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BLACK SOYA BEAN AND BLACK CHIA SEEDS AS A SOURCE OF NUTRIENTS AND BIOACTIVE COMPOUNDS WITH HEALTH BENEFITS

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Abstract: Recent trends in healthy lifestyle and diet made functional foods rich in quality nutrients and bioactive compounds with potential health benefits highly sought after. Some cultivated plants, such as soya and chia can provide a viable source of nutraceuticals with high fibre, protein, and protective antioxidant potential. The whole-grain flours of black soya beans and black chia seeds were used in this study. Potential nutritive and health-benefitting properties of these flours were compared by assessing their chemical composition and antioxidant profile. The content of dietary fibres such as NDF, ADF, ADL and hemicellulose determined in black chia seed sample was higher than in black soya beans which had higher fibre content. The total protein content recorded in black soya beans was almost as twice as high ($42.26 \pm 0.14\%$) as chia protein content ($25.04 \pm 0.20\%$). Black soya beans had the highest content of water soluble proteins ($29.00 \pm 0.13\%$ d.m.) with NSI (nitrogen solubility index) accounting for as much as $70.96 \pm 0.31\%$ of total proteins, while black chia seeds had the highest content of globulins ($14.64 \pm 0.07\%$ d.m.) and NSI $58.48 \pm 0.27\%$ of total proteins. Both black soya bean and black chia contained a high amount of total phenolic compounds (830.66 ± 5.46 and 1201.94 ± 16.29 mg GAE/kg, respectively) and exhibited a considerable total antioxidant capacity, which makes them good contestants for functional food ingredients with potential health benefits.

Key words: chemical properties, phenolics, flavonoids, anthocyanins, fibres, colour

INTRODUCTION

Dietary preferences have changed considerably worldwide in recent years because of the increasing awareness about the strong relation between nutrition and human health. Dietary fibre, proteins, and bioactive non-nutrients, called phytochemicals, which could be either

incorporated into the diet or be a part of the food itself, can be the source for gaining long term health benefits. Functional food products are excellent options as they are aimed to improve life quality by exhibiting some desirable health benefits and preventing nutrition-related

diseases (Cederroth & Nef, 2009; Domínguez Díaz et al., 2020). The popularity of various plant-based functional products such as those made of soya beans and chia seeds, is constantly growing.

Soya bean (*Glycine max*) is the most widely used legume with 336 million metric tons produced worldwide in 2019 (Statista, 2019). Soya bean is composed of macronutrients (proteins, lipids, and carbohydrates), micronutrients (vitamins, and minerals), and phytochemicals which include phytosterols, tocopherols, carotenoids and phenolic compounds (Cederroth & Nef, 2009; Barać et al., 2014).

Soya products have been increasingly becoming important protein-rich food in the human diet because of its nutritional, functional, and even benefiting properties to cardiovascular diseases and overall health due to high-quality proteins, low saturated fats content, and no cholesterol (Tang, 2019).

For centuries, black soya bean has been widely consumed as a medicinal food in Korea, China, and Japan (Xu & Chang, 2008). Their seed coat consists of a high level of anthocyanins: cyanidin, delphinidin, and pelargonidin as 3-O-glucosides (Žilić et al., 2019). The content of anthocyanins and proanthocyanidins located in the epidermal layer of the seed coat is responsible for the difference in colour between yellow (standard) and black soya bean (Žilić et al., 2013). Recently conducted studies have shown that polyphenols and dietary fibre from the soya bean seed coat can be used as bioactive ingredients in functional food and pharmaceutical products aiming at different health problems (Žilić et al., 2020). Favourable biochemical composition is what makes black soya bean valuable source of phenolics and other nutrients, in addition, the impact of phenolics in the seeds is not crucial for the yield of these cultivars (Kiprovski et al., 2019). Significant antioxidant, anti-obesity, and anti-diabetic properties of black soya bean seed coat anthocyanin extract (BSSCE) were reported by Thompson et al. (2016). Anthocyanins have also been investigated because of their ability to reduce the risk of different diseases like atherosclerosis, cancer, diabetes, ischemia, obesity, and neurodegenerative disorders (Kim et al., 2008, 2012).

