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Heat processing of soybean kernel and its effect on lysine availability and protein solubility

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Abstract: Soybean kernels of cultivars Bosa and ZPS 015 were used in the experiment. The contents of available lysine as well as water and salt soluble proteins, were analysed in fresh soybean kernels, soybean products made after the processes of dry extrusion, micronisation, microwave toasting and autoclaving. Utilizing a technological procedure of processing, kernels were exposed to temperatures from 57 to 150°C. The duration of exposure of the soybean kernels to the increased temperatures, ranged from 25-30 seconds in dry extrusion to 30 minutes in autoclaving. All treatments were subjected to different sources of heat, causing different thermodynamic processes to take place in kernels and change their chemical composition; i.e. nutritive quality. The content of water and salt soluble proteins decreased under the influence of higher temperatures in the course of all treatments of processing. The drop of solubility already was drastically effected by temperatures of 100°C in dry extrusion, while there was a gradual decrease in other treatments. The content of available lysine was determined by the modified Carpenter methods with DNFB. The processes of micronisation and microwave toasting showed the greatest effect on the reduction of lysine availability. Dry extrusion and autoclaving, performed within closed systems – in which the increased moisture content has a special effect – resulted in significantly smaller changes of the available lysine content.

Keywords: Soybean, available lysine, soluble proteins, heat treatments

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1 Introduction

The significance of soybean proteins in nutrition depends on their quantity, but also on their quality. The following parameters affect the nutritional quality of soybean proteins: their essential amino acid content, the interrelation of amino acids, their digestibility and the limited availability of proteins due to the presence of anti-nutritional factors. The amino acid composition is the main determinant of protein nutritive value. Methionine and cystine, sulphur-containing amino acids, are limiting amino acids in soybean proteins. However, soybean proteins are very rich in lysine and are often used to supplement cereal proteins that are deficient in this amino acid. Solubility is an important property of soybean proteins. Approximately 72-95% of all proteins are easily water soluble, which is a necessary prerequisite for their digestion and absorption.

Heating of plant protein sources reduces its solubility and digestibility. Digestibility is registered by a primary protein quality index and is related to the usability of amino acids; i.e., proteins cannot be used if they are not digestible. Besides high temperatures, protein digestibility is also affected by the presence of certain biologically active components as well as by a chemical (structural) form of soybean proteins. These factors affect digestibility by modifying a portion of proteins that cannot be hydrolysed to amino acids, or they may form free amino acids, by protein hydrolysis, which is less useable in nutrition. However, Livingston, in 1976, presented experimental data showing that thermally induced decrease of protein solubility increased protein efficacy [1]. Wing and Alexander, (1971), experimentally determined that the protein efficiency was greater in soybean flour obtained after autoclaving and microwave treatments than in flour obtained from both, fresh and dry toasted soybean kernels [2]. Crude soyproteins are resistant to trypsin until they change their internal chemical structure under the influence of high temperature. The heat which is applied, breaks secondary bonds–either disulfide or hydrogen ones–by disturbing the physical arrangement of molecules. This disturbance increases the possibility of contacts with other molecules that can lead to easier enzymatic digestion. All factors affecting the breaking bonds between molecules of proteins as well as within molecules and their structural arrangements, also affect their physical, chemical and functional properties. In the majority of cases, these heat-induced selective structural modifications of proteins are a necessary prerequisite for the utilisation of soybean proteins in food and feed.

The Maillard reaction is a general term used to describe a complex series of reactions between reactive carbonyl groups, such as those of reducing sugars, and free amino groups of proteins [3]. Lysine is the most important carrier of free amino groups in proteins in the form of ε-amino group, and therefore is the most significant amino acid participant in the Maillard reaction. Beside lysine, arginine, tryptophane, and histidine are also carriers of free amino groups. Cladinin and Robllee (1952), noticed that a peptide chain containing modified lysine, was not susceptible to effects of trypsin and therefore was not utilisable in animal diet resulting in unthriftiness [4]. Studying the effect of the Maillard reaction products on protein digestion, Oste *et al.*, (1986), determined that low-molecular-weight compounds developed in the reaction of glucose and lysine inhibited n-amino peptidase [5]. This inhibition resulted in reduced protein absorption in the digestive tract.

