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Composition of Proteins in Okara as a Byproduct in Hydrothermal Processing of Soy Milk

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ABSTRACT: Protein quality, based on its subunit composition, in okara obtained as a byproduct during hydrothermal cooking of soy milk was assessed. The composition of 7S and 11S protein fractions was correlated with the physicochemical properties of protein in okara produced from six soybean varieties. The basic 7S globulin (Bg7S) and 11S protein were two main proteins in okara. Investigated soybean genotypes produced okara with mainly acidic A_5 and basic $B_{1,2,4}$ polypeptides of 11S proteins. Soybean 11S content was not an indicator of okara protein recovery or extractability. Of all tested relationships, extractable soluble protein content of okara was influenced only by soybean Bg7S ($r = 0.86$; $p < 0.05$) and its light subunit contents ($r = 0.93$; $p < 0.05$). Okara protein recovery depended on Bg7S heavy subunit content in soybeans ($r = 0.81$; $p < 0.05$). The high quantity of vegetable protein in okara (around 35%) and very high protein extractability (around 85%) qualify this byproduct for potential application in food preparation as a functional ingredient.

KEYWORDS: *okara, basic 7S globulin, glycinin, hydrothermal cooking, soybean genotype*

INTRODUCTION

Among various soy foods, soy milk and tofu are becoming more popular as low-cost substitutes for traditional dairy products for consumers and an ideal nutritional supplement for lactose intolerants. Okara is a byproduct obtained during processing of soybean for soy milk, which is rarely utilized. The ratio of essential amino acids to total amino acids in okara is similar to those of soy milk and tofu.[1] The high quality of protein fraction of okara suggests that okara protein could be applied in food production. Toda et al.[2] speculated that the basic 7S globulin (Bg7S) is likely the main protein in extracts of okara in hot water. It is known that Bg7S is a cysteine-rich glycoprotein that is composed of two subunits linked by disulfide bonding.[3] Both subunits are synthesized as isoforms. They are designated as "heavy" ($H_{I,II}$) and "light" ($L_{I,II}$) subunits with molecular weights of 27000 and 16000, respectively.[4,5] Other authors[6,7] designated these subunits as α- and β-chains of Bg7S. Yoshizawa et al.[7] elucidated the crystal structure of the Bg7S molecule that contains 12 cysteines in positions to form 6 disulfide bonds (4 in the α- and 2 in the β-chain).

Bg7S binds a 4000 protein from soybean seed, leginsulin; it is also reported under the name of 43000 protein or leginsulin-binding protein.[8] Ligand blotting experiments showed that Bg7S can bind both insulin and insulin-like growth factors I and II.[9] Although Bg7S has no amino acid sequence homology with the human insulin receptor and insulin-like growth factor receptors, there are structural similarities between Bg7S and the human insulin receptor. Both proteins are glycosylated and have a cysteine-rich domain, and both have disulfide-bonded α and β subunit structures. Also, Bg7S was shown to have protein kinase activity in the α-chain, as for the insulin receptor β subunit. In addition, the amino acid sequence between the 41st and 53rd residues in the Bg7S $H_{I,II}$ subunit is homologous to that in the human low molecular mass insulin-binding protein.[10]

Omi et al.[3] described the specific protein release from soybean seeds induced by high-pressure treatment. They isolated the major component of the released proteins and identified it as Bg7S. They discussed possible mechanisms of the pressure-induced protein release on the basis of the results obtained on the Bg7S localization in seed dermal tissue and pressure-induced structural changes of the dermal tissue. These results suggest that a large amount of Bg7S was present in the epidermal tissue of soybean seed cotyledon.

Participation of other proteins of soybean 7S and 11S protein fractions in okara has not been sufficiently studied. β-Conglycinin (7S) and glycinin (11S) are the major soy proteins, representing about 70% of the total protein in soybeans.[11] β-Conglycinin is a trimeric glycoprotein consisting of α', α, and β subunits with different combinations and physicochemical properties. Glycinin is composed of acidic and basic polypeptides, linked by a disulfide bridge. The structural properties of glycinin (dimeric and monomeric form) are influenced by pH values.[12]

The ratio of glycinin/β-conglycinin and their polypeptide composition are indicators of functionality and the nutritive value of soybean proteins.[13−15] Because okara is obtained as a byproduct of soy milk production, understanding its protein composition and respective functionality could be useful for application of okara as a functional food additive. Therefore, the aim of this study was to assess the protein composition of okara prepared from different soybean genotypes by high-pressure hydrothermal processing and to correlate their polypeptide structure with the resulting physicochemical properties.

