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Isoflavone Composition, Phenol Content, and Antioxidant Activity of Soybean Seeds from India and Bulgaria

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Isoflavone levels and isoflavone chemical composition in 11 cultivars of soybean, including 4 Indian and 7 genotypes of soybean grown in Bulgaria, were analyzed as determined by C_{18} reversed phase high-performance liquid chromatography coupled with a photodiode array detector. Antioxidant activity of soybean extracts was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, and total phenolic compounds (TPC) were determined by using Folin−Ciocalteu reagent. The range of total isoflavones (TI) was 558.2−1048.6 μg g^{-1} of soy in Indian cultivars, and it was 627.9−1716.9 μg g^{-1} of soy in the case of Bulgarian cultivars. The highest and lowest total isoflavone contents were observed for Maus-2 (1048.6 μg g^{-1} of soy) and Hardee (558.2 μg g^{-1} of soy), respectively, for the Indian cultivars, and they were observed for Boryara (1716.9 μg g^{-1} of soy) and Line 5 (627.9 μg g^{-1} of soy) for the Bulgarian genotypes. DPPH radical scavenging activity did not differ significantly among the cultivars and did not correlate with TI, whereas TPC correlated well with TI and weakly with DPPH. Malonylglucoside of all the aglycones, total genistein (TGin), and total daidzein (TDin) showed strong correlation with total isoflavones, whereas acetylglucoside and aglycone levels did not significantly correlate with total isoflavone. Profiling of soybean isoflavone is helpful in understanding the regulation of isoflavone biosynthesis for greater improved resistance of crops to disease and greater health benefits for humans. This comparative study of soybean cultivars grown in India and Bulgaria throws light on their composition and nutraceutical value.

KEYWORDS: Soybean cultivars; isoflavones; antioxidant; HPLC

INTRODUCTION

Soybean is known to be a complete food as it contains proteins, fats, essential amino acids, and beneficial secondary metabolites such as isoflavones and phenolic compounds. Soybean seeds contain isoflavones, which are bioactive molecules of low molecular weight, hydrophobic peptides, or fatty acid components that are known to influence the physiological state in animals as well as in humans. Epidemiological studies have shown that consumption of soybeans and soy products reduces the risk of human cancer, osteoporosis, and cardiovascular diseases (*1–3*). Isoflavones are structurally similar to naturally occurring estrogens and show promise in protecting against hormone-dependent cancers (*4*). Therefore, increased consumption of soybeans and their processed products may be beneficial in preventing the incidence of degenerative diseases. In addition, isoflavones belong to a group of active plant defense compounds known as phytoalexins and thereby act as a repellant against insect feeding and pathogenic fungi (*5*). Isoflavones are also involved in nodulation of leguminous plants by inducing the expression of nodulation genes in rhizobial bacteria during symbiosis (*6*).

The three isoflavone aglycones, namely,, genistein, daidzein, and glycitein, are each present in four glucosidic forms in soybeans and soy foods (**Figure 1**). In soybean seeds, the isoflavones occur primarily in their storage form as malonyl glucosides, whereas significant levels of glucoside and low levels of aglycones and acetyl glucosides are present. Isoflavone contents and distribution in soybean seeds are often altered by many factors, including cultivar, tissue type, and growth conditions such as planting location, crop year, temperature, soil nutrition, and storage durations (*7–9*). Low temperatures and high precipitation during seed development have been

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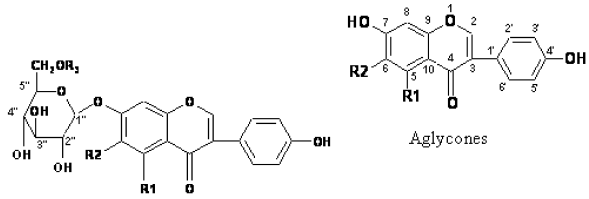

