Food Research International 43 (2010) 323–328

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

A comparative assessment of total phenolic content, ferric reducing-anti-oxidative power, free radical-scavenging activity, vitamin C and isoflavones content in soybean with varying seed coat colour

Vineet Kumar [a,*], Anita Rani [a], Amit Kumar Dixit [a], Devendra Pratap [a], Deepak Bhatnagar [b]

[a] National Research Centre for Soybean (Indian Council of Agricultural Research), Indore, MP, India
[b] School of Biochemistry, Devi Ahilaya Vishav Vidhaylaya, Indore, MP, India

ARTICLE INFO

Article history:
Received 28 March 2009
Accepted 22 October 2009

Keywords:
Yellow soybean
Black soybean
Green soybean
Total phenolic content
Ferric reducing-anti-oxidative power
Free radical scavenging activity
Isoflavones
Vitamin C

ABSTRACT

Reports concerning anti-oxidative properties of yellow, green and black soybean seeds available in the literature are insufficient and limiting. In the present investigation, mature seeds of 18 genotypes, 6 from each type of soybean *i.e.* yellow, green and black, were assessed for total phenolic content (TPC), ferric reducing-anti-oxidative power (FRAP), 2,2-diphenyl-1-picrylhydrazine (DPPH) free radical-scavenging activities (FRSA). Isoflavones and vitamin C, the antioxidants, were also determined in all the soybean genotypes. Significant genotypic variation was observed for TPC, FRAP, FRSA, isoflavones and vitamin C within each type of soybean. Maximum genotypic variation (7.27-fold) was observed for TPC in black soybean. Average FRAP value of yellow soybean was significantly ($p < 0.05$) lower than black and green soybeans. Average FRSA in black soybean was significantly higher than yellow and green soybean group. Average total phenolic content and isoflavones levels were not significant across different types of soybean. Correlation studies indicated significant ($p < 0.05$) positive correlation between FRSA and TPC in black soybean. Our results suggest that antioxidant constituents other than isoflavones contribute significantly to the FRSA in black soybean genotypes.

1. Introduction

Soybean seeds, in general, are with yellow, green and black seed coat. Commercial soybean cultivars in major soybean growing regions are predominantly yellow. However, many of the recently developed vegetable-type soybean genotypes have green seed coat. Soybean genotypes have been utilized as a medicinal food in China and Korea since ancient times (Li, 1999). Recently, inclusion of soybean in daily diet has gained importance worldwide in view of its speculated role in providing protection against the oxidative damage caused by the free radicals, the major cause of killer diseases *viz.* atherosclerosis, diabetes, and cancer (Damasceno, Apolinario, Flauzino, Fernandes, & Abdall, 2007; Nordentoft, Jeppesen, Hong, Abdula, & Hermansen, 2008). This is because of the fact that all types of soybean possess innumerable bio-molecules like isoflavones, tocopherols, vitamin C, soy peptides, lecithin, saponins, and sterols, which quench free radicals species by donating hydrogen atom or an electron (Jun, Kim, & Sung, 2002; Kumar, Rani, Dixit, Bhatnagar, & Chauhan, 2009; Lee, Yang, Xu, Yeung, & Huang, 2005; Sakac, Djilar, & Canadanvic-Brunet, 2000; Tripathi & Misra, 2005). Recent works pertaining to the assessment of anti-oxidative properties *viz.* ferric reducing-anti-oxidative power (FRAP), free radical-scavenging activities (FRSA) and total phenolic content (TPC) in soybean seeds have been largely conducted in the yellow soybean (Prakash, Upadhyay, Singh, & Singh, 2007; Shakthivelu et al., 2008; Xu & Chang, 2008a, 2008b; Xu, Yuan, & Chang, 2007); and such studies in black soybean are scarce (Xu & Chang, 2008a, 2008b; Xu et al., 2007). Xu et al. (2007) in a comparative study of legumes for TPC, FRSA, FRAP value investigated only 2 yellow and 1 black soybean genotype. Xu and Chang (2008a, 2008b) studied TPC, FRAP and FRSA in 30 soybean samples comprising of 28 yellow and 2 black soybeans. In both of the above-mentioned studies, average FRSA, FRAP and TPC have been shown comparatively high in black soybean than the yellow soybean. However, these studies are limiting because the number of black soybean genotypes analysed were too few to give the precise assessment of the genotypic variation for the anti-oxidative properties in black soybean and to compare them with yellow soybean. More importantly, soybean genotypes with green seed coat, which figure prominently among vegetable-type soybean cultivars developed in soybean growing countries, were not included in these two investigations. Furuta et al. (2003) investigated TPC and FRSA in soybean-boiled extracts and the boiled soybean homogenate of

* Corresponding author. Address: National Research Centre for Soybean (Indian Council of Agricultural Research), Khandwa Road, Indore 452 017, India. Tel.: +91 7312362935x132; fax: +91 7312470520.
E-mail address: vineetksahni@yahoo.com (V. Kumar).

0963-9969/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodres.2009.10.019

all three types of soybean; however, studies pertaining to the anti-oxidative properties in the mature seeds of set of green soybean genotypes are not available. In the present study, we selected 6 genotypes from each type of soybean *i.e.* yellow, green and black and conducted a comparative assessment of the anti-oxidative properties, namely, total phenolic content, ferric reducing-anti-oxidative power, DPPH free radical-scavenging activity.

