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Isoflavone Characterization and Antioxidant Activity of Ohio Soybeans

JAEHWAN LEE,[†,‡] MARJORY RENITA,[†] RONALD J. FIORITTO,[§] STEVE K. ST. MARTIN,[§] STEVEN J. SCHWARTZ,[†] AND YAEL VODOVOTZ*,[†]

Department of Food Science and Technology, Parker Food Science and Technology Building, The Ohio State University, 2015 Fyffe Court, and Department of Horticulture and Crop Science, Howlett Hall, The Ohio State University, 2001 Fyffe Court, Columbus, Ohio 43210

Seventeen Ohio soybeans were screened for isoflavone content and antioxidant activity. Isoflavone content was determined by C_{18} reversed phase high-performance liquid chromatography coupled with a photodiode array detector. Antioxidant activities of soybean extracts were measured using 2,2-diphenyl-1-picryl-hydrazyl (DPPH) free radical and photochemiluminescence (PCL) methods. The highest and lowest total isoflavone contents were 11.75 and 4.20 μmol/g soy, respectively, while the average was 7.12 μmol/g soy. Antioxidant activities of soybean extracts ranged from 7.51 to 12.18 μmol butylated hydroxytoluene (BHT) equivalent/g soy using the DPPH method. Lipid and water soluble antioxidant activities of soybean extracts ranged from 2.40 to 4.44 μmol Trolox equivalent/g soy and from 174.24 to 430.86 μmol ascorbic acid equivalent/g soy, respectively, using the PCL method.

KEYWORDS: Soybean cultivars; isoflavones; antioxidant activities

INTRODUCTION

Consumption of soybeans and soy products has been associated with reducing the risks of various cancers, such as prostate and mammary, and several chronic inflammatory diseases (*1*). The health-promoting activity associated with soy consumption is attributed, in part, to the presence of isoflavones. Researchers have postulated that the purported health benefit may be due to isoflavone estrogenic activity or perhaps antioxidant activity (*2*, *3*). The structural similarities of isoflavones to naturally occurring estrogens may protect hormone-dependent cancers by modulating activity of estrogen (*2*).

As compared to Asian diets where soy foods include tofu, miso, natto, and whole soybeans, the Western style diet is lacking in acceptable products containing large amounts of soy (*4*). One strategy to increase the use of soy is to incorporate soy-based ingredients into traditional products in the Western diet. Selecting for soybean cultivars containing high isoflavone content and/or other health-promoting compounds such as antioxidants may enhance the health benefit effects of food products.

Isoflavone content in soybeans and in soy products is reported to range from 1 μg/g in soy sauces to 540 μg/g in tempeh, with soymilk and tofu having the highest isoflavone content (*5*). Isoflavones found in soybeans are in the aglycone, β-glucoside, 6-O″-malonyl-β-glucoside, or 6-O″-acetyl-β-glucoside forms. Raw soybeans contain mostly glucoside forms of isoflavone and low percentage of aglycone forms. Isoflavone content in soybean is influenced by many factors, including genotypes, crop years, crop locations, storage period, and genotype × environment interactions (*6*−*8*).

Antioxidant properties, especially radical scavenging activities, are important due to the deleterious role of free radicals in foods and in biological systems. Excessive formation of free radicals accelerates the oxidation of lipids in foods and decreases food quality and consumer acceptance (*9*). Free radicals have also been associated with the aging process and age-related diseases (*10*). Superoxide anion, which is a reduced form of molecular oxygen, has been implicated in the initiating oxidation reactions associated with aging (*10*). Superoxide anion plays an important role in formation of other reactive oxygen species such as hydrogen peroxide, hydroxyl radical, and singlet oxygen, which induce oxidative damage in lipids, proteins, and DNA (*11*).

In this study, antioxidant activities of soybeans were determined using a DPPH (2,2-diphenyl-1-picryl-hydrazyl) method and a photochemiluminescence (PCL) method. DPPH has been widely used to evaluate the free radical scavenging effectiveness of various flavonoids and polyphenols in food systems (*12*, *13*). PCL measures the luminescence from luminol, a photosensitizer, that generates superoxide anion when exposed to UV light. Antiradical substances react with the superoxide anion, and the remaining luminescence is detected (*14*, *15*). The PCL method has been used to assess antioxidant activity in beverages and herbs such as sage, oregano, and evening primrose. PCL is

* To whom correspondence should be addressed. Tel: 614-247-7696. Fax: 614-292-0218. E-mail: Vodovotz.1@osu.edu.
[†] Department of Food Science and Technology.
[‡] Current address: Department of Food Science and Technology, Seoul National University of Technology, Seoul, Korea.
[§] Department of Horticulture and Crop Science.

