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PROTEIN COMPOSITION IN TOFU OF CORRECTED QUALITY

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Soybeans are an inexpensive, high-quality protein source. Soybeans have long been a staple of the human diet in Asia, especially as tofu, which is prepared from soymilk. In this study, tofu was made using a new production method which includes hydrothermal cooking (HTC) and rennin-pepsin coagulant. The effects of the addition of gallic acid to the slurry during tofu processing were studied. Tofu was made from two soybean genotypes: Lana and Balkan. The observed genotypes are characterized by relatively high content of total proteins in flour, from 45.88% to 48.83%. The prepared tofu samples are characterized by extremely high content of total proteins (52.17% - Lana tofu and 56.08% - Balkan tofu). The presence of gallic acid significantly affects the solubility of tofu protein. The applied modifications of traditional procedure of tofu production significantly improved sensory properties of soybean protein products.

KEYWORDS: Tofu; hydrothermal cooking; proteinases; sensory properties; gallic acid

INTRODUCTION

Soybean is an annual herbaceous and leguminous plant. People of ancient Asia knew for this plant and cultivated it 4 000 years ago. Soya was imported to the European continent during the 18th century, and in our region, arrived sometime in the early 20th century. Modern research, in the first place, emphasizes its beneficial effects on the organism.

Processing of soybeans yields several key products, of which, in our region of a relatively more common use are tofu and soymilk. Tofu is a jellied protein product, with homogeneous composition, cream-colored with mild flavor, which is produced by the coagulation of heated soymilk. Tofu prepared by coagulation of soymilk by $CaSO_4$ or $MgCl_2$ contains about 8% of total proteins, 4-5% lipids and about 2% of carbohydrates on fresh weight basis. Tofu has a special nutritional value due to the presence of dietary fibers (about 1%) and the absence of cholesterol, as well as a very low energetic value. The high content of vitamins and minerals also contributes to the physiological value of the tofu. Analyzing tofu, Wang and Murphy (1) found that it content of total isoflavones is 0.532 mg/g of tofu. However, in our region, a wider use of soy products in human

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nutrition has started only recently. Therefore, the main goal of this study was to make soybean more acceptable to the domestic consumers in the form of products of corrected quality in terms of sensory characteristics that would increase mass consumption. It was noticed that the flavor of „green bean“, so-called „legume smell and taste“ of soybean products prepared in the traditional way does not suit the consumers from our population. These sensory properties, regarding its taste and smell, are a consequence of lipid oxidation - catalyzed by lipoxygenase, during steeping and crushing of the soybeans. Recently, a production process has been developed with the aim to reduce the duration of crushing the soybeans, as well as boiling the crushed grain under pressure, at high temperatures in the shorter period, the so-called hydrothermal cooking (HTC) that decreases the activity of lipoxygenase (2).

With the intention of improving the flavor of the produced cheese tofu was prepared using HTC and enzyme rennet, with the addition of gallic acid. Gallic acid (3,4,5-hydroxyl benzoic acid) is considered to have a strong antiseptic and antioxidant effects due to the presence of three free ortho-phenolic groups in its molecule. Both isolated and in the form of food ingredients the antioxidants prevent oxidation of lipids. They are used in food products in order to prevent perishing, rancidity or discoloration caused by oxidation.

The protein quality of soybean has a direct impact on the protein content of tofu. Dominant storage proteins of soybeans are globulins (3). Based on the coefficient of sedimentation during ultracentrifugation in standard phosphate buffer, they are divided into four fractions: 2S, 7S, 11S, and 15S fraction. Over 70% of soluble proteins of mature soybeans are the components of 7S and 11S fractions. The 7S fraction represents slightly more than 1/3 of total soybean proteins. It consists mainly of β-conglycinin, γ-conglycinin, basic-7S-globulin, lectins, and small quantities of enzymes such as lipoxygenase and β-amylase. β-Conglycinin (7S-globulin, vicilin) as the main protein of the 7S fraction and it makes 90% of the entire fraction. In the soluble proteins it is present with 16.8 to 20.9% and in total proteins of soybean, with about 30%. Glycinin (11S-globulin, legumin) makes 32% of total soybean proteins, and is considered as the main protein of the 11S fraction. When analyzed by PAGE, glycinin is registered with two main zones, one originating from the monomer and the other from the dimmer.