Furthermore, soybean owes its recently acquired 'functional food' status to the presence of isoflavones, the phenolic compounds, concentration of which ranges from 1–3 mg/g in the mature seeds. Though, most of the health-promoting effects of soybean isoflavones *viz.* reduction in breast cancer, prostate cancer, osteoporosis have been attributed to their estrogenic-like activity, however, it is the anti-oxidative activity of soy isoflavones which has been reported to reduce the risk of atherosclerosis and diabetes (McVeigh, Dillingham, Lampe, & Duncan, 2006; Zhuo, Melby, & Watanabe, 2004). In general, soy isoflavones exist in 4 major forms *viz.* as free aglycones (genistein, daidzein and glycitein), as β-glucosides (sugar moiety attached to aglycones) and as malonyl and acetylated derivatives of each of the three β-glucosides. The total 12 isomers of isoflavones present in the seed are converted back to corresponding aglycones in human gut prior to absorption. Aglycones have been reported to possess differential relative anti-oxidative activities (Lee et al., 2005). Furthermore, vitamin C is a strong scavenger of free radicals; and has not yet been investigated in soybean genotypes with varying seed coat color. Therefore, it was felt pertinent to analyse soybean genotypes with varying seed coat color for the above-mentioned antioxidants *viz.* isoflavones and vitamin C, concomitant with anti-oxidative properties.

2. Materials and methods

Six genotypes for each type of soybean *viz.* yellow, green and black were selected randomly from the collection of Indian varieties, land races and exotic germplasm at National Research Centre for Soybean (Indian Council of Agricultural Research), Indore, Madhya Pradesh, India. Among 18 genotypes, Bhatt yellow (yellow genotype), Kalitur (black genotype) and Bhatt black (black genotype) are land races of India. Of the remaining 15 genotypes, 3 genotypes (Hatsataka, G205, AGS436) in green soybean and 1 genotype (AGS328 Whydox) in black soybean group are exotic germplasm accessions while 11 are the Indian cultivars released recently as varieties under All India Co-ordinated Project System on soybean. As evident from pedigree data given in the Table 1, the genotypes selected for the investigation have diverse genetic background. Three replications of each genotype were planted on 27th June 2008 in the three-row plot in randomized complete block design. Each plot was 3 m long with spacing of 0.45 m between rows. Recommended agronomic practices for raising soybean in Malwa region of India were followed. Seeds of all the genotypes were harvested at the time of respective maturity. Genotypes differed in the days required to attain maturity; and categorized as extra early (<85 days), early (85–95 days), medium (95–105 days) and late (>105 days) according to the distinctness–uniformity–stability (DUS) guidelines for soybean crop in India. As the moisture content of harvested seed samples varied in the range of 10–14%, therefore, the seeds were dried in convectional oven at 70 °C till the weight became constant. Moisture free seeds were secured in screw-capped vials for estimation of total phenolic content (TPC), ferric-reducing anti-oxidative power and free radical-scavenging activity (FRSA), individual forms of isoflavones and vitamin C content.

2.1. Chemicals

HPLC-grade solvents (acetonitrile) and individual standards of isoflavones (daidzein, glycitein, and genistein), 2,2 diphenyl-1-picrylhydrazine; 2,4,6-tripyridyl-s-triazine) were procured from Sigma–Aldrich.

2.2. Extraction of antioxidants from the seeds

Oven dried seeds of different soybean genotypes were finely ground into flour and made to pass through 100-mesh sieve. Soy flour (1.0 g) was extracted with 15 ml of 70% aqueous acetone at 25 °C in the dark overnight. The mixture was centrifuged at 3000 rpm for 10 min. The residues were re-extracted with 5 ml of the 70% acetone. Both the extracts were combined and stored at 4 °C in dark for further analyses of total phenolic content, DPPH free radical-scavenging activity and ferric reducing-antioxidant

Table 1
Status of the genotypes, country of origin and pedigree and days-to-maturity of the 18 genotypes selected for the investigation.

Genotype	Color	Status	Country of origin	Pedigree	Days-to-maturity
JS93–05	Yellow	Cultivar	India	Secondary selection from PS73-22	90 (E)
NRC 7	Yellow	Cultivar	India	Selection from S69-96	86 (E)
JS335	Yellow	Cultivar	India	JS78-77 × JS71-5	102 (M)
VLS 59	Yellow	Cultivar	India	(Pb1 × VLS2) × TC3613361	98 (M)
Bhatt yellow	Yellow	Land race	India	Land race	96 (M)
NRC 37	Yellow	Cultivar	India	Gaurav × Pb1 (selection from Nanking)	110 (L)
Hara soya	Green	Vegetable-type genotype	India	Himso 1520 × Bragg	98 (M)
AGS 2	Green	Germplasm accession	Exotic (Taiwan)	NA	96 (M)
Hatsataka	Green	Germplasm	Exotic (Japan)	Not available	86 (E)
JS90-41	Green	Cultivar	India	PS73-7 × Hark	97 (M)
G 205	Green	Germplasm	Exotic (Taiwan)	NA	96 (M)
AGS 436	Green	Germplasm for vegetable-type	Exotic (Taiwan)	NA	78 (EE)
AGS 328 Whydox	Black	Germplasm for vegetable-type	Exotic (Taiwan)	NA	75 (EE)
Kalitur	Black	Indigenous	India	Indigenous material	100 (M)
Birsa soya 1	Black	Cultivar	India	Spontaneous mutant of 'Sepaya black'	101 (EE)
JS76-205	Black	Cultivar	India	Kalitur × Bragg	98 (M)
VLS 1	Black	Cultivar	India	Mutant of Bragg	98 (M)
Bhatt Black	Black	Land race	India	Land race	97 (M)

EE: extra early maturity (<85 days).
E: early maturity (85–95 days).
M: medium maturity (95–105 days).
L: late maturity (>105 days).
NA: not available.

power assay. The extraction and analysis were performed in triplicate.

