JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Phenolic, Flavonoid, and Lutein Ester Content and Antioxidant Activity of 11 Cultivars of Chinese Marigold

WEI LI,[†,§] YANXIANG GAO,*[,†] JIAN ZHAO,[‡] AND QI WANG[†]

College of Food Science & Nutritional Engineering, China Agricultural University, Beijing 100083, China, School of Wine & Food Sciences, EH Graham Centre for Agricultural Innovation, Charles Sturt University, Wagga Wagga, New South Wales 2678, Australia, and College of Biological Engineering, Hubei University of Technology, Wuhan 430068, China

This study analyzed 11 Chinese cultivars of marigold to determine their major phytochemical contents and antixodant activities. Dried marigold flowers were extracted with ethanol, ethyl acetate, and *n*-hexane and the extracts were analyzed by high-performance liquid chromatography–mass spectrometry and chemical methods to determine their lutein esters, phenolic and flavonoid contents, and antioxidant activity, respectively. The different cultivars of marigold showed considerable variations in their lutein ester contents, ranging from 161.0 to 611.0 mg/100 g of flower (dry basis). The lutein esters in marigolds consisted predominantly of six all *trans*-diesters, but small amounts of cis isomers of the respective diesters were also present. The different cultivars of marigold also showed marked variations in total phenols and flavonoids, as well as antioxidant and radical-scavenging activities. Ethanol was confirmed to be the best solvent for extracting both phenols and flavonoids from marigold flowers, while *n*-hexane was the worst. The ethanolic extracts also exhibited the highest antioxidant and radical-scavenging activities. The cultivar *Xinhong* had the highest phenolic and flavonoid contents and radical-scavenging activity, as well as one of the highest lutein contents and antioxidant activities.

KEYWORDS: Marigold; lutein esters; phenols; flavonoids; antioxidant activity; LC-MS; geometric isomers

INTRODUCTION

Marigold (*Tagetes erecta* L.) is a common ornamental plant which bears yellow- to orange-colored flowers and is available in many parts of the world. Apart from its ornamental value, marigold is also well known for its antimicrobial, antiseptic, wound and ulcer healing, and hypotensive properties, and it has a long history of being used as an herbal remedy (*1*). The use of bioactive components extracted from marigold as nutritional and medicinal supplements has been explored. Reported potential benefits include the prevention of cancer and cardiovascular disease, enhanced immune function, inhibition of autooxidation of cellular lipids, and protection against oxidant-induced cell damage (*2*–*5*).

The marigold flower is one of the richest natural sources of lutein, a member of the xanthophyll family of carotenoids, whose health-benefiting functions are increasingly being recognized. Lutein occurs naturally in the acylated form as lutein esters, which are more stable than their non-esterified form against heat and UV light (*6*). Lutein has been identified as a major constituent of the macular pigment of the human retina and has the ability to absorb high-energy blue light from sunlight (*7*). Research has strongly suggested that it has protective activity against the two common eye diseases of aging, cataracts and age-related macular degeneration (*8*), which are the main causes of irreversible loss of vision. A higher intake of lutein may also have other beneficial effects on human health, including protection against cardiovascular disease (*9*), stroke, and UV-radiation-induced skin damage (*10*). These findings have led to an expanding international market for dietary lutein supplements, especially for eye health formulations.

The health-promoting functions of marigold are also related to its content of other secondary metabolites such as flavonoids and phenols, which are typical antioxidant compounds possessing multiple biological functions (*11*). The beneficial effects of flavonoids and phenols on human health are principally related to their antioxidant activity, which protects the human body from free radicals and retards the progress of many chronic diseases (*12*). Moreover, many biological functions such as antimutagenicity, anticarcinogenicity, and antiaging, among others, originate from this property (*13*). These findings have led to an increasing demand for marigold extract for use as a dietary supplement or as a functional ingredient in nutraceutical and pharmaceutical products. As a result, many new marigold cultivars are being bred and cultivated in China, not for their

* Author to whom correspondence should be addressed. Telephone: +86 010-62737034; fax: +86-10-62737689; e-mail gyxcau@126.com.
† China Agricultural University.
‡ Charles Sturt University.
§ Hubei University of Technology.

10.1021/jf071696j CCC: $37.00
Published on Web 09/26/2007

ornamental values but mainly for their perceived better biological functions. However, the content of the major bioactive compounds in these new cultivars as well as their antioxidant activities have not been systematically investigated. Such investigations are necessary because previous studies conducted on European marigolds have found that different species of the plant, as well as different cultivars of the same species, were markedly different in their lutein, phenol, and flavonoid contents and their antioxidant activity (*14*, *15*).

Here, we report a systematic study on the major groups of bioactive compounds and the evaluation of antioxidant activity in 11 different cultivars of the Chinese marigold. Lutein esters were extracted from the flowers and identified and quantified by high-performance liquid chromatography (HPLC) coupled with mass spectrometry (MS). Total phenols and flavonoids were extracted with three solvents (ethanol, ethyl acetate, and *n*-hexane) of varying polarities, and the antioxidant activity of the extracts was evaluated using two established *in vitro* methods.

MATERIALS AND METHODS

Materials and Chemicals. A total of 11 cultivars of the marigold (*Tagetes erecta* L.) flower were used in this study, and they were kindly supplied by Maker Biological Engineering Corp. (Heilongjiang, China). The different cultivars were grown under the same agronomical conditions, and all of the flowers were picked exactly 15 days after the blossom occurred. The flowers were separated from the receptacles, and 500 g samples of each cultivar were freeze-dried. The dehydrated samples were ground to pass through a 0.5 mm sieve and stored at –20 °C until use. Folin-Ciocalteu's phenol reagent, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 6-hydroxyl-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (Trolox), 2,2-azinobis-(3-ethylbenzothiazolin-6-sulfonic acid) (ABTS), lutein, gallic acid, and rutin were purchased from Sigma-Aldrich. Acetonitrile, methanol, and ethyl acetate used in the liquid chromatography work were of HPLC grade; all other chemicals were of analytical grade, unless otherwise stated.

Extraction of Marigold Flowers. Samples (1.0 g, dry weight) of ground freeze-dried marigold flowers were extracted with 30 mL of ethanol, ethyl acetate, or *n*-hexane in 50 mL flasks flushed with nitrogen, at 40 °C for 24 h with periodical shaking. The mixtures were then cooled to room temperature and centrifuged at 3000*g* for 15 min. The supernatants were collected and evaporated to dryness in a rotary evaporator under reduced pressure at 40 °C, and the extracts obtained were stored in amber-colored air-tight containers at –4 °C. For analysis, the extracts were redissolved in a known volume of the same solvent used in the original extraction. The *n*-hexane extracts were used to determine the content and composition of lutein esters in marigold by liquid chromatography–mass spectrometry (LC-MS). All three extracts were further analyzed to determine their total phenols, flavonoids, and antioxidant activities.