Despite more than 90 years research work in this area, the molecular mechanisms are still not well understood, although an increasing number of the Maillard reaction products have been identified recently [6]. Low-molecular-weight products of the Maillard reaction have an exceptionally important role in the formation of flavour, aroma, colour and texture in thermally treated foods. The Maillard reaction partially proceeds during storage. Melanoid products are also formed in the reaction of amino acids or proteins with oxidised lipids. These products are of the similar structure as those developed in the reaction with reducing sugars [7].

Finot (1990), offered a useful summary of physiological and pharmacological effects of the Maillard reaction products [8]. Briefly, these effects include (a) inhibition of processes such as growth, protein and carbohydrate digestion, amino acid absorption and activity of intestinal enzymes including amino peptidases, proteases, and saccharidases, and pancreatic enzymes such as chymotrypsin; (b) induction of cellular changes in the kidneys, liver, and stomach cecum; (c) adverse effects on mineral metabolism (Ca, Mg, Cu, and Zn); and (d) variable effects on allergic response and cholesterol metabolism. However, the Maillard reaction products showed not only adverse effects but also anti-oxidative effects, as well as, antimutagenic, antibiotic and antiallergenic effects [9–11]. Various Maillard reaction products, obtained under strictly controlled conditions, are used as commercial food additives; e.g., as food aromas and antioxidants.

The objective of our study was to evaluate the effects of different temperatures and duration of heat treatments, as well as the effect of kernel moisture and water vapour pressure on quality of soybean products, digestibility and efficiency of biomolecules of soybean grain. It is expected that depending on the source of thermal treatment (infrared rays, microwaves), the water vapour pressure, and the model of the soybean processing systems (open, closed), different thermodynamic processes occur within the grain resulting in greater or smaller changes of chemical components and consequently influence nutritive quality.

The importance of the present study is in the overall approach to the complex effect of different factors on changes of the protein structure of soybean grain.

2 Material and Methods

2.1 Materials

Kernels of a standard chemical composition of commercially used soybean cultivars, Bosa and ZPS 015, developed at the Maize Research Institute, Zemun Polje, were employed in these experiments. The cultivar Bosa belongs to the maturity group 00 and is characterised by high tolerance to drought, while the cultivar ZPS 015 belongs to the maturity group 0 and is of a high yielding potential.

2.2 Heat treatments

After harvest, soybean kernels were cleaned and ground to be used for the analysis of the contents of available lysine, water- and salt-soluble proteins. These parameters also were analysed in products obtained after the application of heat treatments: (a) dry extrusion at 100, 125, 140 and 150°C; (b) micronisation at 100, 125, 140 and 150°C; (c) kernels toasted in the microwave oven of 800 W and 2450 MHz in the intervals of 1, 2, 3, 4 and 5 minutes; (d) autoclaved at the temperature of 120°C and the pressure of 1.4 bar for 10, 20 and 30 minutes. In order to compare results obtained, the sample temperature was checked after kernel toasting by the "thermos bottle test": Bosa (1 min = 57°C, 2 min = 88°C, 3 min= 108°C, 4 min = 121°C, 5 min = 132°C) and ZPS 015 (1 min = 60°C, 2 min = 90°C, 3 min = 108°C, 4 min = 118°C, 5 min = 137°C).

The treatments of extrusion and micronisation were performed at the semi-industrial plant for extrusion and micronisation of the Maize Research Institute, Zemun Polje.

2.3 Micronisation

The Micronizing Co. (UK) has patented the technological process based on the effects of near-infrared rays of wavelength ranging from 1.8 to 3.4 μm emitted by heated ceramic tiles. Penetration of infrared rays into the kernel causes heating and vibration of water molecules. A special kind of simmering occurs in the kernel coat. The heated kernel is subjected to pressures and forces of a flake roller whereby some chemical bonds are disrupted and the organised structure is disturbed. The whole procedure of processing and flake production lasts 2-3 minutes [12].

2.4 Extrusion

This procedure is based on the temperature impacts appearing during friction of the material that is squeezed through a cylinder by a specially designed volute. The whole processing procedure lasts 25-30 seconds. Although extrusion of the whole kernel is possible, soybean kernels were ground by the hammer mill with 3-mm mesh.

2.5 Microwave heating

Is based on the effects of microwaves that are placed in the near infrared spectrum with a wavelength ranging from one millimetre to several centimetres. As microwaves enter the substance they partially penetrate it, then they are partially reflected and partially absorbed. After penetration, microwaves interact with electric dipoles, (positive and negative charges of water molecules), that rotate molecules due to effects of forces of attraction and repulsion between dipoles and the electric field. These motions lead to the disruption of hydrogen bonds between neighbouring water molecules and generate heat by friction.

