DOI:10.1021/jf904278r

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Comparison of Different Strategies for Soybean Antioxidant Extraction

Hyun Chung,[†] Xiangming Ji,[§] Corene Canning,[§] Shi Sun,[§] and Kequan Zhou*[,§]

[†]Department of Food Science and Technology, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061, and [§]Department of Nutrition and Food Science, Wayne State University, Detroit, Michigan 48202

Three extraction strategies including Soxhlet extraction, conventional solid−liquid extraction, and ultrasonic-assisted extraction (UAE) were compared for their efficiency to extract phenolic antioxidants from Virginia-grown soybean seeds. Five extraction solvents were evaluated in UAE and the conventional extraction. The soybean extracts were compared for their total phenolic contents (TPC), oxygen radical absorbance capacity (ORAC), and 2,2-diphenyl-1-picrylhydrazyl radical ($DPPH^{\bullet}$) scavenging activities. The results showed that UAE improved the extraction of soybean phenolic compounds by >54% compared to the conventional and Soxhlet extractions. Among the tested solvents, 50% acetone was the most efficient for extracting soybean phenolic compounds. There was no significant correlation between the TPC and antioxidant activities of the soybean extracts. The extracts prepared by 70% ethanol had the highest ORAC values. Overall, UAE with 50% acetone or 70% ethanol is recommended for extracting soybean antioxidants on the basis of the TPC and ORAC results.

KEYWORDS: Soybean; Soxhlet extraction; ultrasonic-assisted extraction; total phenolic content; antioxidant activity

INTRODUCTION

Consumption of soy-based products has been associated with the reduced risk of hormone-based cancer and cardiovascular diseases (*1*, *2*). Such potential health benefits were in part attributable to beneficial antioxidant compounds in soybean seeds. In this regard, natural antioxidants present in soybeans have received extensive public attention. A wide variety of health-promoting activities have been demonstrated on soybean antioxidants, such as preventing low-density lipoprotein (LDL) from oxidative modification (*3*), inhibiting lipid oxidation (*4*), scavenging oxidative species (*5*), and promoting the expression of antioxidative enzymes in cells (*6*).

Major soybean antioxidants include isoflavones (also known as phytoestrogens), proanthocyanidins, and phenolic acids such as caffeic acid, chlorogenic acid, and ferulic acid (*7*). Most studies on soybean antioxidants have focused on their bioactivities and potential health benefits, whereas less research effort has been devoted to the extraction and preparation of soybean antioxidants. With the increasing popularity of soybean antioxidants for health promotion, it is important to develop a practical and cost-effective process that can efficiently isolate the major antioxidants from soybeans and therefore promote the production of soybean antioxidants for human consumption.

*Address correspondence to this author at the Department of Nutrition and Food Science, 3009 Science Hall, 5045 Cass Ave., Detroit, MI 48202 [telephone (313) 577-3444; fax (313) 577-8616; e-mail kzhou@wayne.edu].

Conventional solid−liquid extraction of natural antioxidants from agricultural products involves water mixtures of different organic solvents. Solvent systems, such as 50% ethanol, 70% methanol, 80% methanol, 50% acetone, and 80% acetone, have been frequently used for extracting natural antioxidants in vegetables, fruits, cereals, and other food products (*8*, *9*). A previous study compared six different solvent mixtures and determined that 50% acetone was the most efficient solvent for extracting phenolic compounds in yellow soybeans (*5*). Other solvents reported for soybean antioxidant extraction included 70% ethanol (*10*), 80% ethanol (*11*), acidified methanol (*4*), and 70% acidified acetonitrile (*12*).