Liquid Chromatography–Mass Spectrometry (LC-MS) Analysis of Lutein Diesters. LC-MS was performed on an Agilent 1100 Series HPLC-MS system equipped with a quaternary pump, a degasser, a thermostatic autosampler, and a diode array detector (DAD). The separation of lutein esters was carried out with a 250 × 4.6 mm i.d. Diamonsil C_{18} analytical column (Dikma Technologies, Beijing) connected to a C_{18} precolumn, and both columns were kept at 30 °C in the column chamber. The HPLC conditions used were based on the procedure of Piccaglia et al. (*15*) with minor modifications. The binary mobile phase consisted of acetonitrile–methanol (9:1, v/v; solvent A) and ethyl acetate (solvent B). Elution was carried out with a gradient program: 50% B to 100% B in 30 min and 100% B to 50% B in 30–45 min; the flow rate was kept at 1.0 mL/min throughout. Samples were filtered through a 0.45 μm syringe filter prior to analysis. The injection volume was 20 μL, and peaks were recorded by the DAD at 332 and 450 nm. The eluate from the HPLC system was directly introduced into the atmospheric pressure chemical ionization (APCI) interface of the mass spectrometer (ion trap) with solvent splitting. The APCI source was heated at 350 °C, and the APCI probe was kept at 400 °C. Nitrogen was used as a sheath and drying gas at 5 L/min. The mass spectrometer was calibrated in the positive ion mode. Mass spectra of all lutein esters were acquired with a *m*/*z* 500–1200 scan range, and ions of lutein esters were also measured using selected-ion monitoring with a retention time of 100 ms per ion. The total lutein ester content in the marigold flower was determined according to the AOAC method 970.64 (*16*) using hot saponification, and the content was expressed as milligrams of xanthophyll (mostly lutein) equivalent (XE) per 100 g of dry marigold flowers. The percentage composition of the lutein diesters in the marigold flower was calculated from their relative peak areas in the DAD chromatograms.

Determination of Total Phenolics. The total phenolic content in the extracts of the marigold flower was determined by the Folin-Ciocalteu method as described by Marquele et al. (*17*) with minor modifications. A 1.0 mL aliquot of the extract was added to 2.0 mL of the 1 M Folin-Ciocalteu reagent, and the mixture was allowed to stand for 5 min before 2.0 mL of 7.5% (w/v) sodium carbonate was added. The mixture was shaken well, kept at room temperature for 30 min, and centrifuged at 2000*g* for 10 min. The absorbance of the supernatant was measured at 760 nm against a reagent blank. A standard curve of gallic acid was used to calculate the total phenolic content in the extract, which was expressed as gallic acid equivalents (mg GAE/g extract).

Determination of Total Flavonoids. Total flavonoids were estimated following the aluminium chloride colorimetric method of Djeridane et al. (*18*). Briefly, aliquots (2 mL) of the marigold extracts were added to 2 mL of a 3% $AlCl_3$ solution in ethanol, and after incubation for 10 min at room temperature, the absorbance was measured at 430 nm. Total flavonoid contents were calculated from a calibration curve of rutin analyzed under the same conditions. The flavonoid content was expressed as milligrams of rutin equivalent (RE) per gram of extract.

Assay of DPPH Free-Radical-Scavenging Activity. The free-radical-scavenging activity of marigold flower extracts was assessed using the DPPH method of Shyu and Hwang (*19*) with minor modifications. Briefly, 2.0 mL of 0.1 mM DPPH in ethanol was added to 2.0 mL of the extracts, and the mixture was shaken well. After incubation at 30 °C for 30 min, the absorbance was measured at 517 nm. A control was measured the same way except that the extract was replaced by ethanol. The DPPH free-radical-scavenging activity was expressed as the inhibition of the DPPH radicals by the sample, which was calculated as

$$\text{DPPH radical scavenging activity (\%)} = [1-(A_1/A_0)] \times 100$$

where A_1 is the absorbance of the sample and A_0 is the absorbance of the control.

Total Antioxidant Activity by the ABTS Assay. The capacity of the extracts to scavenge $ABTS^+$ cation radicals was measured according to the method of Siddhuraju (*20*) with Trolox used as a reference. A stable $ABTS^+$ stock solution was first prepared by mixing 10 mL of 5 mM ABTS with 5 mL of 3.0 mM potassium persulfate, and the mixture was kept overnight. Oxidation of the $ABTS^+$ commenced immediately, but it took approximately 6 h for the absorbance to reach a maximum. The radical cation in the stock solution was stable for more than 2 days in storage in the dark at room temperature. Prior to use, the $ABTS^+$ stock solution was diluted with ethanol to give an absorbance of 0.700 ± 0.020 at 734 nm. Upon adding 4.0 mL of the diluted $ABTS^+$ solution to 30 μL of the extracts or Trolox standard solutions (0.2–2.0 mM), the absorbance at 734 nm was measured exactly 10 min after initial mixing. A reagent control was measured the same way, and the percentage inhibition was calculated for the extracts and the Trolox standard solutions. The antioxidant activity of the samples was expressed as Trolox equivalent antioxidant capacity (TEAC) values.

Statistical Analyses. All experiments were conducted in duplicate, and measurements were done in triplicate. The results were presented as the means ± standard deviation. Data were analyzed by one-way analysis of variance using the SPSS 12.0 package, and significant differences of means were determined using Duncan's multiple-range test. A correlation analysis of antioxidant activity versus the polyphenol and flavanoids content was carried out using the correlation and regression program of Microsoft Excel.