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MATERIALS AND METHODS

Materials. For soy milk and okara preparation six commercial soybean genotypes grown in field conditions were used: Nena and Lana (II maturity group), ZPS-015 (0 maturity group), Krajina (00 maturity group), and Novosađanka and Balkan (I maturity group). Three genotypes (Nena, ZPS-015, and Lana) were selected by the Maize Research Institute Zemun Polje (Belgrade, Serbia) and the others (Krajina, Novosadjanka, and Balkan) by the Institute of Field and Vegetable Crops (Novi Sad, Serbia). Although Novosadjanka was selected as a high seed protein cultivar and Lana lacked the Kunitz type of trypsin inhibitor, all of the genotypes were of food grade and were characterized by the high protein content in grain.

Soy Milk and Okara Processing. Okara was made on a pilot plant scale using a production method that includes hydrothermal cooking (HTC) according to the method of Wang et al.[1] for soy milk preparation. The HTC process according to Wang et al.[1] was modified by Stanojevic et al.[16] Briefly, soybeans were soaked in water (water/soybeans = 5:1) at 5−7 °C for 14 h. Soaked beans were ground and cooked by steam injection system (water/soybeans = 6:1) at 110 °C and 1.8 bar for 8 min (SoyaCow VS 30/40, model SM-30, Russia). The slurry was filtered and squeezed manually to obtain filtrate (soy milk) and a mushy remainder (okara). Samples were stored at 4 °C before further analysis.

Preparation of Samples for Chemical Analyses. Soybean seeds were ground to a coarse powder by a Micro-Mill grinder (Fisher, Germany). The soybean powder and fresh okara were then defatted using hexane (soybean powder or okara/hexane = 1:20). Solvent was evaporated at room temperature, and soybean powder and okara meals were stored at 4 °C until analysis.

From all samples (soy flour, soy milk, and okara), proteins were extracted for 120 min at room temperature with 0.03 M Tris-HCl bufer, pH 8.00 (tris(hydroxymethyl)aminomethane), which contained 0.01 M β-mercaptoethanol.[17] For defatted soy flour the sample to buffer ratio was 1:20; for soy milk and okara the sample to buffer ratio was 1:10. The mixture was centrifuged at 7558g for 15 min at room temperature. The protein extract was used to determine extractable soluble protein content and for sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS-PAGE).

SDS-PAGE. Dissociating electrophoresis for all samples was performed according to the method of Fling and Gregerson,[18] described in detail by Stanojevic et al.[19] using 5% (m/V) (pH 6.80) stacking and 12.5% (m/V) separating gels (pH 8.85). Briefly, the protein extract was diluted to a concentration of 2 mg/mL with sample buffer (pH 6.80), heated at 90 °C for 5 min, and cooled to room temperature. A 25 μL sample was loaded onto each well, and then the gels were run in a buffer solution (pH 8.30) at 80 mA per gel for 6 h to completion. The gels were fixed and then stained with Coomassie brilliant blue R-250 and destained with 18% (v/v) ethanol and 8% (v/v) acetic acid. Molecular weight markers (Pharmacia, Uppsala, Sweden) included phosphorylase B (94000), bovine albumin (67000), ovalbumin (43000), carbonic anhydrase (30000), trypsin inhibitor (20100), and α-lactalbumin (14400).

Densitometric Analysis. The gel was scanned and then analyzed by SigmaGel software version 1.1 (Jandel Scientific, San Rafael, CA, USA). Quantitative estimation of each identified subunit was calculated as the percentage of the corresponding area of the subunit with respect to the total area of the densitogram. The 11S/7S protein fraction ratio in okara was calculated by dividing the sum of acidic and basic glycinin polypeptides with the sum of subunits of proteins belonging to 7S proteins (β- and γ-conglycinin, Bg7S, and lectin) obtained by SDS-PAGE.

Other Analyses. The okara extractable soluble protein (ESP) content in the supernatant was determined according to the procedure of Bradford[20] using bovine serum albumin (BSA, Sigma, USA) as a standard. Okara protein extractability was calculated from the amount of ESP divided by the amount of total protein in okara (calculated on dry weight basis) and multiplied by 100. Total nitrogen content in samples was determined according to the micro-Kjeldahl method,[21] and total protein content was calculated by using a conversion factor of 6.25. Moisture and volatiles contents of samples were determined by using standard AACC procedure.[22] Okara protein recovery rate was expressed by the protein content of produced okara against protein content of soybean seeds, calculated on yield and a dry weight basis. Okara ESP recovery rate was expressed by the extractable soluble protein content of produced okara against the extractable soluble protein content of defatted soybean flour, calculated on yield and a dry weight basis.

Statistical Analysis. Except for electrophoretic analyses (which were duplicated), experiments were performed in triplicate. The data were analyzed using Statistica software version 5.0 (StatSoft Co., Tulsa, OK, USA). Regression analyses were carried out, and all correlations significant at $p < 0.05$ level were analyzed. The significance of differences between means was determined by t-test procedure for independent samples at $p < 0.05$. The results are given as the mean values.

