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Assessment of Soy Genotype and Processing Method on Quality of Soybean Tofu

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ABSTRACT: Protein quality in six soybean varieties, based on subunit composition of their protein, was correlated with quality of the produced tofu. Also, protein changes due to a pilot plant processing method involving high temperature/pressure and commercial rennet as coagulant were assessed. In each soybean variety, glycinin (11S) and β-conglycinin (7S) as well as 11S/7S ratio significantly changed from beans to tofu. Between varieties, the 11S/7S protein ratio in seed indicated genotypic influence on tofu yield and gel hardness ($r = 0.91$ and $r = 0.99$, respectively; $p < 0.05$). Also, the 11S/7S ratio correlated with soymilk pH ($r = 0.89$, $p < 0.05$), leading to a relationship between soymilk pH with protein recovery and yield of tofu ($r = 0.94$ and $r = 0.91$, respectively; $p < 0.05$). The soybean β'-subunit of 7S protein negatively influenced tofu hardness ($r = -0.91$, $p < 0.05$). Seed protein composition and proportion of 7S protein subunits under the applied production method had an important role in defining tofu quality.

KEYWORDS: soybean genotypes, storage protein, processing method, chymosin−pepsin rennet, tofu quality

INTRODUCTION

Soybeans are an inexpensive, high-quality protein source. Because of their economic, nutritive, and dietetic advantages, it is important to develop new soy protein foods or a range of new food formulations with new textures. Soy food has long been a staple of the human diet in Asia, especially as tofu, which is prepared from soymilk. Tofu is formed from soymilk curd. Traditionally prepared soymilk and tofu have painty and beany flavors. In Western societies, this flavor is unacceptable to most consumers and is the major obstacle to widespread acceptance of almost all soy food products, especially soymilk and tofu. Thermal denaturation of soy proteins is a prerequisite for the formation of tofu gel. However, heat treatment will have negative effects on their solubility but mildly heat-treated products will possess strong off-flavors, which is the main problem in developing soy protein foods as such.

A steam-infusion cooking process, known as hydrothermal cooking (HTC) was developed to produce soymilk that has less beany flavor because steam flashing stripped volatiles. It is known that HTC-processed soymilk can be used for manufacturing tofu of superior flavor characteristics and yield.[1] At sufficiently high pressure, soy proteins will be denatured, and at sufficiently high concentration, they will form gels in a similar manner to that induced by high temperature. However, the nature of the high-pressure-induced gels will be very different from those induced by heat, since heat will primarily affect hydrogen-bonded networks while pressure will more effectively disrupt hydrophobic and electrostatic interactions. Soymilk mixed with coagulant can form tofu gel after high-pressure processing without thermal treatment. This gel has strength and a cross-linked network.[2] Coagulation properties of soymilk are critical to achieving high yields and desired texture of tofu. Magnesium or calcium chloride is the most commonly used coagulating agent, but other calcium sources may be used.[3] When Murata et al.[4] were testing coagulation of soymilk protein with commercial proteinases, chymosin and pepsin did not coagulate the protein. Recently, a modified tofu processing method that engages HTC processing of soymilk followed by chymosin−pepsin/thermal coagulation of proteins (curd) was developed.[5] The influence of HTC processing on coagulation of soymilk protein by commercial proteinases that affect protein solubility and protein subunits in tofu is not fully understood.

Yield and quality of tofu are affected by several factors, such as type of tofu processing,[6,7] soybean growing environment,[8] and variety of soybean.[9] Soybean varieties differ in chemical components including proteins, lipids and minerals that may influence yield and quality of tofu.[1] Glycinin (11S protein) and β-conglycinin (7S protein) are the major storage proteins (globulins) in soybeans and soy food. Their content and ratio vary with soybean variety and environment.[6,9−12] β-Conglycinin is a trimeric glycoprotein consisting of three major types of subunits (α', α, and β) with different combinations and physicochemical properties. Glycinin is a hexamer consisting of acidic (A) and basic (B) polypeptides that are linked by disulfide bridges and composed in glycinin subunits. As constituents of glycinin, five subunits are identified: $A_{1a}B_{1b}$ (G_1), $A_{2b}B_{1a}$ (G_2), $A_{1b}B_2$ (G_3), $A_5A_4B_3$ (G_4), and A_3B_4 (G_5). These two proteins, β-conglycinin and glycinin, differ greatly in molecular weight, amino acid composition, surface characteristics, and isoelectric points. It has been suggested that each of the two proteins contributes qualities to the functional properties of soy proteins.[13]

Several studies found relationships of 7S and 11S proteins with tofu texture. The 11S content and 11S/7S protein ratio was reported to correlate positively with tofu hardness in tofu gels prepared from purified soy proteins. The glycinin fraction

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produces stronger gels that have higher water-holding capacity than β-conglycinin, which may be one of the major factors for tofu processing.[14] On the contrary, it was reported that the 7S protein formed harder gels than the 11S protein.[15] Although a negative relationship between tofu hardness and the 11S/7S protein ratio of soybeans was reported,[16] other studies found little correlation between the 11S/7S protein ratio and tofu quality.[17,18] Thus, the understanding of soybean storage protein contributions to tofu texture is contradictory and needs further investigation. Therefore, the aim of this study was to assess the influence of varietal differences in soybean protein structure on changes in composition of major tofu proteins and firmness and yield of tofu produced by a pilot plant method that uses steam injection cooking in combination with commercial chymosin−pepsin rennet.

