mass per volume ratio of 25 mg/mL (water and methanol) and 40 mg/mL (acetone).

To determine the total amount of polyphenols in the studied extracts, the FCR was used, and the measurement was performed at 765 nm with gallic acid as the standard. Results were expressed as milligrams of gallic acid equivalent (GAE).

Flavonoids spectrophotometric determination was done with 5% $NaNO_2$, 10% $AlCl_3 \cdot 6H_2O$, and 1 M NaOH and were measured at 510 nm. The extracts of condensed tannins (procyanidins) with 4% methanol vanillin solution were measured at 500 nm. The total flavanols were estimated using the *p*-dimethylaminocinnamaldehyde (DMACA) method, and then the absorbance at 640 nm was read. (+)-Catechin served as a standard for flavonoids, flavanols, and tannins, and the results were expressed as catechin equivalents (CE). Total ascorbic acid was determined by CUPRAC assay (*23*). The water extract was prepared from 100 mg of lyophilized sample and 5 mL of water. This extract (1 mL) was mixed with 2 mL of 3.0×10^{-3} M of lanthanum(III) chloride heptahydrate. Ethyl acetate (EtAc) was used for extraction of flavonoids to avoid interference. Ascorbic acid was quantified in the aqueous phase. One milliliter of Cu(II)−neocuproine (Nc), in ammonium acetate-containing medium at pH 7, was added to 1 mL of the obtained extract. The absorbance of the formed bis(Nc)−copper(I) chelate was measured at 450 nm.

Determination of Phenolic Acids. *Preparation of Crude Phenolic Extracts*. Dried samples of lotus were hydrolyzed according to a slightly modified method of Falleh et al. (*24*) and others (*25*, *26*). To a lyophilized lotus sample (1 g) were added 40 mL of methanol and then 10 mL of 6 M HCl. The mixture was stirred carefully and then sonicated for 15 min and refluxed in a water bath at 90 °C for 2 h. The obtained mixture was filtered through a 0.45 μm membrane filter and injected (20 μL) to HPLC. The concentration of phenolic compounds extracted from lotus samples was calculated using the developed calibration curve equations.

Preparation of Standard Solutions. Standard solutions of eight phenolic acids and three flavonoids at a concentration of 0.01 mg/mL in methanol were prepared, and several dilutions in mobile phase were made. All standard solutions were filtered through 0.45 μm filters and injected directly.

Chromatographic Conditions. An Agilent 1200 series HPLC system with a DAD detector coupled with ChemStation analytical software was used for analysis. A reverse phase (RP) column (Zorbax Bonus-RP 4.6 × 150 mm, 5 μm) with an extended guard column was used as stationary phase, and column temperature was maintained at 25 °C. The mobile phase was a gradient elution of water containing 0.1% formic acid (solvent A) and methanol (solvent B) at a flow rate of 1 mL/min. The gradient program of solvent B in A (v/v) was as follows: 0 min, 10% B; 10 min, 30% B; 25 min, 30% B; 35 min, 45% B; 45 min, 45% B; 65 min, 100% B; 70 min, 100% B.

Determination of Fatty Acids. Chromatographic analyses were performed using liquid chromatograph series 1200 (Agilent Technology Inc.) equipped with a quaternary pump, an autosampler, a thermostated column compartment, and a diode array detector (*27*, *28*). Analytes were dissolved in a mixture of acetonitrile/acetone (1:1; v/v), and 5 μL of the solution was injected onto the chromatographic column. A mixture of (A) water in (B) ACN + THF (99:1; v/v) were used as a mobile phase in gradient mode at a flow rate of 2.0 mL min^{-1}: gradient at 5 min, 80% B; at 8 min, 85% B; at 20 min, 98% B. The UV detector was operated at 258 nm (DAD in single wavelength mode). All analyses were thermostated at 40 °C. The concentrations of fatty acids in lotus samples were calculated using fatty acid standards and an internal standard (hexadecanoic acid) as a measure of extraction yield. The limit of detection (LOD) was calculated as a signal-to-noise ratio of three, whereas the limit of quantification (LOQ) was defined as 10 times the noise level. Lyophilized lotus samples (∼150 mg) were hydrolyzed with 1 mL of 2 M KOH in $MeOH/H_2O$ (1:1; v/v) at 80−85 °C for 1.5 h in PTFE-linked screw-capped amber-colored tubes (5 mL volume). After cooling, the hydrolysates were acidified with 4 M HCl (∼0.5 mL) to pH ∼2, and the free fatty acids were extracted twice with 1 mL of *n*-heptane. The upper organic layer was separated and dried with Na_2SO_4, and then heptane was removed under a gentle stream of nitrogen at 40 °C. The residue was used for derivatization as below. Fatty acid standards (0.5−100 μg/mL) and fatty acids released by saponification from biological lipid extract were converted to fatty acid *p*-bromophenacyl ester according to the modified method of Wood and Lee (*27*).

To the residue in a PTFE-linked screw-capped amber-colored tube were added 200 μL of *p*-bromoacetophenone solution (10 mg/mL in acetone) and 200 μL of triethylamine solution (10 mg/mL in acetone). The contents were ultrasonicated and heated for 30 min at 50 °C in an ultrasonic bath. The resulting solution was evaporated to dryness under a gentle stream of nitrogen at 40 °C. A 250 μL volume of acetonitrile/acetone (1:1; v/v) was added to the tube. The resulting solution was filtered and injected to the column.

Determination of the Antioxidant Activity. The following 5 tests were used:

(1) 2,2-Azinobis(3-ethyl-benzothiazoline-6-sulfonic acid) diamonium salt ($ABTS^{\bullet+}$) was generated by the interaction of ABTS (7 mmol/L) and $K_2S_2O_8$ (2.45 mmol/L). This solution was diluted with methanol until the absorbance reached 0.7 at 734 nm.