RESULTS AND DISCUSSION

SDS-PAGE Profile of Total Okara Proteins. SDS-PAGE separated 7S and 11S proteins from investigated okara. Electrophoretic patterns of okara proteins from six different soybean varieties are shown in Figure 1. The protein patterns

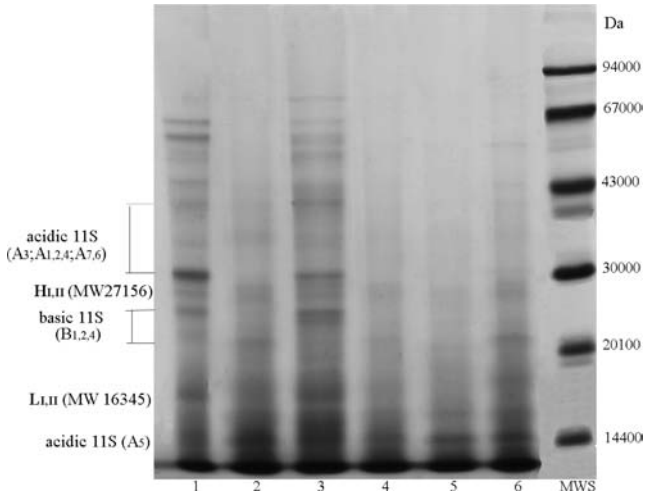

Figure 1. SDS-PAGE analysis of protein composition in okara from investigated genotypes. Lanes: 1, Nena; 2, Krajina; 3, Novosadjanka; 4, Balkan; 5, Lana; 6, ZPS-15; MWS, molecular weight standards.

were similar among all of the okaras from the investigated genotypes. Polypeptides with molecular weights corresponding to α', α, β, and β' subunits of the β-conglycinin (approximately 80000, 70000, 50000, and 42000, respectively) were not detected or were detected in traces. The same was true for γ-conglycinin (molecular weight approximately 64000). These results are in agreement with the work of Toda et al.[2] They also did not report high molecular weight polypeptides in okara extractable protein. It might be assumed that subunits of 7S protein fraction were partly distributed into soy milk during processing and partly hydrolyzed to low molecular weight peptides, which were precipitated in okara. Our results showed that the main proteins in extracts of okara were the ones with molecular weight values of 27000 and 16000. They were identified as "heavy" ($H_{I,II}$) and "light" ($L_{I,II}$) subunits of Bg7S protein.[6,7,23]

Peptides with the molecular weights from approximately 40000 to 32000 were the group of 11S protein acidic polypeptides (A_3, $A_{1,2,4}$, and $A_{6,7}$). The acidic polypeptide A_5 located near the bottom of the gel with a molecular weight of

Table 1. Composition of 7S Proteins in Okara Prepared from Investigated Genotypes[a]

	okara 7S proteins (% extractable protein)				
			Bg7S[b]		
genotype	β-conglycinin	γ-conglycinin	$H_{I,I}$	$L_{I,II}$	total
Nena	5.18 a	3.20 a	6.21 b	19.52 e	25.73 c
Krajina			5.50 d	19.78 d	25.28 d
Novosadjanka	4.89 b	3.09 a	7.83 a	20.54 b	28.37 a
Balkan			4.81 e	20.05 c	24.86 e
ZPS-015			5.93 c	21.57 a	27.50 b
Lana			6.31 b	18.30 f	24.61 e

[a]Means in the same column with different letters are significantly different ($p < 0.05$). [b]Bg7S, basic 7S globulin.

Table 2. Composition of 11S Protein in Okara Prepared from Investigated Genotypes[a]

	okara 11S protein (% extractable protein)					
	acidic					basic
genotype	A_3	$A_{1,2,4,}$	$A_{7,6}$	A_5	total	$B_{1,2,4}$
Nena	2.01 b	3.05 b	5.31 a	15.31 b	25.68 a	10.23 d
Krajina	2.51 b	2.71 c	2.58 b	5.81 e	13.61 e	15.31 b
Novosadjanka	3.55 a	5.58 a	1.93 d	8.73 d	19.79 c	13.32 c
Balkan	1.91 c	2.31 d	1.07 f	10.31 c	15.58 d	15.67 b
ZPS-015	2.01 b	2.24 d	2.40 c	5.32 f	11.97 f	16.52 a
Lana	0.93 d	1.12 e	1.35 e	17.31 a	20.71 b	9.83 e

[a]Means in the same column with different letters are significantly different ($p < 0.05$).

about 15000 was the main acidic polypeptide of the 11S protein. The cluster of protein bands with molecular weights of approximately 23000 were basic $B_{1,2,4}$ polypeptides of glycinin. Although B_3 basic polypeptide was previously detected in soy flour and tofu made from the investigated varieties,[15,19] it was not registered in protein extracts of okara. This indicated that during the preparation of okara B_3 was released from soybean into soy milk. Investigating the same soy genotypes, Stanojevic et al.[19] divided the SDS-PAGE patterns of soybeans and tofu proteins into two regions: the region of bands with MW < 44000 and that with MW > 44000; the first region contained mainly glycinin polypeptides, and the second region contained mainly subunits of β-conglycinin. On the contrary, our results showed that okara proteins from the same genotypes were localized mainly in one region of bands with MW < 40000. This indicated that soluble proteins of higher molecular weight were partly released into soy milk and partly degraded during processing, remaining in okara.