Instrument-assisted techniques such as ultrasonic-assisted extraction (UAE) and supercritical fluid extraction (SFE) (*13*) have recently been applied in natural antioxidant extraction (*14*). UAE has become a popular alternative method to extract antioxidants in food materials, herbs, and other natural products. For instance, aqueous ethanol extractions assisted by the ultrasonic treatment have been investigated to isolate natural antioxidants in fruits, wheat bran, and plants (*8*, *15*−*17*). Other studies reported the use of UAE with different solvent mixtures such as 70% acetone, 40% ethanol, and 60% ethanol to extract isoflavones and other antioxidants in soybean seeds (*4*, *14*, *18*). Although these studies evaluated the applicability of UAE for soybean antioxidant extraction, it is still unknown how efficient UAE is compared to commonly used conventional and Soxhlet extractions for soybean antioxidants because there are currently no reports on direct comparison of the three methods. The ideal

 Published on Web 03/22/2010

method would be the one that can maximize antioxidant extractability from soybean seeds while minimizing solvent and time consumption. Most available reports have focused on the selection of extraction solvents for soybean antioxidants rather than the utilization of different extraction technologies (*5*, *19*, *20*). The objective of this study is therefore to directly compare UAE, conventional solid−liquid extraction, and Soxhlet extraction for their extraction efficiency on soybean antioxidants. This study may lay the foundation for future development of a practical and cost-effective preparation of natural antioxidants from soybean seeds.

MATERIALS AND METHODS

Materials. Three soybean varieties, NC Roy, V00-3493, and V00-3636, were used in this investigation. The soybeans were grown in Warsaw, VA, by a soybean breeding project at Virginia Polytechnic Institute and State University and harvested in 2006. Folin−Ciocalteu reagent, fluorescein (FL), 2,2′-bipyridyl, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), and phenolic acid standards were purchased from Sigma-Aldrich (St. Louis, MO), and 2,2′-azobis(2-aminopropane) dihydrochloride (AAPH) was purchased from Wako Chemicals USA (Richmond, VA). Other reagents and solvents were purchased from Sigma-Aldrich.

Preparation of Soybean Seed Samples. A portion of soybean seeds (15 g) was ground using a Bel-Art micromill grinder (Pequannock, NJ) for 3 min. The ground sample was sieved through a 150 mesh Gilson sieve. The retained residue was ground repeatedly to pass the sieve with a particle size of approximately 110 μm or less. The same process was repeated for three more portions. The ground samples were combined and blended to obtain homogeneous powder for further extraction experiments.

Conventional Solid−Liquid Extraction. The ground seed samples were extracted with five different solvent mixtures: 50 or 80% acetone, 50 or 70% ethanol, or 80% methanol (v/v). The extraction was conducted at the sample mass/solvent ratio of 1:20 (g/mL) under shaking in a dark room at ambient temperature for 15 h. We used this ratio because our previous experiments showed that the soybean seed/solvent ratio of 1:10 (g/mL) for 15 h is sufficient to extract most phenolic compounds by 50% acetone (*21*). Considering the different extraction efficiencies of the solvent mixtures used in this experiment, we reduced the seed/solvent ratio to 1:20 to ensure that the maximal extraction of soybean antioxidants can be achieved. After filtration (Whatman no. 2 filter paper), the extracts were centrifuged by an optima L-90K ultracentrifuge (Beckman Coulter Inc., Brea, CA) at 1500*g* and 4 °C for 10 min. The supernatant was collected and further filtered with a 0.45 μm syringe filter (Acrodisc, Gelmen Science). The clear filtrate was kept in the refrigerator for further antioxidant analysis.

Soxhlet Extraction. Methanol and ethanol were used and compared in Soxhlet extraction. The ground soybean samples were placed in a thimble and extracted with absolute ethanol or methanol (1:20, m/v) in a Soxhlet extractor for 4 h at 80 °C. After cooling, the extracts were filtered and kept in the refrigerator for further antioxidant analysis.

Ultrasonic-Assisted Extraction. The ground soybean samples were placed in a sealed flask containing the extraction solvents (1:20, m/v), 50 or 80% acetone, 50 or 70% ethanol, or 80% methanol, and extracted with an ultrasound liquid processor (Sonicator 3000, Misonix, Farmingdale, NY). The actual power delivered into the extraction system was 40 W (at 20% amplitude) for 3 min (1 min at a time to control temperature). An ultrasonic probe with a tip diameter of 7 mm was fitted into the flask and the tip was inserted at half of the height of the extraction solvent. The extracts were filtered and kept in the refrigerator for further antioxidant analysis.