Compounds	R1	R2	R3	Isoflavones
Aglycones	H	H	-	Daidzein
	OH	H	-	Genistein
	H	OCH_3	-	Glycitein
β-glucosides	H	H	H	Daidzin
	OH	H	H	Genistin
	H	OCH_3	H	Glycitin
Acetylglucosides	H	H	$COCH_3$	Acetyldaidzin
	OH	H	$COCH_3$	Acetylgenistin
	H	OCH_3	$COCH_3$	Acetylglycitin
Malonylglucosides	H	H	$COCH_2COOH$	Malonyldaidzin
	OH	H	$COCH_2COOH$	Malonylgenistin
	H	OCH_3	$COCH_2COOH$	Malonylglycitin

Figure 1. Chemical structures of isoflavones present in soybean.

shown to result in higher isoflavone contents (*10*). In soybean seeds, the cotyledons contain >80–90% of the total isoflavones. Wang and Murphy (*11*) compared the isoflavone composition of eight American and three Japanese soybean cultivars and reported that the crop year affects isoflavone content more than the planting locations. Riedl et al. (*12*) tested six soybean cultivars grown in four locations in Ohio and showed that cultivar, growing location, and cultivar−location interactions all influenced the isoflavone concentration in soybeans.

Isoflavones exert antioxidant properties, especially free radical scavenging activities, thereby significantly strengthening the protective effects against oxidative damage (*13*). Free radicals are deleterious to human health, because of their ability to oxidize biomolecules, leading to mutagenic changes, tissue damage, and cell death. Excessive formation of free radicals accelerates the oxidation of lipids in foods and decreases food quality and consumer acceptance. Free radicals have also been associated with the aging process and age-related diseases (*14*). Antioxidant activities of isoflavones have been reported in 80% methanol extracts of soybeans by Lee et al. (*15*), in vivo and in vitro by Yen and Lai (*16*), and in liposomes and lipoproteins by Patel et al. (*17*).

Various forms of isoflavones have been shown to possess antioxidant activities. Naim et al. (*18*) reported that aglycones have greater antioxidant activities than their glucosides and glycosylation of isoflavones may depress the antioxidant activities of aglycones. However, malonyl isoflavones also have been shown (*19*) to exhibit strong antioxidant activities using a chicken olein storage test, but at the end of the storage, all malonyl forms are in the forms of glucosides. Among the three isoflavone aglycones, genistein is the most potent antioxidant in a carotene bleaching assay. Yen and Lai (*16*) showed that supplementations with either purified isoflavones (genistein and daidzein) or 80% methanol extract from several soy foods, including tofu, inhibited reactive nitrogen species-induced oxidation both in vitro and in vivo. The inhibition was positively correlated with the total isoflavone contents in the extract. The antioxidant activity of soybean extract were attributed to the combined effect of isoflavones and phenolic acids.

Soybean is an extensively cultivated crop in India and an important legume in Bulgaria. However, systematic characterizations of isoflavone content and antioxidant activities of both Indian and Bulgarian cultivars have not been reported. The objective of this study was to determine the isoflavone contents, free radical scavenging activity, and total phenolic content of the selected soybean cultivars grown in India and Bulgaria.

MATERIALS AND METHODS

Materials. Soybean seeds of four Indian cultivars, namely,, Hardee, Khsb-2, Maus-2, and JS 335, were obtained from the Department of Genetics and Plant Breeding, GKVK campus, University of Agricultural Sciences, Bangalore, Karnataka, India. Isoflavones extracted from the soybean seed samples of cultivars and experimental lines grown in Pavlikeni, Bulgaria, were obtained from the Institute of Genetics, Bulgarian Academy of Sciences, Sofia, Bulgaria. Indian genotypes were chosen to represent the dominant cultivars in the southern Indian region. DPPH and gallic acid were purchased from Sigma Aldrich (Bangalore, India). High-performance liquid chromatography (HPLC) grade methanol, acetonitrile, HCl, and acetic acid were purchased from Rankem Laboratories (Mumbai, India). Deionized water from Milli-Q (Millipore Co., Bangalore, India) was used for all extraction and quantification purposes.