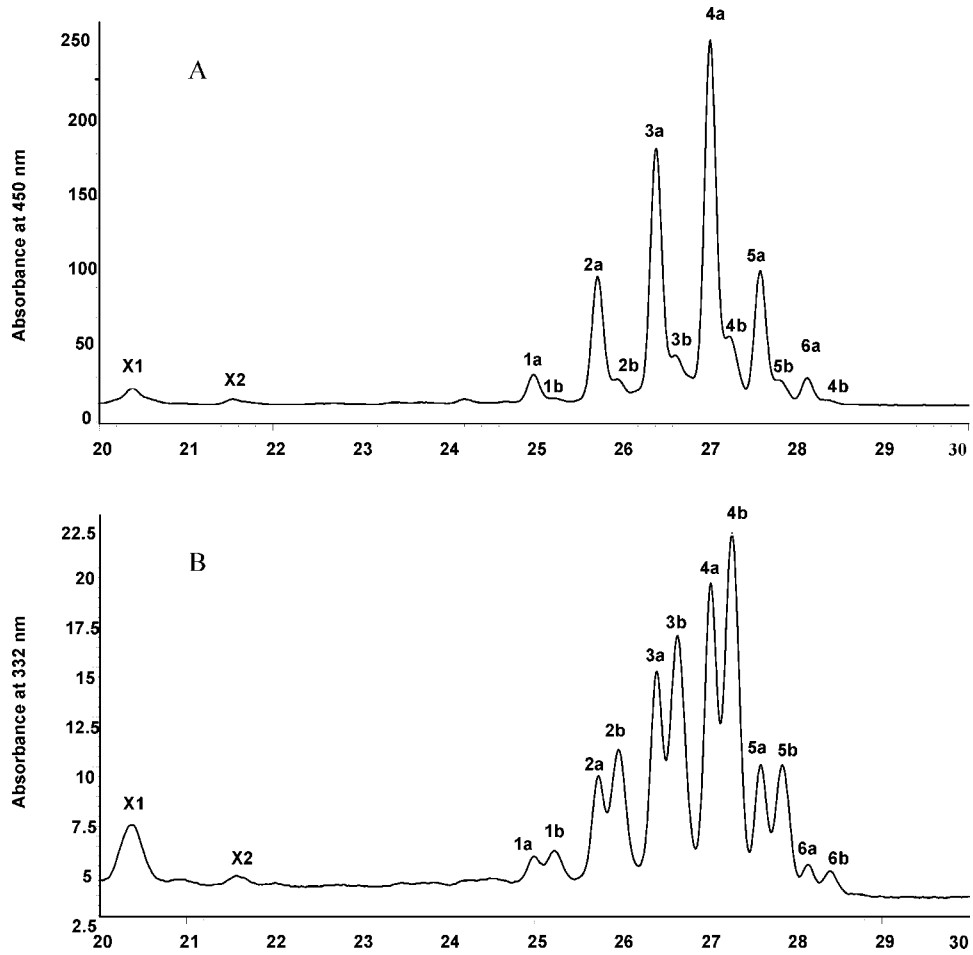

Figure 1. Typical HPLC profiles of the *n*-hexane extract of the marigold flower with detections by DAD at (**A**) 450 nm and (**B**) 332 nm. See **Table 1** for identities of the peaks. Peaks X1 and X2 were unidentified.

Table 1. LC-MS Data of Lutein Esters from Marigold Oleoresin

peak	retention time (min)	identity[a]	M_w	*m/z* (%) $[M + H - FA1]^{+b}$	$[M + H - FA - 92]^+$	$[M + H - FA2]^+$	$[M + H - FA1 - FA2]^+$	$[M + H - FA1 - FA2 - 92]^+$
1a	25.0	all-*trans*-LML	960	733(32)		761(26)	533(100)	441 (8)
1b	25.2	*cis*-LML	960	733(49)		761(70)	533(100)	441(11)
2a	25.7	all-*trans*-DML	988	761(65)	669 (4)	761(65)	533(100)	441(10)
2b	26.0	*cis*- DML	988	761(53)	669 (3)	761(53)	533(100)	441 (5)
3a	26.4	all-*trans*-MPL	1016	761(41)	669 (3)697(4)	789(26)	533(100)	441 (8)
3b	26.7	*cis*-MPL	1016	761(43)	669 (4)697(4)	789(40)	533(100)	441(11)
4a	27.0	all-*trans*-DPL	1044	789(60)	697 (6)	789(60)	533(100)	441(11)
4b	27.3	*cis*-DPL	1044	789(69)	697 (8)	789(69)	533(100)	441(10)
5a	27.6	all-*trans*-PSL	1072	789(28)	725 (4)	817(35)	533(100)	441 (8)
5b	27.9	*cis*-PSL	1072	789(44)	725 (7)	817(60)	533(100)	441(12)
6a	28.2	all-*trans*-DSL	1100	817(88)	725 (8)	817(88)	533(100)	441 (9)
6b	28.4	*cis*-DSL	1100	817(94)	725(23)	817(94)	533(100)	441 (8)

[a] LML, lauroylmyristoyl-lutein; DML, dimyristoyl-lutein; MPL, myristoylpalmitoyl-lutein; DPL, dipalmitoyl-lutein; PSL, palmitoylstearoyl-lutein; DSL, distearoyl-lutein. [b] – FA1 = loss of fatty acid from one end of the lutein diester; – FA2 = loss of fatty acid from the other end of the lutein diester; – FA = loss of fatty acid from either end of the lutein diester; – 92 = loss of tolune.

RESULTS AND DISCUSSION

Analysis of Lutein Diesters in 11 Cultivars of Marigold. The dried marigold flowers were extracted with ethanol, ethyl acetate, and *n*-hexane, and the oleoresins obtained by the *n*-hexane extraction were analyzed by HPLC-MS using a C_{18} reversed-phase column with the APCI-MS operating in the positive-ion mode. **Figure 1** shows typical HPLC chromatograms of marigold flower oleoresins with the peaks detected by DAD at both 450 and 332 nm. The oleoresins from all 11 different cultivars of marigold flowers produced very similar chromatographic profiles characterized by the presence of six major peaks (1a–6a) and six minor peaks (1b–6b) at 450 nm (**Figure 1A**). Peaks 1a–6a showed maximum absorption at 450 nm but little absorption at 332 nm, which was typical of the trans isomers of free lutein and lutein esters (*21*). In contrast, peaks 1b–6b showed an extra absorption at 332 nm (**Figure 1B**), which was typical of the cis isomers of free lutein and lutein esters (*21*). Peaks 1a–6a were identified by comparing their MS and UV spectral data (**Table 1**) with those published in the literature as all *trans*-lauroylmyristoyl-lutein (C12/C14), all *trans*-dimyristoyl-lutein (C14/C14), all *trans*-myristoylpalmitoyl-lutein (C14/C16), all *trans*-dipalmitoyl-lutein (C16/C16),