Chia (*Salvia hispanica* L.) of the family Lamiaceae is an annual herbaceous species that

originates from Central America where it was traditionally cultivated in the pre-Columbian period (Caruso et al., 2018; Oliveira-Alves et al., 2017). The interest in the study of chia seeds is growing nowadays due to their nutritional characteristics and health-promoting properties. Chia seeds are a good source of fibre, proteins, antioxidants, essential fatty acids, vitamins, and minerals. These seeds, by many considered a "superfood" that contribute to human nutrition and fight obesity by helping to increase the satiety index, also exhibit several biological properties (Valdivia-Lopez & Tecante, 2015). Studies showed that dietary intake of bioactive compounds of chia seeds can lead to the reduction of cardiovascular risk factors that include diabetes, hypertension, and inflammation. Chia seeds are also gluten-free, so they can be consumed by persons that suffer from celiac disease (Caruso et al., 2018; Menga et al., 2017). Chia seeds are usually grey in colour with dark spots, but their colour may vary from white to black.

The chia seeds are commonly used ground, like whole-grain or chia fibre flour, as an ingredient in bakery products, refreshing drinks, beverages, fruit juices, and salads (Caruso et al., 2018; Oliveira-Alves et al., 2017).

The aim of this study was to investigate and compare potential nutritive and health-benefitting properties of black soya bean and black chia seed by assessing their chemical composition and antioxidant profile. The whole-grain flours of black soya bean and black chia seeds were used to determine ash, oil, dietary fibre, total protein content, and soluble protein fractions. Content of phenolic compounds and the total antioxidant capacity were determined and colour of the whole-grain flours was measured.

MATERIALS AND METHODS

Black soya bean genotype Black Tokyo was obtained from the Maize Research Institute, Zemun Polje gene bank, and black chia seeds were purchased in a local health food store. The whole-grain flour (particle size < 500µm), obtained by grinding soya bean kernels and chia seeds on a Cyclotec 1093 lab mill (FOSS Tecator, Sweden) was used in the analyses.

Chemical analyses

The oil content was determined according to the Soxhlet standard method (AOAC, 2000). The ash content was determined by the slow

combustion of the sample at 650 °C (AOAC, 1990).

Analysis of dietary fibre

The content of hemicellulose, cellulose, neutral detergent fibre (NDF), acid detergent fibre (ADF), and lignin (ADL) were determined by the Van Soest detergent method modified by Mertens (1992) using the Fibertec system. The method is based on the fibres solubility in neutral, acid, and alkali reagents. NDF practically represents total insoluble fibres (not soluble in water), ADF mainly consists of cellulose and lignin, while ADL is pure lignin. The content of hemicellulose was obtained as a difference between NDF and ADF contents, while the cellulose content was calculated as the difference between ADF and lignin contents. All the results are given as the percentage per dry matter (d.m.).

Protein analysis

The protein content was determined by the Kjeldahl method as the total nitrogen multiplied by 6.25 (AOAC, 1990). The results are expressed in the percentages of proteins per dry matter (d.m.).

Analysis of soluble protein fractions

Successive extractions of the whole-grain flour with a series of solvents (in a ratio of 1:10, w/v) were performed to obtain different protein fractions by the method proposed by Landry and Moureaux (1970), with some modifications. Albumin, globulin, gliadin, and glutelin (G3-glutelin) fractions were extracted by distilled water, 0.5mol/l NaCl, 70% ethanol, and 0.0125mol/l borate buffer, pH 10, containing 5% sodium dodecyl sulphate, respectively. The non-protein nitrogen (NPN) (free amino acids) has been determined as the acid-soluble nitrogen remaining in the supernatant following protein precipitation with 20% trichloroacetic acid (TCA). The protein content was calculated, in each fraction, from the nitrogen content determined by the micro Kjeldahl method, using 6.25 as the conversion factor. The results are given as % of dry matter (d.m.), as well as % of total protein (nitrogen solubility index, NSI) (Žilić et al., 2011).

