

# Distribution of $\beta$ -amylase and lipoxygenase in soy protein products obtained during tofu production

Slađana P. Stanojević, Miroljub B. Barac, Mirjana B. Pesic, Biljana V. Vucelić-Radović

Faculty of Agriculture, Institute for Food Technology and Biochemistry, Department of Chemistry and Biochemistry, University of Belgrade, Belgrade, Serbia

## Abstract

Soybean is considered an important source of human food and animal feed. Okara and tofu whey are the main by-products of soymilk and tofu production. The distribution of enzymes  $\beta$ -amylase and lipoxygenase (Lox) from six soybean genotypes in protein extracts of okara and tofu as well as in soymilk and tofu whey was assessed. SDS-electrophoretic results showed that production process significantly affected high molecular mass protein fractions of soybean by-products. Low amounts of Lox in soymilk were registered, while in okara protein extracts and tofu whey this enzyme was present in trace. In tofu protein extracts, Lox was registered in higher quantity that might be the result of the aggregation process during formation of the tofu gel. Content of  $\beta$ -amylase in all soy protein products was high and similar to the content in soybean genotype. Content of  $\beta$ -amylase in okara depended on respective soybean genotype.

**Keywords:** soybean, hydrothermal cooking, soy protein products,  $\beta$ -amylase, lipoxygenase.

Available online at the Journal website: <http://www.ache.org.rs/HI/>

SCIENTIFIC PAPER

UDC 633.34:66:577.122

*Hem. Ind.* 71 (2) 119–126 (2017)

Soybean is considered as an important source of human food and animal feed and it is also used as raw material in a great variety of industrial non-nutritional products, for example fertilizers [1,2]. Soybean contains 30–40% proteins, 12–24% oil, around 34% carbohydrates, 3–6% mineral matter and some vitamins (above all A and B vitamins) [3]. The most popular soyfoods are soymilk and tofu. Soymilk is a water extract of whole soybean seed. It is a white emulsion/suspension, consisting of water-soluble proteins and carbohydrates, and much of the soybean oil component [3]. Tofu is prepared from soy milk as soymilk curd by the addition of coagulants [4]. The search for alternative food and feed ingredients for man and livestock continues to attract the attention of researchers all over the world [5]. Therefore our interest was directed to by-products of soymilk and tofu production. Okara (also called “soy pulp”) and tofu whey is the main by-products of soymilk and tofu preparation. It is a white-yellowish material consisting of the insoluble parts of the soybean seeds which remain after filtration of soy milk [6,7]. Tofu whey (also called “soybean whey”) is a pale-yellowish liquid with pleasant aroma and taste which remains after tofu squeezing [3].

Traditionally prepared tofu has paint and beany flavors. In Western societies, this flavor is unacceptable to the most consumers and is the major obstacle to widespread acceptance of all soy food products. Thermal denaturation of soy proteins is a prerequisite for the formation of tofu gel. A steam-infusion cooking process, known as hydrothermal cooking (HTC), was developed to produce soymilk that has less beany flavor. It is known that HTC-processed soymilk can be used for manufacturing tofu of superior flavor characteristics [4,8]. Another significant feature of the tofu processing is the type of coagulant. Calcium or magnesium chlorides are usually used for traditional tofu preparation [8]. However, the enzyme rennet may also be used for this purpose [4].

Soy protein products are characterized by a high content of proteins: in soymilk and tofu around 40–50% [4], in okara around 30–40% [7] and in tofu whey around 22–28% [9]. Furthermore, soy beans and soy protein products contain a wide range of bioactive compounds (trypsin inhibitors (TIs), lectins, lysine, phytic acid, isoflavones, lunasin, saponins, phytosterols) exhibiting therapeutic and preventive effects in treatment of diseases of the heart, blood vessels and cancer [10–14], and classifying these products into a category of “functional food”. To be categorized as “functional food” each product containing soy proteins must contain 6.5 g of soy proteins per serving [15]. These bioactive components of soybean protein products are intensively studied due to their effect in the prevention and treatment of different diseases. However, these products also contain other active ingredients, such as enzymes, that beside technological have important nut-

Correspondence: S. Stanojevic, Institute of Food Technology and Biochemistry, Department of Chemistry and Biochemistry, Faculty of Agriculture, University of Belgrade, Nemanjina 6, P.O. Box 14, 11080 Belgrade-Zemun, Serbia.

E-mail: [sladjas@agrif.bg.ac.rs](mailto:sladjas@agrif.bg.ac.rs)

Paper received: 25 May, 2015

Paper accepted: 28 December, 2015

<https://doi.org/10.2298/HEMIND150525021S>

ritional role. Soybean seeds and soybean protein products contain a large number of different enzymes, such as: amylases, glycosidases, hexokinases, lipases, lipoperoxidase, lipoxygenase, peroxidases, phosphatases, transaminases, urease, etc. [3].