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Table 1. Genotypes and Planting Locations of 17 Soybean Cultivars Used in This Study[a]

cultivar or line	pedigree	use	planting location
Dilworth	Chapman × Probst	grain	Hoytville, OH
Dwight[1]	Jack × A86-303014	grain	Hoytville, OH
HC95-1503	DPL 3478 × Sprite 87	grain	Cygnet, OH
HF01-0019	HF92-080 × HS93-6169	food	Wooster, OH
HF02-0218	ORC 9508 × IA 2016	food	Hoytville, OH
HF9667-2-15	General × GXR 9648	grain	Crotton, OH
HF9667-2-4	General × GXR 9648	grain	Crotton, OH
HF99-019	IA 2022 × Archer	grain	Hoytville, OH
HS93-4118[2]	IA 2007 × DSR 304	grain	Hoytville, OH
HS96-3145	HS89-8843 × Ohio FG1	food	Wooster, OH
HS96-3850	HS89-2966 × HS89-8843	food	Wooster, OH
Ohio FG4	Ohio FG1 × HS89-3078	food	Wooster, OH
Ohio FG5	Ohio FG1 × HS89-3078	food	Wooster, OH
HS0-3274	HS93-4118 × Savoy	grain	Hoytville, OH
Ohio FG1[3]	LS301 × HS84-6247	food	Hoytville, OH
Ohio FG3	HS89-8843 × Ohio FG1	food	Hoytville, OH
Pana[4]	Jack × Asgrow A3205	grain	Hoytville, OH

[a] References for the registration of some selected cultivars are as follows: (1) Nickell, C. D.; Noel, G. R.; Cary, T. R.; Thomas, D. J. *Crop Sci.* **1998**, *38*, 1398. (2) St. Martin, S. K.; Mills, G. R.; Fioritto, R. J.; Schmitthenner, A. F.; Cooper, R. L. *Crop Sci.* **2001**, *41*, 591. (3) St. Martin, S. K.; Calip-DuBois, A. J.; Fioritto, R. J.; Schmitthenner, A. F.; Min, D. B.; Tang, T.-S.; Yu, Y. M.; Cooper, R. L.; Martin, R. J. *Crop Sci.* **1996**, *36*, 813. (4) Nickell, C. D.; Noel, G. R.; Cary, T. R.; Thomas, D. J. *Crop Sci.* **1998**, *38*, 1398.

approximately 100−1000 times more sensitive than conventional chemical methods using anion radical compounds (*16*, *17*).

Soybean is one of the important crops in Ohio and the leading grain crop in the area planted (*18*). However, systematic characterizations of isoflavone content and antioxidant activities in Ohio soybean cultivars have not been reported.

The objectives of this study were to screen 17 Ohio soybean cultivars for their isoflavone content and antioxidant activities and thereby identify the soybean cultivars with high isoflavone content and/or high antioxidant activities for further processing into food ingredients of soy-based functional foods.

MATERIALS AND METHODS

Materials. Seventeen soybean cultivars and experimental lines, including Ohio FG1, Ohio FG3, Ohio FG4, Ohio FG5, HS96-3145, HS96-3850, HF01-0019, HS0-3274, HF9667-2-4, HF9662-2-15, HF99-019, HF02-0218, HC95-1503, HS93-4118, Dilworth, Dwight, and Pana, were obtained from the Department of Horticulture of The Ohio State University. Most of the soybean cultivars were developed in the Ohio Agricultural Research and Development Center (OARDC) of The Ohio State University and grown and harvested in 2002 at various sites in Ohio. These genotypes were chosen to represent a diversity of both food and grain type materials. Pedigrees, use type, and locations of production are shown in **Table 1**. All 12 standard isoflavones, including daidzein, glycitein, genistein, daidzin, glycitin, genistin, malonyl daidzin, malonyl glycitin, malonyl genistin, acetyl daidzin, acetyl glycitin, and acetyl genistin, were purchased from LC Laboratories (Woburn, MA). DPPH, butylated hydroxytoluene (BHT), and formononetin were purchased from Aldrich (St. Louis, MO). High-performance liquid chromatography (HPLC)-grade methanol, acetonitrile, HCl, and acetic acid were purchased from Fisher Scientific (Fairlawn, NJ).