(2) The cupric reducing antioxidant capacity (CUPRAC) assay is based on utilizing the copper(II)−neocuproine [Cu(II)−Nc] reagent as the chromogenic oxidizing agent. The absorbance at 450 nm was recorded against a reagent blank.

(3) The ferric reducing/antioxidant power (FRAP) assay measures the ability of the antioxidants in the investigated samples to reduce ferric−tripiridyltriazine (Fe^{3+}−TPTZ) to a ferrous form (Fe^{2+}), which absorbs light at 593 nm.

(4) 1,1-Diphenyl-2-picrylhydrazyl (DPPH) solution (3.9 mL, 25 mg/L) in methanol was mixed with the sample extracts (0.1 mL). The reaction progress was monitored at 515 nm until the absorbance was stable.

(5) The β-carotene−linoleic acid assay was performed by dissolving of 0.5 mg of β-carotene in 1 mL of chloroform and adding 25 mL of linoleic acid together with 200 mg of Tween 40. The chloroform was evaporated. One hundred milliliters of aerated water was added to the residue. To 2.5 mL of this mixture was added 300 mL of each extract (*13*−*19*).

Statistics. The results of the investigation are means ± SD of five measurements. When it was appropriate, differences between groups were tested by two-way analysis of variance (ANOVA). *P* values of < 0.05 were considered to be significant.

RESULTS

Bioactive Compounds. Chemical analyses for Muan, Chungyang, and Garam lotus samples did not show significant differences ($P > 0.05$) in proteins [12.9 ± 0.3, 13.2 ± 0.5, 12.6 ± 0.3 (%)], crude fat [2.3 ± 0.1, 2.3 ± 0.1, 2.2 ± 0.1 (%)], and carbohydrates [65.3 ± 5.5, 64.9 ± 5.3, 66.1 ± 5.9 (%)].

The wavenumbers of FTIR spectra for catechin at 827, 1039, 1115, 1143, 1286, 1478, 1511, and 1610 cm^{-1} were assigned to C—H alkenes, −C—O alcohols, C—OH alcohols, −OH aromatic, C—O alcohols, C—H alkanes, C=C aromatic ring, and C=C alkenes. Gallic acid showed the following wavenumbers: 866, 1026, 1238, 1450, 1542, and 1618 cm^{-1}. Lotus samples in

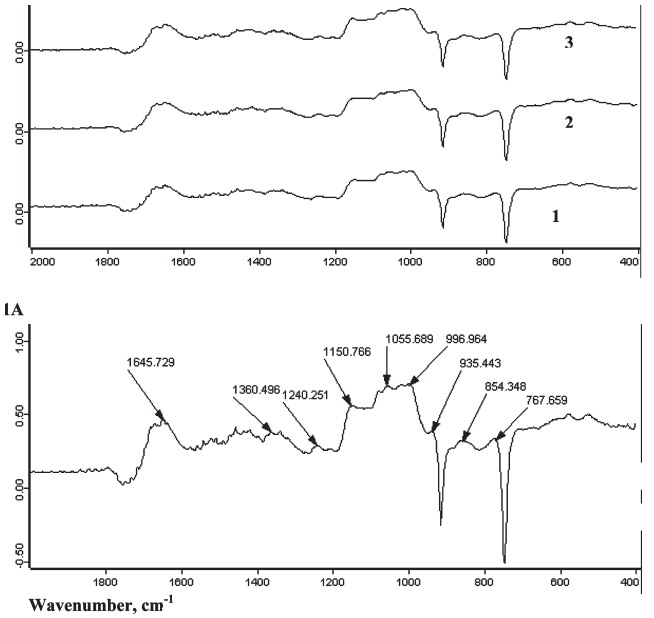

Figure 1. FTIR spectra of (1) Muan, (2) Chungyang, and (3) Garam lotus cultivars; **1A**, spectrum of Chungyang cultivar.

the region of polyphenols showed slightly different bands from the standards, 854, 935, 1056, 1150, 1240, and 1645 cm^{-1}, but the wavelengths of the bands were similar in all lotus samples (**Figure 1**). Other additional bands in the lotus samples in the region of polyphenols (**Figure 1**) were slightly shifted in comparison with the standards (*20*, *21*).

The following data of fluorometric measurements were obtained (**Figure 2**): for polyphenol methanol extracts (**Figure 2A**): Chungyang with two peaks of 281.5 nm and the absorption units (AU) of 159.91 and 300.0 nm and 47.59 AU; Muan with two peaks, 282.0 nm and 121.26 AU and 300.5 nm and 41.77 AU; Garam with two peaks, 299.5 nm and 95.96 AU and 300.5 nm and 35.40 AU. For polyphenol water extracts (**Figure 2B**) the following absorption peaks were found: Chungyang with two peaks of 279.5 nm and the absorption units (AU) of 261.81 and 295.5 nm and 100.42 AU; Muan with two peaks, 280.0 nm and 169.82 AU and 296.5 nm and 59.35 AU; Garam with two peaks, 280.0 nm and 151.70 AU and 296.0 nm and 61.54 AU.

Figure 2C shows the spectrum of lotus sample at emission wavelength of 740 nm and the following two peaks: at 371.5 and 739.5 nm with 977.26 and 562.77 AU and for comparison with catechin at 371.5 and 739.5 nm and 590.86 and 311.90 AU.