MATERIALS AND METHODS

Materials. Six commercial soybean genotypes grown in field conditions were evaluated: Krajina (00 maturity group), ZPS-015 (0 maturity group), Novosadjanka and Balkan (I maturity group), and Nena and Lana (II maturity group). Three genotypes (Krajina, Novosadjanka, and Balkan) were selected by the Institute of Field and Vegetable Crops (Novi Sad, Serbia) and the others (Nena, ZPS-015, and Lana) by the Maize Research Institute Zemun Polje (Belgrade, Serbia). Novosadjanka was selected as a high seed-protein variety and the genotype Lana lacks the Kunitz type of trypsin inhibitor. As coagulating agent, commercial chymosin−pepsin rennet ("Idealka", Rennet workshop, Novo Selo, Serbia) was used.

Tofu Processing. Tofu was made on the pilot-plant scale by the production method that includes hydrothermal cooking according to Wang et al.[1] for soymilk preparation and chymosin−pepsin rennet for coagulation.[5] Soybeans were soaked in water (soybeans:water = 1:5) at 5−7 °C, for 14 h. Soaked beans were ground and cooked by steam injection system (soybeans:water = 1:6) at 110 °C/1.8 bar/8 min (SoyaCow VS 30/40, model SM-30, Russia). The slurry was filtered through a muslin cloth and squeezed manually to obtain filtrate (soymilk). When the cooked milk was cooled, commercial chymosin−pepsin rennet (10 mL of rennet/L of cooked soymilk) was added. The soymilk−coagulant mixture was stirred manually and left for 20 min to stabilize. Afterward, fast and brief manual stirring was repeated again and the mixture was left for 15 min to stabilize. The curd was pressed in a manual press (model SM-30, Russia) for 60 min. The weight of freshly formed tofu was recorded after pressing. Samples were stored at 4 °C before further analysis.

Preparation of Samples for Chemical Analyses. The seeds were ground in a Micro-Mill grinder (Fisher, Germany) to a coarse powder. The powder was then defatted by use of hexane. Solvent was evaporated at room temperature, and dried defatted soybean meal was stored at 4 °C until analysis. Tofu was defatted by the Folch extraction method.[19]

Extractable Soluble Protein Content. To determine extractable soluble protein content, protein was extracted for 120 min at room temperature with 0.03 M Tris-HCl buffer [tris(hydroxymethyl)aminomethane], pH 8.00, which contained 0.01 M β-mercaptoethanol (the sample to buffer ratio was 1:20). The mixture was centrifuged at 7558*g* for 15 min at room temperature. The protein content in the supernatant was determined by the procedure of Bradford[20] with bovine serum albumin (BSA, Sigma) as a standard.

Protein extractability in soy flour and tofu was calculated from the amount of extractable soluble protein divided by the amount of total protein in soy flour and tofu (calculated on a dry weight basis) and multiplied by 100. Protein extractability was calculated by the following formula:[12]

$$\text{protein extractability (\%)} = \frac{\text{extractable soluble protein in sample}}{\text{total protein content in sample}} \times 100 \quad (1)$$

Sodium Dodecyl Sulfate−Polyacrylamide Gel Electrophoresis (SDS−PAGE). Dissociating electrophoresis was carried out according to the procedure of Fling and Gregerson[21] in 1.5 mm thick gels with 12.5% (w/v) separating gels (pH 8.85) and 5% (w/v) stacking gels (pH 6.80). Proteins were extracted from defatted soybean flours in the same way as in the procedure to determine extractable soluble protein content by the Bradford[20] method. For defatted tofu samples, proteins was extracted for 1 h at room temperature with 0.055 M Tris-HCl−glycerin buffer, pH 6.80, which contained 0.64 M β-mercaptoethanol (the sample to buffer ratio was 1:10).

The protein extract was diluted to a concentration of 2 mg/mL with sample buffer, pH 6.80 [0.055 M Tris-HCl, 2% (w/v) SDS, 5% (v/v) β-mercaptoethanol, 0.0025% (w/v) bromophenol blue, 7% (v/v) glycerin], heated at 90 °C for 5 min, and cooled to room temperature. A 25 μL sample was loaded onto each well. The gels were run in a buffer solution of pH 8.30 [0.05 M Tris, 0.19 M glycine, and 0.1% (w/v) SDS] at 80 mA/gel for 6 h to completion. Gels were fixed, stained with 0.23% (w/v) Coomassie brilliant blue R250 [dissolved in 3.90% (w/v) trichloroacetic acid (TCA), 17% (v/v) methanol, and 6% (v/v) acetic acid] for 50 min and destained with 18% (v/v) ethanol and 8% (v/v) acetic acid. Molecular weights of polypeptides were estimated by use of a low molecular weight calibration kit (Pharmacia, Sweden). Molecular weight markers included phosphorylase B (94 000), bovine serum albumin (67 000), ovalbumin (43 000), carbonic anhydrase (30 000), trypsin inhibitor (20 100), and α-lactalbumin (14 400).

SDS−PAGE was performed in electrophoresis unit LKB-2001-100 with power supply LKB-Macrodrive 5 and LKB-Multitemp as a cooling unit (Pharmacia, Sweden). To investigate genotypic and processing method effects, electrophoresis of the storage proteins in six soybean varieties and tofu was performed in duplicate. Two aliquots of the same sample were analyzed at the same time. Two gels were run simultaneously in the same electrophoretic cell.

Densitometric Analysis. The destained gels were scanned and then were analyzed by SigmaGel software version 1.1 (Jandel Scientific, San Rafael, CA). Quantitative estimation of each identified subunit was calculated as the percentage of the corresponding area of the subunits with respect to the total area of the densitogram.