$\beta$ -Amylase ( $\alpha$ -1,4-glucan maltohydrolase; EC 3.2.1.2) catalyzes the liberation of  $\beta$ -anomeric maltose from non-reducing ends of  $\alpha$ -1,4-glucans with inversion of the anomeric configuration (starch, glycogen and maltooligosaccharides). The enzyme isolated from soybean flour consists of a single polypeptide chain of molecular weight of 55000–57000 [16,17]. This monomeric protein has been known to require no essential low molecular mass cofactors for the maintenance of the molecular structure. It is also found in various higher plants and in some gram-positive spore-forming bacteria. Soybean  $\beta$ -amylase was first crystallized by Fukumoto and Tsujisaka in 1954 [18]. From then until today, many characteristics of  $\beta$ -amylase have been examined: heat and pH stability relationships, the pH dependence of its activity, inhibition of its activity by chemical reagents, the mode of action on starch, crystal structure, molecular weight, amino acid composition, N- and C-terminal amino acid residues, behavior of sulfhydryl groups, the comparison of soybean  $\beta$ -amylase with bacterial  $\beta$ -amylase etc [16,17,19–26]. Biotechnologists gave a lot of attention to this enzyme [27–29].

Lipoxygenase (Lox) is an important enzyme in soybean, too. Soybean Lox has molecular mass around 94000–96000 [30,31]. Sensory characteristics of soyfoods are significantly reduced because of the activity of Lox. Lox activity generates off-flavors in soybeans by hydroperoxidation of fatty acids and by interaction with soy-proteins during soaking and grinding [32]. Undesirable flavors, characterized as beany, painty, grassy, green, astringent, and bitter, reduce acceptance of soy foods by many consumers who may prefer a mild flavor of soy products. Nowadays, the production of soy-food with as much as possible inactivated Lox is tried to be achieved. HTC processed soymilk (high temperature/short time/under pressure) has less beany flavors because of the much shorter time for Lox to be active and because the steam flashing removes volatiles.

In order to improve nutritional, sensory and technological properties of soymilk, okara, tofu and tofu whey, as well as their yields, the HTC-process for their preparation was applied in this study. There is a few literature data on the distribution and retention of the enzymes  $\beta$ -amylase and lipoxygenase in soybean protein products which can be obtained during tofu production. Therefore, the aim of this study was to assess the distribution of the two enzymes:  $\beta$ -amylase and lipoxygenase, during the preparation of soy protein

products by hydrothermal cooking of soybeans. This research is a continuation of our previous studies on the characteristics of soy protein products obtained by the HTC process [4,7,33,34].

## MATERIAL AND METHODS

### Chemicals

All chemicals were p.a. grade. Tris,  $\beta$ -mercaptoethanol, glycerin, HCl, acrylamide, bis-acrylamide, ammonium persulfate, acetic acid, ethanol, *n*-hexane, sodium dodecyl sulfate (SDS), bovine serum albumin (BSA), Coomassie brilliant blue (R-250) were purchased from Merck (Germany). Tetramethylethylenediamine (TEMED) is purchased from Sigma (St. Louis, MO, USA). Standards of molecular masses were purchased from Pharmacia (Sweden). Commercial chymosin-pepsin rennet is purchased from „Biserka“ (Belgrade, Serbia).

### Plant materials

For tofu preparation six commercial soybean genotypes were used: Nena and Lana (II maturity group), Krajina (00 maturity group), ZPS-015 (0 maturity group), Novosađanka and Balkan (I maturity group). Three genotypes (Balkan, Novosadjanka and Krajina,) were selected by the Institute of Field and Vegetable Crops (Novi Sad, Serbia) and the others (Lana, Nena, and ZPS-015) by the Maize Research Institute Zemun Polje (Belgrade, Serbia). Soybean genotypes were grown in 2008 on the Zemun and Novi Sad localities, using usual agro technical practice.

### Tofu processing

Tofu was made on the pilot plant scale using the production method which includes hydrothermal cooking according to [8], for soymilk preparation, modified by Stanojevic *et al.* [4]. Chymosin-pepsin rennet was used for soymilk coagulation [4]. Briefly, soy beans were soaked 14 hours in water at 5–7 °C. Soaked beans were ground and cooked by steam injection system (6:1 = water:soybeans) at 110 °C/1.8 bar/8 min. (Soya-Cow VS 30/40, model SM-30, Russia). Thereafter, the slurry was filtered to separate soymilk (filtrate) from okara (residue). Commercial chymosin-pepsin rennet (10 ml rennet/L cooked soymilk) was added. After 35 min the curd was pressed and separate tofu (curd) and tofu whey (filtrate). Finally, samples were stored at 4 °C before further analysis.

### Preparation of Samples for SDS Analyses

The seeds were ground to a coarse powder (Micro-Mill grinder Fisher, Germany). The soybean powder and okara were defatted using *n*-hexane and solvent was evaporated at room temperature. Tofu was defatted by the Folch extraction method [33] and dried defatted samples were stored at 4 °C until analysis.

From defatted soy flour and okara, proteins were extracted at room temperature with 0.03 M Tris-HCl buffer, pH 8.00 (Tris-hydroxymethyl aminomethane) which contained 0.01 M  $\beta$ -mercaptoethanol, for 120 min. The sample to buffer ratio for defatted soy flour was 1:20; for soymilk and okara the sample to buffer ratio was 1:10, and for tofu whey it was 1:1. From defatted tofu samples proteins were extracted with 0.055 M Tris-glycerin-HCl bufer, pH 6.80 which contained 0.64 M  $\beta$ -mercaptoethanol for 1 h at room temperature (the sample to buffer ratio was 1:10). Then the all extracts were centrifuged at 7558g for 15 min at room temperature, and the protein supernatants were used for SDS-analyses.