Extraction of soluble free phenolic compounds

Extracts for the detection of total phenolic compounds, total flavonoids, and phenolic

acids were obtained by continuous shaking of 0.5 g of sample in 5 to 10 ml of 70% (v/v) ethanol for 30 min at room temperature. After the centrifugation at 11200 g for 5 min, the supernatant was used for the experiments. For the detection of soluble free phenolic acids 5 ml of extracts were evaporated under the N₂ stream at 30 °C to dryness. Final residues were redissolved in 1.5 ml of methanol. These methanolic solutions were used for the HPLC analysis. The extracts were kept at -70 °C until the analysis. All extractions were performed in duplicates.

Analysis of total phenolic content (TP)

The total phenolic content was determined by the Folin–Ciocalteu assay and expressed as mg of gallic acid equivalent (GAE) per kg of dry matter (Singleton et al., 1999). Fifty to 150 µL of the extract was transferred into the test tubes and their volume filled up to 500 µL with distilled water. After adding the 0.2 M Folin–Ciocalteu reagent and 20% aqueous Na₂CO₃ solution to the tubes, samples were vortexed. Spectrophotometer Agilent 8453 was used for measurements of the absorbencies of the mixtures at 750 nm after 40 min.

Analysis of total flavonoids (TF)

The total flavonoid content was assessed according to the method proposed by Zhishen et al. (1999) with some modifications. Namely, 50 µL of 5% NaNO₂ were mixed with 200 µL of the extract. After 6 min, 500 µL of a 10% AlCl₃ solution were added. After 7 min, 250 µL of 1 mol/L NaOH were added and the mixture was centrifuged at 5000 × g for 10 minutes. The absorbance of the supernatant was measured at 510 nm against the solvent blank. The total flavonoid content was expressed as milligrams of the catechin equivalent (CE) per kilogram of dry matter.

Analysis of total anthocyanin content (ANC)

Anthocyanins were extracted from 250 mg to 1 g of seeds by mixing with 5 ml of methanol acidified with 1 M HCl (85:15, v/v) and shaken for 30 min at room temperature. The crude extract was centrifuged at 11200 g for 5 min, and absorbance of the supernatant was measured at 535 and 700 nm using Agilent 8453 spectrophotometer to detect anthocyanins, which were then calculated using the molar extinction coefficient of 25 965 Abs/M x cm and a molecular weight of 449.2 g/mol and expressed as

mg of cyanidin 3-glucoside equivalent (CGE) per kg of dry matter (Abdel-Aal & Hucl, 1999).

Analysis of individual phenolic acids

The prepared extracts were analysed for the content of phenolic acids after their filtration through the 0.45 µm nylon syringe filter of the Thermo Scientific Ultimate 3000 HPLC system with the photodiode array detector according to the method described by Žilić et al. (2012). The detected phenolic acids peaks were confirmed and quantified by data acquisition and spectral evaluation using the Thermo Scientific Dionex Chromeleon 7.2. chromatographic software. Standard solutions of p-coumaric acid, ferulic acid, chlorogenic acid, and caffeic acid were used. The results are expressed as mg per kg of dry matter.

Analysis of total antioxidant capacity (TAC)

The antioxidant capacity of black soya bean and black chia was measured according to the direct or QUENCHER method as described by Serpen et al. (2008). The total antioxidant capacity was expressed as the Trolox equivalent antioxidant capacity (TEAC) and given as mmol of Trolox per kg of dry matter (mmol Trolox Eq/kg).