2.3. Determination of TPC

Total phenolic content of the soybean extracts was determined using Folin–Ciocalteu reagent following the method of Singleton and Rossi (1965). An aliquot (0.05 ml) of 70% acetone soy four extract was mixed with 0.5 ml of Folin–Ciocalteu reagent and 0.5 ml of 20% sodium carbonate and the final volume was made to 5 ml with distilled water. The absorbance was measured at 700 nm against distilled water as blank after incubation for 30 min at room temperature. Total phenolic content was expressed as gallic acid equivalents (mg of GAE/g on dry weight basis) through standard calibration curve of freshly prepared propyl gallate.

2.4. Ferric reducing-antioxidant power assay

Total antioxidant capacity of the soybean extract was determined using the ferric reducing-antioxidant power as described by Benzie and Strain (1996). FRAP reagent [3 ml containing 300 mM acetate buffer, 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) in 40 mM HCl and 20 mM $FeCl_3{\cdot}6H_2O$ in the ratio 10:1:1] was mixed with 0.1 ml of soybean extract, incubated for 15 min at 37 °C and absorbance was recorded at 593 nm. FRAP value was expressed as m moles/100 on dry weight basis using the calibration curve of Fe^{2+}. Linearity range of the calibration curve was 0.015–0.075 μM.

2.5. DPPH free radical-scavenging activity

DPPH free radical scavenging capacity of the extract was evaluated using absolute ethanolic solution of DPPH following Mellors and Tappel (1996). The absorbance of sample (A_{sample}) was measured using a spectrophotometer (UV160A, Shimadzu, Japan) at 517 nm against ethanolic blank. A negative control was run after adding DPPH solution to 0.1 ml of the extraction solvent (70% acetone). Decrease in absorbance at 517 nm showed reduction of DPPH radical. The percent inhibition of the DPPH radical by the soybean antioxidant extract was calculated using the formula:

$$\%\ \text{DPPH free radical scavenging activity} = [1 - (A_{sample}/A_{control})] \times 100$$

where $A_{control}$ is absorbance of the control and A_{sample} is the absorbance of the sample after incubation for 10 min at room temperature.

2.6. HPLC determination of isoflavones

2.6.1. Sample preparation

Mature seeds were completely dried in an oven at 70 °C till they were moisture free. Completely dried seeds were finely ground and passed through 100-mesh sieve. Finely ground soy flour (125 mg) was extracted with 80% ethanol (5 ml) and concentrated HCl (1 ml) for 2 h in a boiling water bath using standard method (Vyn et al., 2002), which relies on acid hydrolysis of 12 endogenous isoflavone isomers to their respective aglycone forms *i.e.* daidzein, glycitein and genistein. The suspension resulted after the extraction was centrifuged at 10,000 rpm for 10 min.

2.6.2. HPLC conditions

The supernatant obtained after centrifugation was passed through syringe filter (Whatman 0.5 μ, 13 mm dia.) before loading into the HPLC system. Twenty microliter of the syringe-filtered sample was injected into a Shimadzu chromatograph (LC-10AT VP), equipped with a UV detector (SPD 10AT VP) and oven (CTO-10) housing a C-18 silica column (Phenomenex; 5 μ with dimension of 250 × 4.6 mm), preceded by a guard column (Phenomenex 4.0 × 3.0 mm). The column oven was maintained at 40 °C. The separation and elution of isoflavones was accomplished by employing binary gradient mode with solvent A (10% ACN) and solvent B (38% ACN) at a flow rate of 0.8 ml/min for 25 min. The solvent system was run as follows (% solvent A/solvent B): 0 min (0/100), 5 min (10/90), 20 min (0/100), and 25 min (0/100). The resolution of isoflavones as detected at 260 nm is shown in chromatogram (Fig. 1). A standard curve for daidzein, glycitein, and genistein was generated by injecting varying concentrations of the standards of these isoflavones procured from Sigma–Aldrich. The relative concentration of individual isoflavone in the sample was calculated by software CSW version 1.7 after superimposing the chromatogram of the sample on the standard curve. Individual isoflavone concentration was expressed as μg/g on dry weight basis. Concentrations of aglycones were summed up to compute total isoflavone concentration.