Soy Meal Preparation. Soybeans were dehulled and ground using a coffee grinder (Black & Decker, Trumbull, CO) to make the soy meal with a particle size less than 0.1 cm in length. Soy meals were stored at −20 °C until use.

Isoflavone Analysis. *Isoflavone Extraction.* Soy meal (0.5 g) of each cultivar was mixed with 100 mmol/L HCl (2 mL), acetonitrile (7 mL), and deionized water (3 mL) in a 50 mL centrifuge bottle (Nalge Company, Rochester, NY). Sample bottles were vortexed for 1 min and shaken with a multiwrist shaker (Lab-line Instruments, Inc., Melrose Park, IL) on setting 9 out of 10 scale for 2 h at room temperature before centrifuging at 4500 rpm for 30 min (Sorvall, Kendro Laboratory, CO). An aliquot (1 mL) of supernatant was transferred to a 10 mL glass bottle and dried under nitrogen gas flow at room temperature. Dried samples were stored at −20 °C in the dark until use (*19*). Duplicates of each soybean cultivar were extracted. The reliability of the extraction methods was assessed by extracting after addition of known concentration of formononetin to HF99-019, HF01-0019, and Dwight soybean cultivars as an internal standard and glycitein in 80% MeOH, which is the lowest isoflavone in soybean, to HS93-4118, Ohio FG4, and Ohio FG3 cultivars and determining its recovery. Recovery percentages for formononetin and glycitein were 98.3 ± 5.3 and 97.4 ± 5.9%, respectively ($n = 6$).

HPLC Analysis. A Waters model 2690 HPLC equipped with a Waters 2996 photodiode array detector (PDA) (Waters Associated, Milford, MA) was used to separate, identify, and quantify isoflavones (*20*). Separation of isoflavones was achieved using a 4 μm Waters Novapak C_{18} reversed phase HPLC column (150 mm × 3.9 mm I.D.) with a Novapak C_{18} stationary phase guard column and a 0.5 μm filter from Vydac (Hesperia, CA). One milliliter of 100% methanol was used to resolubilize the samples for injection. The mixture was vortexed and passed through a 0.2 μm syringe filter (Alltech Associates Inc., Deerfield, IL) prior to HPLC injection. The mobile phase consisted of 1% (v/v) acetic acid in water (solvent A) and 100% acetonitrile (solvent B) at a flow rate of 0.6 mL/min. The sample injection volume was 10 μL, and components were eluted using the following solvent gradient: from 0 to 5 min, solvent A was 85%; from 5 to 44 min, solvent A was decreased from 85 to 65%; from 44 to 45 min, solvent A was increased from 65 to 85%; finally, solvent A was reequilibrated at 85% for 5 min. Between each injection, a mixture of 85% solvent A and 15% solvent B was run for 20 min (*21*). The spectra were collected between 240 and 400 nm by PDA, and compounds in the eluate were detected at 260 nm.

Isoflavone Identification. All 12 isoflavones, including daidzein, glycitein, genistein, daidzin, glycitin, genistin, malonyl daidzin, malonyl glycitin, malonyl genistin, acetyl daidzin, acetyl glycitin, and acetyl genistin, were identified by a combination of the retention time in HPLC chromatograms and UV spectra pattern of pure standard isoflavone compounds (*7*, *22*, *23*).

Calibration Curve Preparation and Quantification of Isoflavones. Approximately 1 mg of crystalline standard compounds of daidzein, glycitein, genistein, daidzin, glycitin, genistin, malonyl daidzin, malonyl genistin, acetyl daidzin, acetyl glycitin, and acetyl genistin was dissolved in 80% methanol in water (100 mL) to prepare the stock solutions. The stock solutions were placed in the refrigerator overnight to ensure the complete solubility of isoflavone. Each stock solution of isoflavones was serially diluted with 80% methanol in water. The concentration of working solutions was determined using the Beer−Lambert Law with UV absorbance reading in the range of 240−360 nm and their molar extinction coefficients in 80% methanol using a UV−vis spectrophotometer (Hewlett-Packard 8453) (*23*, *24*). Each isoflavone standard solution was injected into the HPLC, and the peak areas were determined. The relationship between HPLC peak area and concentration of isoflavones from the UV−vis spectrophotometer was calculated and used for the quantification of the isoflavones. The concentration of malonyl glycitin was calculated based on acetyl glycitin standard. Isoflavone content in this study was expressed in μmol/ g soy (*23*). The correlation coefficient (r) of all standard curves for isoflavone standard compounds was over +0.99.