Total Phenolic Content (TPC). The TPC of soybean extracts was determined using Folin−Ciocalteu reagent with gallic acid as phenolic standard (*22*). In brief, appropriate dilutions of the extracts were mixed with Folin−Ciocalteu reagent and 20% sodium carbonate (Na_2CO_3) at ambient temperature. After incubation for 2 h, the blue color developed in each assay mixture was recorded at 760 nm (Thermo Electron Corp., Genesys 10-UV scanning, Madison, WI). The TPC value of the soybean extracts was expressed in micrograms of gallic acid equivalent (GAE) per gram of soybean on a dry weight basis.

Table 1. Extraction Yield of Soybean Seeds with Different Extraction Methods

	extraction yield (g/10 g)		
extraction method[a]	NC Roy	V00-3493	V00-3636
C−50% acetone	1.46	1.33	1.37
C−80% acetone	1.14	0.99	1.16
C−50% ethanol	1.20	1.21	1.14
C−70% ethanol	0.98	1.03	1.06
C−80% methanol	1.07	1.27	1.17
UAE−50% acetone	1.08	0.88	1.13
UAE−80% acetone	0.79	1.04	0.97
UAE−70% ethanol	0.81	0.96	1.08
UAE−80% methanol	0.75	1.03	0.86
Soxhlet−ethanol	1.08	0.97	1.09
Soxhlet−methanol	1.14	1.01	1.07

[a] C and UAE represent conventional solvent extraction and ultrasonic-assisted extraction, respectively.

Oxygen Radical Absorbance Capacity (ORAC). The ORAC assay was conducted to measure the peroxyl radical scavenging activity of soybean samples with Trolox as an antioxidant standard according to the method reported previously (*23*). Trolox is a water-soluble analogue of vitamin E. In brief, a fluorescein stock solution (100 μM) in phosphate buffer (75 mM, pH 7.4) was prepared and kept in the dark at 4 °C. A fresh working fluorescein solution (100 nM) was prepared daily by diluting the stock solution in phosphate buffer. The working fluorescein solution (200 μL) was added to 40 μL of sample or Trolox (20, 40, 80,100, and 200 μM) in a black 96-well plate and incubated for 20 min at 37 °C. The reaction was initiated by adding the peroxyl radical generator prepared in phosphate buffer. Specifically, 35 μL of 0.36 M 2,2′-azobis-2-amidinopropane (AAPH) was added, and the fluorescence was measured (λ_{ex} = 485 nm and λ_{em} = 535 nm) every minute using a Victor multilabel plate reader (Perkin-Elmer, Turku, Finland) maintained at 37 °C until the reading had declined to < 5% of the initial reading. Standards and samples were run in triplicate. Results for ORAC were determined by using a regression equation relating Trolox concentrations and the net area under the kinetic fluorescein decay curve. The ORAC value of each soybean extract was expressed in micromoles of Trolox equivalents per gram of sample on a dry weight basis (μmol/g).

DPPH• Scavenging Activity. DPPH• scavenging assay was carried out in a 96-well plate. In brief, the reaction mixture contained 100 μL of antioxidant soybean extracts and 100 μL of 0.208 mM DPPH• solution. The absorption at 515 nm was determined immediately when the reaction was initiated by gentle shaking. Each plate was read once every minute for 30 min. The relative DPPH• scavenging capacities were expressed as micromoles of Trolox equivalents (TE) per gram of sample on a dry weight basis (μmol of TE/g).

