POTENTIAL OF SECONDARY RAW MATERIAL – SOYBEAN OKARA FOR USE AS FISH FEED

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MOGUĆNOST PRIMENE OKARE KAO SEKUNDARNE SIROVINE U PROIZVODNJI HRANIVA ZA RIBE

Apstrakt

Poslednjih godina istraživanju soje poklanja se velika pažnja zbog povoljnih zdravstvenih efekata. Mnoge studije su utvrdile u zrnu soje prisustvo komponenti poput proteina, dijetalnih vlakana, masnih kiselina, izoflavona i drugih fitohemikalija, koje povoljno deluju na organizam. Sirova okara, poznata i kao "sojina pulpa", je nusprodukt u priozvodnji sojinog mleka. To je belo-žućkasta materija koja se sastoji od nerastvorljivih delova semena soje (uglavnom semenjače) koji ostaju na filteru pri filtriranju usitnjenog kuvanog zrna soje pri proizvodnji sojinog mleka (Jimenez-Escrig *et al*., 2008).

Cilj ovog rada je bio da se proceni uticaj metoda polupogonskog postrojenja koje koristi hidrotermičku obradu sojinog zrna u proizvodnji sojinog mleka (HTC obrada; visoka temperatura i povišen pritisak/kratko vreme) na sadržaj i aktivnost hranljivih komponenti okare, pripremane od šest različitih genotipova soje, kao i da se proceni mogućnost korišćenja okare u pripremi hrane za ribe. Može se pretpostaviti da će primenjeni HTC postupak, koji je sličan procesu ekstrudiranja, koji se najčešće koriste u proizvodnji hrane za ribe (visoka temperatura/kratako vreme), dati okaru povoljnih svojstava, pogodna za ishranu riba. Pored toga, obzirom da je optimalna pH vrednost vode za uzgoj riba od 7.0 do 8.0 (Zhanga *et al*., 2011), različita od izoelektrične tačke (Ip) glavnih proteina okare može se pretpostaviti da se proteini okare neće taložiti u mulju, već će ostati da plutaju u vodi.

Glavne komponente okare dobijene primenjenim HTC postupkom su ugljeni hidrati (51.25-59.25%) i proteini (31.81-40.36%). Mateos-Aparicio *et al*. (2010b) ističu veoma povoljnu antioksidativnu aktivnost okare, koju uglavnom pripisuju polisharidima semenjače sojinog zrna, pre svega pektinima, iako ističu da se ne može isključiti ni doprinos belančevina. Ovakva istraživanja ukazuju na mogućnost delovanja okare kao komponete u ishrani riba u smislu odbrane od antioksidativnog stresa. Visok sadržaj proteina, čini okaru potencijalno dobrim izvorom biljnih proteina, niske cene, za ishranu ljudi i životinja. Visok sadržaj i vrlo dobre funkcionalne karakteristike proteina okare (Mateos-Aparicio *et al*., 2010a), ukazuju na to da mogu biti pogodni kao dopuna hrane za ribu u smislu vezivnog medijuma za druge aktivne i hranljive komponente u proizvodu, pri čemu doprinose nutritivnoj vrednosti hraniva. Štaviše, odnos esencijalnih amino kiselina u ukupnom aminokiselinskom sastavu proteina okare je sličan kao u sojinom mleku i tofuu (Vang i Cavins, 1989). Glavni proteini okare su bazni, 7S globulin (Bg7S; 24.61-28.37%) i glicinin (11S globulin; 28.49-33.11%). Poznato je da je Bg7S glikoprotein bogat cisteinom (Omi *et al*., 1996) što povećava nutritivnu vrednost proizvoda. Proteini soje nisu nutritivno idealni proteini, obzirom da ispoljavaju neželjeni efekat na metabolizam nakon konzumiranja sirove sojine sačme, što se pripisuje prisustvu tripsin inhibitora (TI) i lektina. Tripsin inhibitorska aktivnost ispitivanih uzoraka je veoma mala (4.82-7.99%) što ukazuje da okara ne bi ispoljavala antinutritivni efekat na organizam, tim pre što se veruje da postizanje zadovoljavajućeg nivoa TIA dovoljno smanjuje aktivnost lektina, obzirom da su inhibitori termički stabilniji nego lektini (Friedman i Brendon, 2001).