Antioxidant Activities. *DPPH Method.* The free radical scavenging activities of soybean cultivars were determined using a modification of Ozcelik et al. (*13*). The DPPH method was used to measure the antioxidant activities for food systems. Dried sample extracts were resolubilized in 1 mL of 100% methanol and filtered with a 0.2 μm disk filter. DPPH was dissolved in 100% methanol to a concentration of 0.5 mM. The 3.75 mL of 0.5 mM DPPH was mixed with 0.25 mL of sample extract in methanol. The absorbance changes of the DPPH mixtures were measured at 30 min at 517 nm.

The free radical scavenging activity of these samples was expressed as an equivalent of that of BHT, a well-known radical scavenging

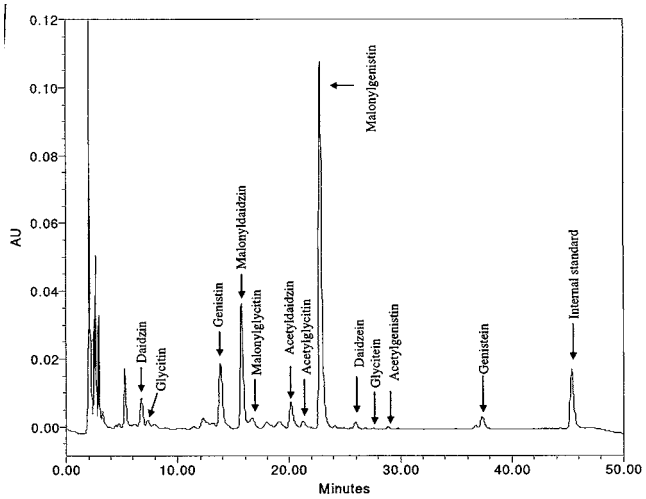

Figure 1. Representative HPLC chromatogram of isoflavones with an internal standard in soybean.

compound used in food systems. A standard curve of the scavenging activity of BHT on DPPH was obtained by measuring the absorbance at 517 nm at 30 min of 3.75 mL of 0.5 mM DPPH mixed with 0.25 mL of 0.05, 0.075, 0.1, 0.25, and 0.5 mM BHT in 100% methanol. The free radical scavenging activities of soybean cultivars were expressed as BHT equivalent as a reference compound. The advantage of using a BHT equivalent expression is to minimize the effects of analysis conditions such as the volume ratio of DPPH and sample or concentration of DPPH.

PCL Method. A PCL detection method using a Photochem (Analytik Jena AG, Jena, Germany) system was used to measure antioxidant activities, especially superoxide anion scavenging activity of soybean extracts (*16*). The PCL method was used to test the antioxidant activities for biological systems. Sample extracts were resolubilized in 1 mL of 100% methanol and filtered through a 0.2 μm disk filter. The antioxidant activity of these samples was measured using "ACL" and "ACW" kits provided, and the procedures were followed as described by the manufacturer. ACL and ACW kits measure integral antioxidative activity of lipid and water soluble substances, respectively. The antioxidant activities of these samples from ACL and ACW kits were reported as Trolox equivalence and ascorbic acid equivalence/g soy, respectively.

Statistical Analysis. Isoflavone analyses of each cultivar were repeated in quadruplicate. All antioxidant analysis was repeated in duplicate or triplicate. The data were analyzed statistically by analysis of variance and *t*-test using StatView (BrainPower Inc., Calabasa, CA). A P value < 0.05 was considered significant.

RESULTS AND DISCUSSION

Isoflavone Analysis of Soybean Cultivars. A typical HPLC chromatogram of isoflavones in soybean is shown in **Figure 1**. Genistein, daidzein, and glycitein as well as their β-glucosides (genistin, daidzin, glycitin), 6-O″-acetyl-β-glucosides (acetylgenistin, acetyldaidzin, acetylglycitin), 6-O″-malonyl-β-glucosides (malonylgenistin, malonyldaidzin, malonylglycitin), internal standard were successfully separated and identified using the applied HPLC conditions. The average coefficient of variations for all 12 isoflavone analyses in each soybean cultivar was less than 5% ($n = 4$).