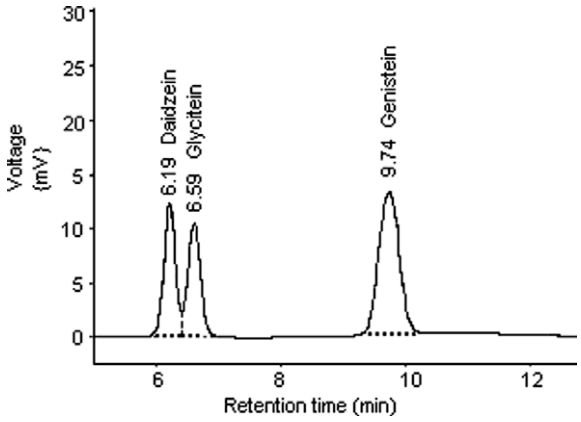

Fig. 1. HPLC chromatogram depicting the separation of daidzein, glycitein and genistein.

2.6.2.1. Vitamin C. Vitamin C was quantified by using standard 2,6 dichlorophenol-indophenol dye method.

2.6.2.2. Statistical analyses. The Data were analysed by analysis of variance (ANOVA). Tukey test was employed to draw the comparison between the means and the significance was accepted at $p < 0.05$. All the statistical analyses were undertaken using SPSS (evaluation version 14).

3. Results and discussion

Data for total phenolic content (TPC), ferric reducing-anti-oxidative power (FRAP), free radical-scavenging activity (FRSA) of the 18 genotypes are presented in Table 2. With in each group of soybean, Genotypic variation for total phenolic content with in 3 groups of soybean *viz.* yellow, green and black was significant ($p < 0.05$). Total phenolic content (TPC) was expressed as mg gallic acid equivalent (GAE)/g. Ranges for yellow, green and black soybean were 1.06–1.54, 0.96–2.89, 0.81–5.89, respectively (Table 3). Though average values for black soybean was higher than green and yellow; however, the differences were non-significant ($p < 0.05$) (Table 4), which may be due to the large variation between genotypes in black and green soybean group. Among all the genotypes analysed, the highest value of TPC was observed in 'Kalitur' and the lowest in 'AGS328 Whydox'. As both these genotypes fall under black soybean group, the variability for TPC was

Table 2
Total phenolic content (TPC), ferric reducing-anti-oxidative power (FRAP) and DPPH free radical-scavenging activity (FRSA) of soybean varying in seed coat color.

Genotype	Total phenolic content (TPC) (mg GAE/g)	Ferric-reducing anti-oxidative power (FRAP) m moles/100 g	Free radical-scavenging activity (FRSA) (% DPPH reduction)
Yellow genotypes			
JS93–05	1.5 ± 0.02ef	2.70 ± 0.03^{b}	30.86 ± 1.41^{def}
NRC 7	1.27 ± 0.06^{d}	3.11 ± 0.02^{b}	34.78 ± 1.60^{f}
JS335	1.50 ± .05^{ef}	1.10 ± 0.01^{a}	16.42 ± 0.08^{a}
VLS 59	1.39 ± 0.03^{de}	2.82 ± 0.13^{b}	44.05 ± 1.21^{g}
Bhatt yellow	1.54 ± 0.07^{ef}	2.83 ± 0.08^{b}	32.02 ± 1.10^{ef}
NRC 37	1.06 ± 0.04^{bc}	2.75 ± 0.04^{b}	28.98 ± 0.87^{de}
Green genotypes			
Hara Soya	1.37 ± 0.08^{de}	4.75 ± 0.03^{d}	21.70 ± 0.97^{b}
AGS 2	1.67 ± 0.07^{f}	4.02 ± 0.24^{c}	32.42 ± 1.20^{ef}
Hatsataka	1.51 ± 0.08^{ef}	6.44 ± 0.28^{h}	21.08 ± 0.78^{b}
JS90–41	2.89 ± 0.09^{h}	5.33 ± 0.32^{ef}	32.55 ± 1.64^{ef}
G 205	1.23 ± 0.04^{cd}	4.77 ± 0.14^{d}	23.44 ± 0.87^{bc}
AGS436	0.96 ± 0.03^{ab}	5.52 ± 0.27^{f}	27.00 ± 1.30^{cd}
Black genotypes			
AGS 328 Whydox	0.81 ± 0.02^{a}	4.77 ± 0.16^{d}	62.75 ± 1.8^{h}
Kalitur	5.89 ± 0.10^{j}	4.79 ± 0.25^{de}	83.00 ± 2.43^{i}
Birsa Soya 1	1.94 ± 0.07^{g}	6.14 ± 0.13^{gh}	82.02 ± 1.50^{i}
JS76–205	0.98 ± 0.04^{ab}	3.03 ± 0.17^{b}	59.71 ± 1.72^{h}
VLS 1	5.43 ± 0.06^{i}	2.87 ± 0. 14^{b}	79.61 ± 1.9^{i}
Bhatt black	1.87 ± 0.04^{g}	5.76 ± 0.23^{fg}	83.47 ± 2.6^{i}

Values given are mean ± standard deviation of triplicate samples; values superscripted with different alphabets in the same column are significantly ($p < 0.05$) different from each other.

maximum (7.27-fold) in black soybean. Reports concerning TPC in soybeans seeds with varying seed coat color in the literature are limited. Xu et al. (2007) estimated total phenolic content in 1 black soybean, which was 3.23-fold higher than the average value of 3 yellow soybean genotypes analyzed in the study. In our results, none of the genotypes from yellow and black soybean group exhibited as high value for TPC as observed by Xu and Chang (2008a, 2008b) who reported a range of 2.07–2.90 in 28 yellow and 8.75–9.01 mg GAE/g in 2 black soybean samples. As the number of black genotypes undertaken in the above-mentioned previous studies were few, hence, it is difficult to conclude from these studies that all black soybean genotypes contain higher levels of TPC than the yellow soybeans. In our study, TPC of some of the black soybean genotypes was less than some green and yellow soybean genotypes. JS90-41, which is a green soybean genotype, exhibited higher values for TPC than 4 black soybean genotypes *viz.* AGS328 Whydox, Birsa Soya 1, JS76-205 and Bhatt black. Similarly, 2 yellow genotypes (JS93-05 and JS335) showed higher values for total phenolic content than 2 black soybean genotypes *viz.* AGS Whydox 328 and JS76-205.