Standardization of Chromatographic Separation Using Reference Phenolic Acids. RP-HPLC-DAD conditions for the qualitative and quantitative profiles of eight major phenolic acids, GA, PCA, *p*-HBA, VA, CafA, FA, *p*-CoumA, and CA, and three flavonoids, Cat, Quer, and Kaempf, in lotus samples were optimized using different proportions of methanol and water as the mobile phase with reverse stationary phase. Variations in pH of the mobile phase are reported to have significant effect on the resolution and tailing of polar compounds in RP-HPLC. Better separation for phenolic acids is achievable in the presence of acids in the mobile phase because these suppress the ionization of acidic groups. A gradient of 0.1% formic acid in water and methanol was optimized as mobile phase as described under Chromatographic Conditions. **Figure 3** shows the chromatograms monitored at two different wavelengths, selected on the basis of maximum absorbance and maximum peak/noise ratio. Regression equations were obtained by the external standard

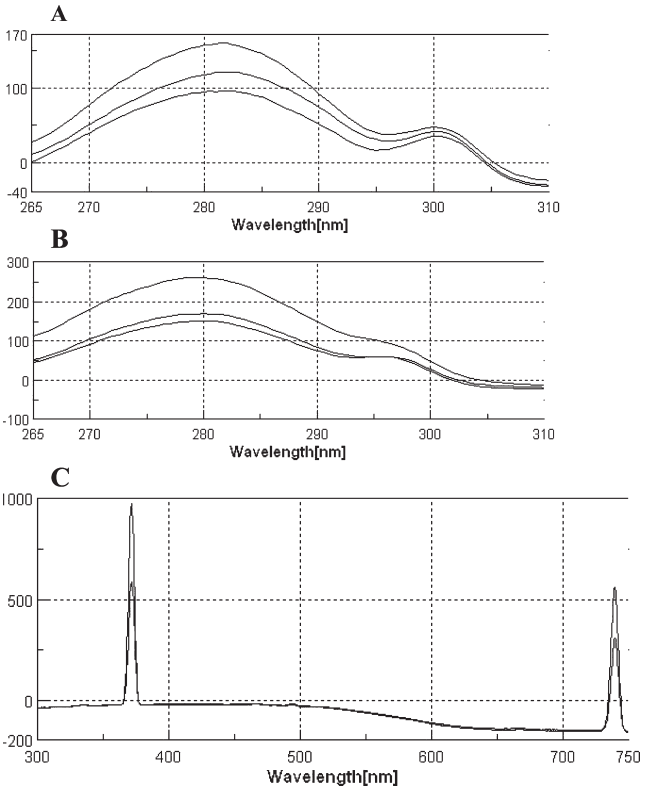

Figure 2. Fluorometric excitation spectra of lotus extracts with emission wavelength at 330 nm recorded over the frequency range from the excitation wavelength to a wavelength of 310 nm: (**A**) methanol extracts of polyphenols (0.1 mg/mL) of Chungyang, Muan, and Garam lotus; (**B**) water extracts of polyphenols (0.1 mg/mL) of Chungyang, Muan, and Garam lotus; (**C**) emission wavelength at 740 nm from 300 to 750 nm.

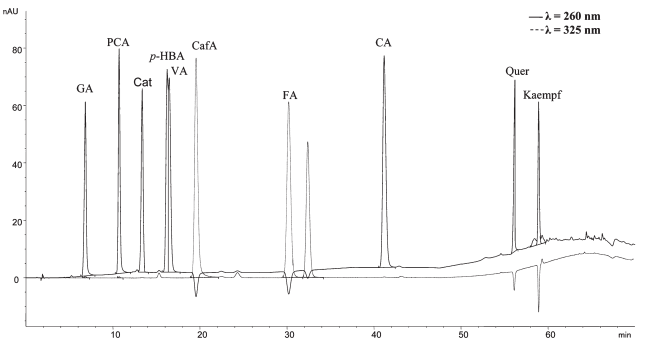

Figure 3. HPLC chromatograms of phenolic acids at two different wavelenghts (λ = 260 and 325 nm): gallic acid (GA), protocatechuic acid (PCA), (+)-catechin (Cat), *p*-hydroxybenzoic acid (*p*-HBA), vanillic acid (VA), caffeic acid (CafA), ferulic acid (FA), *p*-coumaric acid (*p*-CoumA), cinnamic acid (CA), quercetin (Quer), and kaempferol (Kaempf).

method. Retention times (t_R) of reference phenolic acids, the linear range, regression equation, and correlation coefficients of each analyte are summarized in **Table 1**. All of the components showed good linearity ($R \geq 0.998$) in a relatively wide concentration range.

The freeze-dried lotus samples were analyzed for their phenolic acids content. Peaks in the chromatograms were identified by comparing the retention times and UV spectra with those of reference compounds. Hydrolyzed lotus extracts presented a chemical profile composed of 10 identified phenolic compounds including protocatechuic acid, catechin, *p*-hydroxybenzoic acid, vanillic acid, caffeic acid, ferulic acid, *p*-coumaric acid, cinnamic

Table 1. Detection Wavelength (λ), Retention Time (t_R), and Linear Regression Data of Phenolic Compounds

compound	λ (nm)	t_R (min)	regression eq[a]	R^2
gallic acid	260	6.8	$y = 1855.5x - 2.5$	0.9992
protocatechuic acid	260	10.7	$y = 2657.9x - 2.8$	0.9989
catechin	280	13.4	$y = 419.4x - 1.1$	0.9993
p-hydroxybenzoic acid	260	16.2	$y = 4207.5x + 17.4$	0.9953
vanillic acid	260	16.5	$y = 3071.7x + 0.8$	0.9982
caffeic acid	325	19.5	$y = 1214.8x + 2.3$	0.9989
ferulic acid	325	30.2	$y = 5425.7x + 5.3$	0.9981
p-coumaric acid	325	32.4	$y = 7596.3x - 48.9$	0.9972
cinnamic acid	280	41.2	$y = 5740.3x + 14.4$	0.9981
quercetin	260	56.1	$y = 378.5x - 4.4$	0.9972
kaempferol	260	58.9	$y = 924.9x + 31.3$	0.9992

[a] *x*, peak area; *y*, content of analyte (μg).