Isoflavone Analysis. *Isoflavones Extraction.* The soybean isoflavones were extracted using the method of Lee et al. (*7*). Two grams of ground soybean seed with the seed coat was mixed with 2 mL of 0.1 N HCl and 10 mL of acetonitrile (ACN) in a 125 mL screw-top flask, stirred for 2 h at room temperature, and filtered through a Whatman no. 42 filter paper. The filtrate was dried in a vacuum rotary evaporator at a temperature below 30 °C and then redissolved in 10 mL of 80% HPLC grade methanol in distilled water. The redissolved sample was filtered through a 0.45 μm filter unit (Cameo 13N syringe-filter, nylon) and then transferred to 1 mL vials.

HPLC Analysis. The HPLC analysis was conducted following the method of Walsh et al. (*20*). The Shimadzu LC 10-AS high-pressure liquid chromatograph equipped with a dual pump and a UV detector (model SPD-10A) was used to separate, identify, and quantify isoflavones. Separation of isoflavones was achieved by a Bondapak C_{18} reversed phase HPLC column (250 × 4.6 mm and 5 μm internal diameter), and the samples were injected using a Rheodyne 7125 injector. A linear HPLC gradient was used with solvent A (0.1% glacial acetic acid in distilled water) and solvent B (0.1% glacial acetic acid in acetonitrile). Following the injection of 20 μL of the sample, solvent B was increased from 15 to 35% for 50 min and then held at 35% for 10 min. The solvent flow rate was 1 mL/min. The wavelength of the UV detector was set at 256 nm. Solvent ratios were expressed on a volume basis.

Isoflavone Identification. Authentic standards of daidzein, genistin, genistein, and glycitien were purchased from Sigma-Aldrich, Bangalore. Daidzin, glycitin, malonyl daidzin, malonyl genistin, malonyl glycitin, acetyl daidzin, acetyl genistin, and acetyl glycitin were isolated and purified using the modified method of Wang and Murphy (*21*). The 12 reference compounds were chromatographed alone and in mixtures. All 12 isoflavones were identified by their retention times and by cochromatography with standard compounds, and the individual concentrations were calculated on the basis of each peak area.

Free Radical Scavenging Activity. The free radical scavenging activities of soybean cultivars were determined using the DPPH scavenging method (*22*). Dried sample extracts as prepared above were redissolved in 1 mL of 100% methanol and filtered through a 0.2 μm disk syringe filter. DPPH was dissolved in 100% methanol to a concentration of 0.5 mmol L^{-1}. DPPH (3.75 mL of 0.5 mmol L^{-1}) was mixed with 0.25 mL of sample extract in methanol. The initial absorbance was measured at 517 nm and again after 30 min to determine the amount of DPPH scavenged. The free radical scavenging activity of samples was expressed in percentage and each sample was analyzed in triplicate.

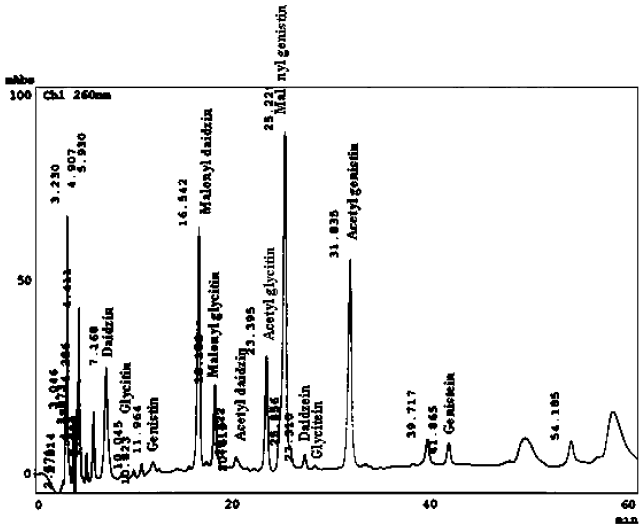

Figure 2. Representative chromatogram of all 12 isoflavone compounds in soybean seeds.