Quantification of 11S and 7S Protein Fractions and Their Subunits. The results of densitometric analysis of the two major storage protein fractions, 11S protein and 7S proteins, and their subunits from okara separated by SDS-PAGE are shown in Tables 1−3.

It was evident that the investigated soybean genotypes produced okara with mainly low molecular weight proteins. These results were in accordance with Zhu et al.,[24] who found that most of the 11S and 7S subunits in okara were hydrolyzed by enzymes to low molecular weight peptides below 20000. Polypeptides A_5 (MW = 15186) and $B_{1,2,4}$ (MW = 23142) were more resistant to the applied treatment and were found in higher concentrations than other 11S polypeptides (Table 2). Polypeptide A_5 constituted 18.67−56.68% of 11S protein, whereas $B_{1,2,4}$ polypeptides contributed 28.49−57.99% (Figure 2C). The concentrations of $H_{I,II}$ and $L_{I,II}$ in okara produced from the six investigated soybean genotypes ranged from 4.81 to 7.83% and from 18.30 to 21.57% of extractable protein,

Table 3. Relationship between Major Storage Proteins in Okara Prepared from Investigated Genotypes[a]

	proteins		
genotype	7S (%)	11S (%)	11S/7S
Nena	34.40 b	35.91 a	1.04 c
Krajina	25.43 d	28.92 e	1.14 b
Novosadjanka	38.08 a	33.11 b	0.87 d
Balkan	24.92 e	31.25 c	1.25 a
ZPS-015	28.81 c	28.49 f	0.99 d
Lana	24.68 e	30.54 d	1.24 a

[a]Means in the same column with different letters are significantly different ($p < 0.05$). 7S proteins included β- and γ-conglycinins, Bg7S, and lectin.

respectively. Basic 7S represented 24.61−28.37% of okara extractable protein (Table 1). On the basis of our results it was evident that Bg7S was one of the main proteins in extracts of okara produced by the HTC processing method. These results confirmed other findings, wherein it has been postulated that Bg7S is likely the main protein in extracts of okara in hot water, but was not detected in the okara extracted in water at room temperature.[2] Also, Asano et al.[25] found Bg7S to be difficult to extract in water at room temperature.

The presence of 12 cysteines in the molecular structure in position to form 6 disulfide bondings in the Bg7S molecules[7] stabilizes the three-dimensional structure of Bg7S. This strong bonding might also contribute to the stability of the Bg7S molecule to high pressure because in this study hydrothermal cooking for soy milk preparation at 1.8 bar was used. Therefore, we might speculate that high Bg7S content in okara extractable proteins was the consequence of stability of its structure to the applied HTC processing method. Stability of Bg7S molecule was also confirmed by the finding that Bg7S is the major component of the proteins released from soybean seeds by extreme high-pressure treatment.[3]

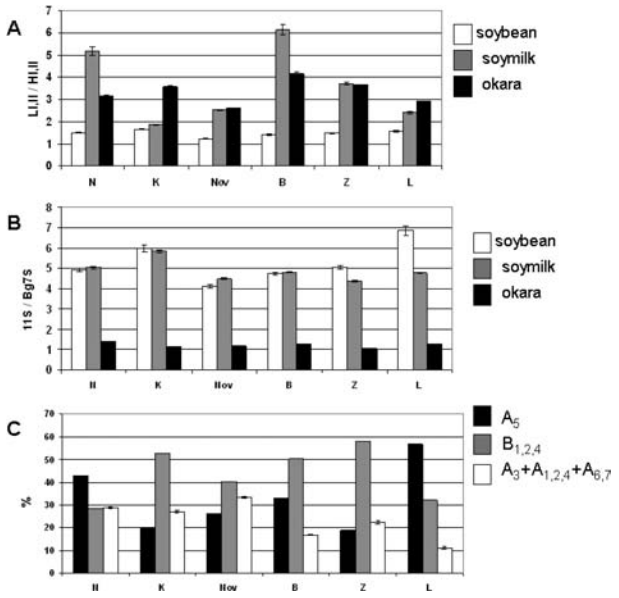

Figure 2. (A) Ratio of $L_{I,II}$ and $H_{I,II}$ protein subunits of basic 7S globulin in extractable protein of soybean, soy milk, and okara; (B) ratio of 11S and basic 7S globulin (Bg7S) in extractable protein of soybean, soy milk, and okara; (C) participation of specific acidic and basic polypeptides in okara 11S protein. Investigated soybean genotypes: N, Nena; K, Krajina; Nov, Novosadjanka; B, Balkan; Z, ZPS-015; L, Lana.