Isoflavone content in 17 soybean cultivars grown in Ohio is shown in **Table 2**. There were significant differences in isoflavone content that included all aglycones and their glucoside conjugates as well as total daidzein, total genistein, total glycitein, and total isoflavone ($P < 0.05$). The highest total isoflavone content was 11.75 μmol/g soy in HF99-019 soybean cultivar, and the lowest was 4.20 μmol/g soy in HF9667-2-4 soybean cultivar, while the average of total isoflavones in the 17 soybean cultivars was 7.12 μmol/g soy. Among the isoflavones, malonyl genistin content was the highest, followed by malonyl daidzin and genistin, and glycitein was the lowest, which agrees with the previous reports (*7*, *8*). Total genistein content was the highest, followed by total daidzein and total glycitein, which was 64.7, 32.9, and 2.4% of total isoflavones, respectively.

Wang and Murphy (*6*) reported that genotypes and planting years had greater effects on isoflavone content in soybeans than planting locations. Hoeck et al. (*7*) suggested that isoflavone content seems to be a quantitative trait and thus soybean cultivars containing high isoflavone content can be bred. Therefore, on the basis of the results of this study, HF99-019, Dwight, HS93-4118, and Ohio FG1 cultivars could be recommended as potential cultivars with higher isoflavone content. If the identified soybean cultivars are planted in the same locations and grown in the same environmental conditions, the planting location effects on the total isoflavone content could be minimized.

Table 2. Isoflavone Contents of 17 Soybean Cultivars Grown in the State of Ohio[a]

	μmol/g of wet wt base soy															
cultivar	DE	DI	ADI[b]	MDI	GE	GI	AGI[b]	MGI	GY	GYI	AGYI	MGYI	TDE	TGYE	TGE	TI
Dilworth	0.048a	0.345a	0.390a	2.449a	0.032a	0.513a	0.039a	4.995a	tr	0.016a	0.034a	0.050a	3.235a	0.100a	5.581a	8.915a
Dwight	0.047a	0.413b	0.455b	2.922b	0.049b	0.643b	0.043b	5.880b	tr	0.025a	0.025b	0.094b	3.838b	0.145b	6.617b	10.602b
HC95-1503	0.054b	0.260c	0.289c	1.000c	0.078c	0.648b	0.032c	3.332c	tr	0.012a	0.024b	0.069c	1.605c	0.105a	4.091c	5.803c
HF01-0019	0.054b	0.198d	0.217d	0.780d	0.094d	0.479a	0.030c	2.345d	tr	0.025b	0.033a	0.057a	1.251d	0.116a	2.947d	4.317d
HF02-0218	0.045ac	0.388b	0.440b	2.000e	0.035e	0.712c	0.036d	5.041a	tr	0.030b	0.020b	0.096e	2.875e	0.148b	5.826a	8.849a
HF9662-2-15	0.022d	0.148e	0.297c	0.875d	0.033d	0.394d	0.045e	3.357c	tr	0.014a	0.019c	0.063c	1.344d	0.097a	3.831c	5.272e
HF9667-2-4	0.015e	0.147e	0.215d	0.573f	0.019f	0.477a	0.068f	2.594d	tr	0.009a	0.023bc	0.023a	0.952f	0.093c	3.160d	4.205d
HF99-019	0.105f	0.607f	0.492e	3.758g	0.063g	0.759c	0.035d	5.817b	tr	0.025ab	0.028b	0.058ac	4.964g	0.112a	6.675b	11.753f
HS93-4118	0.040g	0.434b	0.291c	1.773h	0.037h	0.558a	0.029g	3.110c	tr	0.301c	0.030d	0.507d	2.539h	0.839d	3.735c	7.115g
HS96-3145	0.048a	0.337a	0.292c	0.957c	0.075i	0.769e	0.033h	3.859e	tr	0.042d	0.038e	0.047a	1.635c	0.128e	4.738e	6.503h
HS96-3850	0.040g	0.275c	0.302c	0.899c	0.057j	0.603b	0.030g	2.767f	tr	0.026e	0.037d	0.106b	1.517c	0.170f	3.458d	5.147e
Ohio FG4	0.056b	0.563g	0.366f	1.599i	0.056j	0.937f	0.037h	3.669e	tr	0.026ab	0.032e	0.050a	2.585h	0.109de	4.700e	7.395g
Ohio FG5	0.055b	0.634f	0.353f	1.738h	0.052j	0.952f	0.039a	3.763d	tr	0.045e	0.039f	0.132e	2.782e	0.218g	4.808e	7.809g
HS0-3274	0.042g	0.335h	0.324h	1.685hi	0.041k	0.617b	0.034i	3.989e	tr	0.017ab	0.026b	0.060ac	2.389h	0.103a	4.682e	7.175g
Ohio FG1	0.092h	0.588fg	0.363f	1.504i	0.103l	1.011g	0.035i	3.542c	tr	0.055f	0.024f	0.088bc	2.549h	0.168fh	4.693e	7.411g
Ohio FG3	0.058h	0.424b	0.260c	1.174j	0.073i	0.835h	0.033h	3.184c	tr	0.041d	0.023f	0.079bc	1.918i	0.143f	4.127c	6.188h
Pana	0.043g	0.146e	0.337h	1.356k	0.052b	0.371d	0.030c	4.200g	tr	0.012a	0.014c	0.059ac	1.884i	0.086ac	4.655e	6.626h
average	0.051	0.367	0.334	1.591	0.056	0.664	0.037	3.850	tr	0.042	0.028	0.098	2.345	0.170	4.607	7.123

