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Phenolic Content and Antioxidant Properties of Soybean (*Glycine max* (L.) Merr.) Seeds

Djordje Malenčić [1,*], Milan Popović [1] and Jegor Miladinović [2]

[1] Faculty of Agriculture, University of Novi Sad, Trg Dositeja Obradovića 8, 21000 Novi Sad, Serbia; e-mail: popmilan@polj.ns.ac.yu
[2] Institute of Field and Vegetable Crops, Maksima Gorkog 30, 21000 Novi Sad, Serbia; e-mail: jegor@ifvcns.ns.ac.yu

* Author to whom correspondence should be addressed; E-mail: malencic@polj.ns.ac.yu

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Abstract: The contents and antioxidant ability of various classes of phenolic compounds present in the seeds of twenty soybean hybrids were evaluated. Total phenolics, tannins and proanthocyanidins were determined spectrophotometrically, after extraction of seeds with 70% aqueous acetone. In addition, the flavonoid contents were determined. The antioxidant activity of aqueous acetone extracts was evaluated by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity assay. The highest contents of total phenolics were found in Serbian cultivar 1511 and Chinese cultivar LN92-7369, which also displayed the highest total antioxidant activity. Conversely, genotypes poor in phenolics also showed low levels of DPPH-radical scavenging activity. The results suggested that besides protein and oil contents, the phenolic contents should be also considered as an important characteristic feature of soybean seeds, and as a potential selection criterion for antioxidant activity in soybean.

Keywords: Soybean seeds; total phenols; tannins; flavonoids; proanthocyanidins; antioxidant activity; DPPH assay.

Introduction

Naturally occuring plant phenolics include several groups of compounds that have health-promoting properties. Phenolics may act as antioxidants, thereby reducing the risk of atherosclerosis and coronary heart disease, which can be caused by oxidation of low-density lipoproteins. They also may protect against some forms of cancer [1]. Depending on the substituents of a phenolic hydroxyl group, their antioxidant properties comprise all known mechanisms. This is of importance for humans using them as vitamins and/or protectants against oxidative stress. Phenolic compounds play also an important role in plant resistance and defence against microbial infections which are intimately connected with reactive oxygen species (ROS) [2].

Many phenolic compounds found in plant tissues (in addition to tocopherols) are potential antioxidants: flavonoids, tannins and lignin precursors may all work as ROS-scavenging compounds. Antioxidants act as a cooperative network, employing a series of different redox reactions. Interactions between ascorbic acid and glutathione and ascorbic acid and phenolic compounds are well known [3]. To prevent oxidation of fats and oils, antioxidants are widely used in foods and cosmetics. Because of possible toxicity of the widely used butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), together with consumers` preference for “natural” products, much research on natural antioxidants has been undertaken in the recent past [4].

Soybean [*Glycine max* (L.) Merr., *Fabaceae*] is one of the most important crops for human and animal consumption, and the most important organic components of soybean seed are proteins (about 40%) and oil (about 20%). With the exception of isoflavonoids, few studies have been carried out on the other phenolic classes present in soybean. Thus, the aim of this study was to examine seed extracts of different soybean genotypes and select those with higher phenolic content and/or exibiting increased antioxidant activity, since this may be important for alimentary or pharmaceutical purposes.

Results and Discussion

With regards to total phenolics contents, Chinese cultivar LN92-7369 and domestic (Serbian) 1511, as well as F_1 hybrid 5 x 1, showed the highest levels of these compounds (Table 1). It is worth mentioning that these genotypes also showed the highest DPPH-radicals scavenging activities (48.17, 45.98 and 47.20%, respectively). At the same time, genotypes with the lowest contents of total phenolics, such as Tara, Sava and F_1 hybrid 7 x 8, expressed only about half of the activity of phenolic-rich genotypes (22.87-27.73%). These results are in accordance with the findings of other authors concerning phenolic contents and antioxidant activity in common beans (*Phaseolus vulgaris* L.), another *Leguminosae* species [5]. Antioxidant activity increased proportionally to the phenolic content and a linear relationship between DPPH-radical scavenging activity and total phenolics was established (R^2 = 0.56). No correlation between contents of other examined phenolic classes and antioxidant activity was observed. DPPH activity is a measure of non-enzymatic antioxidant activity. Higher levels of DPPH activity have been correlated with tolerance to different stress conditions [6], but at the same time, they may point to a source of easily accessible natural antioxidants that could be used as a possible food supplement or in the pharmaceutical industry.