Colour measurement

Colour properties of the soya bean and chia were measured with a Chroma Meter (CR-400, Konica, Minolta, Japan), which was calibrated against a white calibration standard (CM – A70).

Statistical analyses

All chemical analyses were performed in two replicates. Results are presented as means ± standard deviation (SD).

RESULTS AND DISCUSSION

The ash, oil and fibres content of the investigated black soya beans and black chia seeds are summarised in Table 1.

Black chia seeds contained a distinctively higher amount of oil 34.66±0.63% in comparison with soya beans 22.43±0.53%, while the ash content of the investigated soya and chia samples was similar: 5.41±0 and 4.72±0%, respectively. Studies have shown that the average oil content of the chia seeds ranged from 25% to 35% and contained high concentrations of polyunsaturated fatty acids,

mainly α-linoleic acid and a low percentage of saturated fatty acids (Taga et al., 1984; Oliveira-Alves et al., 2017; Caruso et al., 2018). Soya bean seeds usually contain around 20% of lipids with a high phospholipids content that is 2-3 times higher than in other plant oils. These phospholipids have exquisite emulsifying properties and are very highly valued in the food and other industries, as well as in medicine (Barać et al., 2014).

Dietary fibre has become an important component in the everyday diet because of its beneficial health effects such as the reduction of cholesterolemia, modification of the glycemic and insulinemic responses, changes in the intestinal function, and the antioxidant activity (Reyes-Caudillo et al., 2008). Furthermore, dietary fibre is a mixture of compounds consisting of plant carbohydrate polymers, e.g. cellulose, hemicellulose, pectic substances, and gums that may be associated with lignin and other non-carbohydrate components. Valdivia-Lopez and Tecante (2015) found that chia seed had 2.3, 2.6, 8.3, and 9.8 times more fibre per 100 g of the edible portion than wheat, oat, maize, and rice, respectively. The values of the individual fibres, depicted in Table 1, show that chia seeds had overall higher fibre content than soya bean seeds. Black chia seeds had a higher content of NDF, ADF, ADL, and hemicellulose (32.18±0.19, 15.84±0.09, 13.18±0.20, 16.44±0.22%, respectively), but a lower content of cellulose (2.87±0.19%), in comparison with black soya bean seeds (5.15±0.24%). The lignin content, (ADL) in black soya beans was the lowest of all investigated fibre fractions (0.70±0.04%). The lignin component supposedly protects unsaturated oils in chia seeds by building a strong and resistant structure and also by the antioxidants it contains. Due to its bile acids absorbing potential, lignin tends to promote the hypocholesterolemic effect associated with fibre intake (Valdivia-Lopez & Tecante, 2015). High neutral detergent fibre (NDF) content (32.18%) indicates the presence of diverse carbohydrates that form the structure of mucilage - a protective layer for the seed embryo that swells in contact with water forming a sticky gelatinous capsule. The utilisable value of mucilage of chia seeds has been growing in recent years due to the need to achieve the desired food product functionality and texture. It is used as a fat substitute in pound cakes, in

ice cream as an emulsifier and stabiliser, in bread as a fat replacer (Punia & Dhull, 2019). The high fibre content determined in black chia seed samples indicates that the consumption of these seeds as a food component could be beneficial from both nutritional and health-promoting standpoints.

The content of total protein and soluble protein fractions in black soya beans and black chia seeds is presented in Table 2.