### Sodium dodecyl sulfate - polyacrylamide gel electrophoresis (SDS-PAGE)

Dissociating electrophoresis for all samples was performed according to the Fling and Gregerson [36] procedure, detailed by Stanojevic *et al.* [4,7] using 12.5% separating (pH 8.85) and 5% (pH 6.80) stacking gels. Briefly, the all protein extracts were diluted with sample buffer (pH 6.80) to a concentration of 2 mg/mL. A 25  $\mu$ L sample was loaded onto each well. The gels were run at 80 mA per gel for 6 h in a buffer solution, pH 8.30. The gels were stained for 50 min with 0.23% Coomassie brilliant blue R-250 and destained with 8 vol.% acetic acid and 18 vol.% ethanol. Standards of molecular masses included: phosphorylase B (94000), bovine albumin (67000), ovalbumin (43000), carbonic anhydrase (30000), trypsin inhibitor (20100) and  $\alpha$ -lactalbumin (14400). Two gels were run simultaneously in the same electrophoretic cell and two aliquots of the same sample were analyzed at the same time. SDS-electrophoresis was performed in electrophoresis unit LKB-2001-100 using power supply LKB-Macrodrive 5 and LKB-Multitemp as a cooling unit (Pharmacia, Sweden).

### Densitometric analysis

All distained gels were scanned and subsequently analyzed by SigmaGel software version 1.1 (Jandel Scientific, San Rafael, CA). Then, the quantity of each identified subunit was calculated as the percentage of the respective area with respect to total area of the densitogram.

### Statistical analysis

All data were assessed by analysis of variance (ANOVA) using the Statistical software version 7.0 (StatSoft Co., Tulsa, OK). Tukey multiple range test was used to separate means. Significance was accepted at  $p < 0.05$ . Regression analyses were also carried out, so that the different parameters were correlated with each other by Pearson two-tailed significance correlation at  $p < 0.05$  level.

## RESULTS AND DISCUSSION

### SDS-PAGE separated proteins from investigated soybean seeds and protein products

Electrophoretic patterns of soybeans proteins extracts from six different soybean genotypes and from HTC processed soymilks are shown in Figures 1 and 2, respectively.

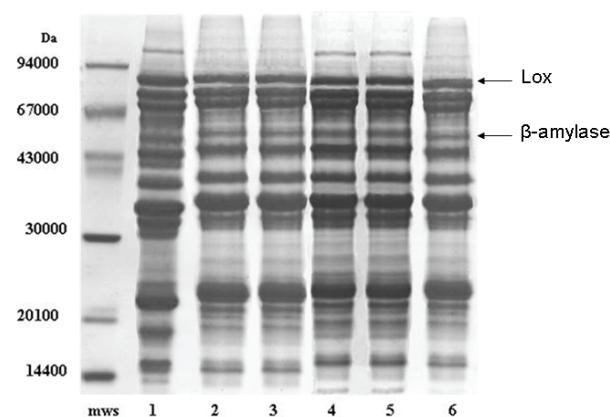


Figure 1. SDS-PAGE analysis of soybeans protein extracts from investigated genotypes; Lanes: mws – molecular weight standards; 1 – Nena, 2 – Krajina, 3 – Novosadjanka, 4 – Balkan, 5 – ZPS-015, 6 – Lana.

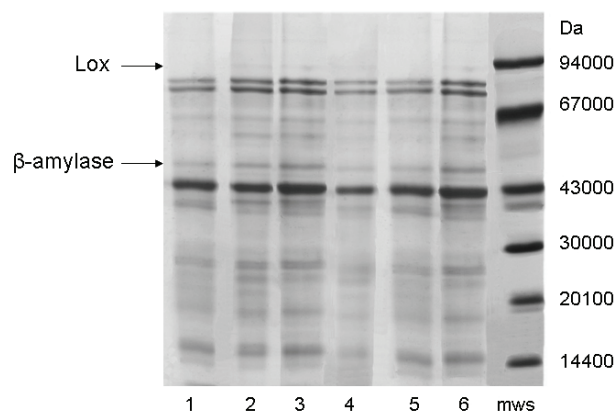


Figure 2. SDS-PAGE analysis of soymilk proteins from investigated genotypes; Lanes: 1 – Nena, 2 – Krajina, 3 – Novosadjanka, 4 – Balkan, 5 – Lana, 6 – ZPS-015, mws – molecular weight standards.

Significant differences in protein composition of soybean and the first protein product after applying HTC process, soymilk, were observed. Figure 3 shows comparative densitometric analysis of pH 8 protein extracts of soybean and soymilk.

