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A screening for the most representative classes of compounds from two varieties of soy

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Abstract

Glycine max (L.) Merr. Sin *Glycine hispida* (Moench) Maxim, known as soy is a plant from the Fabaceae family known for its nutraceutical properties. The product used in the pharmaceutical and food area is the soybean, *Glycine semen*, with a composition formed of proteins 35-40%, lipids 15-20%, sugars 15-35%, isoflavones 0,12-0,3 %.

The consumption of soy products has many health benefits, including protection against breast cancer, prostate cancer, menopausal symptoms, heart disease and osteoporosis. Many of the health benefits of soy are derived from its isoflavones. The chemical structure of isoflavones is very similar to that of our own estrogen. Therapeutic daily dose of genistein is 1.5 to 4.1 mg / person and genistin from 6.3 to 8.3 mg / person. For this study grains from two varieties (B and H) of *Glycine max* (L.) Merr. were considered for quantitative analysis of total lipids (Soxhlet), proteins (Kjeldahl), polyphenols (Folin Ciocâlteu) and isoflavones, daidzin, genistin, daidzein and genistein (HPLC).

Conducted tests show that different varieties of soybean have a different quantitative chemical composition in isoflavones. The concentration of total lipids and proteins fit in the values indicated in literature. The polyphenol concentration in the two samples was similar, 313 and 303 mg/100g. The glycosidic structures of isofavones were present in the ethilic alcoholic solution 90%. H variety has higher concentrations of daidzin 819.0 mg /g and genistin 905.6 mg /g towards B variety 460,48 µg/g daidzin and 364,60 µg/g genistin. H variety is preferred to be used in furtherer studies.

Keywords: soy, isoflavones, polyphenols, lipids, proteins

1. Introduction

Natural products have a long history of use in the service of manking for the prophylaxis and treatment of several diseases. For nearly five millennia soybean, the vegetal product from *Glycine max* (L.) Merr. Sin *Glycine hispida* (Moench) Maxim, family *Fabaceae* has been a staple of the Southeast Asian diet and both its medicinal and nutritional values are deeply rooted in Traditional Chinese Medicine and Herbal Medicine.

A remarkable amount of research regarding the healthy effects of soy consumption was carried out during the past 20 years, and the conclusions were that in most of the cases positive effects can be attributed to the presence of isoflavones, a class of organic compounds produced almost exclusively by the members of the *Fabaceae*/*Leguminosae* (bean) family [1].

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Soybeans are an important global crop, providing oil and protein. Soybeans are native to East Asia but only 45 percent of soybean production is located there.

Consumation of soy food was associated with low incidences of a number of chronic diseases, such as cardiovascular diseases, cancer, and osteoporosis. Suggested as the major bioactive components in soy, isoflavones and soy protein got a considerable attention in the research area. The chemical structure of isoflavones is very similar to that of our own estrogen. Therapeutic daily dose of genistein, the most important soy isoflavonoid is 1.5 to 4.1mg / person and for it's glicozide genistin from 6.3 to 8.3mg / person [2,12]. But, soybeans and soy-based foods have hundreds of phytochemical components. In recent years, accumulating evidence has suggested that the isoflavones or soy proteins stripped of phytochemicals only reflect certain aspects of health effects associated with soy consumption. The benefic effect of soy may be attributed also to other phytochemicals, either alone or in combination with isoflavones or soy protein [3].
It is known the fact that product used in the pharmaceutical and food area is the soybean, *Glycine semen*, with a composition formed of proteins 35-40%, lipids 15-20%, sugars 15-35%, isoflavones 0,12-0,3 % [4,13]. The aim of our study is to determine the most representative classes of compounds, responsible for the therapeutic action, from two varieties of soy: H and B and to see if the results obtained resemble values given by literature in order to decide if the two varieties can be used for further studies.

2. Materials and methods

For this study grains from two varieties (B and H) of *Glycine max* (L.) Merr. were considered for quantitative analysis of total lipids (Soxhlet), proteins (Kjeldahl), polyphenols (Folin Ciocâlteu) and isoflavones- daidzin, genistin, daidzein and genistein (HPLC). The seeds from two different soy varieties G and H were kindly provided from University of Agricultural Sciences and Veterinary Medicine, Timisoara, Romania, Department of Plant Culture.

For the determination of total lipids a Soxhlet device was used. The method involved the repeated and continuous extraction of tested material with a specified volume of solvent.

