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Bioactive Components of Soybean as a Source of Medicinal Products

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ABSTRACT

This article presents the results of qualitative and quantitative composition of biologically active substances of green mass 6 recognized varieties of soybean, soybean seeds, soybean meal after oil extraction and biologically active complex of soy. The presence of 21 amino acids, 7 flavones, 6 isoflavones, 2 coumarin, 4 phenolcarbonic acid, tannins, vitamins (E, K, P, B), polysaccharides was determined. The resource-saving technology of oil production, biologically active complex and protein were developed.

Keywords: Soybean, Biologically active substances, Green mass, Meal, Qualitative composition, Quantitative, Biologically active complex

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INTRODUCTION

Conditions of modern life with its unfavourable ecology, psychological pressures, stresses, unbalanced and unreasonable nutrition lead to deterioration of adaptability of the organism and thus to the development of so-called diseases of civilization: heart and vascular disturbances, cancer, nervous and psychiatric problems, allergy, etc.

A variety of herbal and plant based medicines, biologically active supplements is a growing field in the pharmaceutical industry.

Previous research has shown that soya, such as *Glýcine máxima* (L.) Merr. or *Glýcine híspida* (Moench) Maxim., or *Glýcine soja* Sieb. et Zucc., pea family Fabaceae, subfamily Faboideae, genus *Glycine* L., effectively supplies the organism with required nutrient. According to chemical composition, soybean is an exceptional plant among all herbal products. Soybean seeds contain high level of protein (38-47%), which is equivalent to "ideal" by its amino acid composition, fats (19-25%), and carbohydrates (25-30%). Soybeans carbohydrates include 9-12% of soluble sugars (raffinose, stachyose, and sucrose), 3-9% of starch, 3-6% of cellulose, along with a variety of vitamins, enzymes and minerals [1,2].

Soybean and its products are well known as good nutrition and diet supplements. Today, soya products are widely presented in the world market: soya butter, flour, isolated protein, textured soy protein, soy protein concentrate, fermented soya products (sauce, meat, natto), soy-based food (milk), and lecithin [3].

Soybean is a highly demanded product in Western Europe and Eastern countries. In several Asian countries, soybean has been the basic nutrient for thousand years and represents an adequate substituent for meat, eggs and milk. Soybean based diet is one of the factors of longevity in Japan (83 years old for women whereas men reach 79). It is also used as an additive included in preparation of meat, bread and confectionery products [3].

Recently, researches were oriented on health benefits of soya that can help to prevent and cure a number of diseases. For instance, soya protein and butter have therapeutic and dietetic effects. These products contain phytoestrogens in much higher concentrations than oats, maize and barley. According to Japanese scientists, women who regularly consume soya products have 90 times lower risk of breast cancer development than women in Western countries. Soy milk and products at its basis are recommended for preventing ulcer, liver, kidney and biliary tract diseases, typhoid fever and chronic infectious diseases of gastrointestinal tract. It is included in the diet for children suffering from diathesis and allergies to animal proteins. Soy phosphatides are widely used as emulsifiers in confectionery, pharmaceutical, paint and varnish industries. Today, soybeans found a new use as a mean to increase body's resistance to radiation exposure. Soybean content can protect from ionizing radiation injuries having preventing or mitigating effects on the toxicity levels. Soy lecithin helps in inhibiting cholesterol precipitation in bile [4-7].

The aim of our study was to investigate biologically active substances of phytomass of 6 regional varieties of soybeans, soybean seeds and soybean meal after oil extraction in order to create new medicines and dietary supplements.

At the present stage of economic development of Ukraine, soy is of great importance as a valuable and profitable protein and oilseeds crop. The importance of soybeans for solving food and energy problems clearly can be traced in Ukraine. The sown area has risen from 16,000 hectares in 1996 to 5,82,000 hectares in 2007 and 2.2 million hectares in 2016. Soybean production has increased from 22,000 to 72,0000 tons during the same period. In the next 3-5 years, Ukraine can expect a further increase in the average of soybeans to 2.5-3 million hectares and increase the gross collection up to 5-6 million tons. Ukraine takes the first place in Europe by the soybeans planting acreage [8].

