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OPTIMIZATION OF ANTHOCYANINS EXTRACTION PROCESS FROM BLACK SOYBEAN SEED COAT FOR THE PREPARATION OF MAIZE-BASED FUNCTIONAL FOOD
OPTIMIZACIJA PROCESA EKSTRAKCIJE ANTOCIJANA IZ SEMENJAČE CRNE SOJE ZA PRIPREMU FUNKCIONALNE HRANE OD KUKURUZA

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ABSTRACT

Black soybean seed coat has a considerable content of anthocyanins which can providea positive effect on the health of the consumers through food products enriched with the extracts of these valuable bioactive compounds. Nevertheless, the use of soybean seed coat, a by-product from soybean processing, additionally valorizes this raw material.The possibility of enrichment of sweet maize grains with anthocyanin extract was investigated. Several procedures with acetic acid were applied in the experiments of anthocyanins extraction from black soybean seed coat. During the marination process, the grains were stained, changing their standard yellow color to a crimson red. Since the brines with the addition of citric acid became cloudy (opalescent) after some time, lactic acid was used as an anthocyanin stabilizer in the continuation of the research. Depending on the extraction conditions, the spectrophotometrically determined content of total anthocyanins in the solution varied from 3541.90 to 5387.70 μg CGE/g d.m., and in the marinated maize grain between 179.89 and 286.05 μg CGE/g d.m. After seven days, the total anthocyanin content in the grain did not increase significantly, so this aging period was selected as optimal for marinating maize products.

Keywords: *black soybean, anthocyanins, maize, functional food.*

REZIME

Semenjača crne soje ima značajan sadržaj antocijana, koji mogu pozitivno uticati na zdravlje potrošača kroz prehrambene proizvode obogaćene ekstraktima ovih vrednih bioaktivnih jedinjenja. Istovremeno, upotrebom sojine semenjače, sporednog proizvoda prerade soje, dodatno se valorizuje ova sirovinu. Ispitivana je mogućnost obogaćivanja zrna kukuruza šećerca ekstraktom antocijana. U eksperimentima ekstrakcije antocijana iz semenjače crne soje primenjeno je nekoliko postupaka sa sirćetnom kiselinom. Tokom procesa mariniranja, zrna su promenila boju, menjajući standardnu žutu boju u tamnocrvenu. S obzirom da su posle izvesnog vremena nalivi za mariniranje sa dodatkom limunske kiseline postali zamućeni (opalescentni), u nastavku istraživanja je korišćena mlečna kiselina kao stabilizator antocijana. U zavisnosti od uslova ekstrakcije, spektrofotometrijski utvrđen sadržaj ukupnih antocijana u rastvoru varirao je od 3541,90 do 5387,70 mg CGE/g d.m., a u zrnu mariniranog kukuruza između 179,89 i 286,05 mg CGE/g. Posle sedam dana, ukupan sadržaj antocijana u zrnu nije značajno povećan, pa je ovaj period odležavanja izabran kao optimalan za mariniranje proizvoda od kukuruza.

Ključne reči: *crna soja, antocijani, kukuruz, funkcionalna hrana.*

INTRODUCTION

Maize (*Zea Mays* L.) is the most important field crop in the Republic of Serbia, which is grown on about one million hectares per year which includes about 56% of the arable land sown with cereals (*Babić et al., 2016*). In terms of nutritional value, maize is an excellent source of starch, protein, fat, as well as numerous bioactive compounds important for human health. According to the United States Department of Agriculture (USDA), the total estimated corn production in 2021 was around 1,133.89 million metric tons, and Serbia ranks 16th in the world in terms of production of about 8 million metric tons. (*World Agricultural Production.com, 2021*). The significance of maize is immeasurable due to its various uses in food and feed production, as well as its importance as a raw material for a wide spectrum of products from numerous industries. Apart from being used in the form of cornmeal or flour, cooked baked, or popped, maize grain can also be preserved by canning. Canned vegetables can be just as healthy as fresh vegetables because they remain fresh after processing. Studies have shown that preserved and marinated vegetables retain their freshness after processing and are considered as healthy as fresh vegetables due to the comparable contents of the majority of nutrients after being used in a prepared meal (*Featherstone, 2016; Nikolić et al., 2021*).