Electrophoretic patterns of SDS-PAGE separated proteins from okara protein extracts and tofu whey are shown in Figures 4 and 5. Because of the great compactness of tofu gel higher concentration of  $\beta$ -mercaptoethanol, and Tris-glycerin as well as lower pH of extraction buffer was required in order to release pro-

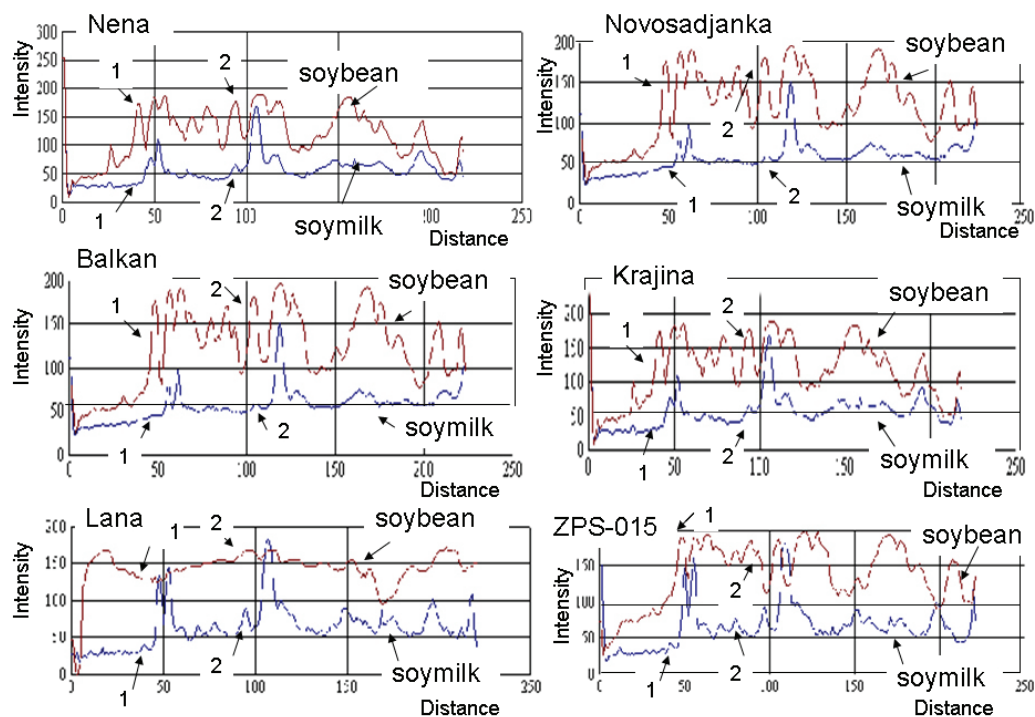


Figure 3. Comparative densitometric analysis of SDS-PAGE-electrophoregrams of soybeans protein extracts and soymilk proteins of investigated genotypes. Lox and  $\beta$ -amylase peak zones are marked as 1 and 2, respectively.

tein molecules from the gel matrix. SDS-PAGE separated proteins from tofu protein extracts are shown in Figure 6.

SDS-electrophoregrams show the separation of all globulin fractions from soybeans and soy protein products. Considering that the aim of this study was distribution of two enzymes,  $\beta$ -amylase and lipoxygenase during the HTC production of tofu, their participation in specific protein products is shown in Tables 1 and 2.

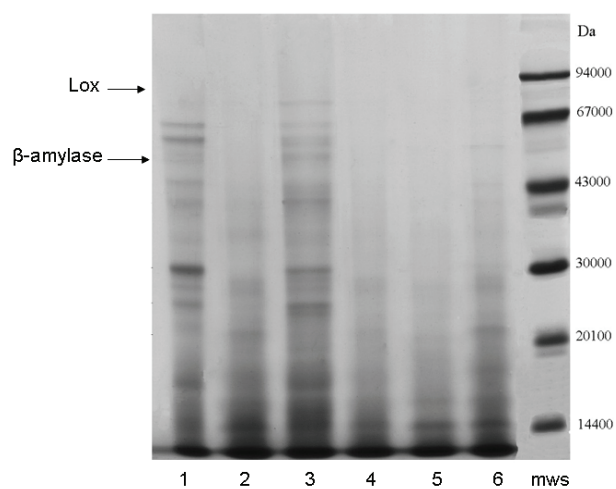


Figure 4. SDS-PAGE analysis of okara protein extracts from investigated genotypes; Lanes: 1 – Nena, 2 – Krajina, 3 – Novosadjanka, 4 – Balkan, 5 – Lana., 6 – ZPS-015, mws – molecular weight standards.

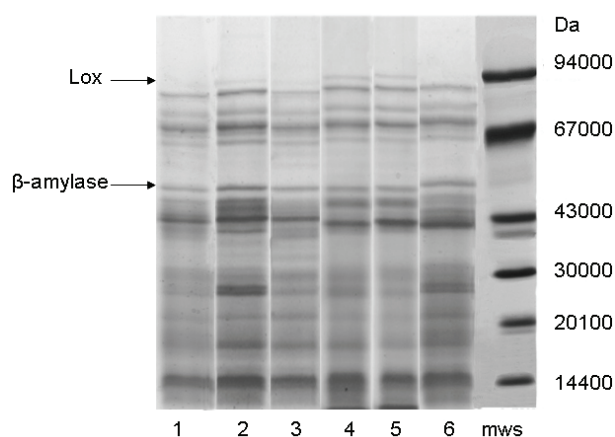


Figure 5. SDS-PAGE analysis of tofu whey proteins from investigated genotypes; Lanes: 1 – Nena, 2 – Krajina, 3 – Novosadjanka, 4 – Balkan, 5 – Lana., 6 – ZPS-015, mws – molecular weight standards.

Protein patterns of all investigated genotypes were similar with clearly separated Lox (93 578) and  $\beta$ -amylase (55 902) bands (Figure 1).