Ključne reči: *okara, hrana za ribe, HTC obrada, nutritivna vrednost*
Keywords: okara, fish feed, HTC processing, nutritional values

INTRODUCTION

In recent years soybean has gained much attention because of its health-promoting properties. Many studies have reported on the beneficial components of soybean such as protein, dietary fiber, fatty acids, isoflavones and other phytochemicals. Soy foods and feeds are becoming more popular as low cost substitutes of traditional products and an ideal nutritional supplement for protein rich products. Raw okara, also called "soy pulp" is a byproduct of the soymilk industry. It is a white-yellowish material consisting of the insoluble parts of the soybean seeds which remains in the filter sack when pureed soybeans are filtered for the production of soymilk (Jiménez-Escrig *et al*., 2008). The main components of okara are protein 25.40-40.36% (O'Toole, 1999; Stanojevic *et al*., 2012) and dietary fiber, 14.5-55.4 g/100 g on dry basis (O'Toole, 1999). High protein content of about 30% makes okara a potential source of low cost plant protein for production of food and feed. Moreover, the ratio of essential amino acid to total amino acids in okara is similar as in soymilk and tofu (Wang and Cavins, 1989). The basic 7S globulin (Bg7S) and 11S protein are two main proteins in okara (Stanojevic *et al*., 2012). It is known that Bg7S is a cysteine-rich glycoprotein (Omi et al., 1996) which increases the nutritional value of the product.

Soy protein is not an ideal protein because adverse nutritional effects following consumption of raw soybean meal have been attributed to the presence of trypsin inhibitors (TI) and lectins and to their poor digestibility. Since the TI are cysteine-rich proteins (Wolf, 1977) heat treatment should aim to preserve their content and to reduce their activity to make them nutritionally valuable. Therefore, we assessed the influence of a pilot plant method that uses high-pressure hydrothermal processing for soymilk production (HTC processing, high temperature and high pressure/short time) on content and activity of nutritional components in okara prepared from six different soybean genotypes and also, the possibility of using okara as a fish feed.

MATERIALS AND METHODS

Materials. For okara preparation six commercial soybean genotypes grown in field conditions were used: ZPS-015, Krajina, Novosađanka, Balkan, Nena and Lana. The genotype Lana lacked the Kunitz type of trypsin inhibitor. **Okara processing.** Okara was made on the pilot plant scale using the production method which includes hydrothermal cooking (HTC; Wang *et al.,* 2003) for soymilk preparation, modified by Stanojevic *et al.,* (2011). **Applied analysis.** Dissociating electrophoresis (SDS-PAGE) for all samples were performed according to the Fling and Gregerson procedure (1986), detailed by Stanojevic *et al*. (2011, 2012). Trypsin inhibitor activity (TIA) of okara was estimated according to the method of Liu and Markakis (1989), and was expressed as residual activity (TIA in percents) relative to defatted soybean flour or in trypsin units inhibited (TUI) per milligram of the dry sample. Total nitrogen content in samples was determined by the micro-Kjeldahl method (AAOC, 2000) and total protein content was calculated (Nx6.25). Total fat content in samples was determined by extraction with diethyl ether in a Soxhlet system (AOAC, 2006). Ash content was calculated by AOAC method (1984). Moisture and volatiles were determined by standard AACC procedure (2000). Carbohydrate content was calculated by subtracting moisture, protein, fat, ash and cellulose values from 100%.

RESULTS AND DISCUSSION

All investigated samples of okara were characterized by high content of total protein (31.81-40.36%; Table 1) what indicated significant nutritional value of these products. The high protein content in okara and very good functional characteristics of okara proteins (Mateos-Aparicio *et al*., 2010a) indicate that okara may be suitable for fish feed supplement in terms of connective medium for active and nutrient components incorporation in the product. In addition to protein, okara contained mainly carbohydrates (51.25-59.25%), Table 1. Mateos-Aparicio *et al*. (2010b) reported that, potential antioxidant activity of okara cell-wall polysaccharides could be attributed to pectins, although the contribution of residual proteins cannot be ruled out. Such research suggests the possibility of okara activity as a component in the fish feed in terms of oxidative stress defense.