Participation of Bg7S in extractable protein of soybean and soy milk was significantly less than in okara (5.18−8.91% for soybeans and 7.79−11.14% for soy milks; Table 4). This was known for soybeans, but for soy milk higher participation of Bg7S in total protein was expected because the applied thermal treatment should have released more of it into solution. Besides the stability of the Bg7S molecule, this may be the result of the localization of Bg7S in soybean seed cotyledon.[3] Because Bg7S is tightly bound to plasma membranes and cell walls,[26] it was not easily extractable and therefore remained in okara. Such a high content of Bg7S in okara is desirable because of its nutritional value as it is a cysteine-rich glycoprotein.[9] Both Bg7S subunits contain more methionine and cysteine than β-conglycinin and glycinin.[4] Also, okara protein has a higher or similar content of all essential amino acids[27] relative to β-conglycinin or glycinin.[28]

Our results showed that the $L_{I,II}$ subunit of Bg7S was prevalent over the $H_{I,II}$ subunit in soybean, soy milk, and okara (Tables 1 and 4). The ratio of $L_{I,II}/H_{I,II}$ protein subunits varied from 1.22 to 1.66 in soybean, from 1.87 to 6.15 in soy milk, and from 2.62 to 4.17 in okara (Figure 2A). The differences in $L_{I,II}$ and $H_{I,II}$ contents and the $L_{I,II}/H_{I,II}$ ratio were due to both genetic differences and processing method effect. Also, $L_{I,II}$ and $H_{I,II}$ contents and the $L_{I,II}/H_{I,II}$ ratio significantly changed from soybean seed to respective soy milk and okara. Moreover, in okara the $H_{I,II}$ content was negatively affected by rising soy milk pH value ($r = -0.92$), but no correlation was registered for okara $L_{I,II}$ content (Table 6). This indicates a much more significant impact of the soy milk pH value on heavy than on light protein subunit contents of Bg7S. Regardless of the lower content of $H_{I,II}$ subunit in soybeans, soy milk, and okara, it influenced more the $L_{I,II}/H_{I,II}$ ratio ($r = -0.89$ in soybeans; $r = -0.94$ in soy milk, $r = -0.92$ in okara) than the $L_{I,II}$ subunit (Figure 3 and Table 6). Smaller and lighter molecules of the

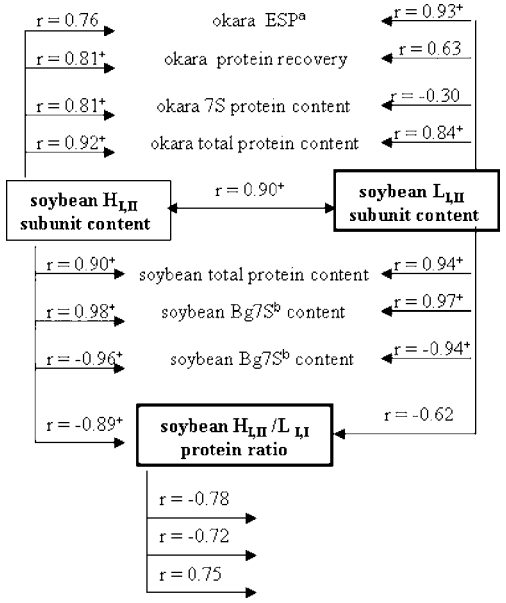

Figure 3. Correlation relationships among soybean basic 7S globulin and protein properties of soy milk and okara. +significant at $p < 0.05$; [a]ESP-extractable soluble protein content; [b]Bg7S, basic 7S globulin.

$L_{I,II}$ subunit were similarly distributed in okara protein extracts. We might speculate that under the applied conditions the $H_{I,II}$ subunit lost its solubility and was not extracted from okara. Also, such a low content of $H_{I,II}$ subunit in okara might indicate that the Bg7S molecule is bound to the plasma membrane and cell wall through this subunit.