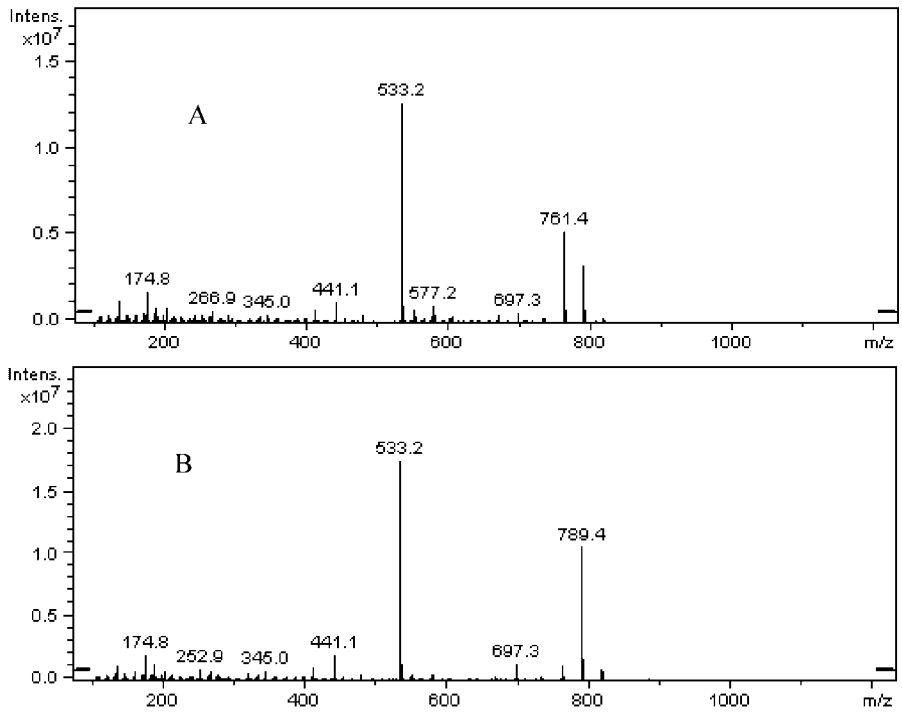

Figure 2. Typical positive-ion APCI mass spectra of lutein diesters. (**A**) Myristoylpalmitoyl-lutein, *m/z* 789 = [M + H − myristic acid]$^+$, *m/z* 761 = [M + H − palmitic acid]$^+$, *m/z* 533 = [M + H − myristic acid − palmitic acid]$^+$; (**B**) dipalmitoyl-lutein, *m/z* 789 = [M + H − palmitic acid]$^+$, *m/z* 533 = [M + H − palmitic acid − palmitic acid]$^+$.

all *trans*-palmitoylstearoyl-lutein (C16/C18), and all *trans*-distearoyl-lutein (C18/C18), respectively. The all *trans*-lutein diester patterns obtained in this study agreed closely with those reported in the literature (*21–23*), although in the latter two studies, the geometric isomers were not identified. Peaks 1b–6b gave very similar fragmentation patterns as peaks 1a–6a, respectively (**Table 1**), and were identified as their respective cis isomers. The position of the cis double bond in the lutein backbone was not determined in this study, but it has been suggested that the most likely positions would be at the 13 or 15 position of the β-ionone ring or the 13′ position of the ε-ring. This is because only a cis double bond in the central region of the lutein backbone would have a dipole moment sufficiently large to produce a significant absorption (*21*).

LC-MS Fragmentation Patterns of Lutein Diesters. The MS data of the lutein diesters showed two general patterns (**Figure 2** and **Table 1**). In the case of lutein acylated with two different fatty acids, the mass spectra showed three major fragments, [M – FA1 + H]$^+$, [M – FA2+ H]$^+$, and [M – FA1 – FA2 + H]$^+$ (FA stands for fatty acid). Two of them resulted from the neutral loss of one fatty acid from the protonated molecule or quasimolecular ion, whereas the third signal represented the lutein backbone after the loss of both fatty acid moieties (**Figure 2A**). In contrast, the mass spectra of lutein acylated with only one type of fatty acid produced only two major fragments; one represented the protonated molecule after the loss of a single fatty acid, while the other corresponded to the lutein backbone (**Figure 2B**). In addition, the loss of *m/z* 92 from the first fragment ion and from the luten backbone ion, which has been attributed to the loss of toluene (*24*), was observed. Under ion trap APCI+ve MS conditions, Young et al. (*24*) also detected the quasimolecular ion [M + H]$^+$ and the quasimolecular ion with the loss of toluene [M + H – 92]$^+$. These ions, however, were not detected in the current study, nor in the study of Breithaupt et al. (*22*) and Tian et al. (*25*). Moreover, Young et al. (*24*) showed that fatty acid or water loss occurred preferentially at the 3′ position in the ε-ring. Using this information, they were able to identify regioisomers of both lutein mono- and diesters in a commercial lutein supplement. However, such preferential fragmentation patterns were not observed in the present study, and therefore the possible existence of regioisomers of lutein diesters in native marigold flower extracts could not be identified. There were also a few small peaks that appeared before the 24th minute in the HPLC chromatograms (**Figure 1**). Two of the peaks (X1 and X2) showed an absorption at 332 nm and gave a fragment ion *m/z* 533 (MS spectra not shown) and therefore could be the cis isomers of lutein monoesters. However, the MS data were not sufficient to allow a positive identification of their chemical structure. No free lutein was detected in any of the marigold extracts, confirming the finding of a previous report (*26*).

Content and Composition of Lutein Esters in the 11 Different Types of Marigold. The 11 cultivars of marigold showed considerable differences in their content of lutein esters, ranging from 161.0 mg/100 g of flowers (dry basis) in the cultivar *Fanmei* 2 to 611.0 mg/100 g in the variety *Chiyu* (**Table 2**). The cultivars of *Xinhong*, *Famei* 1, *Handan*, and *Huangjin* also had relatively high contents of lutein esters (greater than 400 mg/100 g), while the values were much lower in the remaining cultivars. These results are broadly comparable with those reported by Piccaglia et al. (*15*) for the marigold varieties grown in Italy, but some of the very low values (e.g., <20 mg/100 g) reported in their study were not observed in our investigation. Although the different cultivars differed considerably in the total lutein ester content, their lutein ester compositions were rather similar. Dipalmate lutein was the most predominant lutein ester, accounting for 36.3–41.0% of the total lutein esters, followed by myristoylpalmitoyl-lutein (22.0–28.8%), and palmitoylstearoyl-lutein (12.4–19.3%), while distearoyl-lutein (2.4–4.9%) and lauroylmyristoyl-lutein (2.8–4.9%) were the least predominant lutein diesters in the marigold extract. This trend was consistent in all 11 marigold cultivars studied. The lutein diester compositions obtained in the current study were in general agreement with those reported by Piccaglia et