Determination of Total Phenolic Compounds (TPC). TPCs of soybean extracts were determined according to the method of Zielinski and Kozlowska (*23*) with minor modifications. A 0.25 mL methanolic soybean extract was mixed with 4 mL of water and 0.25 mL of Folin−Ciocalteu reagent, vortexed for 30 s, and left to stand for 5 min. To the mixture was added 0.5 mL of saturated sodium carbonate, and the mixture was allowed to stand for 30 min in the dark. The absorbance was measured at 725nm against distilled water as blank. Each sample was analyzed in triplicate, and the results were expressed as gallic acid equivalents (milligrams of GAE per gram of sample) through the calibration curve of gallic acid.

Statistical Analysis. Isoflavone analysis of each cultivar by HPLC was repeated four times with two extracts in each cultivar. Free radical scavenging activity and TPC were repeated in triplicates. The data were analyzed statistically by analysis of variance (ANOVA), and the difference between the means of sample was analyzed by the least significant difference (LSD) test at a probability level of 0.05.

RESULTS AND DISCUSSION

Isoflavones Analysis of Soybean Cultivars. A typical HPLC chromatogram of isoflavones in soybean is shown in **Figure 2**. Genistein, daidzein, and glycitein as well as their β-glucosides (genistin, daidzin, glycitin), acetyl-β-glucosides (acetylgenistin, acetyldaidzin, acetylglycitin), and malonyl-β-glucosides (malonylgenistin, malonyldaidzin, malonylglycitin) were successfully separated and identified using the applied HPLC conditions. The average coefficient of variation for all 12 isoflavone analyses in each soybean cultivar was <5% ($n = 4$). Of the three types of acetyl glucoside and aglycones, only acetyl genistin, daidzein, and genistein could be reliably identified in all samples.

Isoflavone contents of the four Indian cultivars and seven soybean cultivars grown in Bulgaria as analyzed by HPLC are 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 isoflavons, ($P < 0.05$). In this study the range of total isoflavones was 558.2−1048.6 μg g^{-1} of soy in Indian cultivars, and it was 627.9−1716.9 μg g^{-1} of soy in the case of Bulgarian cultivars, whereas the overall range was 558.2−1716.9 μg g^{-1} of soybean. This range is similar to that found in previous papers (*8*–*12*). The highest and lowest total isoflavone contents were observed for Maus-2 (1048.6 μg g^{-1} of soy) and Hardee (558.2 μg g^{-1} of soy), respectively, for the Indian cultivars, and they were observed for Boryara (1716.9 μg g^{-1} of soy) and Line 5 (627.9 μg g^{-1} of soy) for the Bulgarian genotypes. The average of total isoflavones was 811.5 and 1135.8 μg g^{-1} of soy for the Indian and Bulgarian genotypes, respectively, which implies that the Bulgarian genotypes have higher content of individual and total isoflavones compared to the Indian genotypes.

Table 1. Genotypes and Planting Locations of the Soybean Cultivars Used in This Study

cultivar	planting location	year of harvest
Indian genotypes		
Hardee	Central India	2004
Maus 2	South India	2004
Khsb 2	South India	2004
Js 335	Central India	2004
Bulgarian genotypes		
Line 5	Pavlikeni	2005
Line 7	Pavlikeni	2005
Line 9	Pavlikeni	2005
Hodson	Pavlikeni	2005
Dida	Pavlikeni	2005
Biser	Pavlikeni	2005
Boryara	Pavlikeni	2005