Colored, predominantly anthocyanin-pigmented, cereals and legumes hold promise as functional food ingredients (e.g., whole grain products) or functional food colorants (e.g., anthocyanin-rich grain fractions) (*Abdel-Aal et al., 2006*). Unlike carotenoids, i.e. yellow pigments that provide the standard yellow-amber color to the grains, the anthocyanins, characteristic to the aleurone or pericarp are responsible for the color of pigmented grains, which can range from blue to purple, and red (*Ficco et al., 2014*). Anthocyanins are allowed in the European Union, Australia and New Zealand for use as food colorants with the E163 label. According to the Serbian Regulation on food additives, they can be added to the food products in the amount of *quantum satis (Pravilnik o prehrambenim aditivima, 2018).*

The color of soybeans (*Glycine max*) seed coat is determined by the pigment-stimulating metabolites, and can vary from yellow, to brown and black (*Kumar et al., 2022*). Black soybean has been used for centuries in traditional Eastern medicine for the preparation of teas and fermented products with high antioxidant capacity. Black soybean seed coat has a high content of anthocyanins: cyanidin, delphinidin, and pelargonidin in the form of 3-O-glucoside (*Nikolić et al., 2020; Žilić et al., 2019*). The antioxidant properties of these compounds have a positive effect on weight regulation and prevention of diabetes, reducing the risk of various diseases such as atherosclerosis, cancer, diabetes, ischemia, and neurodegenerative disorders (*Kim et al., 2012*). Products enriched with anthocyanins from black soybean seed coats can have a positive effect on the health of consumers, while the use of soybean seed coats, a by-product from soybean processing, additionally valorizes this raw material. Nutritive and health-promoting properties of canned maize products such as sweet maize and baby corn can be improved by enrichment with anthocyanins extracted from black soybean seed coats.

The extraction process is used for the isolation of bioactive compounds from different foods, as well as for application in the pharmaceutical and food industry. The requirements for valuable health-promoting components, such as anthocyanins, in numerous food products are rising, which stimulates the investigation of novel extraction processes. (*Kumar et al., 2022*).

The aim of this study was to optimize the process of anthocyanin extraction from black soybean seed in order to further enrich marinated maize products with bioactive compounds and, therefore, enhance their functional properties.

MATERIAL AND METHODS

Black soybean genotype Black Tokyo from the Maize Research Institute, Zemun Polje gene bank, and sweet maize genotype ZP 555 were grown in the experimental field in Zemun Polje, Serbia (44°52´N, 20°19´E, 81 m s.l.). The seed coats were dissected manually from black soybeans and ground in a Cyclotec 1093 lab mill (FOSS "Tecator", Sweden) (particle size < 500µm). After blanching them for two minutes in boiling water, the sweet maize grains were gently removed by hand from the cobs without damaging the grain surface, and stored in the freezer before use.

Three different anthocyanin extraction procedures with variations have been tested using ground black soybean seed coats. Extraction parameters were varied in order to optimize the extraction process (Table 1). Extractions of anthocyanins from the ground soybean seed coat were done with acetic acid (1.6 or 0.8%), with or without the addition of citric acid (0.5 or 1%) or lactic acid (1 or 1.5%). Three different procedures were tested:

1. "Cold" extraction, at room temperature in a horizontal shaker (MLW Thys 2) for 60 minutes;
2. "Warm" extraction with heating (50˚C) on a magnetic stirrer for 60 minutes, and
3. Extraction with ultrasonic treatment in a sonicator (Model: UZ 4P 220/115V; power 100W; "Iskra", Slovenia) at frequency 30 kHz, temperature 40 ˚C, for 30 minutes.