Table 2. Total Content and Composition of Lutein Diesters in Different Cultivars of Marigold

cultivar	composition (%)						total content (mg XE/100 g of dry flower)[b]
	LML[a]	DML	MPL	DPL	PSL	DSL	
Biza	4.1 ± 0.3	11.7 ± 1.2	25.3 ± 2.2	37.8 ± 2.9	17.1 ± 1.7	3.9 ± 0.4	305.2 ± 23.9 c
Chiju	4.5 ± 0.4	13.1 ± 0.9	27.3 ± 1.8	36.4 ± 3.0	15.4 ± 1.3	3.4 ± 0.3	611.1 ± 43.9 g
Chiyu	4.2 ± 0.4	14.4 ± 1.3	27.5 ± 2.5	37.6 ± 2.9	13.7 ± 1.1	2.6 ± 0.2	360.5 ± 27.7 d
Fanmei 1	3.0 ± 0.3	10.9 ± 0.8	22.7 ± 1.9	39.1 ± 3.3	19.3 ± 1.7	4.8 ± 0.5	458.9 ± 41.7 e
Fanmei 2	3.1 ± 0.4	10.6 ± 0.9	22.0 ± 2.4	41.0 ± 3.8	18.3 ± 1.6	4.9 ± 0.4	161.0 ± 13.5 a
Handan	4.4 ± 0.5	12.1 ± 1.0	25.8 ± 2.2	38.2 ± 3.2	16.1 ± 1.6	3.4 ± 0.2	450.0 ± 44.1 e
Huangjin	2.8 ± 0.3	11.9 ± 1.1	24.7 ± 2.3	39.3 ± 3.1	16.9 ± 1.5	4.4 ± 0.5	416.4 ± 31.7 e
Xinhong	3.9 ± 0.4	14.9 ± 1.1	28.8 ± 2.2	37.5 ± 3.3	12.4 ± 0.9	2.4 ± 0.2	542.6 ± 46.6 f
Shainuo	4.3 ± 0.5	12.3 ± 1.2	26.3 ± 1.9	37.9 ± 3.4	15.6 ± 1.9	3.5 ± 0.3	247.4 ± 17.0 b
Shengza	4.2 ± 0.3	12.9 ± 0.9	26.6 ± 2.4	38.1 ± 3.8	15.2 ± 1.5	3.0 ± 0.3	250.1 ± 17.2 b
Zajiao	4.9 ± 0.4	14.7 ± 1.1	27.9 ± 2.3	36.3 ± 3.5	13.0 ± 1.1	2.6 ± 0.2	210.4 ± 19.1ab

[a] LML, lauroylmyristoyl-lutein; DML, dimyristoyl-lutein; MPL, myristoylpalmitoyl-lutein; DPL, dipalmitoyl-lutein; PSL, palmitoylstearoyl-lutein; DSL, distearoyl-lutein. Data were combined values of the trans and cis isomers of the lutein diesters. [b] XE, xanthophylls (mostly lutein) equivalent; data not followed by the same letter differ significantly ($P < 0.05$).

Table 3. Total Phenolic and Flavonoid Contents and DPPH Radical Scavenging and ABTS Antioxidant Activities in the Extracts of 11 Different Cultivars of Marigold[a]

cultivar of marigold	total phenolic content (mg GAE/g of extract)	total flavonoid content (mg RE/g of extract)	DPPH radical scavenging activity (% RSA)	antioxidant activity (mmol TEAC/g of extract)
		Ethanol Extract		
Biza	144.2 ± 6.7 c	66.9 ± 3.0 d	83.7 ± 4.9 d	15.8 ± 1.4 e
Chiju	165.7 ± 7.1 f	73.4 ± 3.5 ef	89.4 ± 5.0 de	15.0 ± 1.4 de
Chiyu	142.0 ± 5.4 c	58.5 ± 3.0 c	82.5 ± 4.7 d	14.7 ± 1.5 cde
Fanmei 1	159.2 ± 4.9 ef	70.3 ± 3.4 de	87.7 ± 4.3 de	12.1 ± 1.3 bc
Fanmei 2	81.8 ± 3.1 a	33.4 ± 2.2 a	43.8 ± 3.0 a	7.7 ± 0.9 a
Handan	166.4 ± 8.0 f	78.8 ± 3.8 f	88.7 ± 4.0 de	12.8 ± 1.1 bcd
Huangjin	154.7 ± 7.6 de	75.6 ± 3.7 ef	86.2 ± 4.0 de	18.6 ± 1.6 f
Xinhong	223.3 ± 12.0 g	93.3 ± 4.0 g	93.0 ± 4.6 e	16.6 ± 1.6 ef
Shainuo	152.6 ± 5.0 d	54.9 ± 2.9 c	72.5 ± 3.3 c	14.2 ± 1.8 cde
Shengza	109.4 ± 3.9 b	47.9 ± 2.7 b	64.6 ± 3.1 b	18.7 ± 1.8 f
Zajiao	110.3 ± 4.1 b	28.6 ± 1.7 a	39.4 ± 3.0 a	10.6 ± 1.1 b
		Ethyl Acetate Extract		
Biza	35.8 ± 2.5 bc	29.0 ± 1.8 d	31.8 ± 3.0 c	2.2 ± 0.03 d
Chiju	33.9 ± 2.3 b	35.2 ± 2.1 f	42.7 ± 4.0 d	2.8 ± 0.03 g
Chiyu	22.9 ± 2.0 a	22.3 ± 1.6 c	22.5 ± 1.8 b	1.6 ± 0.01 b
Fanmei 1	36.6 ± 2.1 bc	32.4 ± 2.0 ef	41.9 ± 3.8 d	2.3 ± 0.04 e
Fanmei 2	25.1 ± 1.9 a	10.8 ± 1.0 a	7.7 ± 0.9 a	1.0 ± 0.01 a
Handan	38.7 ± 2.6 c	30.9 ± 2.0 de	45.7 ± 3.6 d	2.8 ± 0.03 g
Huangjin	38.7 ± 3.1 c	28.5 ± 2.0 d	44.0 ± 3.4 d	2.6 ± 0.04 f
Xinhong	89.2 ± 3.4 d	40.6 ± 2.4 g	69.5 ± 4.9 e	3.6 ± 0.07 h
Shainuo	25.8 ± 2.4 a	16.1 ± 1.3 b	23.4 ± 1.9 b	1.7 ± 0.02 c
Shengza	25.2 ± 1.7 a	17.6 ± 1.4 b	20.2 ± 1.9 b	1.7 ± 0.02 b
Zajiao	34.1 ± 2.1 b	11.2 ± 1.0 a	8.2 ± 1.1 a	0.9 ± 0.01 a
		n-Hexane Extract		
Biza	3.4 ± 0.1 cd	13.1 ± 1.2 cd	5.8 ± 0.6 a	3.7 ± 0.05 b
Chiju	2.9 ± 0.1 b	20.3 ± 1.5 f	11.7 ± 1.2 c	4.0 ± 0.04 c
Chiyu	2.5 ± 0.2 a	14.5 ± 1.2 d	11.9 ± 1.0 c	4.1 ± 0.04 d
Fanmei 1	3.2 ± 0.2 bc	17.5 ± 1.6 e	9.8 ± 1.2 b	3.7 ± 0.05 b
Fanmei 2	3.3 ± 0.1 bc	9.1 ± 0.9 a	9.1 ± 1.1 b	3.6 ± 0.02 a
Handan	2.6 ± 0.1 a	17.6 ± 1.8 e	6.2 ± 0.7 a	4.0 ± 0.03 c
Huangjin	4.1 ± 0.4 e	15.0 ± 1.4 d	9.7 ± 1.0 b	3.8 ± 0.04 b
Xinhong	3.1 ± 0.2 b	21.4 ± 2.0 f	9.3 ± 1.0 b	4.0 ± 0.04 c
Shainuo	3.6 ± 0.3 d	10.3 ± 0.9 ab	10.2 ± 0.9 bc	4.3 ± 0.03 e
Shengza	3.0 ± 0.1 b	11.7 ± 1.0 bc	11.7 ± 1.0 c	3.8 ± 0.04 b
Zajiao	2.4 ± 0.1 a	9.0 ± 0.8 a	9.2 ± 0.9 b	3.6 ± 0.03 a