Growing year, planting location, and genotype of the cultivars (**Table 1**) have each been shown to play a significant role in determining isoflavone levels, which may be the reason for variation in the total isoflavone content within and among the Indian and Bulgarian cultivars. Wang and Murphy (*11*) reported that genotypes and planting years had greater effects on isoflavones content than planting locations. Similarly, Riedl et al. (*12*) and Hoeck et al. (*24*) also confirmed the influence of planting location and cultivar on the total isoflavones content in four planting locations in the state of Ohio and at eight locations in the state of Iowa, respectively. Furthermore, Hoeck et al. (*24*) suggested that isoflavone content seems to be a quantitative trait, whereas Riedl et al. (*12*) observed that the genetic background of cultivars did not have a clear effect on the seed isoflavone content.

Among the 12 isoflavones studied, malonyl genistin content was the highest, followed by malonyl daidzin and genistin, and glycitein was the lowest, irrespective of Indian or Bulgarian cultivars (**Table 2**). Similar to other results, in this study also malonyl glucosides dominated the isoflavones profiles of soybean seeds followed by glucosides, aglycones, and acetyl glucosides in both the Indian and Bulgarian cultivars.

With increasing TI, the contribution of total genistein, daidzein, and glycitein to that of total isoflavones varied. The total genistein content, which accounts for roughly 59% of TI content at lower TI levels, increased to >66% at higher TI levels. Thus, over the same range of TI values the total genistein content increased by 7%, whereas the total daidzein content decreased by 9% and the total glycitein content increased by about 2% of the TI content (**Figure 3a**). Thus, a shift was observed toward the accumulation of more genistein content at the expense of daidzein, as the isoflavone content increases. This shift was more prominent in Bulgarian cultivars because of its broader range of total isoflavones and large sample numbers compared to the Indian cultivars. The results are in contrast to Riedl et al. (*12*), who observed a shift of isoflavone profiles toward daidzein, at the expense of genistein and glycitein with an increase in total isoflavones content. Although the basis for such a shift at high TI is unclear, the authors cited the environmental conditions under which the plants are grown as the reason for such a shift.

Table 2. Concentration of 12 Isoflavones from Seed Extracts of Indian and Bulgarian Cultivars (Micrograms per Gram of Dry Weight)[a]

	daidzein family					glycitein family					genistein family					
cultivar	Din	Mdin	Adin	Dein	TDin	Glyn	Mglyn	Aglyn	Glyein	TGlyin	Gin	Mgin	Agin	Gein	TGin	TI
Hardee	36.4 c	141.2 cd	5.4 bc	12.9 cd	195.9 cd	12.1 c	19.3 c	1.5 bc	nd	33.0 c	37.2 cd	281.4 cd	2.6 bc	8.2 c	329.4 cd	558.2 cd
Maus 2	60.8 a	266.4 a	8.5 a	23.5 a	359.3 a	26.6 a	32.8 a	3.0 a	nd	62.4 a	87.0 a	518.1 a	6.8 a	15.0 a	627.0 a	1048.6 a
Khsb 2	54.8 ab	230.2 ab	6.1 b	17.8 b	309.0 ab	24.0 ab	33.3 a	2.3 ab	nd	59.6 a	63.6 b	439.3 b	7.1 a	6.9 cd	517.0 b	885.6 b
JS 335	38.6 c	212.8 b	3.9 cd	15.0 c	270.4 bc	15.7 bc	23.3 bc	0.5 cd	nd	39.6 bc	51.2 bc	382.0 bc	1.1 c	9.3 bc	443.6 bc	753.5 bc
Line 5	19.0 d	210.5 cd	3.7 d	8.5 d	241.7 cd	6.7 cd	12.7 d	1.9 c	1.4 c	22.7 d	41.1 cd	314.7 d	3.4 cd	4.4 d	363.5 d	627.9 d
Line 7	30.3 cd	217.2 cd	4.6 cd	11.6 cd	263.8 c	17.3 ab	34.7 cd	0.2 cd	0.4 cd	52.6 c	44.8 c	543.6 c	7.9 cd	11.1 bc	607.4 c	923.7 c
Line 9	37.1 c	277.4 bc	4.8 cd	18.8 bc	338.1 b	10.0 c	19.3 cd	0.4 cd	1.9 cd	31.6 cd	46.4 c	737.0 b	1.1 d	10.1 c	794.6 b	1164.3 bc
Hodson	40.3 c	379.1 a	12.5 b	16.9 bc	448.7 a	20.9 a	40.8 c	1.3 cd	4.3 a	67.4 c	46.4 c	769.7 b	9.1 cd	12.0 bc	837.3 b	1353.4 b
Dida	38.8 c	179.0 d	5.8 cd	13.0 c	236.6 cd	14.5 b	25.8 cd	5.1 b	4.2 a	49.6 cd	42.1 cd	431.2 cd	38.4 a	4.6 d	516.2 cd	802.5 cd
Biser	76.0 a	233.8 c	8.7 c	21.4 b	339.9 b	9.5 c	19.5 cd	0.8 cd	0.4 cd	30.2 cd	87.1 a	890.6 ab	3.8 cd	10.4 c	991.9 ab	1362.1 b
Boryara	66.0 ab	330.6 ab	19.2 a	27.2 a	443.0 a	20.5 a	113.3 a	8.3 a	0.8 c	142.9 a	80.0 ab	1018.5 a	12.8 c	19.7 a	1131.1 a	1716.9 a

