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ISOFLAVONES IN TRANSYLVANIAN SOYBEANS GENOTYPES

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Introduction

Soybean (*Glycine max L.*) is an important crop, grown worldwide as the most valuable plant-based source of protein known to mankind; besides, it is one of the cheapest and most convenient sources of protein available to date. Protein and lipid combined make up more than 60% of soybean seeds on dry weight basis. Besides macronutrients, these seeds contain a wide area of biologically active substances, such as isoflavones, phytates, lipids, phytoalexins, saponins, lectins, vitamins, carbohydrates, phytosterols, carotenoids, unsaturated fatty acids, etc. As a result, soy food intake has been shown to have several beneficial effects, such as those on cardiovascular diseases and cancer risk factors, on lowering the incidence of diabetes and increased tissue sensitivity to insulin, on osteoporosis' prevention, etc. Among the bioactive substances, isoflavones are important phytoestrogens, being associated mainly with women's health and increasingly used in dietary supplements [1, 2].

Research objectives

Since up to date there are no data on the content of these substances in Romanian soybeans genotypes, hence a proper assessment of isoflavones' intake from these was not possible, the major aim of this work was to establish the content of isoflavones from several commercial registered Transylvanian soybeans genotypes, created at the Research & Development Station for Agriculture (RDSA), Turda. The targeted aglycones from the analyzed matrices were genistein, glycitein and daidzein, while the glycosides were daidzin, glycitin and genistin.

Materials & Methods

Representative seeds belonging to 20 soybean genotypes were harvested from the experimental fields of RDSA Turda; ~ 100 g seeds were milled, then ~ 1g from the resulted flour was weighed and extracted with 20 mL ethanol (50%) on a magnetic stirrer (350 rpm, 60^0C, 2 hours). The resulted suspensions were vacuum-filtered, then the volumes were brought to 25 mL with ethanol (50%), being finally filtrated through 0,47 membrane filters then subjected to high performance liquid chromatographic (HPLC) analysis.

Six isoflavones were determined by an optimized HPLC method, using a Flexar system consisting from two UHPLC pumps, a solvent degasser, an autosampler, a column oven and an UV-VIS detector. Baseline separations were accomplished for daidzin, glycitin, genistin, daidzein, genistein and glycitein using a Kinetex column and gradient elution with acetonitrile and water, both with 0.1% acetic acid, in a total run time less than 8 minutes (fig.2). Quantifications were based on the external standard method. A summary of validation parameters is presented in table 1.

Table 1. A summary of validation parameters for the HPLC method

	Daidzin	Glycitin	Genistin	Daidzein	Glycitein	Genistein
Concentration range [mg/L]	1.2- 17	1.3 - 15	1.2 - 16	1 - 18	1.5 - 15	2 -16
Limit of detection [mg/L]	0.008	0.009	0.043	0.031	0.021	0.035
Limit of quantification [mg/L]	0.024	0.027	0.129	0.093	0.063	0.105
Liniarity (R^2)	0.9992	0.9999	0.9998	0.9970	0.9982	0.9977
Recovery	0.9731	0.9859	0.9902	0.9315	0.9577	0.9894