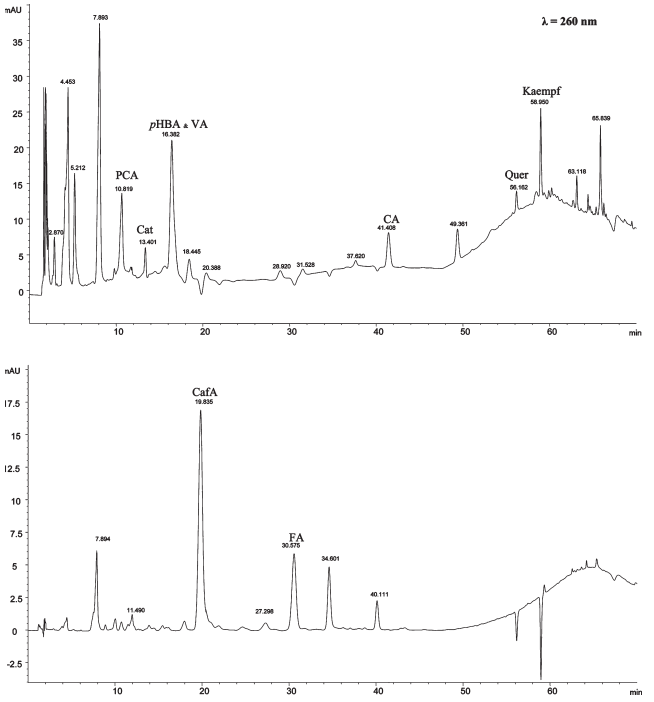

Figure 4. HPLC chromatograms of phenolic compounds of Chungyang lotus cultivar at λ = 260 and 325 nm: protocatechuic acid (PCA), (+)-catechin (Cat), *p*-hydroxybenzoic acid (*p*-HBA), vanillic acid (VA), caffeic acid (CafA), ferulic acid (FA), cinnamic acid (CA), quercetin (Quer), and kaempferol (Kaempf).

acid, quercetin, and kaempferol. The chromatograms also show some other peaks apart from the 11 standards studied (**Figure 4**). The amount of each analyte was calculated from the corresponding calibration curve and represented as the mean ± standard deviation of three analyses. Composition of phenolic acids in the dry matter of lotus cultivars is summarized in **Table 2** and **Figure 4**.

Fatty Acids. The HPLC profiles (**Figure 5**) and **Table 3** revealed that the major unsaturated acid was linoleic, whereas the main saturated acid was palmitic. Myristic and stearic acids were found as well. Palmitic (16:0), oleic (18:1), and linoleic (18:2) acids were among the major fatty acids throughout all lotus samples. The levels of stearic and linoleic fatty acids were significantly different ($P < 0.05$) between the lotus samples.

Bioactive Compounds. Different solvents (water, acetone, and methanol) were used for polyphenol extraction and showed variation in the amounts of bioactive compounds. Polyphenols (mg of GAE/g) in acetone extracts were the following: for

Table 2. Phenolic Compounds in Hydrolyzed Extracts from White Lotus Cultivars (Milligrams per Gram of Dry Matter)[a]

phenolic compound	Garam	Muan	Chungyang
gallic acid	nd	nd	nd
protocatechuic acid	0.062 ± 0.009	0.081 ± 0.011	0.142 ± 0.052
catechin	0.123 ± 0.015	0.074 ± 0.012	0.172 ± 0.031
p-hydroxybenzoic acid	0.083 ± 0.010	0.055 ± 0.005	0.174 ± 0.032
vanillic acid	0.073 ± 0.013	0.125 ± 0.001	0.233 ± 0.009
caffeic acid	0.135 ± 0.008	0.211 ± 0.003	0.412 ± 0.043
ferulic acid	0.011 ± 0.003	0.051 ± 0.001	0.071 ± 0.003
p-coumaric acid	nd	0.050 ± 0.001	nd
cinnamic acid	0.052 ± 0.001	0.061 ± 0.002	0.083 ± 0.001
quercetin	0.174 ± 0.006	0.193 ± 0.012	0.221 ± 0.011
kaempferol	0.101 ± 0.001	0.051 ± 0.001	0.086 ± 0.012
total	0.814	0.952	1.594

[a] Values are mean ± SD (n = 3). nd, not detected.

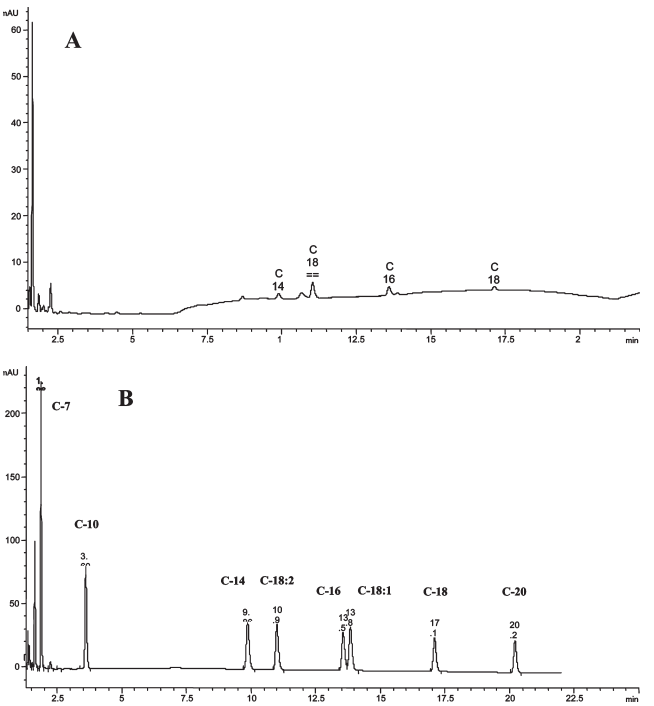

Figure 5. (**A**) Chromatogram of lotus sample; (**B**) typical chromatogram of fatty acids standard solution. Analytes: C-7, enanthic acid; C-10, capric acid; C-14, myristic acid; C18:2, linoleic acid; C-16, palmitic acid; C-18:1, oleic acid; C-18, stearic acid; C-20, arachidic acid.