The crude soybean seed coat extract was centrifuged at 11200 rpm for 5 min and the absorbance was measured from the supernatant. Three days into the marinating process sweet maize grains (0.5 g) were mashed using mortar and pestle, acidified with methanol (6 ml), ultrasonicated for 30 minutes, shaken for 30 minutes, and centrifuged for 3 minutes at 4˚C and 7000 rpm. The 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 (d.m.) (*Abdel-Aal&Hucl, 1999*).

RESULTS AND DISCUSSION

Several procedures with two different concentrations of acetic acid (1.6% and 0.8%), and with or without the addition of different concentrations of citric and lactic acid, shown in *Table 1 and Table 2*, were applied during the experiments of anthocyanins extraction from black soybean seed coat (*Figure 1*).

Table 1. Total anthocyanins content and pH values obtained by varying parameters of extraction procedures in brine with 1.6% acetic acid

Procedure	pH	Total anthocyanins content in brine (µg CGE/g d.m.)	Total anthocyanins content in marinated sweet maize grain (µg CGE/g d.m.)
Cold extraction	3.03	3541.90	179.89
Warm extraction	3.03	3766.67	275.79
Ultrasound extraction	3.03	3625.68	156.89
Cold extraction, 0.5% citric acid	2.64	3594.75	189.43
Warm extraction, 0.5% citric acid	2.64	4953.67	279.79
Ultrasound extraction, 0.5% citric acid	2.64	4658.42	211.99
Cold extraction, 1% citric acid	2.31	3933.56	188.47
Warm extraction, 1% citric acid	2.31	4793.50	256.91
Ultrasound extraction, 1% citric acid	2.31	4888.72	212.47
Cold extraction, 1% lactic acid	2.45	3809.55	191.78
Warm extraction, 1% lactic acid	2.45	5387.70	281.33
Ultrasound extraction, 1% lactic acid	2.45	4420.00	212.47
Cold extraction, 1.5% lactic acid	2.40	4194.94	255.87
Warm extraction, 1.5% lactic acid	2.40	5387.70	286.05
Ultrasound extraction, 1.5% lactic acid	2.40	4995.75	237.78

In general, the warm extraction proved to be the most efficient, hence this type of procedure was further explored with the lower concentration of acetic acid (0.8%), in order to provide a more palatable food product (*Table 2*).

Table 2. Total anthocyanins content and pH values obtained by varying parameters of extraction procedures in brine with 0.8% acetic acid

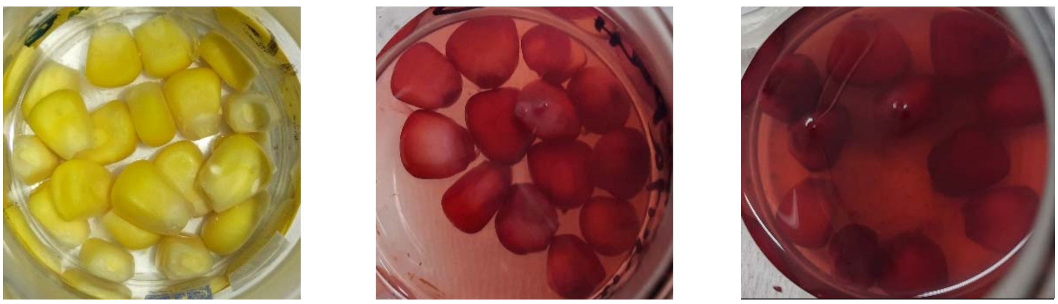
Procedure	pH	Total anthocyanins content in brine (µg CGE/g d.m.)	Total anthocyanins content in marinated sweet maize grain (µg CGE/g d.m.)
Warm extraction,0.5% citric acid	2.85	3753.89	268.88
Warm extraction, 1% citric acid	2.54	3911.56	243.56
Warm extraction, 1% lactic acid	3.04	4057.42	285.25
Warm extraction, 1.5% lactic acid	2.60	3834.80	256.17