Textural Analysis. Texture profiles of curds were evaluated by use of a Höppler texture analyzer (Höppler-Cosistometer, VEB Prufgeräte-werk, Medingen/Dresden, Germany). Before measurement, tofu was allowed to equilibrate to room temperature. Curds were carefully cut into blocks (1 × 1 × 1 cm) and were put between two weights (250 g). Deformity of tofu curds was measured on continuous load of 0.25 kg, in 10th second:[22]

$$F = (Ph/V) \times 9.81 \quad (2)$$

where F (newtons per square centimeter) = hardness, P = 0.25 kg, h = tofu-cut height (centimeters), and V = measure of tofu-cut shortening under deformity (centimeters).

Microstructure of Tofu. A light binocular microscope (Leica DMLS; with camera Leica DC-30) was used to examine the fine structure of tofu. After tofu was made, small slices of fresh tofu (about 1 mm × 1 cm × 1 cm) were taken. For light microscopy the samples were not frozen.

Free α-Amino Nitrogen Analysis. Content of free α-amino nitrogen is an appropriate method to observe the degree of enzyme proteolytic activity. The sample was extracted for 1 h at room temperature from defatted meal or tofu in a 1:20 ratio with 85% ethanol. The mixture was centrifuged at 1073*g* for 15 min at room temperature. The content of free α-amino nitrogen was measured by a colorimetric ninhydrin method by the procedure of Wylie and Johnson [23] at 570 nm.

Other Analyses. Total nitrogen content in samples was determined by the micro-Kjeldahl method,[24] and total protein content was calculated by using a conversion factor of 6.25. Moisture and volatiles content was determined by standard AACC procedure,[25] and 1000 grain mass was measured. Tofu yield was expressed as mass in kilograms of fresh tofu produced per kilogram of dry soybeans. Tofu protein recovery rate was expressed by the protein content of produced defatted tofu against protein content of defatted soybean flour, calculated on a dry weight basis. The pH of soymilk was measured on a Consort-C931 pH meter (Belgium) with automatic temperature compensation.

Statistical Analysis. Experiments were performed in triplicate, except for electrophoretic analysis, which were duplicated. The data were analyzed with Statistica software version 5.0 (StatSoft Co., Tulsa, OK). 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. Regression analyses were also carried out. All correlations were significant at $p < 0.05$ level.

RESULTS AND DISCUSSION

SDS−PAGE Profile of Total Soybean Proteins. SDS−PAGE separated β-conglycinin (α′, α, β, and β' subunits) and glycinin (acidic and basic polypeptides) from soybeans and tofu. Electrophoretic patterns of seed and tofu proteins from six different soybean varieties are shown in Figure 1.

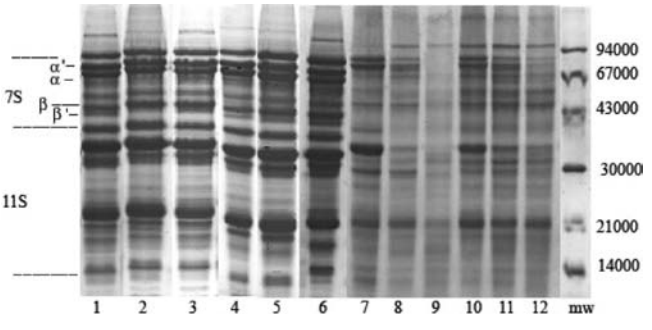

Figure 1. SDS−PAGE analysis of protein composition in soybean varieties (lanes 1−6) and tofu (lanes 7−12): mw, molecular weight standards; lanes 1 and 7, Nena; lanes 2 and 8, Krajina; lanes 3 and 9, Novosadjanka; lanes 4 and 10, Balkan; lanes 5 and 11, ZPS-015; lanes 6 and 12, Lana.

The protein patterns were similar among all the soybean varieties. The molecular weight values of the α′, α, β, and β' subunits of the 7S protein were approximately 80 000, 70 000, 50 000, and 42 000, respectively. Polypeptides with molecular weight of 39 000 are acidic A_3 polypeptides, and the group of polypeptides with molecular weight of about 34 000 is the major group of acidic polypeptides (A_{1a}, A_{1b}, A_2, and A_4). The separation in this region was not clear enough to detect these components separately. The acidic polypeptide A_5 located near the bottom of the gel had a molecular weight of about 15 000. The cluster of protein bands with molecular weight values of approximately 20 000 are basic components of glycinin. The protein band found slightly above the basic components is the B_3 basic polypeptide of glycinin. These results are consistent with earlier reports.[11,12] Accordingly the SDS−PAGE patterns of soybean and tofu protein extracts were divided into two regions: the region of bands with MW < 44 000 and that with MW > 44 000. The first region contained mainly 11S protein subunits, and the second region contained mainly subunits of 7S proteins.[26]

Quantification of 11S and 7S Proteins and Their Subunits. The results of densitometric analysis of the two major storage proteins, 11S and 7S, and their subunits from soybean varieties and tofu separated by SDS−PAGE are shown in Tables 1 and 2.