[a] Abbreviations: Din, daidzin; Mdin, malonyl daidzin; Adin, acetyl daidzin; Dein, daidzein; TDin, total daidzein; Glyn, glycitin; Mglyn, malonyl glycitin; Aglyn, acetyl glycitin; Glyein, glycitein; TGlyin, total glycitein; Gin, genistin; Mgin, malonyl genistein; Agin, acetyl genistein; Gein, genistein; TGin, total genistein; TI, total isoflavone; nd, not detected. Different letters are significant among cultivars ($P < 0.05$).

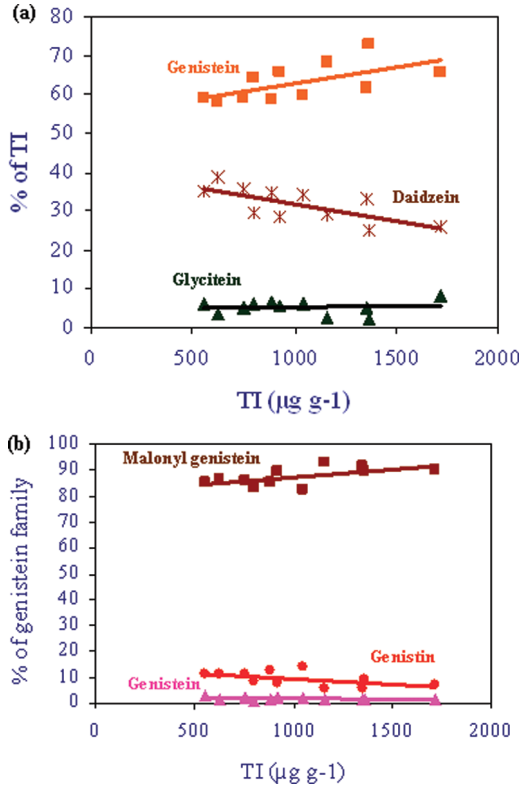

Figure 3. Concentration of (**a**) isoflavone families in soybean seeds of Indian and Bulgarian cultivars as percentage of total isoflavones and (**b**) individual genistein compounds in soybean seeds of Indian and Bulgarian cultivars as percentage of entire genistein family plotted as a function of total isoflavones (TI).