The total product obtained after removal of solvents represented the fats. From the sample we wanted to analyze, we weighed 5 g on an analytical balance and afterwards we placed it in an extraction thimble. Extraction was carried out with acetone in Soxhlet apparatus for 3 hours at a temperature of 90 ºC. After the last siphoning, cartridge was removed from the extract. The extract obtained was concentrated in a rotary evaporator at 50 ºC.

Nitrogenous substances of plant matter are represented in a big proportion of proteins. Nitrogen content of proteic substances from plants rather wide range of limits, but conventional admits an average of 16%. It is considered that a gram of nitrogen (1g N) corresponds to 100/16 = 6.25 g protein [5]. The determination of protein may be reduced to the dosage of organic nitrogen. By multiplying the quantity of organic nitrogen by a factor of 6.25 is obtained the so-called crude protein.

Kjeldahl method was used. This method is based on the decomposition of organic substances with boiling sulfuric acid in the presence of a catalyst (copper sulfate), followed by ammonia release by prior alkalinization with concentrated sodium hydroxide, and capturing it in hydrochloric acid solution (0.1 N) and then back-titration with normal sodium hydroxide solution.

Determination of total nitrogen consists of three operations: digestion, distillation and titration with a solution of sodium hydroxide 0.1 N.

Analysis of total polyphenols was performed by Folin Ciocâlteu spectrophotometric method. The result was expressed as mg fenol/100sample. Phenols reduced fosfopolimolibdenic acid to blue molybdenum witch can be colorimetrically determined. Sensitivity of the method is 0.2 mg phenol/ml.

10 g sample was introduced in a 250 ml Erlenmeyer and extracted with 50 ml NaOH 0.1 N solution by stirring with a magnetic stirrer. The solution was filtered, and was captured in a 100 ml flask. The filter and the flask were washed with 15 ml NaOH 0.1 N three times. Combined solution was completed to the mark with NaOH 0.1 N solution.

From this solution 10 ml was pipetted into a 20 ml graduated tube. It was neutralized first with HCl, 1 N then near the point of neutrality with HCl 0.1 N in presence of litmus paper and filled with water to 20 ml.

It was taken 10 ml from the solution, and added 0.5 ml Folin-Ciocâlteu reagent and 2 ml of sodium carbonate 20%. The solution was stirred for 30 minutes, and afterwards it was inserted 1 minute in a boiling water bath. The sample was cooled immediately in running water.

After 5-10 minutes was measured the extinction spectrophotometricaly at λ = 720 nm, in a 1 cm cuvette. Calibration curve was drawn between 10-50 mg of phenol by 10 to 10 points. Extinction values were multiply by 2 (because we worked with 10 / 2 of the extracted solution) resulting in C concentration of polyphenols.

The most important isoflavonoidic structures in soybeans, in terms of therapeutic action and quantitative ratio are genistein, daidzein and their glycosides. Dry soybeans were putted into powder. Hydroalcoholic extracts were made, containing 10% vegetal product, using as solvent 70% ethanol, ultrasonicated for 10 minutes in the ultrasonic bath.

The experiment was carried out using an Agilent 1100 HPLC system equipped with an degasser, binary pump, autosampler and column thermostat. For the separation of compounds, a reversed-phase Zorbax SB-C18 analytical column (100 x 3.0 mm i.d., 3.5 µm particles) was used. The column thermostat was operated at 48˚C. The mobile phase used for the elution and separation of isoflavons was a mixture of acetic acid 0.1% (V/V) in water (A) and methanol (B), in linear gradient mode, as follows: until 2 min, 20% B, at 10 min 40% B, at 10.5 min 40% B, at 11.5 min 45% B, hold 45% B until 17 min. The flow rate was 1 ml/min. For detection and quantification, the HPLC system was coupled with an Agilent 1100 Ion Trap SL mass spectrometer, operated with an electrospray (ESI) ion source in negative ion mode. The nebulising gas used by mass spectrometer was nitrogen, at 65 psi; the dry gas was also nitrogen at flow rate of 12 L/min and heated at 360 C. The capillary potential was set at +2500 V. The analysis mode of isoflavons was either in single ion monitoring mode (SIM) – for aglycons or in single reaction monitoring mode (SRM) – for glycosides Table 1.

The calibration curves for all isoflavons were built in range of 40-4000 ng/ml. For fitting the calibration curves, a quadratic model and 1/y weighting scheme was used. The accuracy of calibration points, for each compound, was no more than ± 15%.