Statistical analysis showed a significant correlation between $H_{I,II}$ and $L_{I,II}$ subunits of soybean Bg7S and properties of other proteins in soybean, soy milk, and okara (Figure 3). A strong

Table 4. Basic 7S Globulin Content in Soybean and Soy Milk of the Investgated Genotypes[a]

	soybean			soy milk				
	Bg7S[b] (% extractable protein)			Bg7S (% extractable protein)				
genotype	$H_{I,II}$	$L_{I,II}$	total	$H_{I,II}$	$L_{I,II}$	total	total protein (%)	pH
Nena	3.11 b	4.69 b	7.80 b	1.52 e	7.89 a	9.41 d	44.98 d	5.79 b
Krajina	2.54 c	4.21 d	6.75 d	2.71 c	5.08 e	7.79 f	52.03 c	5.99 a
Novosadjanka	4.01 a	4.90 a	8.91 a	3.15 a	7.99 a	11.14 a	56.32 a	5.10 c
Balkan	3.11 b	4.43 c	7.54 bc	1.25 f	7.69 b	8.94 e	54.75 b	6.01 a
ZPS-015	3.00 b	4.46 c	7.46 c	2.05 d	7.58 c	9.63 c	54.83 b	6.02 a
Lana	2.01 d	3.17 e	5.18 e	3.00 b	7.27 d	10.27 b	43.22 e	5.79 b

[a]Means in the same column with different letters are significantly different ($p < 0.05$). [b]Bg7S, basic 7S globulin.

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Table 5. Protein Properties of Soy Grain and Okara Prepared from Investigated Genotypes[a] (%)

	okara					soy grain	
genotype	total protein	protein recovery	ESP[b]	ESP recovery	extractability	total protein	moisture
Nena	35.07 d	32.16 b	31.72 c	45.08 a	90.45 a	38.72 d	5.99 b
Krajina	35.27 c	28.81 bc	31.14 c	36.79 bc	88.29 b	39.03 c	6.21 b
Novosadjanka	40.36 a	36.16 a	32.00 b	38.26 b	79.29 e	41.34 a	6.81 a
Balkan	35.41 c	29.96 b	31.29 c	37.03 bc	88.37 b	39.04 c	5.58 d
ZPS-015	37.32 b	27.28 c	32.53 a	42.20 a	87.17 d	39.41 b	5.79 c
Lana	31.81 e	28.16 bc	27.83 d	36.95 c	87.49 c	34.98 e	5.30 e

[a]Means in the same column with different letters are significantly different ($p < 0.05$). [b]ESP, extractable soluble protein.

dependence was registered between $H_{I,II}$ and $L_{I,II}$ subunit contents in grain total protein ($r = 0.90$; Figure 3). No significant correlation was found between soybean total Bg7S content and the content of this protein in soy milk and okara as well as between total Bg7S content in soy milk and okara Bg7S content (Table 6). These facts indicated that total Bg7S content in okara depended on production method conditions. Although 11S was 4−6-fold higher in grain and in milk than in okara, the 11S/Bg7S ratio in okara was around 1.20 (Figure 2B). As only a negligible amount of other 7S proteins was registered on SDS gels, it can be concluded that the dominant extractable proteins in okara were 11S and Bg7S proteins. Such a low ratio of 11S/Bg7S might also confirm a lower release of Bg7S from crushed grain into soy milk.

β- and γ-conglycinin were registered only in okara from Nena and Novosadjanka (Table 1). In other genotypes these proteins were registered in traces. As all of the investigated varieties synthesize β- and γ-conglycinin, the absence of these proteins in okara points out a partial degradation or distribution into soy milk of these proteins during treatment.

There are few data on the protein composition of the 11S fraction in okara. Our results showed that the investigated soybean genotypes produced okara containing mainly acidic A_5 and basic $B_{1,2,4}$ polypeptides (Table 2). The content of these polypeptides was negatively correlated ($r = -0.92$), which led to a negative influence of $B_{1,2,4}$ polypeptides on total acidic 11S polypeptides content ($r = -0.91$; Table 6). Other acidic polypeptides (A_3, $A_{1,2,4}$, and $A_{7,6}$) in okara were registered in much smaller quantities (Table 2). The acidic polypeptides are oriented to the exterior of the glycinin molecule, more exposed than the basic ones[29] and therefore much more susceptible to heat denaturation. Furthermore, Lakemond et al.[29] reported that 80−90% of the soybean acidic polypeptides remain in the soluble fraction after heat treatment, whereas the basic polypeptides are retained in precipitate. This implies a disruption of the noncovalent bonds (S−S bridge) between the basic and acidic polypeptides. On the basis of that we can assume that larger acidic polypeptides (A_3, $A_{1,2,4}$, and $A_{7,6}$) were mainly released into soy milk, whereas A_5 and basic polypeptides were retained in okara. The content of glycinin acidic polypeptides in okara positively correlated with 11S protein content in okara ($r = 0.90$), whereas basic polypeptides did not show significant effect ($r = -0.64$; Table 6). Also, A_3 was positively correlated with $A_{1,2,4}$ polypeptides ($r = 0.95$). The content of these acidic 11S polypeptides in okara positively correlated with the total protein content of soybeans ($r = 0.92$ and 0.87, respectively) unlike the basic ones ($r = 0.41$; Table 6). The subunit composition of the glycinin molecule in okara might be of industrial relevance because several authors found links between gelation and emulsifying properties with subunit composition of soybean glycinin.[15,30,31]