The concentrations of β-conglycinin (7S) and glycinin (11S) in six soybean varieties ranged from 18.40% to 22.18% and 35.49% to 40.41% of total extractable protein, respectively. These values were similar to the results of Cai and Chang[6] as well as those of Pesic et al.[12] Variety Balkan had the lowest 7S content (18.40%), whereas Novosadjanka had the highest (22.18%). Balkan had the lowest 11S protein content (35.49%), whereas Krajina had the highest (40.41%). Glycinin and β-conglycinin constituted approximately 54−76% of total extractable protein, which was similar to values for 13 soybean genotypes reported by Cai and Chang.[6] No significant correlation was found between total protein content and the content of either major storage protein, 7S or 11S (Table 5). This was in accordance with findings of Pesic et al.[12]

The ratio of 11S/7S soybean protein varied from 1.66 to 1.95 among the varieties, which is similar to the ranges of values that Mujoo et al.[11] found for seven soybean varieties harvested in the year 2000 (1.63−2.05) as well as the values for 12 soybean genotypes grown in 2001 (1.54−20.8) reported by Pesic et al.[12] The differences in 11S and 7S contents were due to both genetic and environmental differences. Balkan and ZPS-015 had the highest 11S/7S protein ratios (1.93 and 1.95), whereas Novosadjanka had the lowest ratio (1.66). The 11S/7S protein ratio of soybean varieties varies greatly in literature. One report showed that the 11S/7S protein ratio ranged from 2.1 to 3.4 in 12 soybean varieties,[16] whereas another study reported the 11S/7S protein ratio ranging from 1.66 to 2.51 in 13 soybean varieties.[6]

The 7S and 11S protein content and the 11S/7S protein ratio of tofu depended on the soybean variety. Also, it significantly changed from soybean seed to respective tofu. The quantity and quality of protein in seed are major biochemical parameters influencing soybean tofu quality.[27] The 11S/7S protein ratio of tofu ranged from 1.64 to 3.30 (Table 2).

The results showed significantly higher 11S than 7S protein content in soybeans and respective tofu (Tables 1 and 2). Higher 11S content in soybeans (35.49−40.41%) led to higher 11S content in tofu (30.82−36.47%). Also, 11S acidic polypeptides showed significantly higher content than basic polypeptides in soybeans and tofu. The results showed the difference in thermal

Table 1. Composition of Soy Protein in Soybeans[a]

	β-conglycinin (7S)						glycinin (11S)				
genotype	α′ (%)	α (%)	β (%)	β′ (%)	α′ + α (%)	β + β′ (%)	acidic (%)	basic (%)	7S (%)	11S (%)	11S/7S
Nena	5.92 a	5.11 c	3.08 b	7.34 c	11.04 a	10.42 c	26.98 a	11.47 f	21.46 b	38.45 b	1.79 b
Krajina	6.39 a	4.91 c	3.15 b	6.93 d	11.30 a	10.08 d	25.09 b	15.32 a	21.38 b	40.41 a	1.89 ab
Novosadjanka	4.97 c	5.47 b	3.30 a	8.44 b	10.44 b	11.74 a	23.93 d	12.84 d	22.18 a	36.77 d	1.66 c
Balkan	5.41 b	5.65 a	2.92 c	4.42 f	11.06 a	7.34 f	22.77 e	12.72 e	18.40 e	35.49 e	1.93 a
ZPS - 015	5.30 b	5.72 a	2.60 d	5.73 e	11.02 a	8.33 e	24.27 c	13.41 c	19.35 d	37.68 c	1.95 a
Lana	5.21 b	4.15 d	2.16 e	8.61 a	9.36 c	10.77 b	20.92 f	14.61 b	20.13 c	35.53 f	1.76 b

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

Table 2. Composition of Soy Protein in Tofu[a]

	β-conglycinin (7S)						glycinin (11S)				
genotype	α′ (%)	α (%)	β (%)	β′ (%)	α′ + α (%)	β + β′ (%)	acidic (%)	basic (%)	7S (%)	11S (%)	11S/7S
Nena	7.05 a	5.01 a	4.90 d	4.34 b	12.06 a	9.24 b	23.13 c	11.71 d	21.30 a	34.84 d	1.64 f
Krajina	5.01 b	3.09 d	6.19 a	5.54 a	8.10 b	11.73 a	22.27 e	13.19 a	19.83 b	35.46 c	1.79 e
Novosadjanka	0.50 f	1.01 e	4.91 d	2.91 d	1.51 d	7.82 c	22.43 d	8.39 e	9.33 e	30.82 f	3.30 a
Balkan	4.93 c	3.08 d	4.53 e	3.05 c	8.01 b	7.58 e	19.64 f	12.29 c	15.59 c	31.93 e	2.05 d
ZPS-015	4.05 d	3.94 b	5.67 b	2.01 e	7.99 b	7.68 d	24.25 a	11.72 d	15.67 c	35.97 b	2.29 c
Lana	3.37 e	3.38 c	5.31 c	1.02 f	6.75 c	6.33 f	23.50 b	12.97 b	13.08 d	36.47 a	2.79 b

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

stability between 11S and 7S proteins. It is known that the denaturation temperature of 11S protein (92 °C) is approximately 20 °C higher than that of 7S protein (71 °C) and there is a possibility to conduct thermal denaturation of 7S protein while 11S protein's native structure is preserved.[7] It may be assumed that, due to thermal denaturation, a significant portion of 7S protein fraction was retained in okara, which resulted in nonuniform enhancement of 11S/7S protein ratio in the obtained tofu in comparison with respective soybeans. Okara is the residue left from ground soybeans after separation of water extractable fraction used to produce soymilk.

Novosadjanka had the lowest protein ratio in soybeans, whereas Novosadjanka tofu had the highest 11S/7S protein ratio (Tables 1 and 2). This may be a result of the processing method and coagulating agent used in this study. Namely, the two most important steps in tofu making are soymilk extraction and coagulation. In this study, high temperature/pressure treatment was used for soymilk extraction and commercial chymosin−pepsin rennet was used for coagulation. They affected tofu storage protein compositions. Moreover, Cai and Chang[28] reported that some soybean varieties were more sensitive to changes in coagulation conditions (coagulant concentration, stirring speed, and duration). They summarized[6] that different varieties may require different coagulation conditions to maximize their tofu yield and quality.