Fig. 1. Black soybeans (left) and seed coat (right)

"Warm" extraction was performed on a magnetic stirrer at a temperature of 50˚C for 60 minutes to test the extraction efficiency. "Cold" extractionwas performed at room temperature in a horizontal shaker in order to avoid thermal decomposition of bioactive compounds, however, this method of extraction proved to be the least effective. Extraction in an ultrasonic water bath was applied in order to decompose the material, i.e. soybean seed coat, as quickly as possible, and increase the availability of anthocyanins. Warm extraction proved to be the most suitable for obtaining the highest possible anthocyanin yield, as seen in *Table 1*, and was used in further experiments using lower concentration acetic acid (0.8%) in order to obtain a food product with sensory properties more acceptable for the consumers (adequate pH).

Studies have shown that organic acids such as citric and lactic acid stabilize anthocyanins in solution (*Minatel et al., 2017*). Although during the process of acidification – i.e. lactic acid fermentation of vegetables such as cabbage, cauliflower or cucumber, lactic acid is synthesized as the primary metabolite of lactic acid bacteria, lactic acid was added to the acidified solution (brine) to better stabilize bioactive compounds (anthocyanins) and thus maintain colors of the final product. The results showed that the process of enrichment of the sweet maize grains with anthocyanins extract was efficent, and the value of the total anthocyanins in the grain ranged from 179.89 and 286.05 µg CGE/g d.m (*Table 1, Table 2*). This optimized procedure opens up new possibilities for the fortification of sweet maize and other marinated vegetables with anthocyanins and the production of various functional food products. According to the data, among the berries, chokeberry has the highest content of anthocyanins from 2,000 to 10,000 µg CGE/g d.m, followed by black and red currants from 800 to 4,000 µg CGE/g d.m, blueberries from 250 to 5,000 µg CGE/g d.m, blackberries from 800 to 3,000 µg CGE/g d.m and finally raspberries from 100 to 600 µg CGE/g d.m (*Žilić et al., 2019*). Of the above-mentioned berries rich in anthocyanins, in 2019, raspberries were mostly grown in. Furtheremore, it can be emphasized that the increased content of anthocyanins in the marinated sweet maize with black soybean seed coat extract can be achieved by adding only about 2.5% of soybean seed coat in the preparation of the brine, i.e. about 4 g of soybean seed is needed for 350 g of the product (350 ml glass jar).

Samples of sweet maize grains that were immersed for seven days in the marinating brine with anthocyanins extract changed their color from standard yellow *(Figure 2a)* to crimson red, as seen in *Figure 2b* and c. The marinating brine shown in *Figure 2b* was prepared with the addition of 1% lactic and did not become cloudy, compared to the brine shown in *Figure 2c* prepared with the addition of 1% citric acid that became opalescent during that period.



a *b* *c*

Fig. 2. Samples of sweet maize; a) prior to marination process; b) with the addition of 1% lactic acid in anthocyanins extract; c) with the additoin of 1% citric acid in anthocyanins extract

CONCLUSION

The study conducted in order to optimize the parameters of the procedure for the extraction of anthocyanins from black soybean seed coat showed that the most suitable process for higher anthocyanin yields was the warm extraction. Even though the highest extracted total anthocyanins content (5387.70 µg CGE/g d.m.) in the brine used for marination of the sweet maize kernels was achieved with 1.6% acetic acid and 1% lactic acid during the warm extraction conducted at 50˚C for 60 minutes, the pH value was quite low (2.45). The more sensory acceptable pH value (3.04) of the brine was achieved by applying 1% lactic acid in 0.8% acetic acid during the warm extraction conducted at 50˚C for 60 minutes, and the total anthocyanins content was also high (4057.42 µg CGE/g dm). The studies directed toward the enrichment of maize products with bioactive compounds for the preparation of maize-based functional food need to be further expanded and will continue in the near future.

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