Muan, 0.22 ± 0.02 b; for Chungyang, 0.26 ± 0.02 c; and for Garam, 0.19 ± 0.01 a, where values with different letters are significantly different ($P < 0.05$). Water was the most effective solvent in the extraction yield of bioactive compounds.

The results of the determination of the contents of polyphenols extracted from three lotus samples with methanol and water are summarized in the **Table 4**. As can be seen, the content of polyphenols in Chungyang was significantly higher than in the other two cultivars and in its water extract significantly higher than in its methanol extract (15.85 ± 0.5 vs 7.80 ± 0.5 mg of GAE/g, respectively, $P < 0.05$).

The amounts of ascorbic acid (mg of Asc.ac/g) were 10.1 ± 0.9 b, 12.6 ± 1.1 c, and 8.6 ± 0.7 a for water extracts of Muan, Chungyang, and Garam cultivars, respectively, where values with different letters are significantly different ($P < 0.05$). The major bioactive compounds were significantly higher in the Chungyang cultivar than in other cultivars ($P < 0.05$). In water extracts the contents of polyphenols, flavanols, and

Table 3. Fatty Acid Composition in Lotus Samples[a]

	fatty acids (μg/g of dry wt)							
white lotus sample	enanthic acid C7:0	capric acid C10:0	myristic acid C14:0	palmitic acid C16:0	stearic acid C18:0	arachidic acid C20:0	oleic acid C18:1	linoleic acid C18:2
Muan	nd	nd	3.95 ± 0.66 a	9.94 ± 0.75 a	6.71 ± 0.82 b	nd	2.41 ± 0.43 a	17.18 ± 0.68 b
Chungyang	nq	nd	4.27 ± 0.08 a	8.13 ± 0.67 a	3.5 ± 0.39 a	nd	nd	11.48 ± 097 a
Garam	nq	nd	nd	7.55 ± 1.03 a	7.55 ± 0.89 b	nd	3.00 ± 0.56 a	10.79 ± 1.01 a

[a] Each value is the mean ± SD of triplicate extractions, derivatization, and determinations. nd, not detected; nq, below the limit of quantification.

Table 4. Some Bioactive Compounds Extracted with Methanol and Water from Lotus Roots[a]

white lotus sample	polyphenols[b] (mg of GAE/g)	flavonoids[b] (mg of CatE/g)	flavanols[b] (μg of CE/g)	tannins[b] (mg of CE/g)	polyphenols (mg of GAE/g)[c]	flavonoids[c] (mg of CatE/g)	flavanols[c] (μg of CE/g)	tannins[c] (mg of CE/g)
Muan	3.92 ± 0.3 a	0.63 ± 0.05 a	450.8 ± 32.1 b	0.62 ± 0.05 a	14.54 ± 0.3 a	1.14 ± 0.05 a	878.2 ± 43.1 b	7.49 ± 0.05 b
Chungyang	7.80 ± 0.5 b	1.01 ± 0.01 b	580.7 ± 4 2.3 c	3.12 ± 0.03 b	15.85 ± 0.5 b	2.56 ± 0.01 b	1144.5 ± 52.3 c	7.15 ± 0.03 b
Garam	3.75 ± 0.3 a	0.40 ± 0.03 a	213.1 ± 18.9 a	0.52 ± 0.04 a	13.78 ± 0.3 a	0.76 ± 0.03 a	671.4 ± 28.9 a	5.49 ± 0.04 a

[a] Values are means ± SD of five measurements, per gram of dry weight. Values in columns with different letters are significantly different ($P < 0.05$). [b] Extracted at room temperature in concentration of 25 mg of lyophilized sample in 1 mL of methanol. [c] Extracted at room temperature in concentration of 25 mg of lyophilized sample in 1 mL of water.

ascorbic acid are significantly higher in the Chungyang cultivar ($P < 0.05$).

Antioxidant Activity. The antioxidant activity determined by different antioxidant scavenging methods and in methanol, acetone, and water extracts was in most cases significantly higher ($P < 0.05$) in Chungyang samples than in other cultivars (**Table 5**). The percentage of inhibition by β-carotene was 45.4 ± 3.9 a, 48.2 ± 4.9 b, and 44.9 ± 4.2 a for Muan, Chungyang, and Garam cultivars, respectively. The highest antioxidant activity for Chungyang cultivar was estimated in the water extract, using all of the antioxidant methods, as well as the β-carotene−linoleic assay.

DISCUSSION

The main aim of this investigation was to determine the nutritional and bioactive values of different extracts of Korean white lotus cultivars (Muan, Chungyang, and Garam). To fulfill this aim the bioactive compounds and the antioxidant activity of different extracts of these cultivars were determined. It was found that the contents of the basic nutritional compounds (protein, crude fat, and carbohydrates) were comparable in all three studied cultivars of the Korean white lotus. The contents of protein, crude fat, and carbohydrates are in agreement with the data recorded by other authors (*9*, *28*−*30*): on average, 14.5% protein, 2.4% crude fat, and 66.3% carbohydrates. The lotus samples displayed the level of linoleic acid approximately 43% of the total fatty acid content. Oleic acid was not detected in Chungyang lotus. The percentage of oleic acid in other cultivars was about 6% for Muan and 10% for Garam lotus in comparison with others (*28*), whereas in lotus samples the amount of oleic acid was 9.3%. These results show that lotus has moderate levels of fatty acids. This information can be used by nutritionists and food technologists to improve the nutrition of local people and develop food products that would be beneficial to human health.