Genotypic variation was also observed for the FRAP within each type of soybean (Table 2). FRAP, expressed in m moles/100 g, ranged from 1.10 to 3.11 for yellow; 4.02 to 6.44 for green and 2.87 to 6.15 for black soybean. Among all the genotypes analyzed, 'Hatsataka', a green soybean genotype, exhibited the highest (6.44) while 'JS335', a yellow genotype, the lowest FRAP value (1.10). Average

Table 3
Individual forms of isoflavones (μg/g), total isoflavones (μg/g) and vitamin C content (mg/100 g) in soybean genotypes varying in seed coat color.

Genotype	Daidzein	Glycitein	Genistein	Total isoflavones	Vitamin C
Yellow soybean					
JS93–05	304.4 ± 15^{fg}	254.4 ± 18^{ef}	518 ± 22^{fg}	1076.8^{fg}	3.54 ± 0.03^{a}
NRC 7	97.2 ± 12^{a}	240.0 ± 11^{def}	153.2 ± 6^{a}	490.4^{ab}	3.94 ± 0.10^{ab}
JS335	317.6 ± 10^{g}	517.1 ± 13^{j}	615.2 ± 19^{h}	1449.9^{jl}	5.61 ± 0.14^{de}
VLS 59	122.7 ± 7^{ab}	181.2 ± 10^{b}	303.8 ± 16^{b}	607.7^{b}	3.52 ± 0.08^{a}
Bhatt yellow	228.6 ± 19^{cd}	394.4 ± 10^{h}	390.0 ± 12^{c}	1013.0^{ef}	3.66 ± 0.05^{a}
NRC 37	624.4 ± 20^{i}	270.2 ± 14^{f}	739.9 ± 36^{j}	1634.5^{k}	4.30 ± 0.11^{bc}
Green soybean					
Hara Soya	138.1 ± 8^{b}	312.7 ± 8^{g}	382.9 ± 12^{c}	833.7^{cd}	4.51 ± 0.12^{c}
AGS 2	381.1 ± 9^{h}	216.4 ± 15^{cd}	485.9 ± 18^{ef}	1083^{fg}	4.53 ± 0.18^{c}
Hatastaka	209.1 ± 14^{e}	185.0 ± 9^{bc}	387.5 ± 14^{c}	781.6^{c}	5.50 ± 0.12^{de}
JS90–41	607.4 ± 12^{j}	243.1 ± 16^{def}	942.2 ± 30^{j}	1792.7^{l}	5.99 ± 0.17^{ef}
G205	386.7 ± 17^{h}	315.0 ± 13^{g}	556.6 ± 2^{g}	1258.4^{hi}	6.34 ± 0.15^{f}
AGS436	209.1 ± 8^{c}	53.0 ± 4^{a}	117.7 ± 5^{a}	379.9^{a}	4.58 ± 0.13^{c}
Black soybean					
AGS 328 Whydox	157.0 ± 8^{b}	170.9 ± 12^{b}	137.03 ± 6^{a}	464.9^{a}	4.80 ± 0.23^{c}
Kalitur	277.0 ± 14^{ef}	443.7 ± 13^{i}	454.8 ± 6de	1175.5^{gh}	5.44 ± 0.21^{d}
Birsa Soya 1	255.0 ± 12de	234.1 ± 7de	430 ± 11^{ed}	919.1^{de}	7.47 ± 0.34^{g}
JS76–205	302.1 ± 10^{fg}	558.4 ± 15^{k}	519.1 ± 15^{fg}	1379.6^{ij}	6.49 ± 0.25^{f}
VLS 1	122.4 ± 12^{ab}	265.3 ± 10^{ef}	700.2 ± 27^{i}	1087.9^{fg}	5.78 ± 0.14^{de}
Bhatt black	389.6 ± 12^{h}	478.0 ± 11^{i}	611.2 ± 12^{h}	1478.8^{j}	5.64 ± 0.23^{de}

Values given are mean ± standard deviations of triplicate samples; values superscripted with different alphabets in the same column are significantly ($p < 0.05$) different from each other.

Table 4
Average value of ferric reducing-anti-oxidative power, free radical-scavenging activity, total phenol content, individual and total isoflavones content, and vitamin C content in yellow, green and black soybean.