Due to the implementation of the manufacturing process that is significantly different from the traditional, and the application of gallic acid, it is realistic to expect the changes in protein composition of the obtained product, as well as the changes of sensory characteristics. The aim of this study was to examine the changes in the content and composition of the main protein fractions of the produced tofu after obtaining satisfactory sensory evaluation results.

EXPERIMENTAL

Soymilk processing. Two soybean genotypes grown in field conditions were evaluated. One genotype (Balkan) was selected by the Institute of Field and Vegetable Crops (Novi Sad, Serbia) and the other (Lana) by the Maize Research Institute Zemun Polje (Belgrade, Serbia). Soymilk and тofu were made using the new production method which

includes HTC (2) with slight modifications and rennin-pepsin coagulant. Soybeans were soaked in water (soybeans:water =1:5) at 5-7°C, for 14 h. Soaked beans were ground and cooked in water (soybeans:water = 1:6) at 110°C, and 1.8 bar, for 8 min. (SoyaCow VS 30/40, model SM-30, Russia) (4). The slurry was filtered through a muslin cloth and squeezed manually to obtain filtrate (soymilk).

Tofu processing and quality modifications by gallic acid. The prepared soymilks were separated in two equal volume parts. When the cooked milk was cooled, to one part gallic acid (1ml gallic acid/l soymilk; 100ppm) was added, and afterwards commercial rennin-pepsin rennet (rennet-″Idealka″, Rennet workshop-Novo Selo, Serbia) 10 ml/l of cooked soymilk was added. To the second part, commercial rennin-pepsin rennet was added only. The soy milk-coagulants was stirred manually and kept 20 min. Afterwards, fast and brief manual stirring was applied and the content left for 15 min. The curds was pressed with manual press (model SM-30, Russia) for 60 min. The weight of freshly formed tofu was recorded after pressing (5). Samples were stored at 4ºC before analysis.

Extractable soluble protein content. To determine soluble protein it was extracted for 1h at room temperature from defatted meal and tofu in a 1:20 ratio with 0.03 M Tris-HCl buffer, pH 8, which contained 0.01 M β-mercaptoethanol. The mixture was centrifuged at 17000 x *g* for 15 min at room temperature. The protein content in the supernatant was determined by the procedure of Bradford (6) at 595 nm.

Protein solubility in tofu was calculated from the amount of extractable soluble protein divided by the amount of total protein and multiplied by 100. Protein solubility was calculated using the following formula (7):

$$\text{Solubility (\%)} = \text{soluble protein content/total protein content} \times 100 \qquad [1]$$

Polyacrylamide gel electrophoresis (PAGE). PAGE was performed according to the method of Davis (8). The separating gels were 7% (wt/vol), pH 8.9 and stacking gels were 5% (wt/vol), pH 6.7. A 25 µl sample of the extract (2 mg protein/ml) diluted with sample buffer, pH 8.0 [0.03M Tris-HCl buffer with 0.01M β-mercaptoethanol, 10% (vol/vol) glycerol, 0.0025% (wt/vol) bromophenol blue] was loaded per well. The gels were run in a buffer solution, pH 8.3 [0.05M Tris (hydroxymethyl) aminomethane, 0.19M glycine] at 90 mA for 4 h to completion. Gels were fixed, stained with 0.1% (wt/vol) Coomassie blue R-250 [dissolved in 12% (vol/vol) acetic acid, and 50% (vol/vol) methanol] for 45 min and destained with 7% (vol/vol) acetic acid and 10% (vol/vol) methanol for 48 h. PAGE was performed on the electrophoresis unit LKB-2001-100 in connection with the power supply LKB-Macrodrive 5 and LKB-Multitemp as a cooling unit (LKB, Sweden). Electrophoresis of tofu was performed in duplicate. Namely, two aliquots of the same sample were analyzed at the same time. Two gels were run simultaneously in the same electrophoretic cell. The identification was done using 7S and 11S protein fractions obtained according to the procedure of Than and Shibasaki (9).