RESULTS AND DISCUSSION

Total Phenolic Content. The extraction yields by different solvents and technologies are shown in **Table 1**. **Table 2** shows the TPC values of the soybean samples by different extraction methods. For the conventional solid−liquid extraction, the TPC values of soybean extracts prepared by five different solvents were significantly different for each soybean variety, ranging from 3.13 to 3.42 mg of GAE/g for NY Roy, from 2.75 to 3.22 mg of GAE/g for V00-3493, and from 2.50 to 3.45 mg of GAE/g for V00-3636. The result suggested a significant effect of solvents on the extraction of phenolic compounds in soybean seeds. The TPC of the Virginia soybeans under our experimental conditions were comparable to previous studies. Xu and Chang reported a TPC of 2.67 mg of GAE/g in yellow soybean prepared by 50% acetone extraction, whereas Lin and Lai reported a TPC of 4.5 mg of GAE/g in a soybean sample by 80% methanol extraction (*5*, *24*, *25*). It should be noted that the difference of TPC values may be in part due to their soybean varietal difference because the previous study suggested that soybean varieties have significant impact on their total phenolic content (*21*). Therefore,

to avoid the interference of soybean varieties, we examined three soybean samples for comparison in this study. For NC Roy and V00-3493 soybean seeds, the extracts prepared by 50% ethanol and 50% acetone showed the highest TPC, followed by 70% ethanol, 80% methanol, and 80% acetone. For V00-3636 soybean seeds, the extract prepared by 50% acetone showed significantly higher TPC than the extracts prepared by other solvents ($P < 0.01$). Overall, 50% acetone and 50% ethanol are more efficient than other selected solvents for extracting phenolic compounds in soybean seeds. For the UAE method, five different solvent mixtures used were examined for their extraction efficiency. The extract prepared by 50% ethanol was very turbid even after 0.45 μm filtration and therefore was not included for this investigation. The results showed that the UAE−50% acetone extract had the highest TPC (6.93−7.80 mg of GAE/g of seeds) regardless of the soybean variety, which was significantly higher than the extracts prepared by other solvents (4.49−6.34 mg of GAE/g). On average, the UAE−70% ethanol extracts had the second highest TPC values followed by UAE−80% methanol and UAE−80% acetone extracts. The results were comparable to 4.9 mg of GAE/g for a soybean sample previously extracted by UAE−70% acetone (*7*). Our results also showed that UAE methods extracted 54−139% more phenolic compounds than the conventional solid−liquid extraction. The Soxhlet extraction had TPC values from 2.11 to 4.16 mg of GAE/g, which are comparable to the conventional solvent extraction. In the Soxhlet extraction, ethanol extracted significantly more phenolic antioxidants than methanol. Overall, the results from the conventional extraction and UAE suggest that 50% acetone is the most efficient solvent to extract soybean phenolic antioxidants. The ultrasonic extraction can be completed within 30 min and therefore is considerably less time-consuming compared to the 15 h conventional solvent extraction.

Table 2. Total Phenolic Content of Soybean Seeds with Different Extraction Methods

	total phenolic content (mg of gallic acid equiv/g)		
extraction method[a]	NC Roy	V00-3493	V00-3636
C−50% acetone	3.26 ef ± 0.45	3.15 e ± 0.36	3.45 d ± 0.05
C−80% acetone	2.90 f ± 0.25	2.90 ef ± 0.10	2.50 g ± 0.13
C−50% ethanol	3.42 e ± 0.13	3.22 e ± 0.17	2.97 e ± 0.10
C−70% ethanol	3.22 ef ± 0.11	2.75 f ± 0.16	2.69 f ± 0.13
C−80% methanol	3.13 ef ± 0.16	2.88 ef ± 0.17	2.93 ef ± 0.25
UAE−50% acetone	7.80 a ± 0.02	7.05 a ± 0.02	6.93 a ± 0.02
UAE−80% acetone	5.46 d ± 0.04	4.49 c ± 0.09	5.05 bc ± 0.62
UAE−70% ethanol	6.34 b ± 0.04	5.40 b ± 0.44	5.65 b ± 0.04
UAE−80% methanol	6.05 c ± 0.18	4.68 c ± 0.18	4.62 c ± 0.02
Soxhlet−ethanol	2.41 g ± 0.06	4.16 d ± 0.01	3.02 e ± 0.24
Soxhlet−methanol	2.11 h ± 0.12	2.24 g ± 0.25	2.22 h ± 0.07

[a] C and UAE represent conventional solvent extraction and ultrasonic-assisted extraction, respectively. The data in each column marked by the same letter are not significantly different ($P < 0.05$).