Considerable variability in the contents of all other phenolic classes was observed. As shown in Table 1, the total tannins contents varied over a wide range between 0.88 and 2.06 g catechin/kg dry

plant material. The highest level of tannins was recorded in domestic genotype 1511, which also had the highest level of total phenolics. Proanthocyanidin contents ranged from 1.04-3.31 g leucoanthocyanidin/kg dry plant material. The highest content was observed in the U.S. Lori genotype, which also showed elevated DPPH activity (46.71%), although its total phenolic content was significantly lower, compared to phenolic-rich genotypes. It seems that the antioxidant activity of the seed extract of this genotype is due to high levels of proanthocyanidins. The amount of total flavonoids in the seed extracts of the examined genotypes, only reached up to 0.61 g rutin/kg dry plant material. This is considerably less than that observed in many wild growing plant species which are flavonoid-rich [7]. Flavonoids can directly scavenge molecules of active oxygen, including hydrogen peroxide, singlet oxygen, and superoxide, hydroxyl, and peroxyl radicals. They are also effective scavengers of peroxynitrite, a highly reactive oxidant formed when superoxide reacts with nitric oxide [8].

Table 1. Phenolic contents and antioxidant activity in extracts of investigated soybean genotypes.

Sample	Total polyphenols[a]	Tannins[a]	Proantho-cyanidins[b]	Flavonoids[c]	DPPH values[d]
1. LN92-7369[e]	4.66±0.28	1.70±0.12	2.24±0.23	0.48±0.02	48.17±2.78
2. 1581/99	4.07±0.21	1.71±0.09	1.90±0.07	0.53±0.06	35.55±1.47
3. 1511	4.88±0.19	2.06±0.06	1.93±0.22	0.53±0.06	45.98±2.65
4. 1499/99	3.15±0.08	1.36±0.01	2.58±0.22	0.50±0.04	43.79±2.53
5. Lori	3.38±0.10	1.14±10.49	3.31±0.15	0.49±0.05	46.71±2.69
6. Linda	3.40±0.16	1.38±0.09	2.42±0.20	0.55±0.02	35.04±2.02
7. Balkan	3.17±0.17	1.33±10.18	2.06±0.11	0.53±0.04	31.39±1.81
8. BL-8	3.60±0.19	1.29±0.05	1.80±0.09	0.51±0.01	36.98±2.13
9. Alisa	2.88±0.15	1.26±0.11	1.64±0.09	0.61±0.05	29.68±1.71
10. Tara	2.70±0.15	1.09±2.52	1.79±0.13	0.56±0.08	27.73±1.60
11. Meli	3.40±0.24	1.19±0.13	1.98±0.24	0.48±0.08	29.92±1.72
12. Sava	3.04±0.13	1.17±0.04	2.00±0.26	0.32±0.06	22.87±1.32
13. Venera	3.52±0.16	1.38±0.14	1.27±0.18	0.55±0.01	34.55±1.99
14. Morava	4.23±0.17	1.51±0.04	1.09±0.19	0.45±0.06	43.80±2.53
15. 1 x 2[f]	3.84±0.10	1.46±0.06	1.61±0.03	0.47±0.03	40.15±2.32
16. 4 x 2	3.54±0.21	1.76±0.14	1.12±0.03	0.61±0.05	45.25±2.61
17. 4 x 3	3.30±0.21	0.88±0.07	1.04±0.05	0.57±0.06	45.25±2.61
18. 5 x 1	4.72±0.07	1.51±0.16	1.53±0.07	0.50±0.05	47.20±2.72
19. 6 x 1	3.34±0.12	1.33±0.02	2.06±0.14	0.51±0.02	43.06±2.48
20. 7 x 8	3.38±0.18	1.09±0.06	1.40±0.20	0.49±0.03	24.33±1.40

[a] Expressed as g catechin/kg dry plant material.

[b] Expressed as g leucoanthocyanidin/kg dry plant material.

[c] Expressed as g rutin/kg dry plant material.

[d] Expressed as % of control.

[e] cultivars.

[f] F_1 hybrids.

Plants consumed by humans may contain thousands of different phenolic compounds. The effects of dietary phenolics are of great current interest, due to their antioxidative and possible anticarcinogenic activities. A popular belief is that dietary phenolics are anticarcinogens because they are antioxidants, but direct evidence supporting this supposition is lacking [9]. Phenolics may inhibit carcinogenesis by affecting the molecular events in the initiation, promotion, and progression stages. Isoflavones and lignans from soybean may influence tumor formation by affecting estrogen-related activities. They also modulate the growth of benign and malignant prostatic epithelial cells *in vitro* [10]. The bioavailability of the dietary phenolics has been discussed extensively, because the tissue levels of the effective compounds determine the biological activity. Epidemiological studies concerning consumption of phenolics and human cancer risk suggest the protective effects of certain food items and phenolics, but more studies are needed to reach clear-cut conclusions [11].