State programs were developed in Ukraine: "Soya in Ukraine 2008-2015" and "Development and implementation of technologies for production of soy products". These programs expanded the range of soybean use in food processing industry, medical and pharmaceutical industries, and forage production [9,10].

Ukrainian soybean varieties of natural selection have benefits on the world market over GM varieties that are grown in North and South America. Therefore, interest in the domestic varieties of soybean is growing [11].

MATERIALS AND METHODS

According to the Registry of Plant Varieties of Ukraine, for phytochemical studies were selected the following soybean varieties: Kharkovskaya 35, Kharkovskaya 66, Kharkovskaya zernokormovaya (3K), Romance, Dream and Horizon. Varieties Kharkovskaya 35, Kharkovskaya 66 were created in Ur'iev institute by hybridization of different varieties; Kharkovskaya zernokormovaya (3K), Romance, Dream and Horizon by chemical mutagenesis followed by multiple individual selections [12]. Objectives of our study were above ground part (phytomass) of mentioned above soybean varieties collected in the period of milky-wax-ripeness, soybean seeds, soybean meal after oil extraction and biologically active soybean complex. Samples of plant were provided by Institute of Animal Production of Ukrainian National Academy of Agrarian Science (NAAS).

Examined samples were macerated in 80% water-ethanol solution for 16-18 h at room temperature, then blended with water (1:1) and evaporated. The obtained crude extract was treated with organic solvents of varying polarity. The following resulting fractions were obtained: chloroform fraction containing coumarins and triterpenoids; ethyl acetate fraction containing flavonoid aglycones and their monocytes; butanol fraction containing flavonoid glycosides; water fraction containing flavonoid biocides and hydroxycinnamic acid.

Separation of isolated compounds was performed on columns packed with silica gel and polyamide. Monitoring was carried out by Thin-layer Chromatography (TLC) on plates of Sorbfil and Silufol in various solvent systems: chloroform-ethyl alcohol (9:1), and Paper Chromatography (PC) in the following solvent system: 5% solution of acetic acid–n-butanol–water (4:1:2).

Water-alcohol solutions were used for the extraction of biologically active polyphenol compounds. Methods of adsorption and distribution by chromatography were employed for separation employing different sorbents (silica gel, polyamides, aluminium oxide). The structure of obtained compounds was defined by their physicochemical properties. Results obtained by paper and thin layer chromatography, UV-Vis and FT-IR spectroscopies were compared to valid standards of flavonoids and their derivatives.

The content of free amino acids was determined in water alcohol extracts of all mentioned above samples on amino acid analyzer AAA 881 (Czech Republic); their presence was confirmed by ninhydrin reaction [13].

Fatty acids composition was determined for soybean oil on GLC chromatograph "Color 500" with a flame ionization detector. Components identified by reference materials (Sigma) methyl esters of fatty acids and their relative retention times. Hitachi F4010 spectrophotometer was used for determination of the Polyphenols, flavonoids and carotenoid content in water-ethanol extract of soybean phytomass.

RESULTS AND DISCUSSION

Qualitative analyses were carried out using paper and thin layer chromatography allowed to identify 7 flavonoids (quercetin, kaempferol and their glycosides), 6 isoflavonoids (genistein, formononetin, daidzein and their glycosides), 4 phenolcarbonic acids (chlorogenic acid, neochlorogenic, ferulic, π-coumaric), organic acids, coumarins (umbelliferone, scopoletin), tannins. Besides that 21 amino acids (10 essential), water and oil soluble vitamins (group B–B_1, B_2, B_3, B_4, B_6, vitamins E, K, P) were identified.

Determination of the quantitative content, depicted in Table 1, shows that soybean seeds of investigated varieties contain 36-42% of protein, 21-28% of fats.