Content of these enzymes in soybean protein extracts was around 4–5% (Tables 1 and 2) which is consistent with literature data [37]. After heat treatment, much more significant reduction of Lox content than  $\beta$ -amylase content in soymilk could be observed (Figure 2). Content of  $\beta$ -amylase reduced from around 4% in the soybean protein extracts to approximately 3% in soymilks, while the content of Lox was 4–5 times lower

in soymilk compared to soybean protein extracts (Tables 1 and 2). These results lead to the conclusion that the applied HTC treatment significantly affected protein fractions of higher molecular masses, causing a considerable degree of their degradation (Figure 3).

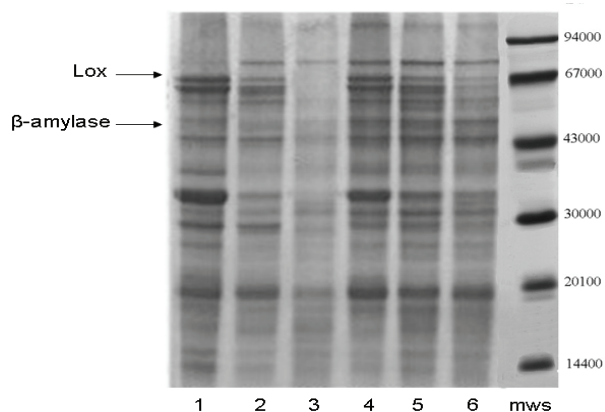


Figure 6. SDS-PAGE analysis of tofu protein extracts from investigated genotypes; Lanes: 1 – Nena, 2 – Krajina, 3 – Novosadjanka, 4 – Balkan, 5 – Lana., 6 – ZPS-015, mws – molecular weight standards.

After extracting soymilk from soybeans, okara remained in the precipitate (Figure 4). Lox content in okara protein extracts from investigated genotypes was low ranging from 0.02 to 0.53% (Table 2), which is in agreement with literature data [34].

According to Ren *et al.* [38], the relative content of Lox and  $\beta$ -amylase gradually decreases with the increase of temperature and almost disappears at 80 °C in soluble fractions, so the significant amount of these enzymes exceeds in milk. However, our results indi-

cated that contents of  $\beta$ -amylase in protein extracts of okara following the applied HTC method varied significantly depending on genotype. In protein extracts of okara from genotypes Balkan, ZPS-15 and Lana the presence of  $\beta$ -amylase was not registered. This is consistent with our previous results which showed that the majority of extracted proteins from okara produced by hydrothermal processing of soymilk were low molecular mass peptides with molecular masses below 40000 [7]. However, in okara protein extracts from three other genotypes (Nena, Krajina and Novosadjanka), the presence of  $\beta$ -amylase was registered ranging from 2.71 to 7.17% (Table 1). This could be the result of genotypic differences in deposition spot of  $\beta$ -amylase molecules upon biosynthesis and/or the effect of its surrounding in soy grain. Correlation analysis showed that the contents of the Lox and  $\beta$ -amylase in okara protein extracts were in a very strong dependence ( $r = 0.92$ ;  $p = 0.05$ ). This data could be important for technology practice. The applied technological process should preserve  $\beta$ -amylase content because it has favorable nutritional properties [27–29]. On the other hand, one of the aims of technological process is the reduction of Lox activity and content since it undesirably affects the sensory properties of products. Considering that their contents are in strong positive correlation it is needed to balance technological process so that the reduction of the content of Lox does not significantly decrease the content of  $\beta$ -amylase.

During tofu processing up to 30% of the soybean may be lost as waste mainly in the form of okara and tofu whey. Content of Lox in tofu whey as well as in okara protein extracts was in traces (Figure 5; Table 2).

Table 1.  $\beta$ -amylase content of soybeans protein products from investigated genotypes (% extractable protein); means in the same row with different letters are significantly different ( $p < 0.05$ )

Genotype	Soybean	Soymilk	Tofu	Tofu whey	Okara
Nena	4.25±0.03 a	3.10±0.02 b	4.65±0.02 c	4.94±0.05 c	3.20±0.09 b
Krajina	4.99±0.04 a	3.21±0.06 b	5.69±0.02 c	5.60±0.05 c	2.71±0.08 d
Novosadjanka	4.43±0.04 a	3.38±0.03 b	4.43±0.07 a	5.19±0.02 c	7.17±0.03 d
Balkan	4.20±0.09 a	3.41±0.08 b	4.15±0.03 a	4.99±0.05 c	–
ZPS - 015	4.18±0.02 a	3.98±0.07 b	5.47±0.03 c	5.38±0.04 d	–
Lana	3.94±0.03 a	3.52±0.07 b	5.74±0.07 c	5.33±0.07 d	–

Table 2. Lipoxygenase content of soybeans protein products from investigated genotypes (% extractable protein); means in the same row with different letters are significantly different ( $p < 0.05$ )

Genotype	Soybean	Soymilk	Tofu	Tofu whey	Okara
Nena	4.94±0.03 a	1.31±0.04 b	2.64±0.01 c	0.10±0.000 d	0.21±0.002 e
Krajina	5.43±0.04 a	1.15±0.03 b	4.20±0.05 c	0.21±0.001 d	0.30±0.001 e
Novosadjanka	5.09±0.03 a	1.39±0.03 b	6.17±0.03 c	0.15±0.000 d	0.53±0.002 e
Balkan	4.31±0.02 a	1.43±0.05 b	6.16±0.04 c	0.30±0.001 d	0.02±0.001 e
ZPS - 015	5.50±0.06 a	1.75±0.04 b	3.64±0.04 c	0.25±0.001 d	0.06±0.001 e
Lana	5.63±0.04 a	1.63±0.04 b	5.40±0.05 c	0.08±0.000 d	0.20±0.000 e

This low content of Lox in tofu whey indicated that the activity of this enzyme was negligible and similar to Lox activity in okara protein extracts [34]. Namely, Lox is denatured at 69–74 °C [39].