The results of the determination of the studied bioactive compounds in the methanol, acetone, and water extracts of the Korean white lotus cultivars show that the highest contents of polyphenols, flavonoids, flavanols, tannins, and vitamin C were in the water extracts of Chungyang cultivar.

It is not surprising that other investigators of lotus extract (*16*) found methanol as the solvent yielding highest extraction, because they used only the DPPH and β-carotene bleaching assays. This result is seemingly in contradiction with our findings,

Table 5. Antioxidant Activity of Lotus Root Cultivars (μMTE/g) in Methanol, Water, and Acetone Extracts[a]

	Muan	Chungyang	Garam
ABTS[b]	28.7 ± 1.9 b	53.5 ± 4.8 c	20.8 ± 1.7 a
ABTS[c]	90.2 ± 8.1 a	108.0 ± 9.5 b	92.9 ± 8.3 a
ABTS[d]	3.12 ± 0.2 a	4.61 ± 0.3 b	3.34 ± 0.12 a
CUPRAC[b]	19.4 ± 1.6 b	33.7 ± 2.6 c	14.0 ± 0.9 a
CUPRAC[c]	54.0 ± 4.7 b	49.2 ± 4.1 b	26.5 ± 1.8 a
CUPRAC[d]	2.38 ± 0.2 a	2.94 ± 0.2 a	2.47 ± 0.2 a
FRAP[b]	10.5 ± 0.8 a	25.7 ± 1.9 b	8.5 ± 0.7a
FRAP[c]	23.5 ± 1.7 b	31.1 ± 2.3 c	19.2 ± 1.6 a
FRAP[d]	1.01 ± 0.1 a	1.90 ± 0.2 c	1.50 ± 0.1 b
DPPH[b]	8.6 ± 0.7 a	21.2 ± 2.5 b	6.8 ± 0.4 a
DPPH[c]	146.4 ± 12.5 b	157.0 ± 12.9 b	99.7 ± 8.3 a
DPPH[d]	1.04 ± 0.1 a	1.45 ± 0.1 b	1.11 ± 0.1 a

[a] Values are means ± SD of five measurements, per gram of dry weight. Values in rows with different letters are significantly different ($P < 0.05$). [b] Extracted at room temperature in concentration of 25 mg of lyophilized sample in 1 mL of methanol. [c] Extracted at room temperature in concentration of 25 mg of lyophilized sample in 1 mL of water. [d] Extracted at room temperature in concentration of 40 mg of lyophilized sample in 1 mL of acetone.

but in fact, it is not. The radical species used in the DPPH assay is typically soluble in organic solvents (lipophilic antioxidants) and does not work well for hydrophilic antioxidants. In the previous cited research (*16*) the polyphenolics extraction powers of a number of solvents of different polarities merely by using the DPPH assay were compared. It is inevitable that methanol will win over water as the DPPH assay essentially detects lipophilic antioxidants. On the other hand, the Folin assay works well only for hydrophilic antioxidants. The only antioxidant assay that works equally well for both hydrophilic and lipophilic antioxidants is the CUPRAC assay (*14*).

There are no data about the phenolic acids in lotus roots; therefore, it is difficult to compare with our results. The results cited in the literature showed the amount of the bioactive compounds in lotus flowers. It was reported (*31*, *32*) that in purified ethyl acetate soluble fraction of the stamens of *N. nucifera* Gaertn were determined 13 flavonoids, including kaempferol and 7 of its glycosides, quercetin, and catechin. The amount of quercetin in flowers was about 0.68 mg/g (*31*), which is

higher than in root samples. According to all five antioxidant tests used, the antioxidant activity was significantly higher in Chungyang cultivar, particularly in its water extract. Comparison of the bioactivities of lotus cultivars can be done only on the basis of the very short presentation of the literature data (*13*, *16*, *17*, *33*, *34*). These authors (*8*) found that lotus boiling water extract exhibited stronger antioxidant activity than other extracts with organic solvents. The antioxidant activities are dose-dependent and reached a plateau (about 80% inhibition) when the concentration of lotus liquor exceeded 25 μg in a modified linoleic acid peroxidation induced by Hb. The effect of lotus boiling water extract on DNA damage in human lymphocytes was investigated (*8*).

The data of the report on 12 common vegetables (*13*) that were evaluated by FRAP, DPPH, and β-carotene−linoleic acid emulsion assay exactly correspond with our results. A definite correlation existed between these three test methods, and in our case all five of the used methods well correlated with the determined polyphenols. Lotus in comparison with other plants showed relatively high antioxidant activity and was placed after amaranth. Total phenol contents of each vegetable had a big difference. Amaranth had the largest amounts of total phenol content (163.71 $\pm$ 4.47 mg/100 g), and our results were slightly higher in methanol and lower in acetone fractions (**Table 4**). The highest amount of phenolic acids was in Chungyang cultivar, followed by Muan and Garam, including procatechuic and caffeic acids, which play most important (*22*, *24*−*26*) roles as natural antioxidants in these plants (**Table 2**). Our results can be compared with other investigations (*17*) wherein FRAP was used to measure the antioxidant capacity of vegetables. The lotus root was the strongest in antioxidant capacity among vegetables and followed by ginger, rape, garlic bulb, white onion, white radish, and broccoli. The antioxidant capacity, vitamin C content, total polyphenol content, and total flavonoid content differed greatly among different vegetables. There was a correlation between antioxidant capacity and total polyphenol or total flavonoid content. However, there was no correlation between the antioxidant capacity and vitamin C content, as was found here. The total polyphenols for white cabbage varied from 2.4 to 4.9 mg of GAE/g (*18*), which was similar to Muan cultivar (**Table 4**). ABTS and DPPH antioxidant activities (μM TE/g) ranged for cabbage from 2.7 to 8.2 and from 2.4 to 5.4 and correspond with our data (**Table 5**). The obtained results corresponded with garlic polyphenols, and the antioxidant activities of the methanol fraction in the Chungyang cultivar were similar to those of white onion (*19*).