Oxygen Radical Absorbance Capacity (ORAC). Table 3 shows the ORAC values of soybean antioxidant extracts prepared by different technologies. For the conventional extraction, the ORAC values of the antioxidant extracts were significantly different for each soybean variety, ranging from 248.5 to 427.2 μmol of TE/g for NC Roy, from 208.4 to 457.2 μmol of TE/g for V00-3493, and from 180.4 to 319.1 μmol of TE/g for V00-3636. The results were higher than ORAC values reported by others, which ranged from 38.7 to 228.6 μmol of TE/g in soybean extracts (*21*, *26*−*28*). For all three soybean samples, the extracts prepared by 70% ethanol exerted the highest ORAC (319.1−457.2 μmol of TE/g), whereas the extracts prepared by 80% methanol showed the lowest ORAC (180.4−274.8 μmol of TE/g). Interestingly, there was no significant correlation between the ORAC and TPC of the soybean extracts. We originally anticipated that the extracts prepared by 50% acetone would have the highest ORAC because they had the highest TPC, which are the major antioxidants in soybean seeds (*18*). Research has also showed that the antioxidant activity of soybean seeds was significantly correlated with their TPC value (*28*). To confirm our observations, we repeated the TPC and ORAC experiments. The results were similar, and no correlation was found between ORAC and TPC of the soybean extracts. With those observations, we suspected that the phenolic profiles of the soybean extracts prepared by different solvents are different and that this difference may significantly affect their antioxidant activity. Therefore, higher total phenolic content does not necessarily suggest higher ORAC. The soybean phenolics from 70% ethanol may be more effective against peroxyl radicals and therefore exert higher ORAC than other extracts. For UAE, the ORAC values of the soybean extracts were also significantly different in each soybean sample, ranging from 203.7 to 392.2 μmol of TE/g for NC Roy, from 208.9 to 438.6 μmol of TE/g for V00-3493, and from 144.7 to 393.5 μmol of TE/g for V00-3636. These ORAC values were comparable to those prepared by the conventional extraction, although the TPC levels of the UAE extracts were significantly higher than the conventional extracts. The discrepancy might be attributable to a heating process in the ultrasonic extraction, which might degrade soybean phenolic components. For NC Roy, the UAE−80% methanol extract had significantly higher ORAC (392.2 μmol of TE/g), followed by the UAE−70% ethanol (341.8 μmol of TE/g), UAE−80% acetone (264.7 μmol of TE/g), and UAE−50% acetone extracts (203.7 μmol of TE/g).

Table 3. ORAC Value of Soybean Antioxidants with Different Extraction Methods

	ORAC value (μmol of TE/g)		
extraction method[a]	NC Roy	V00-3493	V00-3636
C−50% acetone	270.92 cd ± 71.64	275.39 e ± 3.95	276.70 cd ± 26.13
C−80% acetone	339.06 b ± 9.64	295.21 de ± 40.69	288.20 cd ± 47.96
C−50% ethanol	248.52 d ± 49.24	307.68 d ± 22.39	240.35 de ± 27.96
C−70% ethanol	427.20 a ± 21.92	457.21 a ± 5.10	319.14 bc ± 33.09
C−80% methanol	274.82 c ± 11.09	208.38 f ± 0.40	180.41 f ± 18.50
UAE−50% acetone	203.71 d ± 42.08	217.00 f ± 12.68	227.92 e ± 14.26
UAE−80% acetone	264.69 cd ± 26.20	208.87 f ± 13.84	144.67 g ± 3.10
UAE−70% ethanol	341.85 bc ± 47.40	438.63 b ± 7.22	393.47 a ± 17.34
UAE−80% methanol	392.25 ab ± 17.75	298.44 d ± 4.61	223.54 ef ± 34.84
Soxhlet−ethanol	144.44 e ± 58.04	221.33 f ± 11.97	168.96 f ± 10.16
Soxhlet−methanol	324.22 bc ± 17.54	345.10 c ± 1.64	295.14 bc ± 16.96

[a] C and UAE represent conventional solvent extraction and ultrasonic-assisted extraction, respectively. The data in each column marked by the same letter are not significantly different ($P < 0.05$).