The fresh tofu whey is widely used as beverage to improve the convalescent's health, so the low content and the activity of this enzyme are preferred [3]. Unlike the Lox, content of  $\beta$ -amylase in tofu whey was relatively high (Table 1) and was in a strong dependence ( $r = 0.84$ ;  $p = 0.05$ ) with the contents of  $\beta$ -amylase in tofu protein extracts.

Electrophoretic analysis indicated (Figure 6) that the content of  $\beta$ -amylase in tofu protein extracts was similar to the contents of this enzyme in soybean protein extracts, soymilk and tofu whey (in soybean protein extracts 3.94–4.99%; in soymilk 3.10–3.98%, in soy whey 4.94–5.60% and in tofu protein extracts 4.15–5.74%; Table 1). Such high  $\beta$ -amylase content in protein products is desirable, but not because of the activity of  $\beta$ -amylase since the  $\beta$ -amylase is denatured at temperatures near 70 °C [40], but because of the amino acid composition of this enzyme. The  $\beta$ -amylase protein chain contains among the others 8 essential amino acids including methionine as well as cysteine [19]. It is known that soybean protein products are deficient in these amino acids. Contents of Lox in tofu protein extracts after HTC method were different depending on the soybean genotype (2.64–6.17%; Table 2). These contents may be the result of different aggregation during the formation of tofu curd. Considering that the temperature of the applied HTC processing was 100 °C it could be expected that this enzyme is inactivated which is a prerequisite for good sensory properties of these cheeses.

## CONCLUSION

Based on presented results, it can be concluded that the applied HTC treatment severely affected protein fractions of higher molecular mass causing a significant degree of their degradation. The content of Lox in soluble fractions was significantly reduced after the HTC process. Hence the low amounts of Lox in soymilk were registered while in okara protein extracts and tofu whey this enzyme was present in traces. The exception was tofu protein extract where depending on the soybean genotype Lox was registered in higher quantities. This could be the result of different aggregation during the formation of tofu gel. Content of  $\beta$ -amylase in tofu protein extracts and in soymilk and tofu whey was relatively high and similar to the soybean. This is preferred due to the amino acid composition of this enzyme which includes the sulfur containing amino acids. The exception was okara protein extract where content of  $\beta$ -amylase depended on soybean genotype. Finally, we can summarize that the HTC

treatment enabled the more effective use of soymilk and tofu as well as by-products, okara and tofu whey, which may be a source of sensory and nutritionally acceptable plant seed products for feed and food preparation.

## Acknowledgements

The authors are grateful to the Serbian Ministry of Education, Science and Technological Development for the financial support (Project No.: TR 31022) and the EU FP7 project Grant Agreement No.316004 (REGPOT-AREA). We are indebted to Maize Research Institute, Zemun Polje, Serbia and Institute of Field and Vegetable Crops, Novi Sad, Serbia, for providing soybean genotypes.

## REFERENCES

- [1] C. Fujino, S. Wada, T. Konoike, K. Toyota, Y. Suga, J. Ikeda, Effect of different organic amendments on the resistance and resilience of the organic matter decomposing ability of soil and the role of aggregated soil structure, *Soil. Sci. Plant Nutr.* **54** (2008) 534–542.
- [2] A.M. Jándula, N.V. Álvarez, M.L. Genta, Development of nutritional products with high energy and protein content with raw materials from northwest region of Argentina (NOA), *Univ. Nat. Tucum Inf.* **15** (1991) 5–6.
- [3] A.K. Smith, J.S. Circle, Chemical composition of the seed, In: K.A. Smith, S.J. Circle (Eds.), *Soybeans: chemistry and technology*, AVI Pub. Co. INC, Westport, CN, 1972, pp. 61–92.
- [4] S.P. Stanojevic, M.B. Barac, M.B. Pesic, B.V. Vucelic-Radovic, Assessment of soy genotype and processing method on quality of soybean tofu, *J. Agric. Food Chem.* **59** (2011) 7368–7376.
- [5] P. Siddhuraju, K. Becker, P.S. Harinder, S.P.H. Makkar, Studies on the nutritional composition and antinutritional factors of three different germplasm seed materials of an under-utilized tropical legume, *Mucuna pruriens* Var. *Utilis*, *J. Agric. Food Chem.* **48** (2000) 6048–6060.
- [6] D.K. O'Toole, Characteristics and use of okara, the soybean residue from soy milk production – A review, *J. Agric. Food Chem.* **47** (1999) 363–371.
- [7] S.P. Stanojevic, M.B. Barac, M.B. Pesic, V.B. Vucelic-Radovic, Composition of proteins in okara as a byproduct in hydrothermal processing of soymilk, *J. Agric. Food Chem.* **60** (2012) 922–9228.
- [8] C. Wang, L.A. Johnson, L.A. Wilson, Calcium coagulation properties of hydrothermally processed soymilk. *J. Am. Oil Chem. Soc.* **80** (2003) 1225–1229.
- [9] S.P. Stanojevic, Biochemical properties of proteins and carbohydrates of modified soy protein products, Doctoral Thesis, Faculty of Agriculture, University of Belgrade, 2007.
- [10] M. Friedman, Nutritional value of proteins from different food sources – A Review, *J. Agric. Food Chem.* **44** (1996) 6–29.