However, other investigators reported that the methanol extract was the most effective (*16*) in comparison with solvents of different polarities (acetone, dichloromethane, and petroleum ether) employed for extraction of lotus powder. Antioxidant activities of the extracts were evaluated by a DPPH assay and a β-carotene bleaching assay and compared with that of BHA and ascorbic acid. Methanol showed the highest extracted yield among all of the solvents. Although the acetone extract had the highest total phenolics content, the methanol extract had the highest total phenolics recovery from lotus powder (20.1 mg of catechin equiv/100 g of lotus powder). These data correspond with the phenolics in acetone and in methanol fractions (**Table 4**). Extracts of either methanol or acetone demonstrated the highest DPPH scavenging activity at both 66.7 and 133.3 mg/L. These results are in accordance with our estimated antioxidant activities by DPPH for acetone fraction (**Table 5**). It was concluded that the properties of the solvents significantly affected the yield, total phenolics content, and antioxidant activity of lotus rhizome extracts. In our opinion, the discrepancy of these authors' and our data is connected to the used solvents and the different extraction conditions.

Total phenols (mg of GAE/g) were slightly lower (**Table 4**) than the methanol (*31*) in raw garlic (9.00) and acetone (2.65) extracts. DPPH (*31*) in the methanol extract was about 9.00—similar to Muan (**Table 5**) and in the acetone fraction of Chungyang.

In conclusion, all three studied Korean white lotus cultivars and particularly their water extracts are rich in basic nutritional and bioactive compounds as are other widely used vegetables such as garlic, white onions, and cabbage. The cultivars exercise a high level of antioxidant activity. Among them the most active is Chungyang. Therefore, all studied Korean white lotus cultivars and particularly Chungyang could be recommended as effective nutritional and bioactive natural products, however, after investigation on humans.

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LITERATURE CITED

(1) Shen-Miller, J. Sacred lotus, the long-living fruits of China Antique. *Seed Sci. Res.* **2002**, *12*, 131–143.

(2) Hu, M.; Skibsted, L. H. Antioxidative capacity of rhizome extract and rhizome knot extract of edible lotus (*Nelumbo nucifera*). *Food Chem.* **2002**, *76*, 327–333.

(3) Xue, J.; Zhuo, L.; Zhou, S. Genetic diversity and geographic pattern of wild lotus (*Nelumbo nucifera*) in Heilongjiang province. *Chin. Sci. Bull.* **2006**, *51*, 421–432.

(4) Lin, J.-Y.; Lai, Y.-S.; Liu, C.-J.; Wu, A.-R. Effects of lotus plumule supplementation before and following systemic administration of lipopolysaccharide on the splenocyte responses of BALB/c mice. *Food Chem. Toxicol.* **2007**, *45*, 486–493.

(5) Sohn, D. H.; Kim, Y. C.; Oh, S. H.; Park, E. J.; Li, X.; Lee, B. H. Hepatoprotective and free radical scavenging effects of *Nelumbo nucifera*. *Phytomedicine* **2003**, *10*, 165–169.

(6) Wang, L.; Yen, J. H.; Liang, H. L.; Wu, M. J. Antioxidant effect of methanol extracts from lotus plumule and blossom (*Nelumbo nucifera* Gertn). *J. Food Drug Anal.* **2003**, *11*, 60–66.

(7) Wu, M. J.; Wang, I.; Weng, C. Y.; Yen, J. H. Antioxidant activity of methanol extract of the lotus leaf (*Nelumbo nucifera* Gertn). *Am. J. Chin. Med.* **2003**, *31*, 687–698.

(8) Lee, H. K.; Choi, Y. M.; Noh, D. O.; Suh, H. J. Antioxidant effect of Korean traditional lotus liquor (*Yunyupju*). *Int. J. Food Sci. Technol.* **2005**, *40*, 709–715.

(9) Bhat, R.; Sridhar, K. R. Nutritional quality evaluation of electron beam-irradiated lotus (*Nelumbo nucifera*) seeds. *Food Chem.* **2008**, *107*, 174–184.

(10) Kuo, Y. C.; Lin, Y. L.; Liu, C. P.; Tsai, W. J. Herpes simplex virus type 1 propagation in HeLa cells interrupted by *Nelumbo nucifera* *J. Biomed. Sci.* **2005**, *12*, 1021–1034.

(11) Ono, Y.; Hattori, E.; Fukaya, Y.; Imai, S.; Ohizumi, Y. Antiobesity effect of *Nelumbo nucifera* leaves extract in mice and rats *J. Ethnopharmacol.* **2006**, *106*, 238–244.

(12) Yu, J.; Hu, W. S. Effects of neferine on platelet aggregation in rabbits. *Yaoxue Xuebao* **1997**, *32*, 1–4.

(13) Yang, D.; Jin, Y.; Ke, L.; Jiang, J.; Huang, X.; Ying, T. Antioxidant activities of 12 common vegetables. *Zhongguo Shipin Xuebao* **2007**, *7*, 24–29.

(14) Apak, R.; Guclu, K.; Ozyurek, M.; Karademir, S. E. Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method. *J. Agric. Food Chem.* **2004**, *52*, 7970–7981.

(15) Ozgen, M.; Reese, R. N.; Tulio, A. Z.Jr.; Scheerens, J. C.; Miller, A. R. Modified 2, 2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) method to measure antioxidant capacity of selected small fruits and comparison to ferric reducing antioxidant power (FRAP) and 2,2′-diphenyl-1-picrylhydrazyl (DPPH) methods. *J. Agric. Food Chem.* **2006**, *54*, 1151–1157.