Total Protein and Extractable Soluble Protein Content. The examined genotypes are characterized by relatively high total protein content in defatted soy flour (45.88−58.02%; Table 3). Genotype Novosadjanka contains very high total protein content, 58.02%, whereas the lowest total protein content was recorded in genotype Lana (45.88%). When compared with other findings, the values are similar to those of 45.88−54.49%, reported in the study for 12 different soybean varieties by Pesic et al.[12] and to 46.30−51.17% reported in the study for four different soybean varieties by Khatib et al.[29] Variations in protein content could be related to differences in soybean genotypes[29] and/or to differences in location and year effects.[30] Although the examined genotypes are not selected as so-called "tofu genotypes", produced tofu gels are characterized by high total protein content (43.12−56.15% of defatted tofu) that is in accordance with literature data.[29,31] As shown in Table 5, soybeans and tofu total protein contents are significantly correlated ($r = 0.95$).

Soybean protein extractability and extractable soluble protein content were significantly different among the investigated genotypes (Table 3). Total protein and extractable soluble protein content from the examined soybean genotypes were positively but not significantly correlated ($r = 0.60$). Soybean extractable protein content was approximately 23−31% and soybean protein extractability was about 50%. When compared with other reported values, a large range of values and even the extractability of around 80% could be noticed.[12] The differences in these values can be attributed mostly to environmental influence. Poysa et al.[30] reported that location and year effects were generally much larger for protein content then the genotype effect.

Among the soybean varieties, tofu extractable soluble protein content varied from 12.31% to 28.88% and extractability varied from 23.24% to 53.15% (Table 4). Similarly to the beans, protein extractability and extractable soluble protein content of tofu were positively correlated ($r = 0.94$). The increase of extractable soluble protein content in seed led to an increase in tofu protein extractability. On the contrary, a negative correlation existed between 11S/7S protein ratio in tofu and tofu protein extractability ($r = -0.95$) as well as between 11S/7S ratio and tofu extractable soluble protein content ($r = -0.84$). Moreover, positive correlation was found between tofu protein extractability and β-conglycinin concentration ($r = 0.88$) and also with α′ + α subunits content ($r = 0.89$), Table 5. Thus, the increase in β-conglycinin concentration and particularly in α′ + α subunits resulted in an increase of extractable soluble protein leading

Table 3. Chemical Properties of Investigated Soybean Genotypes and pH of Soymilk[a]

genotype	total protein (%)	ESP[b] (%)	extractability (%)	moisture (%)	free α-amino N[c] (mg/mL)	soymilk pH
Nena	47.21 e	25.01 d	52.98 b	11.92 c	37.5 c	5.81 b
Krajina	54.49 d	27.00 c	49.55 cd	11.94 b	37.5 c	5.98 a
Novosadjanka	58.02 a	31.00 a	53.4 3 a	11.90 c	40.0 b	5.09 c
Balkan	56.85 c	27.90 b	49.07 d	15.03 a	40.0 b	6.00 a
ZPS-015	56.88 b	23.24 e	40.86 e	8.58 e	37.0 d	6.01 a
Lana	45.88 f	23.33 e	50.85 c	11.09 d	42.5 a	5.79 b

[a] Means in the same column with different roman letters are significantly different ($p < 0.05$). [b] ESP = extractable soluble protein content. [c] Free α-amino nitrogen in soybean ESP.

Table 4. Chemical and Physical Properties of Tofu Prepared from Investigated Genotypes[a]

genotype	total protein (%)	protein recovery (%)	ESP[b] (%)	extractability (%)	moisture (%)	yield[c]	1000 grain mass (g)	hardness (N/cm^2)	free α-amino N[d] (mg/mL)
Nena	45.93 d	42.43 b	24.41 b	53.15 a	75.01 c	1.74 e	137.16 c	6.32 c	4.73 e
Krajina	53.72 b	48.80 a	24.74 b	46.05 c	75.26 bc	2.01 b	165.42 b	6.97 b	4.99 d
Novosadjanka	52.97 c	35.65 c	12.31 e	23.24 f	74.25 c	1.52 f	131.62 e	5.03 e	6.33 b
Balkan	56.03 a	45.89 a	28.88 a	51.54 b	76.00 b	1.94 c	138.53 c	7.74 a	5.83 c
ZPS-015	56.15 a	45.01 a	21.78 c	38.79 d	78.22 a	2.11 a	187.59 a	7.79 a	4.66 e
Lana	43.12 e	44.26 ab	14.20 d	32.93 e	74.93 c	1.88 d	139.78 d	5.92 d	6.99 a

[a] Means in the same column with different roman letters are significantly different ($p < 0.05$). [b] ESP = extractable soluble protein content. [c] Kilograms of fresh tofu per kilogram of dry soybeans. [d] Free α-amino nitrogen (milligrams per milliliter) in tofu ESP.

Table 5. Correlation Coefficients between Soybeans and Soymilk Characteristics and Tofu Properties

relationship	r	relationship	r
soybean total protein−soybean ESP[a]	0.60[b]	soybeans β′−tofu ESP[a]	−0.84[c]
soybean total protein−tofu total protein	0.95[c]	soymilk pH−tofu hardness	0.88[c]
soybean total protein−soybeans 7S	−0.15[b]	soymilk pH−tofu protein recovery	0.94[c]
soybean total protein−soybean 11S	0.05[b]	soymilk pH−tofu yield	0.91[c]
soybean 11S/7S ratio−tofu yield	0.91[c]	tofu 11S/7S ratio−tofu hardness	−0.65[b]
soybean 11S/7S ratio−tofu hardness	0.99[c]	tofu 11S/7S ratio−tofu ESP[a]	−0.84[c]
soybean 11S/7S ratio−soymilk pH	0.89[c]	tofu 11S/7S ratio−tofu extractability	−0.95[c]
soybean α-amino N[d]−tofu α-aminoN[d]	0.98[c]	tofu extractability−tofu ESP[a]	0.94[c]
soybean 7S−tofu hardness	−0.78[b]	tofu extractability−tofu 7S	0.88[c]
soybean β′−tofu hardness	−0.91[c]	tofu extractability−tofu α′ + α	0.89[c]
soybean β + β′−tofu hardness	−0.94[c]	1000 grain mass−tofu yield	0.80[c]