[a] Abbreviations: DE, daidzein; DI, daidzin; ADI, acetyl daidzin; MDI, malonyl daidzin; GE, genistein; GI, genistin; AGI, acetyl genistin; MGI, malonyl genistin; GY, glycitein; GYI, glycitin; AGYI, acetyl glycitin; MGYI, malonyl glycitin; TDE, total daidzein; TGYE, total glycitein; TGE, total genistein; TI, total isoflavone; tr, trace amount; TI, sum of TDE, TGYE, and TGE. Different letters are significant among cultivars ($P < 0.05$). [b] Compounds coelute with unknowns.

Table 3. Antioxidant Activities of 17 Ohio Soybean Cultivars

	antioxidant activities		
cultivar	DPPH method[a,d]	PCL method lipid-soluble[b,d]	PCL method water-soluble[c,d]
Dilworth	7.51a	3.19	211.02
Dwight	12.18b	4.44	260.96
HC95-1503	10.82c	4.17	353.46
HF01-0019	9.25d	4.58	430.86
HF02-0218	9.61de	3.55	223.08
HF9662-2-15	9.54de	3.43	203.28
HF9667-2-4	10.14ef	3.43	190.00
HF99-019	9.45de	3.42	232.38
HS93-4118	11.79b	3.10	183.96
HS96-3145	10.05ef	2.85	320.24
HS96-3850	10.46fg	3.07	300.08
Ohio FG4	10.59g	3.07	274.44
Ohio FG5	10.77g	3.84	338.12
HS0–3274	9.73e	2.40	192.24
Ohio FG1	9.58de	3.76	320.40
Ohio FG3	10.31fg	3.02	191.28
Pana	10.42fg	3.00	174.24
average	10.13	3.43	255.65

[a] unit: μmol BHT equivalent/g soy, [b] unit: μmol Trolox equivalent/g soy, [c] unit: μmol ascorbic acid equivalent/g soy. [d] Coefficient of variations of each antioxidant analysis was less than 5%. Different letters are significant among varieties (P<0.05).

Antioxidant Activities. *DPPH Method.* The antioxidant activities of the 17 soybean cultivars are shown in **Table 3**. There are significant differences in free radical scavenging activities among soybean cultivars ($P < 0.05$). Soybean cultivars Dwight and Dilworth had the highest and lowest free radical scavenging activities, which were 12.18 and 7.51 μmol BHT equivalent/g soy, respectively, while the average free radical scavenging activity of 17 soybean cultivars was 10.13 μmol BHT equivalent/g soy.