Table 4. DPPH• Scavenging Activity of Soybean Antioxidants with Different Extraction Methods

	DPPH• scavenging activity (μmol of Trolox equiv/g)		
extraction method[a]	NC Roy	V00-3493	V00-3636
C−80% acetone	57.90 c ± 0.84	63.47 b ± 1.11	65.41 b ± 5.58
C−70% ethanol	12.26 f ± 0.48	4.62 g ± 5.30	12.29 e ± 2.08
C−80% methanol	61.06 b ± 5.11	41.21 d ± 1.26	51.11 c ± 1.58
UAE−80% acetone	39.35 e ± 0.79	13.48 f ± 1.84	11.46 f ± 1.31
UAE−70% ethanol	50.97 d ± 0.89	34.65 de ± 5.03	37.33 d ± 8.06
UAE−80% methanol	62.37 b ± 1.17	56.09 c ± 0.13	54.82 bc ± 17.93
Soxhlet−ethanol	35.53 e ± 4.40	29.64 e ± 0.90	53.24 c ± 0.71
Soxhlet−methanol	104.43 a ± 0.12	99.99 a ± 0.81	114.74 a ± 1.25

[a] C and UAE represent conventional solvent extraction and ultrasonic-assisted extraction, respectively. The data in each column marked by the same letter are not significantly different ($P < 0.05$).

For V00-3493 and V00-3636, the UAE−70% ethanol extracts had the highest ORAC, followed by UAE−80% methanol, UAE−50% acetone, and UAE−80% acetone extracts. Again, no significant correlation was identified between the ORAC and TPC values of the UAE extracts. For the Soxhlet extraction, the methanol extracts of all three soybean varieties had significantly higher ORAC (295.2−345.1 μmol of TE/g) than the ethanol extracts (144.4−221.3 μmol of TE/g) despite the lower TPC in the methanol extracts. On average, the 70% ethanol extracts in both the UAE and conventional extraction exerted the highest ORAC.

DPPH• Scavenging Activity. Table 4 shows the DPPH• scavenging activities of the soybean extracts by different extractions. The extracts prepared by 50% acetone and 50% ethanol were not included for this comparison because the extracts were too turbid even after 0.45 μm membrane filtration. For the conventional extraction, the DPPH• scavenging activities of the extracts were significantly different for each soybean variety:, ranging from 12.3 to 61.1 μmol of TE/g for NC Roy, from 4.6 to 63.47 μmol of TE/g for V00-3493, and from 12.3 to 65.4 μmol of TE/g for V00-3636. The activities were considerably higher than previously reported soybean extracts prepared by 50% acetone (1.2 μmol of TE/g) (*26*) and 70% ethanol (2.1 μmol of TE/g) (*5*). For NC Roy, the extracts prepared by 80% methanol and 80% acetone had comparable antioxidant activities (61.1 and 57.9 μmol of TE/g, respectively), which were significantly higher than the extracts prepared by 70% ethanol (12.3 μmol of TE/g). For V00-3493 and V00-3636, the extracts prepared by 80% acetone had the highest antioxidant activity, followed by 80% methanol and 70% ethanol extracts. The DPPH• scavenging activities of the soybean extracts were not correlated with their ORAC or TPC values. For UAE, the DPPH• scavenging activities were also significantly different for each soybean variety, ranging from 39.4 to 62.4 μmol of TE/g for NC Roy, from 13.5 to 56.1 μmol of TE/g for V00-3493, and from 11.5 to 54.8 μmol of TE/g for V00-3636, respectively. The UAE−80% methanol extracts had the highest antioxidant activity, followed by the UAE−70% ethanol and UAE−80% acetone extracts. The DPPH• scavenging activities of the NC Roy extracts (but not V00-3493 and V00-3636) were significantly correlated to their ORAC. For the Soxhlet extraction, the extracts prepared by methanol showed significantly higher DPPH• scavenging activities than those prepared by ethanol. Overall, the Soxhlet−methanol extracts had the highest DPPH• scavenging activities among the soybean extracts.