It is known that beside temperature in the processing procedures, duration of heat treatments, as well as kernel moisture and water vapour pressure, affect quality of soybean products. Results obtained in the present study show effects of certain factors on quality of soybean products, so the selection of the stated processing treatments was made.

2.6 Chemical analyses

The content of available lysine was determined after the Booth modification of the Carpenter method [13], which is based on the lysine conversion with a reactive -NH_2 group into yellow coloured -dinitrophenyl-lysine (DNP-L). Absorbance is measured at 435 nm in relation to water, while -DNP-L is calculated from the sample absorbance to standard absorbance ratio and is expressed in g/16gN.

Water soluble proteins were determined after the Michael Blum modification of the Osborne method [14]. After water extraction, solutions were steamed and dried and proteins were determined in the residue by the Kjeldahl method. The content of total and water soluble proteins is expressed as a percentage on a dry matter basis. The content of salt soluble proteins was determined by the method of Lowry [15], after extraction in the Na-phosphate buffer and protein precipitation with 10% trichloracetic acid. The content of salt soluble proteins is expressed in mg/g total proteins.

2.7 Statistical analyses

All chemical analyses were performed with four replicates and the results were statistically analysed. Statistical significance of differences of means of observed chemical parameters was determined by the LSD test after the analysis of variance for trials set up according to the RCB design was performed. An interrelation of observed parameters under effects of a certain heat treatment is expressed in percents.

3 Results and discussion

The content of soluble proteins was reduced under the influence of high temperatures in all applied treatments. Dry extrusion resulted in a maximum drop of the of water-soluble proteins content already at 100°C (73.5%). On the other hand, micronisation at 100°C did not cause any significant reduction in the content of water-soluble proteins. The same was noticed in one-minute microwave toasting (57°C, 60°C). In comparation with shorter microwave treatment, the maximum drop of solubility occurred after three minutes of microwave toasting (108°C), and the content of water-soluble proteins in kernels of the cultivar Bosa, i.e. ZPS 015 amounted to 9.23%, i.e. 12.33%, respectively. Twenty minutes of autoclaving caused a maximum drop of solubility in comparison with the shorter autoclave treatment. This drop amounted to 52.8% and 47.4% in cultivars Bosa and ZPS 015, respectively. The content of water-soluble proteins decreased with higher temperatures in all heat treatments, but the percentage decrease in relation to the previous treatment (low temperature/short time) was significantly lower. The drop in protein solubility in soybean grits of the cultivar Bosa amounted to 6.2% after dry extrusion at the temperatures 140°C and 150°C. Water-soluble protein content in toasted kernels of the same cultivar was not affected after four (121°C, 118°C) and five (132°C, 137°C) minutes of microwave treatment (Table 1).

Treatment	T/T	Water-soluble proteins (%)		Salt-soluble proteins (mg g^{-1})	
		Bosa	ZPS 015	Bosa	ZPS 015
Fresh kernel		28.90	29.25	106.84	99.80
Micronisation	100	27.66	28.56	97.50	110.83
	125	18.68	23.14	69.13	80.69
	140	13.95	15.87	49.46	45.28
	150	10.98	8.87	24.81	12.25
Microwave toasting	1	27.04	28.84	100.42	100.54
	2	19.46	21.98	54.67	57.23
	3	9.23	12.33	25.0	25.00
	4	5.42	8.37	6.35	6.89
	5	5.49	5.74	5.31	3.00
Dry extrusion	100	7.67	7.86	29.53	26.89
	125	7.03	7.87	20.35	25.86
	140	5.67	6.16	13.59	20.06
	150	5.32	5.86	10.21	19.83
Autoclaving	10	23.17	21.82	72.38	71.02
	20	10.94	11.49	36.39	33.52
	30	5.86	6.35	13.04	12.50
LSD 0.05		0.562		1.041	
CV (%)		2.23		1.44	

T/T-temperature/time (°C/min); LSD-Least significant difference; CV-coefficient of variation

Table 1 Contents of soluble protein after grain technological processing treatments of the soybean cultivars Bosa and ZPS 015.