Free radical scavenging activities of extracts from soybeans and soy products on DPPH have been reported as relative inhibition percentages (*25*, *26*) or seed coat weight for a 50% decrease in absorbance at 520 nm (*27*). Soybean extracts were found to possess free radical scavenging activities, which were influenced by genetic and environmental difference (*25*). Processed soy products such as tofu had approximately 50% of free radical scavenging activity as compared to that of raw soybeans, which indicates that some processing methods affect free radical scavenging activity in soybeans (*26*).

Free radical scavenging activities of soybean extracts (10.13 μmol BHT equivalent/g soy) are relatively lower than those of other plant materials such as 50% ethanolic extracts of fermented red bean extracts (approximately 34 μmol BHT equivalent) (*28*) and of raw red bean extracts (approximately 53 μmol BHT equivalent) (*29*). This variability may be due to differences in species or in extraction methods.

PCL Method. Antioxidant activities measured using the PCL methods are shown in **Table 3**. There were differences in both lipid soluble and water soluble antioxidant activities among soybean cultivars ($P < 0.05$). Soybean cultivars HF01-0019 and HS0-3274 had the highest and the lowest lipid soluble antioxidant activities, corresponding to 4.58 and 2.40 μmol of Trolox, respectively, and the average lipid soluble antioxidant activity of tested soybean cultivars was 3.43 μmol Trolox equivalent/g soy. The highest and the lowest water soluble antioxidant activities were 430.86 and 174.24 μmol ascorbic acid equivalent/g soy in soybean cultivars HF01-0019 and Pana, respectively, and the average water soluble antioxidant activity was 255.65 μmol ascorbic acid equivalent/g soy. Soybean extracts resolubilized in methanol had higher water soluble antioxidant activities than lipid soluble antioxidant activities ($P < 0.05$), which may be due to solubility differences of the different compounds.

This study is the first report on antioxidant activities of soybean extracts using the PCL detection system. Because of the various antioxidant test methods available, direct comparison of antioxidant activities of soybean extract with those of other fruits, beverages, and herbs is not always possible. For example, Wang et al. (*30*) reported total antioxidant capacity of fruits and vegetables, including strawberry, plum, orange, and tomato, as 15.36, 9.49, 7.50, and 1.89 μmol Trolox equivalent/g fruit, respectively, using the oxygen radical absorbance capacity method. These values are approximately 4.4, 2.7, 2.2, and 0.5 times, respectively, of the lipid soluble antioxidant activities of average soybean extracts. Toit et al. (*31*) reported green tea and rosemary had 2.23 and 1.03 mmol ascorbic acid equivalent/g dry weight, respectively, using DPPH methods. These values are approximately nine and four times higher than water soluble antioxidant activities of average soybean extracts, respectively. Kim et al. (*32*) reported the antioxidant activities of fresh apples as 7.7−11.6 μmol ascorbic acid equivalent/g apple depending on the test systems, which is approximately 22−33 times less than water soluble antioxidant activities of average soybean extracts.

Antioxidant activities of isoflavones have been known to depend on the concentrations and structures of isoflavones. For example, the glucose linkage to the aglycone reduced the antioxidant activities of isoflavones approximately 50−100 times (*33*). In this study, less than 2.1% (0.15 μmol out of 7.12 μmol/g soy) of isoflavones in soybean are in aglycone form, which may cause the relatively low antioxidant activities of soybean extracts. It has been reported that at least part of the antioxidant activities of soybean extracts may arise from other polyphenolic and flavonoid compounds (*34*, *35*).

CONCLUSION

Seventeen Ohio soybean cultivars were screened for isoflavone content and antioxidant activity. Soybean cultivars HF99-019, Dwight, HS93-4118, Ohio FG1, and HF01-0019 were identified as possible food ingredients due to their high isoflavone content and/or high antioxidant activities. Soybean cultivar HF99-019 had the highest total isoflavone, total genistein, total daidzein, daidzein, acetyl daidzin, and malonyl daidzin content. Soybean cultivar Dwight had the highest DPPH scavenging activities and the highest malonyl genistin content. Soybean cultivar HS93-4118 had the highest total glycitein, acetyl glycitin, and malonyl glycitin content and the second highest DPPH scavenging activities. Soybean cultivar Ohio FG1 showed the highest genistein and genistin content, and HF01-0019 had the highest lipid and water soluble antioxidant activities determined by the PCL method among tested samples. These identified soybean cultivars, after further processing into food ingredients, may enhance the health benefits of soy-containing foods.

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