[a] ESP = extractable soluble protein content. [c] Significant at $p < 0.05$. [b] Not significant at $p < 0.05$. [d] α-Amino N = free α-amino nitrogen.

to the increase in protein extractability from tofu. These facts indicate that tofu containing higher levels of β-conglycinin (precisely α′ + α subunits) would have higher extractability than the others. This is probably due to differences in the nature of chemical bonds and interactions that stabilize glycinin and β-conglycinin molecules in the gel network. Glycinin molecules are connected through electrostatic interactions and strong disulfide bonds in protein gels. Weaker molecular forces such as hydrogen-bonding and hydrophobic interactions are important for formation of β-conglycinin gel networks.[15] This enhances extractability of β-conglycinin from tofu. Furthermore, β-conglycinin is a glycoprotein with about 5% carbohydrate moieties. It is known that the carbohydrate moieties contribute to solubility. Moreover, β-conglycinin has lower surface hydrophobicity than glycinin.[32]

Soymilks prepared from the examined genotypes exhibited pH values from 5.09 to 6.01. Optimal pH range for chymosin activity is 5.50−6.20 and for pepsin is 1.20−2.30, the latter being much lower than pH values registered in soymilks. Chymosin activity depends on the presence of two specific reactive residues of aspartic acid that are located near the active center and participate in formation of enzyme−substrate complex. Carić et al.[33] reported that aspartic acid residues from chymosin bind histidine from substrate. Protein subunits from major storage proteins in soybeans (glycinin and β-conglycinin) all contain histidine residue (β-conglycinin α, 3.5%; α′, 1.4%; and β, 1.9%; glycinin G1, 1.7%; G2, 0.9%; G3, 1.3%; G4, 2.8%; and G5, 3.2%). All these subunits might be involved in binding for histidine.

Due to a very high protein content in seed, tofu prepared from Novosadjanka genotype had high total protein content (52.97%) but significantly lower protein recovery (35.65%; Table 4). For all other soybean varieties there was approximately 42−48% protein recovery rate in tofu. This may be the result of protein distribution to other protein coproducts that were obtained during tofu processing (okara and whey). Further work is needed

to characterize this protein distribution during tofu processing. The protein recovery rate would be an indication of protein extraction efficiency as well as protein quality in different varieties. Also, differences in protein recovery rate could be caused by the procedures used in the applied processing method. This indicates that soybean varieties were different in protein recovered due to differences in their coagulating susceptibility.[29] pH values of soymilks (5.98−6.01; Table 3) that were prepared from soybean varieties with higher tofu protein recovery were in the range optimal for chymosin activity. For that reason, coagulating agent could exhibit maximal activity. Furthermore, higher pH values of soymilks were closer to the isoelectric point of 11S protein fraction (p*I* = 6.40), which might have enhanced its coagulation and contributed to gel formation. On the basis of our results, it was evident that the investigated soybean genotypes produced soymilks with very different pH. Differences between soymilks for almost one whole pH unit drastically changed conditions for enzymatic coagulation, which produced tofu gels with different properties, and it was hard to distinguish which properties of tofu gels were the consequence of genotypic effect and which were caused by the applied tofu processing method. This suggests that modification in producing tofu gels, taking account of genotypic differences of soybeans, is needed.

Content of Free α-Amino Nitrogen. The content of free α-amino nitrogen (4.66−6.99 mg/mL) reflected the content of free amino acids and low molecular weight peptides that could be extracted from tofu. It was about 6−8 times lower, compared to the initial component, soy flour (37.0−42.5 mg/mL; Table 4). Low content of α-amino nitrogen in tofu indicated that a fraction of the low molecular weight peptides and free amino acids was transmitted into the whey during its preparation. On the other hand, ZPS-015 tofu had the lowest content of low molecular weight peptides and free amino acids, but also the highest firmness due to very compact and uniform protein microstructure was registered. This may be the result of strong integration of low molecular weight peptides into the tofu matrix, which leads to better textural characteristics of tofu.

The tofu of genotype Lana shows the highest content of extractable low molecular weight peptides and free amino acids (6.99 mg/mL) compared to other investigated soy genotypes. That is in accordance with the highest content of free α-amino nitrogen (42.50 mg/mL) registered in the flour of this genotype (Tables 3). The correlative and regressive analysis shows that there is a very strong dependence (r = 0.98; Table 5) between flour and tofu free α-amino nitrogen contents. It is obvious that the genotype significantly affected the free α-amino nitrogen content of tofu.

Tofu Yield. According to the obtained results, a high degree of utilization could be noticed as well as an extreme economy of the soy genotype ZPS-15, which produced the highest tofu yield. Namely, 2.11 kg of fresh tofu was produced from 1 kg of dry soybeans. The other genotypes were characterized by slightly lower although still high yields (1.52−2.01 kg of tofu/kg of dry beans).