Densitometric analysis. The destained gels were scanned and then analyzed by SigmaGel software version 1.1 (Jandel Scientific, San Rafael, CA). The 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.

Headspace gaschromatography (GC) analyses were performed on a Hewlett-Packard Model 5890 Series II GC, on an EC-5-capillary column (30m x 0,53mm) with film of 1.2 μm. A volume of 25 ml of gaseous phase from 5% tofu dispersion, preheated to 45ºC was analyzed. Helium flow was 3 ml/min. Electron-ionization detector, set on 35-350 m/z was used. Components identification was done by comparision whit appropriate standards.

The total protein content in the samples was determined by the micro-Kjeldahl method (10). A nitrogen to protein conversion factor of 6.25 was used and calculated on the dry matter basis. The total proteins content in defatted flours was 45.88% for Lana flour and 48.83% for Balkan flour (11). Moisture content was determined by a standard AACC procedure (12). Soybean seeds were dehulled and ground in a mill. The meal was then defatted with n-hexane (meal:n-heksan=1:20 wt/V) for 2 hours, at room temperature and air-dried. Tofu was defatted by the Folch method (13), by the extraction with methanol/chloroform mixture (methanol:chloroform = 1:20; tofu: methanol/chloroform mixture=1:10) for 2 hours, at room temperature and air-dried.

Sensory evaluation. The sensory quality of tofu was evaluated by point system (from 1 to 5). Estimate was done by a panel consisting of 5 members. They gave the average marks of tofu quality and average corrected mark of sensory characteristics. The average mark of tofu quality is the average value of 6 quality parameters sum (smell, taste, color, cut, consistency and general appearance of tofu). Average mark corrected for quality parameter significance was obtained by dividing the corrected mark with the number of panel members. The corrected mark was obtained by multiplying marks for single tofu quality parameter with the appropriate coefficient of importance (for the appearance of tofu, consistency and cut - coefficient is 2; for color and smell - coefficient is 3; for taste - coefficient is 8). Then that sum of the results was divided with 20 (sum of coefficients). The mark for smell and taste is an average sum of marks for smell and mark for taste. Samples for sensory evaluation were stored at 4ºC and warmed to room temperature before evaluation. Tofu was cut into cubic samples (~6 cm × 6 cm × 4.5 cm) and placed on a plastic plate. Replicated samples were evaluated on different days.

Statistical analysis. Experiments were performed in triplicates, except for electrophoretic analysis, which was carried out in duplicates. The data were analyzed using Statistical software version 5.0 (StatSoft Co., Tulsa, OK).

RESULTS AND DISCUSSION

The way of tofu preparation essentially determines the content and composition of proteins in the final product, which has a direct impact on its application in nutrition. Considering that in this study hydrothermal cooking process, which is significantly different from the traditional way of preparation of tofu, as well as coagulation of proteins by enzyme coagulant (rennin and pepsin) was used, the changes in content and in composition of major proteins could be expected. It was expected that the applied method of tofu modification (addition of gallic acid) would lead to the changes both in composition of manufactured product and its sensory characteristics.

The investigated genotypes are characterized by a relatively high content of total proteins in defatted flour (Table 1) (11) which indicates that they are suitable for the use in production of protein products. The high content of proteins in grain is very important, given that, the quality and production of tofu directly depend on the characteristics of soybean as a starting material and conditions of production (14). In addition to high content of total proteins in seeds, the investigated varieties are characterized by a favorable content of soluble proteins, which results in very high solubility (Table 1). Due to the favorable total protein content of soybeans, the prepared tofu is characterized by an extremely high content of total proteins (52.17% - Lana tofu and 56.08% - the Balkans tofu; Table 2).