The total protein content determined in black soya bean was almost twice as high ($42.26 \pm 0.14\%$) as chia protein ($25.04 \pm 0.20\%$) that is in accordance with the results of Žilić et al. (2011) and Valdivia-Lopez and Tecante (2015). The non-protein nitrogen content accounted for 6.63 ± 0 and $5.35 \pm 0\%$ d.m. in the samples of soya beans and black chia seeds, respectively. Albumins soluble in the water had the highest content of all soluble proteins in black soya beans $29.99 \pm 0.13\%$ d.m. with NSI accounting to $70.96 \pm 0.31\%$ of total

protein, while black chia seeds had the highest content of globulins $14.64 \pm 0.07\%$ d.m. and NSI $58.48 \pm 0.27\%$ of total protein. Gliadins soluble in ethanol had the lowest content of all soluble proteins in both investigated species ($0.80 \pm 0.07\%$ in soya and $0.94 \pm 0\%$ in chia), and the content of glutenins soluble in borate buffer was slightly higher ($1.65 \pm 0.07\%$ in soya and $2.72 \pm 0\%$ in chia) (Table 2). Even though soya bean storage proteins are a good source of essential amino acids, they are deficient in sulphur-containing amino acids such as methionine, cysteine, and possibly threonine. Chia seeds contain all the essential amino acids, unlike most of vegetable protein sources that lack two or more amino acids, so they have a better protein quality than cereals and the other seeds (Nitrayova et al., 2014; Valdivia-Lopez & Tecante, 2015).

Total phenolic compounds content determined in black soya bean extracts was 830.66 ± 5.46 mg GAE/kg (Table 3).

Table 1.

Ash, oil and fibre content in whole-grain flour of black soya bean and black chia seed

	Black soya bean	Black chia seed
Ash (%)	5.41±0	4.72±0
Oil (%)	22.43±0.53	34.66±0.63
<i>Fibres</i>		
NDF (%)	10.58±0.70	32.18±0.19
ADF (%)	5.85±0.20	15.84±0.09
ADL (%)	0.70±0.04	13.18±0.20
Hemicellulose (%)	4.74±0.50	16.44±0.22
Cellulose (%)	5.15±0.24	2.87±2.87

Table 2.

Content of total protein and soluble protein fractions in whole-grain flour of black soya bean and black chia seed

Variety	Protein		NPN		Albumins		Globulins		Gliadins		Glutenins	
	(1)	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)	
Black soya bean	42.26±0.14	6.63±0.00	15.69±0.00	29.99±0.13	70.96±0.31	1.27±0.07	3.02±0.16	0.80±0.07	1.89±0.16	1.65±0.07	3.91±0.16	
Black chia	25.04±0.20	5.35±0.00	21.37±0.00	1.38±0.00	5.53±0.00	14.64±0.07	58.48±0.27	0.94±0.00	3.76±0.00	2.72±0.00	10.84±0.00	

NPN non-protein nitrogen; (1) % d.m.; (2) % of total protein

Table 3.

Phenolic compounds and total antioxidant capacity of whole-grain flour of black soya bean and black chia seed

	Black soya bean	Black chia seed
TP (mg GAE/kg)	830.66±5.46	1201.94±16.29
TF (mg CE/kg)	170.86±44.69	233.98±8.42
ANC (mg CGE/kg)	583.35±26.34	n.d.
<i>Phenolic acids</i>		
<i>p</i> -Coumaric acid (mg/kg)	30.09±1.90	14.10±0
Ferulic acid (mg/kg)	93.83±12.17	39.17±0
Chlorogenic acid (mg/kg)	n.d.	121.89±0
Caffeic acid (mg/kg)	n.d.	21.23±0
TAC (mmol Trolox Eq/kg)	92.74±1.47	31.60±1.57

TP – total phenolic compound; TF – total flavonoids; ANC – total anthocyanins; TAC – total antioxidant capacity