The highest tofu yield of ZPS-15 variety can be explained by the largest bean size of this genotype compared to other examined genotypes. It is evident when one takes into account the 1000 grain mass (187.59 g; Table 4). In industrial practice, large size soybeans are preferred for tofu making. The total amount of seed coat in small soybeans is higher than in large soybeans. The higher amount of seed coat in small soybeans is considered by tofu producers to have a negative effect on yield and quality of tofu. Soybean seed size and tofu yield showed a strong dependence (r = 0.80; Table 5) in a linear regressive analysis. Moreover, the higher pH of soymilk prepared from ZPS-15 variety could expose more protein hydrophobic regions to exterior and make them more susceptible to enzyme action. When the hydrophobic interactions are more predominant, aggregation could be induced and yield increased. This assumption might be supported by registering a positive correlation between pH of soymilk with protein recovery (r = 0.94) and also with the resulting tofu yield (r = 0.91).

The genotype ZPS-15 was characterized by lower bean moisture (8.58%) compared to other genotypes (15.03% and 11.09%; Table 3), whereas the moisture content of this variety was the highest in the final product (78.22% in tofu; Table 4). This showed that beans of genotype ZPS-15 took a higher amount of water during soaking that later resulted in higher yield. Sun and Breene[10] reported that a water/bean ratio in the range of 11−12:1 during soaking gave maximum tofu yield, but a ratio of 10:1 gave the best quality of tofu. Aiming for better tofu quality, a water/bean ratio in the range of 5:1 during bean soaking was used in this study. The lower tofu yield than in other reports may be attributed to the water/bean ratio used during soaking.[11,29]

Textural Characteristics of Tofu. The hardness of tofu prepared from investigated varieties decreased as the protein content in soybeans decreased (Tables 3 and 4). These results were in general agreement with other findings, where it has been reported that soybean varieties high in protein content produced tofu with firmer texture.[34] The exception was Novosadjanka variety, which has high total protein content in soy flour (58.02%) but the hardness of its tofu was the lowest (5.03 N/cm^2). That might be due to the pH of Novosadjanka soymilk (5.09), which was not optimal for either chymosin (5.50−6.20) or pepsin (1.20−2.30) activity. For that reason, the coagulating agent might not have exhibited maximal activity. Thus, our results showed that hardness of tofu was affected by the activity of the coagulating agent and protein content of soybeans.

Significant differences in tofu hardness among soybean genotypes have been found. The lowest hardness was registered in tofu genotypes Novosadjanka (5.03 N/cm^2) and Lana (5.92 N/cm^2), whereas genotypes Balkan and ZPS-15 were characterized by the highest hardness (Balkan = 7.74 N/cm^2 and ZPS-15 = 7.79 N/cm^2). These results were in agreement with the microstructure of tofu. Figure 2a shows a fine and uniform protein network structure of tofu made from ZPS-015 genotype, and very similar microstructure is seen for Balkan tofu (Figure 2b). Also, these samples were characterized by the highest hardness. On the other hand, the network of Novosadjanka-tofu (Figure 2f; with lowest hardness) became porous and had a great number of fine uniform cavities (ø = 115−160 μm) and large nonuniform cavities (ø = 335 μm) in the tofu matrix. Lana tofu had similar hardness and network structure to Novosadjanka tofu, with smaller (ø = 140 μm) and larger (ø = 300 μm) cavities. Furthermore, protein network structure in tofu made from Krajina and Nena genotypes was uniform, with a few cavities in tofu matrix (ø =100 μm; Figure 2c,d) but not as compact as ZPS-015 and Balkan tofu.

Registered tofu hardness was correlated with 11S/7S protein ratio of soybeans (r = 0.99; Table 5). Positive correlations between 11S content and 11S/7S protein ratio with tofu hardness have been reported previously.[6] Concentration of 7S protein β'-subunit in seed negatively influenced tofu extractable soluble protein content (r = −0.84) and tofu hardness (r = −0.91),