[a] For the same solvent, data within the same column not followed by the same letter differ significantly ($P < 0.05$).

al. (*15*), although they could only identify five lutein diesters (without lauroylmyristoyl-lutein). Piccaglia et al. (*15*) also reported the presence of free lutein and three lutein monoesters in marigold flower, while Young et al. (*24*) identified four regioisomers of lutein monoesters in a lutein supplement. Other researchers (*21, 22*) did not report the presence of these components in the marigold flower. In the present study, we also failed to positively identify the presence of either free lutein or lutein monoesters, although there were a few small peaks that could be due to lutein monoesters. We did detect the presence of cis isomers of lutein diesters in the marigold flower, but their concentration was generally five- to seven-fold lower than that of their all-trans counterparts (data not shown).

Total Phenolic and Flavonoid Content and Antioxidant Activity of Marigold Flower Extracts. Table 3 presents the contents of total phenolics and flavonoids together with the antioxidant activities of the three different solvent extracts of the marigold flower. The phenolic and flavonoid contents of the ethanol extracts were markedly higher than those of the ethyl acetate and *n*-hexane extracts (**Table 3**). This is expected as

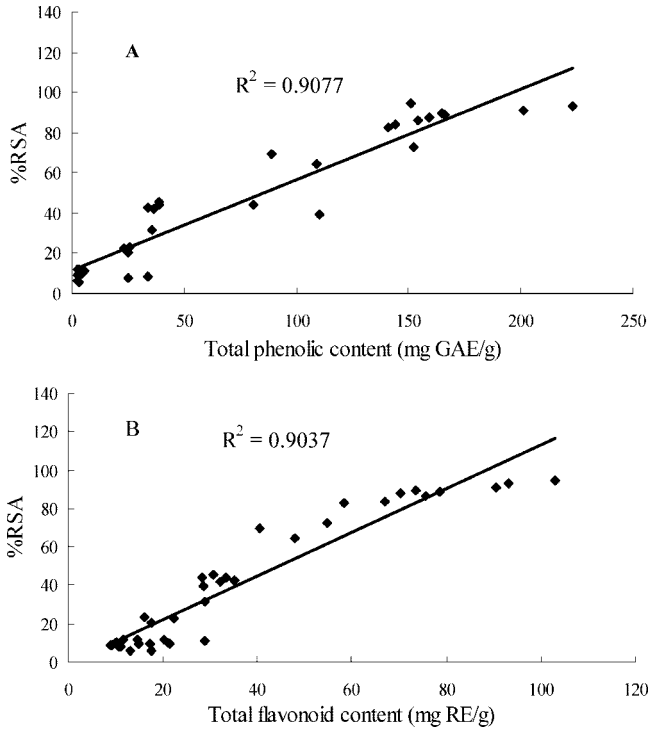

Figure 3. Linear correlation of radical-scavenging activity (RSA) with respect to (**A**) total phenolic and (**B**) flavonoid contents of 11 different cultivars of marigold flower.

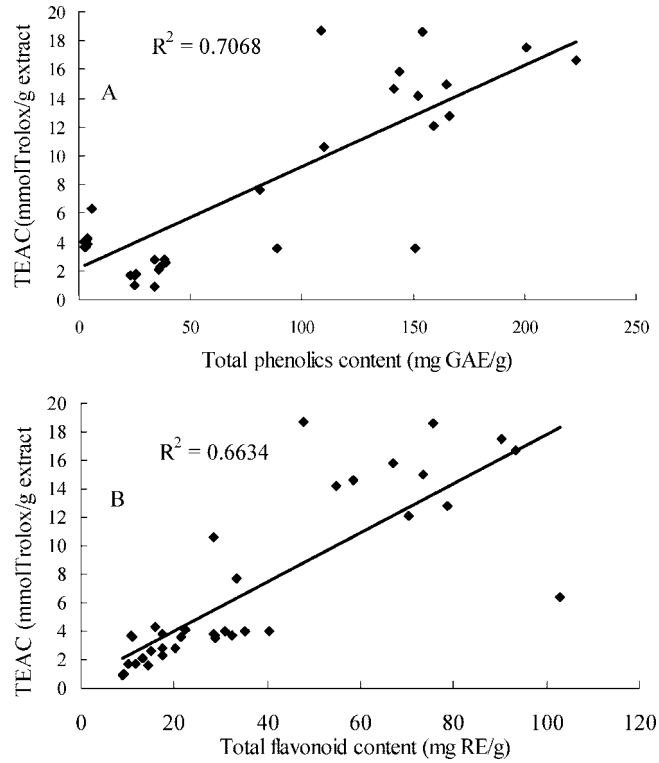

Figure 4. Linear correlation of Trolox equivalent antioxidant capacity (TEAC) with respect to (**A**) total phenolic and (**B**) flavonoid contents of 11 different cultivars of marigold flowers.