In summary, we showed that UAE improved the extraction of phenolic compounds in soybean seeds by 54−139% compared to the corresponding conventional solid−liquid extraction. The selection of extraction solvents also significantly affected TPC and antioxidant activities of the soybean extracts. Fifty percent acetone was most efficient to extract phenolic compounds in soybean seeds in both UAE and the conventional extraction. However, the antioxidant activities of the soybean extracts were not significantly correlated with their TPC. The 70% ethanol extracts by UAE and the conventional extraction had the highest ORAC, whereas the Soxhlet−methanol extracts had the highest DPPH• scavenging activities.

LITERATURE CITED

(1) Lin, Y.; Spitznagel, E. L. Meta-analysis of soy food and risk of prostate cancer in men. *Int. J. Cancer* **2005**, *117*, 667–669.

(2) Taku, K.; Umegaki, K.; Sato, Y.; Taki, Y.; Endoh, K.; Watanabe, S. Soy isoflavones lower serum total and LDL cholesterol in humans: a meta-analysis of 11 randomized controlled trials. *Am. J. Clin. Nutr.* **2007**, *85*, 1148–1156.

(3) Tikkanen, M. J.; Wähälä, K.; Ojala, S.; Vihma, V.; Adlercreutz, H. Effect of soybean phytoestrogen intake on low density lipoprotein oxidation resistance. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 3106–3110.

(4) Takahashi, R.; Ohmori, R.; Kiyose, C.; Momiyama, Y.; Ohsuzu, F.; Kondo, K. Antioxidant activities of black and yellow soybeans against low density lipoprotein oxidation. *J. Agric. Food Chem.* **2005**, *53*, 4578–4582.

(5) Xu, B. J.; Chang, S. K. C. A comparative study on phenolic profiles and antioxidant activities of legumes as affected by extraction solvents. *J. Food Sci.* **2007**, *72*, 159–166.

(6) Suzuki, K.; Koike, H.; Matsui, H.; Ono, Y.; Hasumi, M.; Nakazato, H.; Okugi, H.; Sekine, Y.; Oki, K.; Ito, K.; Yamamoto, T.; Fukabori, Y.; Kurokawa, K.; Yamanaka, H. Genistein, a soy isoflavone, induces glutathione peroxidase in the human prostate cancer cell lines LNCaP and PC-3. *Int. J. Cancer* **2002**, *99*, 846–852.

(7) Malenčić, D.; Popović, M.; Miladinović, J. Phenolic content and antioxidant properties of soybean (*Glycine max* (L.) Merr.) seeds. *Molecules* **2007**, *12*, 576–581.

(8) Wang, L.; Weller, C. L. Recent advances in extraction of nutraceuticals from plants. *Trends Food Sci. Technol.* **2006**, *17*, 300–312.

(9) Zhou, K.; Yu, L. Effects of extraction solvent on wheat bran antioxidant activity estimation. *Lebensm. Wiss. Technol.* **2004**, *37*, 717–721.

(10) Heimler, D.; Vignolini, P.; Galardi, C.; Pinelli, P.; Romani, A. Simple extraction and rapid quantitative analysis of isoflavones in soybean seeds. *Chromatographia* **2004**, *59*, 361–365.

(11) Georgetti, S. R.; Casagrande, R.; Vicentini, F.; Verri, W. A.; Fonseca, M. J. V. Evaluation of the antioxidant activity of soybean extract by different in vitro methods and investigation of this activity after its incorporation in topical formulations. *Eur. J. Pharm. Biopharm.* **2006**, *64*, 99–106.

(12) Lee, J.; Renita, M.; Fioritto, R. J.; St. Martin, S. K.; Schwartz, S. J.; Vodovotz, Y. Isoflavone characterization and antioxidant activity of Ohio soybeans. *J. Agric. Food Chem.* **2004**, *52*, 2647–2651.