Different authors reported significant variations in total phenolic compounds of soya bean. These values are not comparable with our data due to the different methods used and different plant material investigated. For example, Jokić et al. (2009) studied the effect of ethanol concentration and temperature on extraction yield of phenolic compounds from soya bean which ranged from 2.21 ± 0.19 to 4.50 ± 0.06 mg GAE/g, depending on the parameters of the experiment. Slavin et al. (2009) found that 50% acetone extracts exhibited higher total phenolic compounds content than the 70% ethanol extracts and ranged from 2.1 to 2.6 mg GAE/g of seeds, while Chung et al. (2008) reported significant difference between nine soybean varieties ranging from 2.9 to 3.9 mg of GAE/g of seeds. The corresponding total phenolic compounds value in black chia seed extracts, amounted to 1201.94 ± 16.29 mg GAE/kg, that is the content was slightly higher than the one reported by Reyes-Caudillo et al. (2008) who found that the total content of phenolics of chia seed in crude extracts was 0.9211 ± 0.040 mg GAE/g for Jalisco and 0.8800 ± 0.008 mg GAE/g for Sinaloa variety of chia (i.e. 921.1 and 880.0 mg GAE/kg). The total flavonoids content detected in black chia seeds (233.98 ± 8.42 mg CE/kg) was higher than in black soya beans (170.86 ± 44.69 mg CE/kg). While total anthocyanins amounted to 583.35 ± 26.34 mg CGE/kg in black soya beans, the anthocyanins were not detected in black chia seeds, which is in accordance with the

results obtained by Reyes-Caudillo et al. (2008). In a study conducted by Malenčić et al. (2012), flavonoids content of black soya beans ranged from 1.16 ± 0.29 to 2.19 ± 0.46 mg rutin/g, while anthocyanins content varied between 0.57 ± 0.22 and 0.65 ± 0.46 mg CGE/g of dry material. The content of p-coumaric acid and ferulic acid was higher in soya beans (30.09 ± 1.90 mg/kg; 93.83 ± 12.17 mg/kg) than in chia seeds (14.10 ± 0 mg/kg; 39.17 ± 0 mg/kg). The content of chlorogenic acid and caffeic acid was not detected in soya beans, while in chia seeds it was 121.89 ± 0 and 21.23 ± 0 mg/kg. In comparison, Reyes-Caudillo et al. (2008), detected 0.102 mg/g chlorogenic acid and 0.0030 mg/g caffeic in crude chia flour extracts. Valdivia-Lopez and Tecante (2015) reported that rosmarinic acid was the most abundant (0.9267 mg/g), while protocatechuic, caffeic, gallic acids and daidzin were phenolic compound identified and quantified in the chia extracts. After basic hydrolysis and extraction, Žilić et al. (2013) measured the high content of protocatechuic acid and the flavonoid catechin in black soya bean seed coat. Black soya beans showed a higher total antioxidant capacity (92.74 ± 1.47 mmol Trolox Eq/kg) compared to chia seeds (31.60 ± 1.57 mmol Trolox Eq/kg).

The results indicate that both black soya and black chia have a high antioxidant capacity and represent a good source of phenolic compounds that could be incorporated into the human diet.

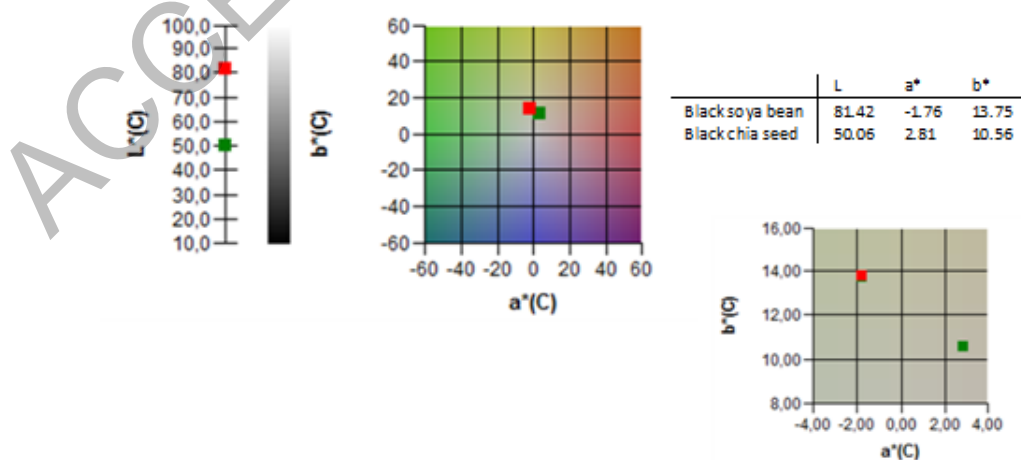


Figure 1. CIE L* a* and b* chromaticity diagram for wholegrain flour of black soya bean and black chia seed (red square marker-black soya flour; green square marker-black chia flour)