most phenolics and flavonoids are hydrophilic compounds that have better solubility in polar solvents such as ethanol. The amount of total phenolics varied considerably in the 11 cultivars of the marigold flower, ranging from 81.8 to 223.3 mg, 22.9 to 89.2 mg, and 2.4 to 4.1 mg GAE/g of extract in the ethanol, ethyl acetate, and *n*-hexane extracts, respectively. Total flavonoids varied from 28.6 to 93.3 mg, 10.8 to 40.6 mg, and 9.0 to 21.4 mg RE/g of extract in the ethanol, ethyl acetate, and *n*-hexane extracts, respectively. The highest phenolic and flavonoid contents were found in the cultivar *Xinghong*, and the contents were lowest in *Fanmei* 2 (phenolic content) and *Zajiao* (flavonoid content). Previous research has observed large variations in the total phenolic and flavonoid contents between two different species of marigold (*14*). Our results showed that wide variations (almost 4-fold) in the contents of the two groups of compounds also existed among the different marigold cultivars of the same species.

In general, most phenolics and flavonoids possess some degree of antioxidant activity. Therefore, extracts with a higher phenolic or flavonoid content would generally show higher antioxidant activity, and some good correlations have been found among these parameters (*26*). Among the extracts of the marigold flower, the highest DPPH radical-scavenging-activity (RSA) was found in the ethanol extracts, followed by the ethyl acetate extracts, while the *n*-hexane extracts exhibited the lowest activity (**Table 3**). A regression analysis showed good correlations between total phenolic and flavonoid contents and RSA with regression coefficients greater than 0.90 ($P < 0.01$, **Figure 3**), indicating that phenols and flavonoids are major contributors to the RSA of the extracts. This result is in agreement with previous reports that the phenols and flavonoids contribute significantly to the RSA in different plants (*27*, *28*). For the ethanol extracts, the cultivar of *Xinhong* exhibited the highest RSA with an inhibition rate of 93.0%, while *Zajiao* had the lowest activity at 39.4%. For the ethyl acetate extracts, these two cultivars also exhibited the highest and lowest RSA, respectively.

The total antioxidant activity of phenols and flavonoids is mainly due to their redox properties, which make them act as reducing agents, hydrogen donors, and singlet oxygen quenchers (*29*). The extracts of marigold flowers showed significant antioxidant activities through their ability to scavenge the $ABTS^+$ radical cation. The $ABTS^+$ assay was calibrated with the water-soluble α-tocopherol analogue, Trolox, and the antioxidant activities of the extracts were expressed as TEAC. The TEAC values of the ethanolic extracts of marigold flowers varied from 7.7 to 18.7 mmol of Trolox /g of extract, which were much higher than the values of the ethyl acetate (1.0–3.6 mmol) and *n*-hexane (3.6–4.3 mmol) extracts. Surprisingly, the TEAC values of the ethyl acetate extracts were generally lower than the corresponding *n*-hexane extracts, although the phenolic and flavonoid contents of the former were considerably greater than the latter. Furthermore, the correlation coefficients (R^2) between TEAC values and the total phenols and flavonoids were 0.7068 and 0.6634 ($P < 0.01$, **Figure 4**), respectively, which were considerably smaller than the corresponding correlation coefficients for RSA. These results appeared to suggest that, apart from phenols and flavonoids, other compounds in the extracts, for example, lutein esters, may also contribute significantly to the antioxidant activity of marigold extracts. However, when a correlation analysis was carried out on the lutein diester content and RSA of the extracts, a significant correlation was not found ($P < 0.05$). Of the 11 cultivars of marigold, *Shengza* had the highest TEAC value of 18.7 mmol of Trolox/g of extract, followed closely by *Huangjin* (18.6 mmol) and *Xinhong* (16.7 mmol), while *Fanmei* 2 had the lowest value of 7.6 mmol of Trolox/g of extract.

In conclusion, the current study showed that variations of up to several-fold in the lutein ester content existed in different cultivars of marigold of the same species. LC-MS analysis

confirmed that the lutein esters in marigold consisted predominantly of six all-*trans*-diesters, and the lutein ester compositions were rather similar in all of the 11 marigold cultivars examined. LC-MS coupled with spectral analysis showed that the marigold oleoresin also contained small amounts of cis isomers of lutein diesters. The different cultivars of marigold also showed considerable variations in their total phenols and flavonoids, as well as antioxidant and radical-scavenging activities. The cultivar *Xinhong* was found to have the highest phenolic and flavonoid contents and DPPH radical-scavenging activity, as well as one of the highest lutein contents and total antioxidant activities and therefore is a promising candidate for the production of marigold extracts.

LITERATURE CITED

(1) Vasudevan, P.; Kashyap, S.; Sharma, S. Tagetes: a multipurpose plant. *Bioresour. Technol.* **1997**, *62*, 29–35.

(2) Zitterl-Eglseer, K; Sosa, S.; Jurenitsch, S.; Schubert-Zsilavecz, J.; Loggia, M.; Tubaro, R.; Bertoldi, A.; Franz, M. Anti-oedematous activities of the main triterpendiol esters of marigold (*Calendula officinalis* L.). *J. Ethnopharmacol.* **1997**, *57*, 139–144.

(3) Soon, P. J.; Chew, B. P.; Wong, T. S. Dietary lutein from marigold extract inhibits mammary tumor development in BALB/c mice. *J. Nutr.* **1998**, *128*, 1650–1656.

(4) Chew, B. P.; Wong, M. W.; Wong, T. S. Effects of lutein from marigold extract on immunity and growth of mammary tumors in mice. *Anticancer Res.* **1996**, *16*, 3689–3694.

(5) Zhang, L. X.; Coney, R. V.; Bertram, J. S. Carotenoids enhance gap functional communication and inhibit lipid peroxidation in C3H/10T/2 cells: relationship to their cancer chemopreventive action. *Carcinogenesis* **1991**, *12*, 109–114.

(6) Subagio, A.; Naofumi, M. Prooxidant activity of lutein and its dimyristate esters in corn triacylglyceride. *Food Chem.* **2003**, *81*, 97–102.