(13) Rostagno, M. A.; Araújo, J. M. A.; Delcio, S. Supercritical fluid extraction of isoflavones from soybean flou. *Food Chem.* **2002**, *78*, 111–117.

(14) Rostagno, M. A.; Palma, M.; Barroso, C. G. Ultrasound-assisted extraction of soy isoflavones. *J. Chromatogr., A* **2003**, *1012*, 119–128.

(15) Adam, M.; Dobiá, P.; Eisner, A.; Ventura, K. Extraction of antioxidants from plants using ultrasonic methods and their antioxidant capacity. *J. Sep. Sci.* **2009**, *32*, 288–294.

(16) Hromádková, Z.; Kost'álová, Z.; Ebringerová, A. Comparison of conventional and ultrasound-assisted extraction of phenolics-rich heteroxylans from wheat bran. *Ultrason. Sonochem.* **2008**, *15*, 1062–1068.

(17) Ma, Y.-Q.; Chen, J.-C.; Liu, D.-H.; Ye, X.-Q. Effect of ultrasonic treatment on the total phenolic and antioxidant activity of extracts from citrus peel. *J. Food Sci.* **2008**, *73*, T115–T120.

(18) Malencic, D.; Maksimovic, Z.; Popovic, M.; Miladinovic, J. Polyphenol contents and antioxidant activity of soybean seed extracts. *Bioresourc. Technol.* **2008**, *99*, 6688–6691.

(19) Lin, F.; Giusti, M. M. Effects of solvent polarity and acidity on the extraction Efficiency of isoflavones from soybeans (*Glycine max*). *J. Agric. Food Chem.* **2005**, *53*, 3795–3800.

(20) Murphy, P. A.; Barua, K.; Hauck, C. C. Solvent extraction selection in the determination of isoflavones in soy foods. *J. Chromatogr., B* **2002**, *777*, 129–138.

(21) Chung, H.; Hogan, S.; Zhang, L.; Rainey, K.; Zhou, K. Characterization and comparison of antioxidant properties and bioactive components of Virginia soybeans. *J. Agric. Food Chem.* **2008**, *56*, 11515–11519.

(22) Zhou, K.; Su, L.; Yu, L. Phytochemicals and antioxidant properties in wheat bran. *J. Agric. Food Chem.* **2004**, *52*, 6108–6114.

(23) Moore, J.; Hao, Z.; Zhou, K.; Luther, M.; Costa, J.; Yu, L. Carotenoid, tocopherol, phenolic acid, and antioxidant properties of Maryland-grown soft wheat. *J. Agric. Food Chem.* **2005**, *53*, 6649–6657.

(24) Lin, P. Y.; Lai, H. M. Bioactive compounds in legumes and their germinated products. *J. Agric. Food Chem.* **2006**, *54*, 3807–3814.

(25) Riedl, K. M.; Lee, J. H.; St. Renita, M.; Martin, S. K.; Schwartz, S. J.; Vodovotz, Y. Isoflavone profiles, phenol content, and antioxidant activity of soybean seeds as influenced by cultivar and growing location in Ohio. *J. Sci. Food Agric.* **2007**, *87*, 1197–1206.

(26) Xu, B.; Chang, S. K. C. Characterization of phenolic substances and antioxidant properties of food soybeans grown in the North Dakota−Minnesota region. *J. Agric. Food Chem.* **2008**, *56*, 9102–9113.

(27) Slavin, M.; Cheng, Z.; Luther, M.; Kenworthy, W.; Yu, L. Antioxidant properties and phenolic, isoflavone, tocopherol and carotenoid composition of Maryland-grown soybean lines with altered fatty acid profiles. *Food Chem.* **2009**, *114*, 20–27.

(28) Xu, B. J.; Yuan, S. H.; Chang, S. K. C. Comparative analyses of phenolic composition, antioxidant capacity, and color of cool season legumes and other selected food legumes. *J. Food Sci.* **2007**, *72*, 167–177.

Received for review December 3, 2009. Revised manuscript received February 16, 2010. Accepted February 25, 2010.