Within each isoflavone family, as the total isoflavone increases the percentage accounted for by malonyl glucoside increases, whereas the simple glucoside decreases and the amount of aglycone and acetyl glucoside remained fairly constant. This is clearly evident in the genistein family, where the increase in malonyl genistin (10%) was at the expense of genistein, which decreased by 8% over the TI range in both the Indian and Bulgarian cultivars (**Figure 3b**). The individual daidzein and glycitein members also showed similar trends, but not at the level of statistical significance.

Accumulation of daidzein and daidzein conjugates has been associated with exposure to specific elicitors such as *Phytophthora sojae*, whereas increased synthesis of genistein and its conjugates is often related to a general defense response against stress conditions such as light exposure, high temperature, and pathogen attack (*25*). The fact that the analyzed soybean cultivars and experimental lines from both India and Bulgaria are subjected to drought tolerance studies may have led to an increase in total genistein content at the cost of daidzein. Our finding is supported by the earlier studies of Caldwell et al. (*26*), who reported that individual isoflavones often had different responses to the various growth conditions during seed maturation, modifying the proportions of the principal isoflavones. They observed that addition of drought stress and elevated CO_2 levels increased total isoflavone content, especially by their promotive effects on malonylgenistin and genistin levels. Because malonyl glucosides are the main storage form in soybean seeds, a relative increase of the malonyl glucoside form over the aglycone form is expected under increased isoflavone content.

Although sharp differences or variations were observed for the total isoflavone contents between the Indian and Bulgarian genotypes, the ratio of the individual compounds to the total isoflavones and their range of distribution and concentration showed a pattern of similarity irrespective of the geographical location in which they were cultivated. The isoflavone levels of cultivars from both countries were in the order malonyl glucoside > glucoside > aglycone > acetyl glucoside. Also, among the three types of isoflavones derivatives in which the R1 and R2 side groups are constant (**Figure 1**), the level of genistein derivatives was highest followed by derivatives of daidzein and glycitein, which were 63, 32, and 5% of total isoflavones, respectively. Irrespective of Indian and Bulgarian genotypes, malonylglucoside ($r = 0.99$, $P = 0.001$), TGin ($r = 0.99$, $P = 0.001$), and TDin ($r = 0.91$, $P = 0.001$) showed strong correlation with total isoflavones, whereas acetylglucoside and aglycone were not significantly correlated with total isoflavone. The above findings are supported by earlier reports of Kim and Chung (*27*), Riedl et al. (*12*), and Lee et al. (*28*). Although the total isoflavone content is influenced by environment, cultivar, and their interactions, the biosynthetic pathway of isoflavones leading to the accumulation of individual isoflavone compounds is not much altered.

DPPH Scavenging. The antioxidant activities of the Indian and Bulgarian cultivars and experimental line soybean seed extracts are shown in **Table 3**, as a percentage of radical scavenging activity of DPPH. There was no significant difference in free radical scavenging activities among the soybean cultivars ($P < 0.05$). Soybean cultivars Hardee and JS 335 had the highest and lowest free radical scavenging activities of 89.40 and 52.63%, respectively, whereas the average free radical

Table 3. Free Radical Scavenging Activity and Total Phenolic Contents of Seed Extracts from Indian and Bulgarian Cultivars[a]

cultivar	DPPH[b] (% of antioxidant activity)	TPC[c] (mg of GAE/g of soybean)
Hardee	89.40	1.51
Maus 2	87.36	1.89
Khsb 2	61.57	1.80
JS 335	52.63	2.17
Line 5	70.00	2.39
Line 7	69.47	3.47
Line 9	81.05	2.18
Hodson	79.47	2.95
Dida	80.00	4.36
Biser	82.63	3.89
Boryara	79.47	5.06

[a] Coefficient of variation of each analysis and contents was <5%. [b] 2,2-Diphenyl-1-picrylhydrazyl. [c] Total phenolic contents.

scavenging activities of Indian and Bulgarian cultivar were 72.74 and 77.44%, respectively, and the overall average of 11 soybean cultivars was 75.73%.