(7) Junghans, A.; Sies, H.; Stahl, W. Macular Pigments Lutein and zeaxanthin as blue light filters studied in liposomes. *Arch. Biochem. Biophys.* **2001**, *391*, 160–164.

(8) Olmedilla, B.; Granado, F.; Blanco, I.; Vaquero, M. Lutein, but not alpha-tocopherol, supplementation improves visual function in patients with age-related cataracts: a 2-year doubleblind, placebo-controlled pilot study. *Nutrition* **2003**, *19*, 21–24.

(9) Dwyer, J. H.; Navab, M.; Dwyer, K. M.; Hassan, K.; Sun, P.; Shircore, A.; Hama-Levy, S.; Hough, G.; Wang, X.; Drake, T.; Mer, C. N.; Fogelman, A. M. Oxygenated carotenoid lutein and progression of early atherosclerosis: The Los Angeles Atherosclerosis Study. *Circulation* **2001**, *103*, 2922–2927.

(10) Dermatol, J. I.; Stahl, W.; Heinrich, U.; Jungmann, H.; Sies, H.; Tronnier, H. Carotenoids and carotenoids plus Vitamin E protect against ultraviolet light-induced erythema in humans. *Am. J. Clin. Nutr.* **2000**, *71*, 795–798.

(11) Lu, Y.; Foo, L. Y. Antioxidant and radical scavenging activities of polyphenols from apple pomace. *Food Chem.* **2000**, *68*, 81–85.

(12) Rakic, S.; Povrenovic, D.; Tesevic, V.; Simic, M.; Maletic, R. Oak acorn, polyphenols and antioxidant activity in functional food. *J. Food Eng.* **2006**, *74*, 416–423.

(13) Lampart-Szczapa, E.; Korczak, J.; Nogala-Kalucka, M.; Zawirska-Wojtasiak, R. Antioxidant properties of lupin seed products. *Food Chem.* **2003**, *83*, 279–285.

(14) Cetkovic, G. S.; Djilas, S. M.; Canadanovic-Brunet, J. M.; Tumbas, V. T. Antioxidant properties of marigold extracts. *Food Res. Int.* **2004**, *37*, 643–650.

(15) Piccaglia, R.; Marotti, M.; Grandi, S. Lutein and lutein ester content in different types of *Tagetes patula* and *T. erecta*. *Ind. Crops Prod.* **1998**, *8*, 45–51.

(16) *Official Methods of Analysis*, 15th ed.; Association of Official Analytical Chemists: Arlington, Virginia, 1990; pp 1048–1049.

(17) Marquele, F. D.; Di Mambro, V. M.; Georgetti, S. R.; Casagrande, R.; Yara, M. L.; Valim, Y. M. L.; Fonseca, M. J. V. Assessment of the antioxidant activities of Brazilian extracts of propolis alone and in topical pharmaceutical formulations. *J. Pharm. Biomed. Anal.* **2005**, *39*, 455–462.

(18) Djeridane, A.; Yousfi, M.; Nadjemi, B.; Boutassouna, D.; Stocker, P.; Vidal, N. Antioxidant activity of some algerian medicinal plants extracts containing phenolic compounds. *Food Chem.* **2006**, *97*, 654–660.

(19) Shyu, Y. S.; Hwang, L. S. Antioxidant activity of the crude extract of crude extract of lignan glycosides fromunroasted Burma black sesame meal. *Food Res. Int.* **2002**, *35*, 357–365.

(20) Siddhuraju, P. The antioxidant activity and free radical-scavenging capacity of phenolics of raw and dry heated moth bean (*Vigna aconitifolia*) (Jacq.) Marechal seed extracts. *Food Chem.* **2006**, *99*, 149–157.

(21) Tsao, R; Yang, R; Young, J. C.; Zhua, H; Manolis, T. Separation of geometric isomers of native lutein diesters in marigold (*Tagetes erecta* L.) by high-performance liquid chromatography-mass spectrometry. *J. Chromatogr., A* **2004**, *1045*, 65–70.

(22) Breithaupt, D. E.; Wirt, U; Bamedi, A. Differentiation between lutein monoester regioisomers and detection of lutein diesters from marigold flowers (*Tagetes erecta* L.) and several fruits by liquid chromatography-mass spectrometry. *J. Agric. Food Chem.* **2002**, *50*, 66–70.

(23) Gregory, G. K.; Chen, T. S.; Philip, T. Quantitative analysis of lutein esters in marigold flowers by high performance liquid chromatography. *J. Food Sci.* **1986**, *51*, 1093–1094.

(24) Young, J. C.; Abdel-Aal, E. M.; Rabalski, I.; Blackwell, B. A. Identification of synthetic regioisomeric lutein esters and their quantification in a commercial lutein supplement. *J. Agric. Food Chem.* **2007**, *55*, 4965–4972.

(25) Tian, Q.; Duncan, C. J. G.; Schwartz, S. J. Atmospheric pressure chemical ionization mass spectrometry and in-source fragmentation of lutein esters. *J. Mass Spectrom.* **2003**, *38*, 990–995.

(26) Rivas, J. D. L. Reversed-phase high-performance liquid chromatographic separation of lutein and lutein fatty acid esters from marigold flower petal powder. *J. Chromatogr.* **1989**, *464*, 442–447.

(27) Parejo, I.; Viladomat, F.; Bastida, J.; Rosas-Romero, A.; Saavedra, G.; Murcia, M. A.; Jimenez, A. M.; Codina, C. Investigation of Bolivian plant extracts for their radical scavenging activity and antioxidant activity. *Life Sci.* **2003**, *73*, 1667–1681.

(28) Su, M.-S.; Silva, J. L. Antioxidant activity, anthocyanins, and phenolics of rabbiteye blueberry (*Vaccinium ashei*) by-products as affected by fermentation. *Food Chem.* **2006**, *97*, 447–451.

(29) Peschel, W.; Sanchez-Rabaneda, F.; Diekmann, W.; Plescher, A.; Gartzia, I.; Jimenez, D.; Lamuela-Raventos, R.; Buxaderas, S.; Codina, C. An industrial approach in the search of natural antioxidants from vegetable and fruit wastes. *Food Chem.* **2006**, *97*, 137–150.

Received for review June 10, 2007. Revised manuscript received August 6, 2007. Accepted August 7, 2007. This research was supported by the Chinese Postdoctoral Fund (No. 20060400534).

JF071696J