Table 1. Total nitrogen and protein content and soluble protein content of soybean defatted flour (11)

Content (%)	Lana	Balkan
total nitrogen	7.34±0.05	7.81±0.03
total protein	45.88±0.33	48.83±0.19
soluble protein	23.33±0.41	27.90±0.02
solubility	51.10±1.02	57.14±0.18
moisture	15.03	11.09

Table 2. Total and soluble protein content of tofu

Content (% d.b.)	Lana - tofu		Balkan -tofu	
	without gallic acid	with gallic acid	without gallic acid	with gallic acid
total protein	52.17±0.3	52.18±0.10	56.08±0.67	65.07±0.19
soluble protein	14.20±0.06	27.48±0.30	28.88±0.08	41.55±0.32
solubility	27.22	51.52	52.66	63.85

The procedure of addition of gallic acid does not significantly change total protein content but it affects the content of soluble proteins. Specifically, soluble protein content in tofu prepared with the addition of gallic acid is significantly different from the samples without it. This might be explained by acidic hydrolysis of tofu proteins by gallic acid, during which smaller molecular weight protein molecules are formed, that increases the content of soluble proteins.

The produced tofu shows significant cultivar differences in terms of the content of soluble proteins, where much higher content of soluble proteins is registered in the tofu prepared from Balkan variety. Proteins of the Balkan tofu are characterized by higher protein solubility compared to the protein solubility of Lana tofu Table 2). These data confirm that in addition to the technological process, the variety and quality of soybean significantly affect the characteristics of manufactured products.

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Original scientific paper

By electrophoresis separation of soybean flour protein extracts the major protein bands, with the following R_f-values: 0.01; 0.03; 0.08; 0.16 and 0.26 are registered. The remaining part is made of minor components and proteins belonging to the protein fraction 2S (Figure 1).

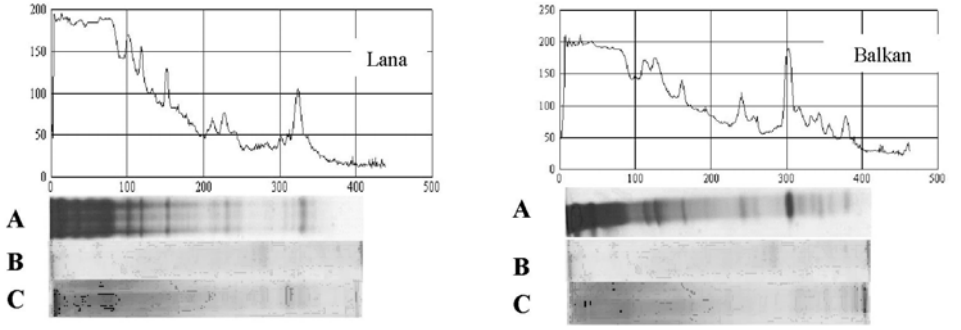

Figure 1. (**A)** Densitometric analysis of PAG electrophoregrams of soluble soybean flour and PAG electrophoregrams of soluble tofu proteins made without (**B**) and with (**C**) gallic acid

Right at the entrance into the gel a protein band is registered. It corresponds to the polymorphous form of β-conglycinin (Bo) and high molecular weight polymers of the dominant protein fraction (R_f=0.01). Both varieties are characterized by a high content of protein polymers (31.70% for Lana and 36.68% for Balkan). Such high percentage of this protein fraction indicates a high degree of association of the dominant proteins, so the part of the protein fraction which corresponds to β-conglycinin simply „remained trapped" at the entrance into the gel. Then follows the zone of β-conglycinin (R_f=0.03) and glycinin zone, which is characterized by two protein bands. The band with smaller electrophoresis mobility corresponds to the glycinin dimer form, while the next band corresponds to the monomeric form of the molecule (Table 3). In the Ornstein-Davis discontinuous electrophoresis system, β-conglycinin migrates more slowly than glycinin, while γ-glycinin has a higher relative mobility in comparision to glycinin. Based on that fact, the band with R_f-value of 0.03 was identified as β-conglycinin, while the band with R_f-value 0.26 corresponds to γ-glycinin.

The key components of the 7S fraction represent 10.11% to 13.00% of flour soluble proteins; β-conglycinin is present with 3.68-4.29%, and γ - conglycinin with 5.82-9.40%. The values obtained for the participation of γ - conglycinin in soluble proteins of flour correspond to the obtained literature data while the literature data indicate a much larger share of β-conglycinin in soluble proteins of flour (15). However, in this study, a large quantity of polymorphic forms of β-conglycinin was registered.