The DPPH scavenging did not correlate with TI ($R^2 = 0.07$, $P = 0.10$) and only weakly with TPC ($R^2 = 0.38$, $P = 0.001$), in cultivars from both countries. Isoflavones are less potent in the DPPH assay compared to some phenolic acids (*29*) also present in soybeans. In addition, the glucose linkage to the aglycone reduced the antioxidant activities of isoflavones approximately 50–100 times (*18*). In this study, <5% of isoflavones in soybean are in aglycone form, and the most potent DPPH scavenger of the isoflavones, genistein, accounts for <1.5% of TI, which may cause the relatively low antioxidant activities of soybean extracts. However, the scavenging activity of the predominantly glucosidic isoflavones may have relevance for oxidative stability of unfermented soy foods. It is possible that phenolic acids and some other compounds present in soybean seeds were responsible for much of the DPPH scavenging activity detected in this study. Because isoflavones were the focus of this study we did not attempt to find the exact source of DPPH scavenging activity. Free radical scavenging activities of extracts from soybeans and soy products on DPPH have been reported as relative inhibition percentages (*15*) or seed coat weight for a 50% decrease in absorbance at 520 nm (*30*). Soybean extracts were found to possess free radical scavenging activities, which were influenced by genetic and environmental difference (*15*). Processed soy products such as tofu had approximately 50% of the free radical scavenging activity of raw soybeans, which indicates that some processing methods affect free radical scavenging activity in soybeans (*30*).

Total Phenolic Compounds. The TPCs of Indian and Bulgarian soybean cultivar seed extracts are shown in **Table 3**. Because isoflavones are themselves phenolics and likely major contributors to TPC values, the TPC correlated with TI ($R^2 = 0.38$, $P = 0.001$) as expected. The trend in TPC with cultivars also parallels that of TI, where the TPCs were higher for the Bulgarian cultivars than for the Indian cultivars. The cultivars Maus-2, Hodson and experimental Line 9, despite showing high antioxidant activity and high TI, had abnormally low TPC values. Although TPC correlated well with TI, it correlated weakly with DPPH. This may be due to the domination of isoflavones in TPC value, thereby obscuring a correlation between DPPH and some minor nonisoflavonoid phenolics. Soybean contains several phenolic acids such as syringic, ferulic, sinapic, coumaric, gentisic, vanillic, hydroxybenzoic, caffeic, and chlorogenic acids (*31*). Among these, caffeic and chlorogenic acids are strong DPPH scavengers (*32*).

Eleven cultivars including four Indian and seven Bulgarian cultivars were screened for their isoflavone content, total phenolic compounds, and antioxidant activity. This is the first report on soybean isoflavone characterization of Indian and Bulgarian cultivars to the best of our knowledge. The total isoflavone content in soybean seeds showed much variation, between and within the Indian cultivars, and cultivars grown in Bulgarian, with the latter recording more isoflavone content than the other. Irrespective of Indian or Bulgarian cultivars, a shift in the isoflavone profile was noticed toward the accumulation of genistein and genistein conjugates with increase in isoflavone contents, which was more pronounced in the Bulgarian cultivars. DPPH scavenging activity was relatively constant for all 11 soybean seed extracts, whereas TPCs simply correlated with the total isoflavones.

Soybean isoflavone profiling may help to identify cultivars for further breeding programs to enhance isoflavone content and also to identify environmental variables associated with isoflavone production and lead to improve stress tolerance of soybean crops for disease, pathogen attack, drought, etc. Furthermore, isoflavone profiling and antioxidant activities of soybean seeds will help to select suitable cultivars for their utilization in the preparation of processed soy products, thereby enhancing the health benefits of soy and soy-based foods.

ABBREVIATIONS USED

TI, total isoflavones; TDin, total daidzein; TGin, total genistein; DPPH, 2,2-diphenyl-1-picrylhydrazyl; TPC, total phenolic compounds.

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