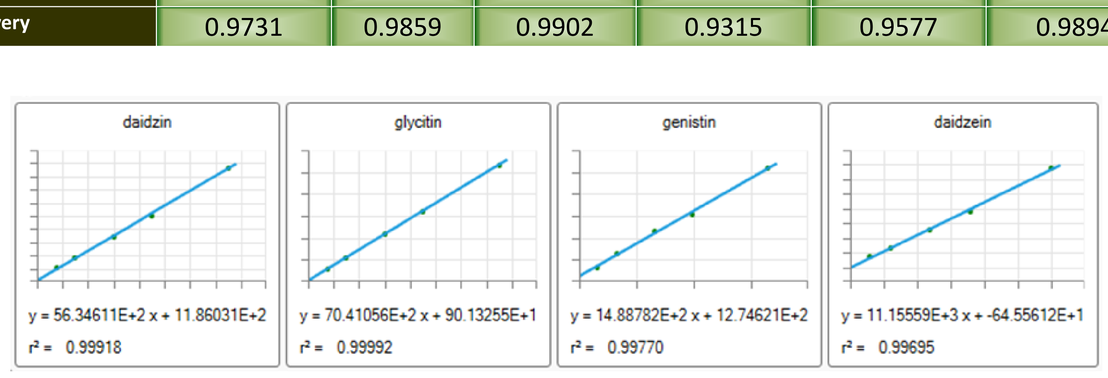

Figure 1. Calibrations for four of the target isoflavones

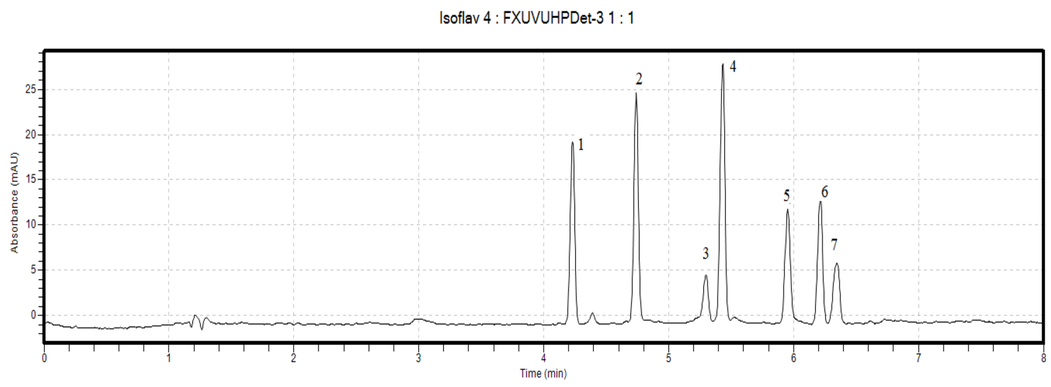

Figure 2. HPLC chromatogram of isoflavones (standard mix). *P*eak ID's: 1-daidzin, 2-glycitin, 3-genistin, 4-daidzein, 5-glycitein, 6-genistein, 7 - formonetin

Results

The analyzed genotypes showed particular isoflavone patterns, depending on genetic factors; the concentrations of the studied isoflavones recorded relative important variations.

The major isoflavones (table 2) were found to be the glycosides glycitin (up to 1017.60 mg/ 100 g) and daidzin (up to 691.68 mg/ 100 g), while genistin, daidzein, glycitein and genistein were found in smaller amounts (10.24 – 273.25 mg/ 100 g).

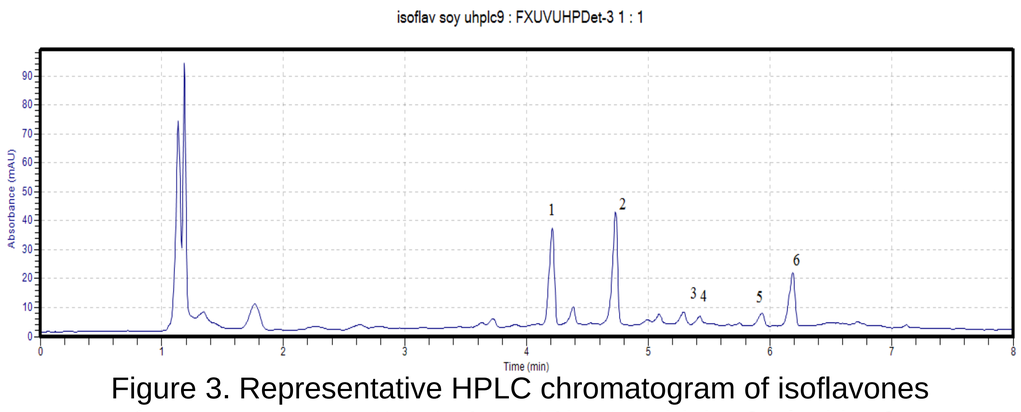

Figure 3. Representative HPLC chromatogram of isoflavones from a soybean extract. Peak ID's: 1-daidzin, 2-glycitin, 3-genistin, 4-daidzein, 5-glycitein, 6-genistein

Table 2. Descriptive statistics for isoflavones from soybean [mg/ 100 g]

	Daidzin	Glycitin	Genistin	Daidzein	Glycitein	Genistein
Average	359.43	564.65	47.85	58.98	36.37	130.23
Min	178.08	157.95	19.54	59.45	10.24	48.43
Max	691.68	1017.60	146.36	245.25	244.44	273.25

Conclusions

- A simple, reliable, fast and sensitive method has been developed for the analysis of isoflavones from soybeans using high performance liquid chromatography, accomplished with a Perkin Elmer Flexar UHPLC system with UV detection, enabling the separation of targeted compounds in less than 8 minutes.
- This study should provide a framework for new applied researches for both plant breeding as well as a new method for quality control of soybeans' products.

References

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