Antioxidant property/ antioxidant	Yellow soybean (n = 18)	Green soybean (n = 18)	Black soybean (n = 18)
FRAP (m moles/100 g)	2.55[a]	5.14[b]	4.56[b]
FRSA (% DPPH reduction)	31.18[a]	26.36[a]	75.09[b]
TPC (gallic acid equivalent/g)	1.04[a]	1.61[a]	2.82[a]
Daidzein (μg/g)	282.5[a]	321.93[a]	250.5[a]
Glycitein (μg/g)	309.6[a]	220.9[a]	358.4[a]
Genistein (μg/g)	453.4[a]	478.8[a]	475.4[a]
Total isoflavones (μg/g)	1045.4[a]	1021.5[a]	1084.3[a]
Vitamin C (mg/100 g)	4.1[a]	5.24[ab]	5.94[b]

Values with different superscripts in the same row are significantly different from each other.

FRAP value for yellow, green and black soybean genotypes were 2.55, 5.14, and 4.56, respectively. FRAP for green and black soybean genotypes were significantly higher than yellow soybean while the differences between black and green were not significant (Table 4). Xu et al. (2007) showed FRAP to the magnitude of 9.43 m moles/100 g for one black soybean genotype and 1.24 m moles/100 g as average value of three yellow soybean genotypes. Xu and Chang (2008a, 2008b) investigated FRAP for 28 yellow and 2 black soybean samples. The authors reported a range from 0.83 to 1.34 for yellow soybean and 13.05 to 14.01 m moles/100 g for black soybean. In both the above studies, though the yellow soybean genotypes undertaken for the study were sufficient; however, the number of samples for black soybean was few. Furthermore, green soybean was not undertaken in these two investigations. Though, Furuta et al. (2003) assessed anti-oxidative properties like TPC and FRSA in boiled soybean extract and boiled soybean homogenate of seeds of varying seed coat color; however, the authors did not determine the FRAP value in their study. Therefore, we did not find any reference to compare the FRAP value of green soybean obtained in our results with other studies.

Significant genotypic differences ($p < 0.5$) were observed for DPPH free radical-scavenging activity among the genotypes within each type of soybean *i.e.* yellow, green and black seed coat (Table 2). It is also evident from Table 4 that black soybean genotypes exhibited average DPPH free radical-scavenging activity significantly ($p < 0.05$) higher than green and yellow soybean genotypes. Average DPPH free radical-scavenging activity of six black soybean genotypes was about 2.45–2.85-fold higher than the corresponding values of yellow and green soybean. In the recent studies, a comparative account of FRSA between black and yellow soybean has been reported (Xu & Chang, 2008a, 2008b; Xu et al., 2007). Xu et al. (2007) showed 14.5 times higher FRSA value in one black soybean genotype than the average value of three yellow soybean genotypes. In a subsequent study from the same laboratory, Xu and Chang (2008a, 2008b) showed average FRSA of two black soybean genotypes 17-fold higher than the highest value of 29 yellow genotypes. However, in our study, we did not observe any black genotype exhibiting so high FRSA value than yellow genotypes as reported in the above-mentioned studies. Furthermore, Xu and Chang (2008a, 2008b) could not detect any DPPH free radical-scavenging activity in three soybean genotypes ('LaMore', '5389', '51C10') in the group of 29 yellow genotypes. However, all the 18 soybean genotypes, which we analyzed, exhibited measurable value of DPPH free radical-scavenging activity. Furuta et al. (2003) investigated DPPH free radical-scavenging activity in boiled homogenates of yellow, green and black soybeans. The study showed the leakage of appreciable levels of FRSA in the extract of boiled seeds; and hence, the FRSA values of homogenates of boiled seeds cannot be compared with the values of mature seeds obtained in our study. However, a higher value of FRSA in the homogenate of boiled black soybean than green and yellow soybean in the study supports our results.

Genotypic variation was observed for individual forms of isoflavones and total isoflavones content in three types of soybean genotypes (Table 3). No significant differences were observed for individual forms of isoflavones and total isoflavones contents across the seed coat color (Table 4). Though, the ratio of individual isoflavones was different in three types of soybean. Ratio of average values of daidzein, glycitein, and genistein was: 1:1.1:1.5, 1.5:1:2.2 and 1:1.4:1.9 for yellow, green and black soybeans.

Role of vitamin C in scavenging free radicals is well established. Significant genotypic variation was observed for vitamin C content with in three types of soybean (Table 3). Ranges for the vitamin for yellow, green and black genotypes were: 3.52–5.61; 4.51; 6.34 and 5.44–7.47 mg/100 g for, respectively. Average vitamin C content in black soybean was significantly ($p < 0.05$) higher than the yellow soybean (Table 4). In general, all the yellow genotypes exhibited comparatively low value for vitamin C than the black soybean genotypes. However, some of the green soybean genotypes ('G205', 'JS90-41', 'Hatsataka') exhibited vitamin C value at par or even higher value than some of the black soybean genotypes.

Table 5 exhibits significant correlation coefficients observed among anti-oxidative properties, isoflavones and vitamin C content in three groups of soybean. Significant positive ($p < 0.05$) correlation was observed between total ferric reducing-anti-oxidative power (FRAP) and DPPH free radical-scavenging activity in yellow soybean. Yellow soybean also exhibited significant ($p < 0.01$) positive correlations between FRAP value and vitamin C content. A positive association between total phenolic content and isoflavones was expected, as the isoflavones are phenolic compounds. However, barring green soybean, which exhibited moderate positive correlation ($p < 0.05$) between total phenolic and genistein content, the other two types of soybean did not show any significant correlation of any of the individual forms of isoflavones and/or total isoflavones content with total phenolic content. Riedel et al. (2007) also did not observe any significant correlation between total isoflavones and total phenol content. Probably, non-isoflavonic components *viz.* phenolic acids like gallic acid, coumeric acid, cinnamic acid and anthocyanins might contribute significantly to

Table 5
Significant correlation coefficient observed among FRAP, FRSA, daidzein, glycitein, genistein, total isoflavones and vitamin C in soybean with varying seed coat color.