Table 1: Chemical composition of soybean seeds, % dry matter

Varieties of soybeans	Protein, %	Fat, %	Ash, %
Horizon	37-38	23-24	4.5
Mechta	38-39	22-23	4.8
Romantic	39	23	4.9
Kharkovskaya 35	36-38	21-22	4.6
Kharkovskaya 66	36-37	22-23	4.8
Kharkovskaya 3K	40.3	28	5.2
Whole seeds	40	21	4.7
Seed-lobe	42.6	22.5	4.9
Seed coat/hulls	8.5	0.9	4.2
Germ (nucleus)	40.5	1.1	4.3

As depicted in Table 1, protein content in soybean seeds of Kharkovskaya 3K is 40.3%, fats –28% that exceeds standard values of 4.5% and 1.5% respectively. The extractives content is 15.41% and 26.45% in the grass (phytomass), seeds, and ashes content is 2.21 and 5.2% respectively.

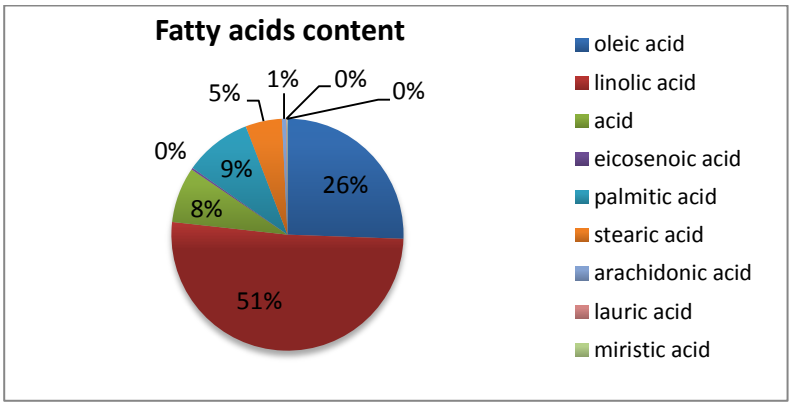
We have quantified the content of seven groups of biologically active substances in the following breeds: Horizon, Mechta, Romantic, Kharkovskaya 3K. We found the content of flavonoids (0.27, 0.19, 0.32, 0.59 respectively), hydroxy-cinnamic acids (1.19, 2.25, 2.12, 1.87), tannins (1.01, 1.43, 1.21, 1.44), organic acids (1.4 1.37, 1.31, 1.22), ascorbic acids (0.064, 0.06, 0.58, 0.054), polysaccharides (8.51, 10.39, 12.87, 15.33), total pectin content (7.18, 7.05, 6.89, 5.87).

The highest amino acid and protein content was found in the soybean grass (phytomass) collected in the period of milky-wax-ripeness in investigated varieties. Besides that investigated varieties contain up to 3% of lipophilic substances, 11-13% of alcohol-soluble polysaccharides and 5-7% of water-soluble polysaccharides, 11-12% of pectin substances and almost 40% hemicellulose.

The total protein content (on dry matter) in soybean seeds is 37.64%, and 45.30% in soybean meal after oil extraction, 17.07% in phytomass.

The biggest quantity of glutamic acid (7.45, 7.89, 6.75%), aspartic acid (4.85, 5.81, 4.98%) and leucine (2.95, 3.13, 2.08%) were found in soybean seeds, soybean meal and biologically active complex respectively.

The composition of soybean oil includes neutral lipids represented by triglycerides or triacylglycerols with different fatty acids (saturated, unsaturated and polysaturated fatty acids). Fatty acids content of soybean oils obtained from Kharkovskaya 3K presented in Scheme 1.



Scheme 1: Fatty acids content of soybean oils obtained from Kharkovskaya 3K

Additionally, soybean lipids contain phospholipids (1.5-2.5%), carotenoids (1.0-3.1%), tocopherols (0.15-0.21) and sterols (0.19-0.30). The main component of phospholipids is phosphatidylcholine, tochopherols-α-tocopherol, sterins-β-sitosterol.

Polyphenols, flavonoids and carotenoid content of soybean phytomass water-ethanol extract was determined on Hitachi F4010 spectrophotometer (Figure 1).