- [11] M. Friedman, D.L. Brandon, Nutritional and health benefits of soy proteins, *J. Agric. Food Chem.* **49** (2001) 1069–1086.
- [12] D. Devine, Soya and health 2002 – clinical evidence, dietetic applications, British Nutrition Foundation, *Nutrit. Bull.* **27** (2002) 195–198.
- [13] M. Barać, S. Stanojević, M. Pestic, Biologically active components of soy bean - A review, *Acta Periodica Technol.* **36** (2005) 155–169.
- [14] I. Meteos-Aparicio, A.C. Redondo, M.J. Villanueva-Suárez, A. M. Zapata-Revilla, Soybean, a promising health source, *Nutr. Hosp.* **23** (2008) 305–312.
- [15] FAO/WHO Expert Committee on food additives, Summary and conclusions, In Proceedings of 53<sup>rd</sup> Meeting, Roma, June, 1990, pp. 1–10.
- [16] E. Mori, B. Mikami, Y. Morita, B. Jirgensons, Circular dichroism and the conformational properties of soybean  $\beta$ -amylase, *Arch. Biochem. Biophys.* **211** (1981) 382–389.
- [17] N.Y. Kang, A. Tanabe, M. Adachi, S. Utsumi, B. Mikami, Structural analysis of threonine 342 mutants of soybean  $\beta$ -amylase: Role of a conformational change of the inner loop in the catalytic mechanism, *Biochemistry* **44** (2005) 5106–5116.
- [18] J. Fukumoto, Y. Tsujisaka, *Kagaku to Kogyo*, Osaka, *Sci. Ind.* **28** (1954) 282–287.
- [19] Y. Moriota, F. Yagi, S. Aibara, Chemical composition and properties of soybean  $\beta$ -amylase, *Biochemistry* **79** (1976) 591–603.
- [20] B. Mikami, M. Degano, E.J. Hehre, C.J. Sacchetti, Crystal structures of soybean  $\beta$ -amylase reacted with  $\beta$ -maltose and maltal: Active site components and their apparent roles in catalysis, *Biochemistry* **33** (1994) 7779–7787.
- [21] E. Sarikaya, T. Higasa, M. Adachi, B. Mikami, Comparison of degradation abilities of  $\alpha$ - and  $\beta$ -amylases on raw starch granules, *Process Biochem.* **35** (2000) 711–715.
- [22] H. Ren, F.J. Thompson, J.T. Madison, Biochemical and physiological studies of soybean  $\beta$ -amylase, *Phytochemistry* **33** (1993) 541–545.
- [23] M. Adachi, B. Mikami, T. Katsube, S. Utsumi, Crystal structure of recombinant soybean  $\beta$ -amylase complexed with  $\beta$ -cyclodextrin, *J. Biol. Chem.* **273** (1998) 19859–19865.
- [24] B. Mikami, K. Nomura, Y. Morita, Two sulfhydryl groups near the active site of soybean  $\beta$ -amylase, *Biosci. Biotech. Biochem.* **58** (1994) 126–132.
- [25] A. Hirata, M. Adachi, A. Sekine, Y.N. Kang, S. Utsumi, B. Mikami, Structural and enzymatic analysis of soybean  $\beta$ -amylase mutants with increased pH optimum, *J. Biol. Chem.* **279** (2004) 7287–7295.
- [26] A. Gertler, Y. Birk, The role of sulphhydryl groups in soybean  $\beta$ -amylase, *Biochim. Biophys. Acta* **11** (1996) 898–105.
- [27] F. Franks, Introduction. In: Characterization of proteins, Franks F., Eds.; Publisher: Cambridge, UK: Pafra, Ltd., (1988) 1–8.
- [28] C. Fukazawa, Method for separation of beta-amylase, Patent number 05294341, Tsukuba, 1994.
- [29] M. Miwa, L.Z. Kong, K. Shinohara, M. Watanabe, Soybean trypsin inhibitor and  $\beta$ -amylase stimulate macrophages, *Biochem. Biophys. Res. Commun.* **173** (1990) 296–301.
- [30] S. Iwabuchi, F. Yamauchi, Electrophoretic analysis of whey proteins present in soybean globulin fractions, *J. Agric. Food Chem.* **35** (1987) 205–209.
- [31] H. Hirano, H. Kagawa, Y. Kamata, F. Yamauchi, Structural homology among the major 7S globulin subunits of soybean seed storage proteins, *Phytochemistry* **26** (1987) 41–45.
- [32] J.J. Rackis, D.J. Sessa, H.D. Honig, Flavor problems of vegetable food proteins, *J. Amer. Oil Chem. Soc.* **56** (1979) 262–271.
- [33] S.P. Stanojevic, M.B. Barac, M.B. Pestic, V.S. Jankovic, B.V. Vucelic-Radovic, Bioactive proteins and energy value of okara as a byproduct in hydrothermal processing of soy milk, *J. Agric. Food Chem.* **61** (2013) 9210–9219.
- [34] S.P. Stanojevic, M.B. Barac, M.B. Pestic, S.M. Zilic, M.M. Kresovic, B.V. Vucelic-Radovic, Mineral elements, lipoxigenase activity and antioxidant capacity of okara as a byproduct in hydrothermal processing of soy milk, *J. Agric. Food Chem.* **62** (2014) 9017–9023.
- [35] W.W. Christie, Extraction of lipids from samples, *Lipid Technol.* **5** (1993) 18–19.
- [36] P.S. Fling, S.D. Gregerson, Peptide and protein molecular weight determination by electrophoresis using a high-molarity Tris-buffer system without urea, *Anal. Biochem.* **155** (1986) 83–88.
- [37] S.M. Žilić, M.B. Barać, M.B. Pešić, S.D. Mladenović-Drinić, D.D. Ignjatović-Mićić, M. Srebrić, Characterization of proteins from kernel of different soybean varieties, *J. Sci. Food Agric.* **91** (2010) 60–67.
- [38] C.L. Ren, M. Tang Zhang, S. Guo, Interactions between whey soybean protein (WSP) and  $\beta$ -conglycinin (7S) during the formation of protein particles at elevated temperatures, *Food Hydrocolloid.* **23** (2009) 936–941.
- [39] P. Lopez, J. Burgos, Lipoxigenase inactivation by manothermosonication: effects of sonication physical parameters, pH, KCl, sugars, glycerol, and enzyme concentration, *J. Agric. Food Chem.* **43** (1995) 620–625.
- [40] N. Yoshigi, Y. Okada, H. Maeba, H. Sahara, T. Tamaki, Role of the C-terminal region of  $\beta$ -amylase from barley and additivity of mutational effects of intragenic amino acid replacements on the thermostability of  $\beta$ -amylase, *J. Appl. Glycosci.* **43** (1996) 227–235.