(16) Yang, D.; Wang, Q.; Ke, L.; Jiang, J.; Ying, T. Antioxidant activities of various extracts of lotus (*Nelumbo nuficera* gaertn) rhizome. *Asia Pacific J. Clin. Nutr*. **2007**, *16*, 158–163.
(17) Chen, Y.; Guo, Ch.; Yang, J.; Wu, C.; Hu, L. The antioxidant capacity of common 25 vegetables. *Henan Gongye Daxue Xuebao, Ziran Kexueban* **2007**, *28*, 37–41.
(18) Kusznierewicz, B.; Bartoszek, A.; Wolska, L.; Drzewiecki, J.; Gorinstein, S.; Namieśnik, J. Partial characterization of white cabbages (*Brassica oleracea* var. *capitata* f. *alba*) from different regions by glucosinolates, bioactive compounds, total antioxidant activities and proteins. *LWT−Food Sci. Technol*. **2008**, *41*, 1–9.
(19) Gorinstein, S.; Leontowicz, H.; Leontowicz, M.; Namiesnik, J.; Najman, K.; Drzewiecki, J.; Cvikrova, M.; Martincova, O.; Katrich, E.; Trakhtenberg, S. Comparison of the main bioactive compounds and antioxidant activities in garlic and white and red onions after treatment protocols. *J. Agric. Food Chem*. **2008**, *56*, 4418–4426.
(20) Chen, Y. M.; Wang, M. K.; Huang, P. M. Catechin transformation as influenced by aluminum. *J. Agric. Food Chem*. **2006**, *54*, 212–216.
(21) Sinelli, N.; Spinardi, A.; Di Egidio, V.; Mignani, I.; Casiraghia, E. Evaluation of quality and nutraceutical content of blueberries (*Vaccinium corymbosum* L.) by near and mid-infrared spectroscopy. *Postharvest Biol. Technol*. **2008**, *50*, 31–36.
(22) Kanakis, C. D.; Tarantilis, P. A.; Polissiou, M. G.; Diamantoglou, S.; Tajmir-Riahi, H. A. Antioxidant flavonoids bind human serum albumin. *J. Mol. Struct*. **2006**, *798*, 69–74.
(23) Ozyurek, M.; Guclu, K.; Bektasoglu, B.; Apak, R. Spectrophotometric determination of ascorbic acid by the modified CUPRAC method with extractive separation of flavonoids−La(III) complexes. *Anal. Chim. Acta* **2007**, *588*, 88–95.
(24) Falleh, H.; Ksouri, R.; Chaieb, K.; Karray-Bouraoui, N.; Trabelsi, N.; Boulaaba, M.; Abdelly, C. Phenolic composition of *Cynara cardunculus* L. organs, and their biological activities. *C. R. Biol*. 2008, *331*, 372−379.
(25) Zadernowski, R.; Naczk, M.; Czaplicki, S.; Rubinskiene, M.; Szalkiewicz, M. Composition of phenolic acids in sea buckthorn (*Hippophae rhamnoides* L.) berries. *J. Am. Oil Chem. Soc*. **2005**, *82*, 175–179.
(26) Arimboor, R.; Kumar, K. S.; Arumughan, C. Simultaneous estimation of phenolic acids in sea buckthorn (*Hippophaë rhamnoides*) using RP-HPLC with DAD. *J. Pharm. Biomed. Anal*. **2008**, *47*, 31–38.
(27) Wood, R.; Lee, T. High-performance liquid chromatography of fatty acids: quantitative analysis of saturated, monoenoic, polyenoic and geometrical isomers. *J. Chromatogr*. **1983**, *254*, 237–246.
(28) Miyatani, Sh.; Yamamoto, K.; Nakayama, T.; Kinoshita, A.; Shibahara, A. Occurrence of *cis*-vaccenic acid in root vegetables. *Nippon Shokuhin Kagaku Gakkaishi* **2002**, *8*, 184–188.
(29) Indrayan, A. K.; Sharma, S.; Durgapal, D.; Kumar, N.; Kumar, M. Determination of nutritive value and analysis of mineral elements for some medicinally valued plants from Uttaranchal. *Curr. Sci*. **2005**, *89*, 1252–1255.
(30) Ogle, B. M.; Dao, H. T. A.; Mulokozi, G.; Hambraeus, L. Micronutrient composition and nutritional importance of gathered vegetables in Vietnam. *Int. J. Food Sci. Nutr*. **2001**, *52*, 485–499.
(31) Samee, W.; Vorarat, S. Simultaneous determination of gallic acid, catechin, rutin, ellagic acid and quercetin in flower extracts of *Michelia alba*, *Caesalpinia pulcherrima* and *Nelumbo nucifera* by HPLC. *Thai Pharm. Health Sci. J*. 2007, *2*, 131−137.
(32) Lim, S. S.; Jung, Y. J.; Hyun, S. K.; Lee, Y. S.; Choi, J. S. Rat lens aldose reductase inhibitory constituents of *Nelumbo nucifera* stamens. *Phytother. Res* 2006, *20*, 825−830.
(33) Gorinstein, S.; Jastrzebski, Z.; Leontowicz, H.; Leontowicz, M.; Namiesnik, J.; Najman, K.; Park, Y.-S.; Heo, B.-G.; Cho, J.-Y.; Bae, J.-H. Comparative control of the bioactivity of some frequently consumed vegetables subjected to different processing conditions. *Food Control* **2009**, *20*, 407–413.
(34) Prakash, D.; Singh, B. N.; Upadhyay, G. Antioxidant and free radical scavenging activities of phenols from onion (*Allium cepa*). *Food Chem*. 2007, *102*, 1389−1393.