Table 1. The retention time of isoflavons and their mass spectrometry detection parameters

No.	Compound	Retention time (min)	Detection mode*	Parent m/z ion $[M-H]^-$	Quantified m/z ion
1	Daidzin	3.7	SRM	415	253
2	Genistin	5.5	SRM	431	268, 269
3	Ononin	8.9	SRM	429	267
4	Daidzein	9.2	SIM	253	253
5	Genistein	11.0	SIM	269	269
6	Formononetin	14.4	SIM	267	267

*SRM= single reaction monitoring; SIM = single ion monitoring

3. Results and Discussion

For the determination of lipid content from the total weight of the balloon witch contains the crude fat we minus the weight of the flask. The result is reported in percent. Lipid concentration value for the two soybean samples are presented in Table 2.

Table 2. Total lipids/100g sample

Analysed sample	Total lipids/100g sample %
Soy B	18,8
Soy H	21,4

H-type soybeans have higher lipid content than soybeans type B. In soybeans, the literature presents a lipid concentration up to 20-22%, with minimum values of 15.4% and maximum 25.3% The amount of lipid present in seeds depends on climatic conditions of the culture of the plant [6,7]. Both types of seed have lipidic concentration that correlate with values given by the literature. Soy type H has a greater content in lipids than soy type B

Protein concentration value for the two soybean samples are presented in Table 3.

Table 3. Protein concentration value for the two soybean samples

Analysed sample	Protein %
Soy B	36,40
Soy H	39,40

H-type soybeans have higher protein content than soybeans type B. In soybeans, the literature shows a concentration of nutritional proteins witch values up to 40%, with minimum values of 35.0% and maximum values of 46.83%. The amount of protein present in seeds depends on climatic conditions of the culture of the plant [8,9].

Both types of seed have a protein concentration that correlate with the literature values. Values of total polyphenol concentration for the two samples are presented in Table 4.

Table 4. Concentration of total polyphenols in soybeans

Analysed sample	Total polyphenols mgP/100g sample
Soy B	313
Soy H	303

The two types of soy beans have a similar concentration of total polyphenols. These structures, by the complexity of their mechanism of action are responsible for the antioxidant, anti-inflammatory, antimicrobial and antitumor action [10].

The values of isoflavonic structures present in soybeans are presented in Table 5.

Table 5. The concentration of isoflavones in soybeans structures. HPLC analyze

No.	Compound name	Sample Soy B µg/ml	Sample Soy H µg/ml	Sample Soy B µg/g	Sample Soy H µg/g
1	Daidzin-glicozide of daidzeine	46.048	81.90	460.48	819.0
2	Genistin-glicozide of genistein	36.46	90.56	364.6	905.6
3	Ononin	0	0	-	-
4	Daidzein-aglicon	0	0	-	-
5	Glycitein	0	0	-	-
6	Genistein-aglicon	7.33	11.90	73.3	119.0
7	Coumestrol	0	0	-	-
8	Formononetin	0	0		

Agliconic structures (daidzein, genistein) are present in low concentration in the hydroalcoholic extract, with a low solubility in the chosen solvent extraction. Daidzein was not detected in the extractive solution. The concentration of glycosidated isoflavonic structures differ in the two analyzed groups. Soybean seed in group H were found to have significantly higher concentrations compared to group B.

Literature mentionates values of total isoflavonoidic structures that range from 2.038% to 9.514%. Other data suggest a concentration of isoflavonic structures of 3g/kg soybean [11,12, 14].

The concentration of flavonoids in group H 918,38µg/g ranges values mentioned in literature, but the concentration of flavonoids in group B 1.843,6µg/g it's under the limit value presented in literature. Thus, in various subsequent studies, that require a high concentration of isoflavonoids for a certain therapeutical action it will use soybeans from group H.

4. Conclusion

Conducted tests show that different varieties of soybean have a different quantitative chemical composition. The results from present study show the fact that booth types of soy (H and B) present a concentration of lipids and proteins that fit values from literature, but soy type H presents a greater content of these structures so it is preferred to be used in furtherer studies. The total polyphenolic concentration in the two samples was similar, 313 and 303 mg/100g. The glycosidic structures of isofavones were present in the ethilic alcoholic solution 90%. H variety has higher concentrations of daidzin 819.0 mg /g and genistin 905.6 mg /g towards B variety 460,48 µg/g daidzin and 364,60 µg/g genistin. In terms of content in isoflavonoidic structures, soy type H fits values mentioned in literature but soy type B has a content of isovlavones that are slightly below the lower limit, so furtherer studies *in vitro* on cell lines and *in vivo* on animal model will use the extracted active principles from soy type H.

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