Total Protein and Extractable Soluble Protein Content. The examined genotypes are characterized by relatively high total protein content in full-fat soy grain (34.98−41.34%; Table 5). Genotype Novosadjanka was selected as a high seed protein variety and contained the highest total protein content (41.34%). The lowest total protein content was recorded in genotype Lana (34.98%), whereas the other varieties had relatively uniform values of total protein content (38.72−39.41%). Soybean total protein content significantly influenced total protein content in soy milk ($r = 0.82$) and okara ($r = 0.94$; Figure 4). In soy milk as well as in okara the highest total protein content was in products from Novosadjanka genotype and the lowest total protein content was in soy milk and okara from Lana genotype, whereas other varieties had relatively elevated values of total protein content within soy milks and within okara samples (Tables 4 and 5). That is in accordance with the results of other authors.[32−35] These results showed that total protein content in soy milk and okara highly depended on soy variety. All investigated soy milks and okara samples were characterized by high contents of total protein, which indicated significant nutritional value of these products.

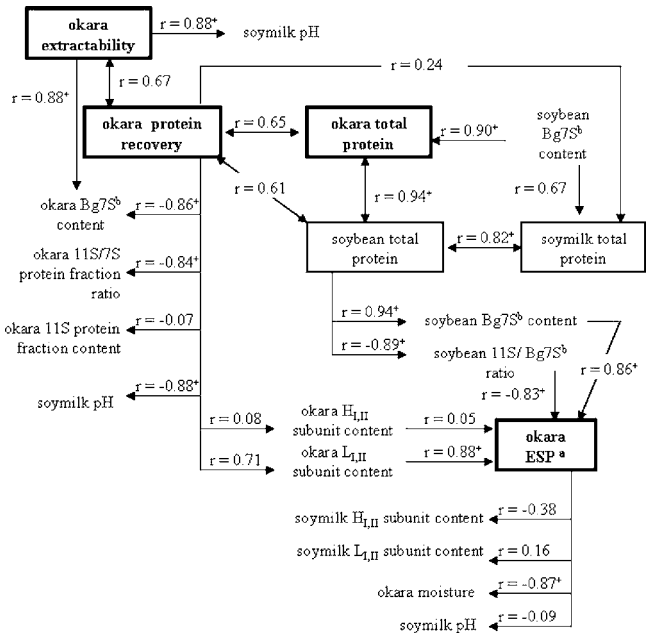

Figure 4. Correlation relationships among okara protein properties with proteins of soybean, soymilk and okara; +Significant at $p < 0.05$; [a]ESP- extractable soluble protein content; [b] Bg7S − basic 7S globulin.

Bg7S content was lowest in seeds, much higher in soy milks, and several times higher in okara. Our previous studies of the same soybean varieties indicated that Bg7S was not the main

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Table 6. Correlation Coefficients between Soybeans and Soy Milk Characteristics and Okara Properties

relationship	r	relationship	r
soybean Bg7S[a]–soy milk Bg7S	0.25	okara $H_{I,II}$–okara $L_{I,II}$/ $H_{I,II}$ ratio	0.92^{+}
soybean Bg7S–okara Bg7S	−0.48	okara $L_{I,II}$–okara $L_{I,II}$/ $H_{I,II}$ ratio	0.30
soybean 11S–okara extractability	0.24	okara A_5–okara $B_{1,2,4}$	-0.92^{+}
soybean 11S–okara protein recovery	−0.08	okara A_5–okara 11S acidic	0.83^{+}
soybean 11S/Bg7S–okara extractability	0.45	okara $B_{1,2,4}$–okara 11S acidic	-0.91^{+}
soybean 11S/Bg7S–okara protein recovery	−0.68	okara 11S acidic–okara 11S[b]	0.90^{+}
soy milk Bg7S–okara Bg7S	0.63	okara 11S basic–okara 11S	−0.64
soy milk pH–okara $H_{I,II}$	-0.92^{+}	okara A_3–okara $A_{1,2,4}$	0.95^{+}
soy milk pH–okara $L_{I,II}$	−0.04	okara A_3–soybean total protein	0.92^{+}
soy milk $H_{I,II}$–soy milk $L_{I,II}$/ $H_{I,II}$ ratio	-0.94^{+}	okara $A_{1,2,4}$–soybean total protein	0.87^{+}
soy milk $L_{I,II}$–soy milk $L_{I,II}$/ $H_{I,II}$ ratio	0.55	okara $B_{1,2,4}$–soybean total protein	0.41
		okara total protein–soybean 11S	0.13

$^{+}$Significant at $p < 0.05$. [a]Bg7S, basic 7S globulin. [b]11S, 11S proteins.