**IZVOD****DISTRIBUCIJA  $\beta$ -AMILAZE I LIPOKSIKGENAZE U SOJINIM PROTEINSKIM PROIZVODIMA DOBIJENIH TOKOM PROIZVODNJE TOFUA**

Slađana P. Stanojević, Miroljub B. Barać, Mirjana B. Pesić, Biljana V. Vucelić-Radović

*Poljoprivredni fakultet, Institut za prehrambenu tehnologiju i biohemiju, Katedra za hemiju i biohemiju, Univerzitet u Beogradu, Beograd, Srbija*

(Naučni rad)

Sojino zrno je danas veoma često korišćena sirovina u proizvodnji funkcionalne hrane, kao i u stočnoj ishrani. Tokom obrade sojinog zrna u mleko i tofu, do 30% od sojinog zrna može biti izgubljeno kao otpad, uglavnom u vidu okare i tofu surutke. Okara je nus-proizvod u proizvodnji sojinog mleka. To je bledo-žučkasti nerastvorljivi deo sojinog semena koji zaostaje nakon filtracije sojinog mleka. Tofu-surutka je bledo-žuta tečnost, prijatnog ukusa, koja se izdvaja nakod ceđenja mlečnog gruša (tofua). Tradicionalni način pripreme sojinog mleka i tofua može imati za posledicu pojavu tzv. „leguminoznog“ ukusa i mirisa, koji su neprihvatljivi u ishrani ljudi. Zato se danas primenjuju termički postupci kojima se ova neprijetna aroma u proizvodima znatno smanjuje. U ovom radu je korišćen postupak pripreme tofua, od šest sorti soje, pod uslovima visoke temperature/kratko vreme/pod pritiskom, tzv. „hidrotermičko kuvanje“ (HTC). Razmatrana je distribucija enzima:  $\beta$ -amilaze i lipoksigenaze (Lox) u proteinskim proizvodima (sojino mleko, tofu, okara i surutka) nakon primene HTC postupka. Rezultati SDS-PAG-elektroforeze proteinskih ekstrakata proizvoda ukazuju na to da je primenjeni HTC postupak imao snažan efekat pre svega na proteinske frakcije velike molekulske mase. Zato su veoma mali sadržaji Lox registrovani u sojinom mleku, dok se ovaj enzim nalazi praktično u tragovima u okari i surutki. U proteinskim ekstraktima tofua zabeležene su izvesne količine ovog enzima, koje mogu biti posledica reakcija agregacija u toku formiranja gela. Može se očekivati da je aktivnost ovog enzima u svim proizvodima zanemarljiva, obzirom da se Lox denaturišu na 80 °C, što je poželjno sa aspekta organoleptike ovih proizvoda. Sadržaj  $\beta$ -amilaze u proteinskim ekstraktima svih proteinskih proizvoda je relativno visok i sličan sadržaju u sojinom semenu. Izuzetak je okara, gde sadržaj ovog enzima značajno zavisi od genotipa sojinog zrna. Dobijeni rezultati ukazuju na to da primenjeni HTC postupak u proizvodnji tofua omogućuje dobijanje proizvoda koji mogu imati široku primenu kako u ishrani ljudi tako i u ishrani životinja.

*Ključne reči:* Sojino zrno • Hidrotermičko kuvanje • Sojini proteinski proizvodi •  $\beta$ -Amilaza • Lipoksigenaza