Soybean type	Parameters	Correlation coefficient (r)
Yellow		
	FRAP and FRSA	0.822*
	FRAP and vitamin C	0.903**
	Daidzein and genistein	0.923**
	Glycitein and vitamin C	0.793*
Green soybean		
	Total phenol and genistein	0.813*
	Diadzein and genistein	0.882*
	Daidzein and total isoflavones	0.900**
	Genistein and total isoflavones	0.813*
Black soybean		
	FRSA and total phenol	0.896**
	Glycitein and total isoflavones	0.885*
	Genistein and total isoflavones	0.800*

* Indicates significance at $p < 0.05$.
** Indicates significance at $p < 0.01$.

the TPC. Furthermore, total phenolic content are known to influence the FRSA. In our results, only black soybean group exhibited significant ($p < 0.01$) correlation between total phenolic and free radical-scavenging activity (FRSA). However, we did not observe any relationship between isoflavones, well-known phenolic antioxidants, and FRSA in any of the three types of soybean.

Phenolic compounds and the vitamin C content are very efficient free radical scavengers (Riedel et al., 2007; Takahata, Ohnishi-Kameyama, Furuta, Takahashi, & Suda, 2001). In our study, no significant differences were found for vitamin C and isoflavones contents between green and yellow soybean group (Table 4), however, green soybean exhibited approximately 2-fold higher FRAP than yellow soybean. This may be attributed to the total carotenoid and lutein content, which have been reported to be significantly high in green soybeans than yellow soybean (Michiko, Junji, Masayoshi, & Koichi, 1994). Similarly, isoflavones did not contribute to the higher FRAP in black soybean than yellow soybean, though vitamin C content may be one of antioxidant constituents contributing to the higher FRAP for black soybean. Significant higher free radical-scavenging activity in the black soybean than yellow and green soybean could not be attributed to the isoflavones; though vitamin C content appeared to contribute to it. Furthermore, anthocyanins *viz*. cyaniding-3-glucose and peonidin–3-glucose, which are found exclusively in black soybean, are well known for free radical-scavenging activity and have been found to be present in the seed coat, which is the major region of black pigmentation (Astadi, Astuti, Santoso, & Nugraheni, 2008). Therefore, significant differences for free radical-scavenging activity among three groups of soybean may be contributed by anthocyanins content. Phenolic acids have also been reported to possess high free radical-scavenging activities (Choung et al., 2001); however, they were not estimated in the present investigation.

4. Conclusions

Our results, though for one year study, indicated that soybean genotypes with black seed coat exhibited higher FRSA (free radical-scavenging activity) than yellow and green soybean; while yellow soybean exhibited comparatively lower ferric reducing-anti-oxidative power (FRAP) value than the black and green soybean. Results also showed that antioxidant constituents other than isoflavones contribute to the higher values of free radical-scavenging activity in black soybean. Furthermore, contrary to the earlier suggestions (Furuta et al., 2003; Xu & Chang, 2008a, 2008b), our results showed that every black genotype does not necessarily contain higher level of total phenolic content than yellow or green soybean. Interestingly, some of the black genotypes despite having low total phenolic content than some of the green soybean genotypes showed correspondingly higher levels of free radical-scavenging activity (FRSA). It would be interesting to investigate the genotypes for the contents of anthocyanins and phenolic acids. Significant genotypic variation observed for anti-oxidative properties within each group of soybean provides opportunities for the plant scientists to identify and develop special soybean genotypes for maximizing the nutraceutical value of soy foods.

Acknowledgement

Director, National Research Centre for Soybean is duly acknowledged for providing the facilities for carrying out the research work.

References

Astadi, I. R., Astuti, M., Santoso, U., & Nugraheni, P. S. (2008). *In vitro* antioxidant activity of anthocyanins of black soybean seed coat in human low density-lipoprotein. *Food Chemistry, 112*(3), 659–663.

Benzie, I. F., & Strain, J. J. (1996). The ferric reducing ability of plasma as a measure of 'antioxidant power': The FRAP assay. *Analytical Biochemistry, 239*, 70–76.

Choung, M. G., Baek, I. Y., Kang, St., Han, W. Y., Shin, D. C., Moon, H. P., et al. (2001). Isolation and determination of anthocyanins in seed coats of black soybean. *Jounal of Agricultural and Food Chemistry, 49*, 5848–5851.

Damasceno, N. R. T., Apolinario, E., Flauzino, F. D., Fernandes, I., & Abdall, D. S. P. (2007). Soy isoflavones reduce electronegative low-density lipoprotein (LDL) and anti-LDL auto-antibodies in experimental atherosclerosis. *European Journal of Nutrition, 46*, 125–132.