protein in grain 7S fraction. According to these results the main 7S protein in grain was β-conglycinin.[19] Neither 11S nor 7S proteins were correlated with total grain protein in investigated genotypes.[19] The results of this study showed that soybean Bg7S content was correlated with total protein content in grain (r = 0.94; Figure 4). Soybean 11S/Bg7S ratio was negatively correlated with total protein in grain (r = −0.89; Figure 4). Our results for okara indicated a strong correlation between okara total protein content and Bg7S content in soybeans (r = 0.90; Figure 4), which is understandable considering that Bg7S was one of the two main proteins in okara (Table 3). It is interesting that okara total protein content was not correlated with 11S content in soybeans (r = 0.13; Table 6), although 11S is the other major protein in okara. Relatively low 11S/7S ratios ranging from 0.87 to 1.25 (Table 3) did not necessarily reflect a lower nutritive value in essential amino acids because the major 7S protein in okara was Bg7S, which is higher in sulfur-containing amino acids than β-conglycinin.[4]

Okara protein recovery rate ranged from 27.28 to 36.16% (Table 5) and did not depend either on total soybean or on okara protein (Figure 4). Okara protein recovery was correlated with $H_{I,II}$ subunit content in grain (r = 0.81; Figure 3) but was not significantly dependent on $H_{I,II}$ and $L_{I,II}$ subunit contents in okara (Figure 4). However, okara protein recovery was negatively correlated with okara total Bg7S content (r = −0.86; Figure 4). Okara protein recovery did not depend on content of okara 11S protein (r = −0.07; Figure 4), but significant negative dependence between okara 11S/7S protein fraction ratio and okara protein recovery was registered (r = −0.84; Figure 4). Whereas okara protein recovery depended on soybean heavy Bg7S subunit content, okara ESP depended on light soybean Bg7S subunit content (r = 0.93; Figure 3). From all tested relationships ESP was influenced only by soybean Bg7S (r = 0.86; Figure 4) and its light subunit contents (Figure 3). Unlike okara protein recovery, which was dependent on grain heavy Bg7S subunit, okara ESP content was dependent on grain light Bg7S subunit (Figure 3), which led to a positive correlation between soybean total Bg7S content and ESP of okara (Figure 4). Also, light Bg7S subunits in okara were positively correlated with okara ESP (r = 0.88; Figure 4). These results of correlation analysis indicated that low molecular weight subunit of Bg7S increased okara ESP content (27.83–32.53%), which led to high ESP recovery (36.79–45.08%) and very high okara extractability (79.29–90.45%; Table 5). Okara extractability was significantly correlated with okara total Bg7S content (r = 0.88; Figure 4). This could be explained by Bg7S glycoprotein structure[3,9] as it is known that the carbohydrate moieties contribute to solubility.[11] Relatively close values for okara total proteins and okara ESP content (Table 5) indicated that almost all okara proteins were obtained in soluble form. This could be explained by the effect of treatment, which both caused protein degradation and changed their solubility properties, which resulted in the presence of low molecular weight proteins, below 40000, as the main proteins in okara. Also, the pH of soy milk was responsible for different properties of okara proteins. Whereas a positive correlation was registered between soy milk pH and okara extractability (r = 0.88), pH negatively influenced okara protein recovery (r = −0.88). Soy milk pH did not affect okara ESP (Figure 4). No significant correlation was found between 11S and 11S/Bg7S in soybeans with okara protein recovery and extractability (Table 6). The effect of soybean genotype on okara protein properties was evident in differences of the Bg7S content in grain. From all tested relationships only a higher Bg7S content in soybeans indicated a higher total protein content and higher ESP of okara (Figure 4). High values for ESP content and protein extractability may be prerequisites for good nutritional and functional properties of okara protein. Moreover, good protein solubility generally correlates with optimal emulsifying, gelation, and foaming activities.[36,37]

In summariy, the majority of protein subunits in 7S and 11S protein fractions in okara produced by hydrothermal processing of soy milk were low molecular weight peptides with molecular weights below 40000. Bg7S was one of two main proteins in extracts of okara produced using hydrothermal treatment. Also, the investigated soybean genotypes produced okara with mainly acidic A_5 and basic $B_{1,2,4}$ polypeptides of 11S proteins. The content of Bg7S in soybeans indicated total protein content in okara. Higher $H_{I,II}$ content in grain indicated better okara protein properties (total protein and protein recovery), whereas $L_{I,II}$ indicated higher extractable protein. 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 products because plant proteins are deficient in these amino acids. The characteristic low molecular protein pattern might indicate good nutritive and functional characteristics such as emulsifying and foaming properties of okara proteins. Moreover, the high quantity of vegetable protein in okara (>35%) and very high protein extractability (>80%) prove this byproduct to be very interesting for potential application in food fortification as a functional ingredient.

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