As far as colour results are concerned, the average CIE L*, a*, and b* values of flour samples are presented in Figure 1.

The colour of black soya flour was considerably darker than black chia flour. CIE L values were 81.42 and 50.06, respectively. The lightness of common soya flour is defined by CIE L value of about 62 (Žilić et al., 2014). Although yellow is dominant colour in both flours (CIE b* = 13.75 and 10.56), it is not intense. Positive CIE b* value for common soya flour is about 26 (Žilić et al., 2014).

CONCLUSIONS

Very high fibre content determined in the black chia seed sample indicates that the consumption of these seeds as a food component could be beneficial from both nutritional and health-promoting standpoints. High content of oil and fibre properties of seeds recommend chia as a fat substitute in pound cakes, in ice cream as an emulsifier and stabilizer, in bread as a fat replacer. The total protein content determined in black soya bean seeds was almost twice as high (42.26±0.14%) as the chia protein content (25.04±0.20%), which is considered to have a better protein quality than cereals and the other seeds. Both, black soya bean and black chia seed contained a considerable amount of phenolic compounds with high antioxidant potential, which makes them good contestants for functional food ingredients with potential health benefits. However, further, more extensive studies are required in order to determine their full potentials and to ensure safe levels of their use.

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ЦРНА СОЈА И ЦРНА ЧИЈА КАО ИЗВОР ХРАНЉИВИХ САСТОЈАКА И БИОАКТИВНИХ ЈЕДИЊЕЊА СА ЗДРАВСТВЕНИМ ПРЕДНОСТИМА

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Сажетак: Нови трендови у савременом здравом начину живота и исхрани довели су до повећане потражње за функционалним намирницама богатим хранљивим материјама и биоактивним једињењима која могу позитивно да утичу на здравље. Неке гајене биљке, као што су соја и чија могу бити добар извор нутрацеутика са високим садржајем влакана, протеина и заштитним антиоксидативним потенцијалом. У овом истраживању коришћена су интегрална брашна црне соје и црне чије. Потенцијална хранљива и здравствено корисна својства ових брашна упоређивана су на основу хемијског састава и антиоксидативног профила. Садржај прехранбених влакана као што су NDF, ADF, ADL и хемицелулоза у узорку црне чије био је виши него у црној соји која је имала виши садржај целулозних влакана. Укупни садржај протеина одређен у црној соји био је скоро двоструко виши ($42,26 \pm 0,14\%$) у односу на садржај протеина чије ($25,04 \pm 0,20\%$). Црна соја имала је највиши удео протеина растворљивих у води ($29,99 \pm 0,13\%$ с.м.) и индекс растворљивости азота (NSI) $70,96 \pm 0,31\%$ од укупних протеина, док је семе црне чије имало највише глобулина ($14,64 \pm 0,07\%$ с.м.) и NSI $58,48 \pm 0,27\%$ од укупних протеина. У узорцима црне соје и црне чије утврђен је висок садржај укупних фенолних једињења ($830,66 \pm 5,46$ и $1201,94 \pm 6,29$ mgGAE/kg, редом) као и значајан укупни антиоксидативни капацитет, због чега се могу сматрати dobrim кандидатима за производњу функционалне хране која може имати позитиван утицај на здравље.

Кључне речи: хемијска својства, фенолна једињења, флавоноиди, антоцијанини, влакна, боја

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