Furuta, S., Takahashi, M. T., Takahata, Y., Nishiba, Y., Oki, T., Maasuda, M., et al. (2003). Radical-scavenging activities of soybean cultivars with black seed coats. *Food Science Technology Research, 9*(1), 73–75.

Jun, H. S., Kim, S. E., & Sung, M. K. (2002). Protective effect of soybean saponins and major antioxidants against aflatoxins B1-induced mutagenicity and DNA adduct formulation. *Journal of Medicinal Foods, 5*(4), 235–244.

Kumar, V., Rani, A., Dixit, A., Bhatnagar, D., & Chauhan, G. S. (2009). Relative changes in tocopherols, isoflavones and antioxidative properties of soybean during different reproductive stages. *Journal of Agricultural and Food Chemistry, 57*, 2705–2710.

Lee, C. H., Yang, L., Xu, J. Z. X., Yeung, S. Y. V., & Huang, Y. (2005). Relative antioxidant activity of soybean isoflavones and their glycosides. *Food Chemistry, 90*(4), 735–741.

Li, S.Z. (1999). Black soybeans. Parts on cereals. In Y.J. Wang, Ben-Cao-Gang-Mu (Eds.) (an outline treatise of medicinal herbs written in 100 A.D.), Bejing, China: People's Hygiene Publishing Company. pp. 1344–1349.

McVeigh, B. L., Dillingham, B. L., Lampe, J. W., & Duncan, A. M. (2006). Effect of soy protein varying in isoflavone content on serum lipids in healthy young men. *American Journal of Clinical Nutrition, 83*, 244–251.

Mellors, A., & Tappel, A. L. (1996). The inhibition of mitochondrial peroxidation by ubiquinon and ubiquinol. *Journal of Biological Chemistry, 241*, 4353–4356.

Michiko, M., Junji, T., Masayoshi, S., & Koichi, C. (1994). Carotenoid components in soybean seeds varying with seed color and maturation stage. *Bioscience, Biotechnology and Biochemistry, 58*(5), 926–930.

Nordentoft, I., Jeppesen, P. B., Hong, J., Abdula, R., & Hermansen, K. (2008). Increased insulin sensitivity and changes in the expression profile of key insulin regulatory genes and beta cell transcription factors in diabetic kkAy-mice after feeding with a soybean protein rich diet high in isoflavones content. *Journal of Agricultural and Food Chemistry, 56*, 4377–4385.

Prakash, D., Upadhyay, G., Singh, B. N., & Singh, H. B. (2007). Antioxidant and free radical scavenging activities of seeds and agri-wastes of some of the varieties of soybean. *Food Chemistry, 104*, 783–790.

Riedel, K. M., Lee, J. H., Renita, M., Martin, S. K., St., Schwartz, S. J., & Vodovotz, Y. (2007). Isoflavones profiles, phenol content and antioxidant activity of soybean seeds as influenced by cultivar and growing location in Ohio. *Journal of the Science of Food and Agriculture, 87*, 1197–1206.

Sakac, M. B., Djilar, S. M., & Canadanvic-Brunet, J. M. (2000). Anioxidants of soybean grain and full fat soybean seeds. *Niton Yuka Gakka Nenkai, Koen Yoshisha, 39*, 160–163.

Shakthivelu, G., Akithadevi, M. K., Giridhar, R., Ravishankar, G. A., Niklova, M. T., Angelov, G. B., et al. (2008). Isoflavone composition, phenol content, anioxidative activity of soybean seeds from India and Bulgaria. *Journal of Agricultural and Food Chemistry, 56*, 2090–2095.

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdate and phosphotungstic acid reagent. *American Journal of Enology and Viticulture, 16*, 144–158.

Takahata, Y., Ohnishi-Kameyama, M., Furuta, S., Takahashi, M., & Suda, I. (2001). Highly polymerized proanthocyanidins in brown soybean seed coat with high free radical scavenging activity. *Journal of Agricultural Food Chemistry, 49*, 5843–5847.

Tripathi, A. K., & Misra, A. K. (2005). Soybean: A consummate functional food. *Journal of Food Science and Technology, 2*, 42–46.

Vyn, T. J., Yin, X., Bruulsema, T. W., Jackson, J. C., Rajcan, I., & Bruder, S. M. (2002). Potassium fertilization effects on isoflavones concentrations in soybean. *Journal of Agricultural and Food Chemistry, 50*, 3501–3506.

Xu, B., & Chang, S. K. C. (2008a). Antioxidant capacity of seed coat, dehulled bean and whole black soybeans in relation to their distributions of total phenolics, phenolic acids, anthocyanins and isoflavones. *Journal of Agricultural and Food Chemistry, 56*, 8365–8373.

Xu, B., & Chang, S. K. C. (2008b). Characterization of phenolic substances and antioxidant properties of food soybeans grown in North Dakota-Minnesota region. *Journal of Agricultural Food Chemistry, 56*, 5102–9113.

Xu, B. J., Yuan, S. H., & Chang, S. K. (2007). Comparative analyses of phenolic composition, antioxidant capacity, and color of cool season legumes and other selected food legumes. *Journal of Food Sciences, 72*(2), S167–176.

Zhuo, X. G., Melby, M., & Watanabe, S. (2004). Soy isoflavone intake lowers serum LDL cholesterol: A meta-analysis of 8 randomized controlled trials in humans. *Journal of Nutrition, 134*(9), 2395–2400.