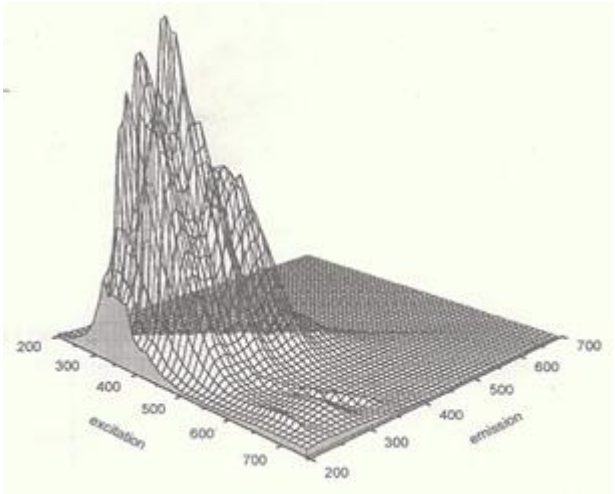


Figure 1: Content of polyphenols, flavonoids and carotenoids in hydrophilic extract of soybean phytomass

The results showed that hydrophilic extract of soybean phytomass contains flavonoids, which are characterized by peaks in regions of fluorescence excitation λexc 240-290 nm and emission λem 390 nm. A polyphenol compounds shows fluorescence excitation at λexc 290-355 nm and emission λem 365-410 nm. A series of peaks in the fluorescence excitation λexc regions 230, 280 and 575 nm and emission λem 675 nm are characteristic for carotenoids.

Soy and its products are widely used as complementary food supplements and curative care. We have developed a combine technological scheme of the oil production and biologically active complex.

High quality of soy oil and favorable combination of fatty acids (oleic, palmitic, linoleic and stearic) allow using it for production of margarine and other products in food industry.

Biologically active complexes of soybean oil (phospholipids, triglycerides, and others) have a regulating effect on vascular tone and their metabolic processes, provide a protective effect in the development of alcoholic liver cirrhosis and prevent catabolism of protein, and possess antioxidant properties etc. [14].

Biologically active complex is a new vegetable anabolic, containing amino acids, polysaccharides, phospholipids, and proteins etc., obtained from a soybean meal after oil extraction. It is recommended as a supplement for enhancing protein-energy processes, regulating acid-base balance and increases the body defense power. The current treatment of protein-energy deficiency by hormonal anabolic has inherent side effects. Therefore, even long term application of soybean complex having anabolic effect and absence of side effects is beneficial. Soybean complex can be used in surgical, nephrology or therapeutic practice as a remedy that affects metabolism and immune system.

Soybean protein is used as a supplement for a protein fortification of bread, pastry and sausage products, potato products (crisps), mayonnaise, soymilk and meat (close to the composition of cow's milk). The use of the nutritional soy products allows, on one hand, to increase the nutritional value of the human diet due to protein content, and on the other, to give it in medical and preventive purposes. Thus, consumption of soy products helps to reduce cholesterol levels, quickly compensate loss of energy, increase muscle mass, significantly reduce the risk of obesity and cardiovascular diseases. Soy products, due to their nutritional value, can be used by military, students, sportsmen. They should be included in the daily diet, preventive nutrition, which is recommended for chronic liver, kidney, heart and others diseases.

Thus, these results indicate therapeutic and prophylactic properties of soy products and prospects of their application in the complex therapy of various diseases, the necessity of the development for this purpose range of special products having directional effects based on soybeans biologically active substances.

All of the above is the justification for a comprehensive study of soybean biologically active substances for creation highly effective drugs of various kinds of action, as well as for use in the dietary therapy.

CONCLUION

We have conducted a phytochemical study of herbs collected in the period of milky-wax ripeness seed meal (waste) after extraction of soybean oil, biologically active complex of 6 regionalized soybeans varieties. The most promising varieties for the profound pharmacological study are Dream, Horizon, Romance and Kharkovskaya zernokormovaya. The following biologically active substances were defined in the investigated objects: 21 amino acids, 7 flavonoids (quercetin, kaempferol and their glycosides), 6 isoflavonoids (genestein, formononetin, daidzein and their glycoside), 2 coumarins (umbelliferone, scopoletin), 4 phenolcarboxylic acid (chlorogenic, neochlorogenic, ferulic, π-coumaric), tannins, vitamins (E, K, P, group B), polysaccharides. New technology was proposed for the production of soybean oil, biologically active complex and soy protein. The use of biologically active substances of soybean enables to enhance the range of nutrients and health-care products for the human organism.

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