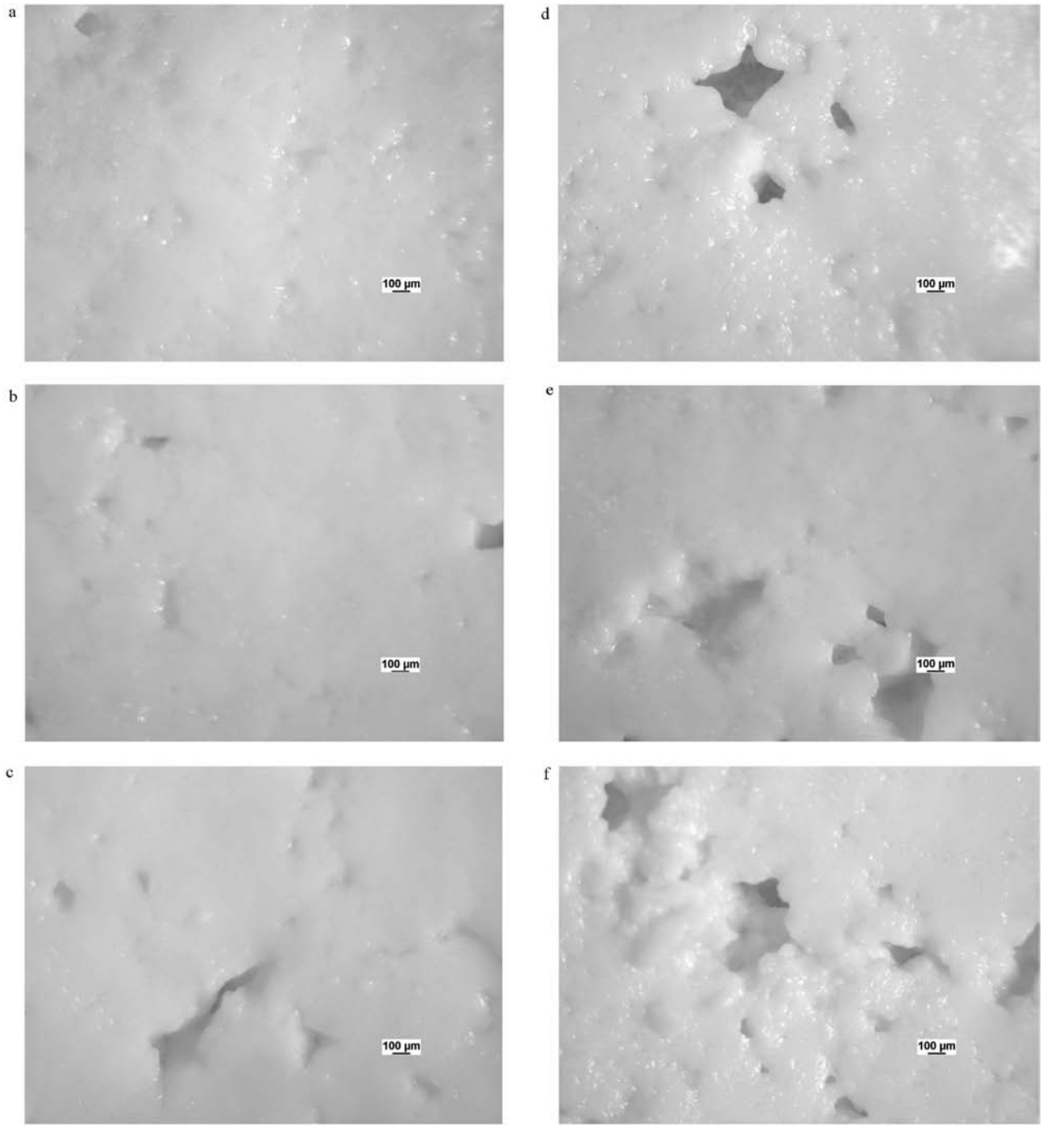

Figure 2. Microstructure of fresh tofu gels produced from investigated genotypes: (a) ZPS-015; (b) Balkan; (c) Krajina; (d) Nena; (e) Lana; (f) Novosadjanka.

leading to negative correlation of soybean $\beta + \beta'$ subunits sum ($r = -0.94$) as well as 7S soybean protein concentration ($r = -0.78$) with these properties (Table 5). These facts indicated that genotypes with higher levels of β-conglycinin, particularly those that synthesize β'-subunit, would produce lower tofu hardness. The examined genotypes had significantly higher 11S than 7S protein content (Table 1), which was more favorable for good textural characteristics of tofu than varieties with higher 7S protein content. According to Mujoo et al.,[11] higher soybean 7S fraction content means a proportionally lower 11S protein content, which results in a softer tofu gel. Saio et al.[14] reported that the 11S protein tofu was harder than 7S tofu, because the sulfhydryl group content in 11S tofu was higher than that in 7S tofu. Tofu texture is considered to be a direct consequence of microstructure, determined by chemical composition and physical forces. Both 11S and 7S proteins form gels upon heating and addition of coagulant. Electrostatic interactions and disulfide bonds in the 11S proteins are important in the formation of three-dimensional networks of the protein gel, whereas hydrogen-bonding and hydrophobic interactions are important for 7S protein gel networks. When

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the 11S/7S ratio increases, more covalent bonds can be produced through disulfide bonding, since 11S protein contains more total cysteine groups than 7S protein. Therefore, it is assumed that stronger molecular forces such as covalent bonding increase tofu hardness. Also, higher 11S/7S ratio in tofu is nutritionally beneficial due to higher content of sulfur-containing amino acids.

Surprisingly, 11S/7S protein ratio differed very much between soybean genotypes and respective tofu. Furthermore, 11S/7S ratio in beans positively influenced tofu hardness ($r = 0.99$). On the contrary, 11S/7S tofu ratio was negatively but not significantly correlated with tofu hardness ($r = -0.65$). Most probably, 7S protein to a certain extent contributed to tofu elasticity. Also, the pH of produced soymilk depended on 11S/7S ratio in beans ($r = 0.89$), which in further production process resulted in higher tofu hardness ($r = 0.88$), Table 5.

In this study, heat treatment/high pressure was used for soymilk preparation and thermal/enzymatic coagulation was used for tofu production. According to literature, in comparison with heat-induced gels, high pressure induces less browning and generally gives a softer and smoother texture.[35] Prepared tofu was not brown and had a good hardness. This pointed to the greater effect of temperature than pressure, but the pressure applied during soy milk preparation still had some beneficial effect.

Summarizing, genotypic differences caused different responses of soybeans to heat/pressure treatment during extraction, leading to variation in pH of soymilks, which in turn changed conditions of tofu processing. The investigated soybean genotypes in such conditions all formed lightly colored tofu gels. Genotypes varying in seed 11S/7S protein ratio produced tofu with significantly different yield, texture, microstructure, and 11S/7S protein ratio. Our present study pointed out the 11S/7S protein ratio in seed as a good indicator for tofu yield and textural properties, based on total protein analysis. Furthermore, β'-subunit of 7S seed protein was negatively related to tofu hardness, and genotypes containing a higher proportion of β'- and β-subunits gave softer tofu gels. On the other hand, it seems that α subunits of seed 7S protein compensated this negative relationship to a certain extent, since the negative influence of 7S protein was lower. Under the applied production method, seed protein composition and proportion of 7S protein subunits had important roles in defining tofu quality. Further work is needed to characterize this role. Better understanding of the relationship between seed protein subunit composition and tofu rheological properties might have applications in the food industry in formulating new soy-based products with desired texture.

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