Table 1. Proximate composition of okara flour[1] (%)

Genotype	Moisture	Solids	Protein	Ash	Fat	Carbohydrate[2]
Nena	6.50^{c}	93.50	35.07^{d}	0.86^{c}	0.25^{d}	57.32
Kraijna	6.71^{a}	93.29	35.27^{c}	1.02^{b}	0.50^{c}	56.50
Novosadjanka	6.62^{b}	93.38	40.36^{a}	0.90^{bc}	0.52^{c}	51.60
Balkan	6.53^{c}	93.47	35.41^{c}	5.69^{a}	1.03^{b}	51.25
ZPS - 015	6.54^{c}	93.46	37.32^{b}	1.13^{b}	0.91^{b}	54.10
Lana	6.63^{b}	93.37	31.81^{e}	1.06^{b}	1.25^{a}	59.25

[1] Means in the same column with different superscript roman letters are significantly different ($p<0.05$), all analyzes were carried out applied to the defatted okara flour; [2] Carbohydrate by difference

One of the most advanced technological operation in fish feed production is the extrusion process. It increases digestibility of diet components thus enhancing nutritional value of fish feed (Sørensen *et al.*, 2005). Based on this, it can be assumed that the high temperature and high pressure in short time that were applied in our HTC procedure gave okara with favorable properties for use in fish feed. In addition, the optimal pH value of water for fish cultivation is from 7.0 to 8.0 (Zhanga *et al.*, 2011), which is different from the isoelectric point (Ip) of okara major proteins, Bg7S (24.61-28.37%) and 11S globulins (28.49-33.11%), Stanojevic *et al.*, (2012), Figure 1, B. It can be assumed that okara proteins wouldn't settle down in the mud, but would tend to float in the water. Moreover, our previous results (Stanojevic *et al.*, 2012) showed that okara extractable soluble protein (ESP) content was 27.83−32.53%, which led to very high okara protein extractability (79.29−90.45%). High values for ESP content and protein extractability may be prerequisites for good nutritional and functional properties of okara protein.

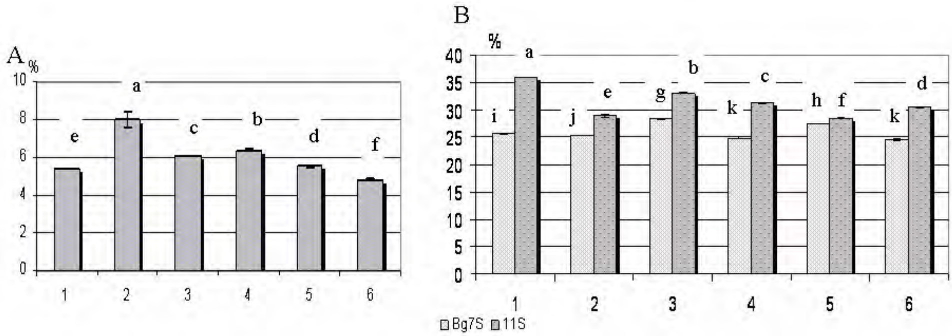

Figure 1. A. Trypsin inhibior activity of okara from investigated genotypes; **B.** Content of the major storage proteins in okara prepared from investigated genotypes; Bars with different letters are significantly different ($p<0.05$); 1 - Nena, 2 - Krajina, 3 - Novosadjanka, 4 - Balkan, 5 - ZPS-15, 6 - Lana.

Our results for trypsin inhibitor activity (TIA; Figure 1, A) in okara that were much lower (4.82-7.99% or 4.61-14.93 TUI/mg) than some literature data might be the consequence of the applied HTC processing. For example, Vishwanathan *et al.* (2011) reported significantly higher levels of TIA in okara produced using membrane technology (33.75 TUI/mg). Such low TIA in okara suggested that okara obtained by hydrothermal cooking could be applied for human consumption and for the fish feed, too, since it featured less than 20% of residual TIA. Friedman and Brandon (2001) reported that the TIA below 20% isn't antinutritional. Since the inhibitors are more heat-stable than the lectins, it is believed that by achieving a satisfactory level of TIA sufficiently decreases the activity of lectins (Friedman and Brandon, 2001).

CONCLUSIONS

The investigated soybean genotypes all produced by HTC process okara with high contents of protein and carbohydrates, as well as with very balanced contents and activity of bioactive components, such as trypsin inhibitors and lectins. Less than 20% of residual TIA indicated that okara was heated adequately to inactivate antinutritional factors. Higher content of sulfur-containing amino acids and lysine in okara protein than in β-conglycinin or glycinin favors its use for supplementation of different food and feed products because plant proteins are deficient in these amino acids. Moreover, the high quantity of plant protein in okara (>35%) and very high protein extractability (>80%) prove this byproduct to be very interesting for potential application in food or feed fortification as a functional ingredient. In addition, it favors okara use as ingredient for extruded aquafeed production.

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