Based on the presented data (Table 3) it is clear that there are differences between the genotypes regarding participation of major proteins. The most notable difference is in the participation of monomeric and dimeric forms of glycinin.

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Table 3. Soluble protein composition of soy flours (%)

Protein	R_f	Lana	Balkan
Bo and large molecule polimers	0.01	31.7±0.05	36.68±0.48
β – conglycinin	0.03	4.29±0.12	3.60±0.01
glycinin dimeric	0.08	8.59±0.55	3.24±0.03
glycinin monomeric	0.16	17.2±0.11	18.76±0.55
R_f = 0.21		8.61±0.10	5.86±0.05
γ – conglycinin	0.26	5.82±0.19	9.40±0.22
R_f = 0.32 - 0.47		7.15±0.06	4.86±0.05

The results obtained by densitometry of polyacrylamide gel electrophoresis of tofu, in native conditions, indicate a very low solubility of tofu proteins (Figure 1). Namely, in the analyzed cultivars, the presence of only one or two protein bands is identified (Table 4). Different solubility of tofu proteins under the conditions of PAGE may indicate the different nature of the links (which are primarily characterized by different strength) in the gel structure obtained from the tested varieties. These results showed that the solubility of the major tofu proteins is very low, indicating their very strong incorporation into the gel matrix. This can lead to the conclusion that in the formation of the gel solid participate covalent bonds (such as disulfide bonds).

By PAGE of the extract of the tofu prepared with the addition of gallic acid a large number of components is separated, which cannot be observed on the electrophoregrams of unmodified tofu (Figure 1). It is obvious that the addition of galic acid led to both hydrolysis and changes in surface properties of major proteins resulting in increase of their solubility. Right at the entrance into the gel is recorded a much higher amount of high molecular weight proteins in Balkan tofu (74.96%) then Lana tofu (9.64%). On the other hand, Lana tofu has shown a significant content of glycinin dimeric form (57.43%), while in the Balkan tofu this component was present in traces (0.69%, Table 4). Such high content of Bo-isoform in β-conglicinin in Balkan tofu can be explained by a significant decrease in solubility of the other protein components, as evidenced by low values of their contents, some of which being found in traces.

Table 4. Soluble protein composition of tofu (%)

Protein	Rf	Lana tofu		Balkan tofu	
		without gallic acid	with gallic acid	without gallic acid	with gallic acid
Bo and largemolecule polimers	0.01	27.86±0.03	9.64 ±0.47	27.86±0.03	74.96 ±0.49
β – conglycinin	0.03	/	6.28 ±0.23	/	5.82 ±0.03
glycinin dimeric	0.08	/	57.43 ±1. 89	/	0.69 ±0.01
glycinin monomeric	0.16	/	7.97 ±0.1	/	0.17 ±0.01
γ – conglycinin	0.26	/	4.07 ±0.14	/	4.64 ±0.07
Rf = 0.28		66.76±0.02	2.10 ±0.03	/	0.17 ±0.01

In Lana tofu, a significantly increased solubility of the 11S fraction is registered, where the compact diametric form of glycinin is dominant. In terms of γ-conglycinin content, there are no differences among the varieties. On the electrophoregrams of these samples a diffuse zone is revealed with very close R_f values to that of γ-conglycinin (R_f=0.28). It can be assumed that a protein fraction of very similar molecular weights γ-conglycinin was formed by acidic hydrolysis. This protein fraction, as well as other major protein components, was not registered in the samples of tofu prepared without gallic acid (Table 4). Therefore, it can be concluded that the presence of gallic acid significantly affects the structure of tofu protein.