Conclusions

Our results on soybean seed extracts suggest that phenolic content should be considered as an important feature of soybeans, besides protein and oil contents. Soybeans are widely accepted as a "healthy food" and some of their pharmacological effects could be attributed to the presence of these valuable constituents. Results showed that Chinese and Serbian genotypes were rich in total phenolics and tannins, and a U.S. genotype in proanthocyanidins. For this reason, we propose that the biological source of the plant material needs to be more precisely defined, as observed antioxidant activities and phenolic contents were greatly dependent on plant material source. In the same time, it seems that total phenolic content, alone or in combination with other phenolic constituents, is a potential candidate as a selection criterion for antioxidant activity in soybeans.

Experimental

General

Twenty soybean genotypes (domestic and introduced cultivars and their F_1 hybrids, Table 1) were grown on experimental fields at the Institute of Field and Vegetable Crops at Rimski Šančevi, near Novi Sad. Seeds for the *in vitro* experiments were collected at the full maturity stage. Collected plant material was then dried in a shaded and well-ventilated place and kept refrigerated in dark all-glass containers until extracted. Plant material (1 g per sample) was ground to a fine powder in a mill and extracted for 20 min with 70% aqueous acetone (50 mL) under sonication in an ultrasonic bath at ambient temperature. The extracts were rapidly vacuum-filtered through a sintered glass funnel and kept refrigerated until assayed.

Total phenolics were determined by the Folin-Ciocalteu procedure [12]. Aliquots (0.1 mL) of aqueous acetone extracts were transferred into test tubes and their volumes made up to 0.5 mL with distilled water. After addition of Folin-Ciocalteu reagent (0.25 mL) and 20% aqueous sodium carbonate solution (1.25 mL), tubes were vortexed and after 40 min the absorbance of the resulting blue colored mixtures was recorded at 725 nm against a blank containing only extraction solvent (0.1 mL). The amount of total phenolics was calculated as a catechin equivalent from the calibration curve

of catechin standard solutions (covering the concentration range between 0.1 and 1.0 mg mL^{-1}), and expressed as g catechin/kg dry plant material.

Total tannin content was determined by the Folin-Ciocalteu procedure as above, after removal of tannins by adsorption on an insoluble matrix (polyvinylpolypyrrolidone, PVPP). Insoluble, cross-linked PVPP (Sigma, Germany; 100 mg) was weighed into test tubes and aqueous acetone extracts (1.0 mL) added. After 15 min at 4 °C, tubes were vortexed and centrifuged for 10 min at 4350 g. Aliquots of supernatant (0.2 mL) were transferred into test tubes and nonabsorbed phenolics determined as described. Calculated values were subtracted from total phenolic contents and total tannin contents expressed as g catechin/kg dry plant material.

Proanthocyanidins were determined by a butanol-HCl assay [12]. In brief, aliquots of prepared extracts (0.5 mL) were transferred into test tubes. After addition of butanol-HCl reagent (95:5 butanol-HCl, 3.0 mL) and 2% ferric reagent (2% ferric ammonium sulfate in 2.0 M HCl, 0.1 mL), test tubes were vortexed and placed in a boiling water-bath for 60 min. After cooling, absorbances were recorded at 550 nm against a blank containing solvent (0.5 mL) instead of the extract. Proanthocyanidins were expressed as g leucoanthocyanidin/kg dry plant material, assuming that the specific absorbance of leucoanthocyanidin was 460 nm.

For the determination of flavonoids, powdered plant material (1 g) was homogenized with extracting solvent (140:50:10 MeOH-H_2O-CH_3COOH, 20 mL) and filtered into volumetric flasks. Volumes were adjusted to 100 mL by addition of additional extracting solvent. To prepare the solutions for analysis aliquots (2.5 mL) were transferred into 50 mL volumetric flasks and their volumes made up with water. To each 10 mL of analysis solution, water (2 mL) and $AlCl_3$ reagent (133 mg crystalline aluminium chloride and 400 mg crystalline sodium acetate dissolved in 100 mL of extracting solvent, 5 mL) were added and absorbances recorded at 430 nm against a blank (10 mL of analyzed solution plus 5 mL of water). The amount of flavonoids was calculated as a rutin equivalent from the calibration curve of rutin standard solutions, and expressed as g rutin/kg plant material [13].

For this investigation, the total potential antioxidant activity of the investigated aqueous acetone soybean seed extracts was assessed based on their scavenging of 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals, using a modified DPPH assay [14]. An aliquot of extract (0.5 mL) was mixed with a ethanolic DPPH solution (0.5 mmol, 0.25 mL) and acetate buffer (100 mmol, pH 5.5, 0.5 mL). After standing for 30 min, the absorbance of the mixture was measured at 517 nm against a blank containing absolute ethanol (0.5 mL) instead of a sample aliquot. DPPH-radical scavenging activity is expressed as % of control.

All measurements were done in triplicate. Results were expressed as mean ± standard error. Statistical comparisons between samples were performed with Student`s t-test for independent observations. Differences were considered significant at $P < 0.05$. Correlation between total phenolics content and antioxidant activity was established by regression analysis.

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Sample availability: Available from the authors.