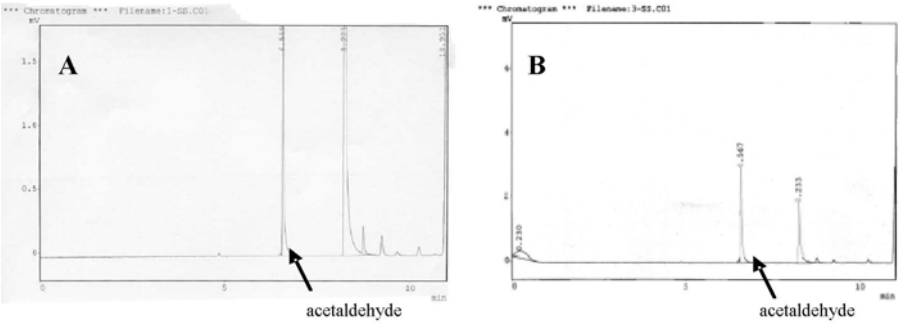

Figure 2. Gas-chromatograms of tofu made with gallic acid of Lana (A) and Balkan (B) soy genotypes

Gallic acid is an excellent antioxidant, and it has been added in the preparation of tofu primarily to reduce the unwanted odor of legumes (16). This objective was achieved, taking into account that, GC showed only several components registering acetaldehyde as major volatile (Fig. 2). The improvement of sensory characteristics is also confirmed by favorable sensor evaluation grades given to the samples prepared with the addition of gallic acid (Table 5).

Table 5. Results of tofu sensory evaluation (the mean marks of tofu quality)

Genotypes		Lana	Balkan	Lana	Balkan
the category of tofu quality		without gallic acid		with gallic acid	
the average marks	smell and taste	3.25±0.07	2.66±0.13	3.30±0.11	3.64±0.13
	quality	3.43±0.19	3.38±0.62	3.65±0.31	3.87±0.17
	corrected mark	3.15±0.40	3.26±0.65	3.55±0.55	3.86±0.26

CONCLUSION

The applied technological process of production has a significant impact on the protein content and composition of storage of soy proteins in tofu, as well as on its sensory characteristics. The supplement of gallic acid in the production of tofu, in addition to

improving the nutritional characteristics, reduces significantly the so-called leguminous smell and taste of produced cheese, as confirmed by gas chromatographic analysis of tofu volatiles, since mainly acetaldehyde was registered. In addition to improvement of the nutritional and sensory properties, gallic acid significantly improves the solubility of the main protein components of tofu. For this study the tofu was prepared from local varieties of soybean, which are not selected as so-called "tofu varieties", but they are very suitable for the tofu production since both of them gave a high-protein product. Because of its exceptional sensory and nutritive values, the tofu might be accepted by larger number of consumers.

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ПРОТЕИНСКИ САСТАВ ТОФУА КОРИГОВАНОГ КВАЛИТЕТА

Слађана П. Станојевић, Мирољуб Б. Бараћ, Мирјана Б. Пешић, Мирјана М. Миловановић и Биљана В. Вуџелић-Радовић

Тофу је желирани протеински производ, који се добија коагулацијом загрејаног сојиног млека. Потрошачима наше популације не одговара арома „зеленог зрна“, такозвани „легуминозни мирис и укус“, који се осећа у производима соје припремљеним на традиционални начин. Са намером побољшања ароме добијених сирева, припремљен је тофу применом хидротермичког третмана и ензима за коагулацију млека (химозин-пепсин) уз додатак галне киселина, која је јак антисептик и антиоксидант.

Захваљујући високом садржају укупних протеина сојиног семена (45,88-48,83% у обезмашћеном брашну) и припремљени тофу се карактерише високим садржајем укупних протеина (52,17% с.м.- Лана тофу и 65,08 % с.м. - Балкан тофу). Поступак додатка галне киселине мења садржај растворљивих протеина (27,48% - Лана тофу и 41,55% - Балкан тофу) који се значајно разликује у односу на узорке без ње (14,20% - Лана тофу и 28,88% - Балкан тофу). Полиакриламидном-гел електрофорезом екстракта тофуа припремљеног уз додатак галне киселине раздвојен је већи број компонената, које се не уочавају на електрофореграмима тофуа немодификованог квалитета.

Гаснохроматографском анализом испарљивих компонената тофуа регистрован је углавном ацеталдехид, тако да је постигнут циљ ублажавања легулинозног мириса. Побољшање сензорних карактеристика потврђују и повољне сензорне оцене које су добили узорци припремљени са галном киселином (3,65 – Лана тофу и 3,87 - Балкан тофу).

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