According to our studies, the changes in contents of salt-soluble proteins under heat treatments were similar to those of water-soluble proteins. The maximum drop of contents of salt-soluble proteins occurred already at 100°C under the treatment of dry extrusion and amounted to 72.6% and 73.1% in cultivars Bosa and ZPS 015, respectively. Similar significant changes were observed after three (at 108°C) and four (at 121°C and 118°C) minutes of microwave irradiation when the content of salt soluble proteins dropped by average 74%. These changes were significantly smaller in other treatments in relation to the previous one (low temperature/short time). It is interesting that the content of salt-soluble proteins measured in soybean flakes of the cultivar ZPS 015 made by micronisation at 100°C and in 1-minute microwave toasted soybean kernels (60°C) was statistically significantly higher than the content in fresh soybean kernels. The content in these products amounted to 110.83 mg/g and 100.54 mg/g vs. 98.11 mg/g determined in fresh soybean kernels of the cultivar ZPS 015 (Table 1). The results obtained by Utsumi *et al.*, (1984), can be used to explain these changes. These authors heated a mixture of 7S and 11S sequences of soybean proteins for two and for 30 minutes at 80°C and they determined that the heat treatment caused the occurrence of soluble macromolecules formed by binding 7S globulins with base subunits of 11S sequence [16].

Factors affecting the decrease of soybean proteins solubility are numerous, and they cause complex processes that result in degradation, denaturation and polymerisation of proteins. Boatright and Hattiarchchy (1995), point out that protein solubility is affected by internal factors–hydrophilic and hydrophobic characteristics of protein molecules, their size and charge, as well as the interaction with other kernel components, and also by external factors– temperature, pH value, type and ionic strength of different salts [17]. Hager, (1984), determined that extrusion decreases the content of soluble proteins of soybean kernel from 60-66% to 25-30% [18]. According to this author low-temperature (<150°C) extrusion in the protein structure forms primarily intermolecular disulfide bridging that is accompanied by changes in noncovalent bonding. Higher temperature (>180°C) extrusion may lead to protein polymerisation by forming intermolecular peptide bonds and these products are known to lower the protein solubility.

These studies encompass the analyses of changes in the content of available lysine in soybean kernels that occurred under influences of heat processing treatments.

The processes of micronisation and microwave toasting showed the highest effect on the decrease of lysine availability. The temperature increase in the course of these two treatments resulted in a gradual drop of the content of available lysine. However, the content of available lysine was higher in flakes, although not significantly, after micronisation at 100°C than in fresh kernels. Based on these results, and considering the fact that a certain, lower percentage of lysine in proteins of fresh soybean gain remains unavailable, it can be supposed that the disturbance of the quaternary structure of soybean proteins under the effect of temperatures led to "revealing" of lysine that became more accessible i.e. available. Similar results were obtained by Lawrence, who studied effects of micronisation on digestibility of soybean kernels and determined that the content of available lysine after micronisation insignificantly increased in relation to the content in fresh kernels [19]. This content amounted to 2.18% after micronisation, while its value in fresh kernels was 1.76%. the drop of the available lysine content after soybean kernel micronisation at 150°C amounted to 21.5% and 44.7% in the cultivars Bosa and ZPS 015, respectively, in relation to the content in fresh kernels. On the other hand, the content of available lysine after five-minute kernel microwave toasting amounted to 69.5%, i.e. 60%, respectively, in relation to the content of available lysine in fresh kernels (Table 2).

Due to higher moisture, dry extrusion and autoclaving caused significantly less changes in the available lysine content. This content in autoclaved kernels of the cultivar Bosa, as well as, in soybean grits of the cultivar ZPS 015, produced by dry extrusion at 100°C, 125°C and 140°C, remained almost the same in relation to the content in fresh kernels (Table 2).

Treatment	T/T	Moisture (%) Bosa	Moisture (%) ZPS 015	Available lysine (g 16gN^{-1}) Bosa	Available lysine (g 16gN^{-1}) ZPS 015
		Bosa	ZPS 015	Bosa	ZPS 015
Fresh kernel		6.75	6.26	4.078^{def}	4.193^{ab}
Micronisation	100	5.10	5.43	4.165^{abcd}	4.216^{a}
	125	3.94	4.53	3.879^{h}	4.123^{abcde}
	140	3.34	3.54	3.368^{j}	3.241^{k}
	150	2.58	2.18	3.203^{k}	2.321^{o}
Microwave toasting	1	6.43	5.69	4.005^{fg}	3.734^{i}
	2	5.56	5.12	3.918^{gh}	3.764^{i}
	3	4.34	4.21	3.989^{fg}	3.721^{i}
	4	3.06	3.01	3.055^{l}	3.275^{k}
	5	2.95	2.24	2.837^{m}	2.518^{n}
Dry extrusion	100	6.67	6.27	3.943^{gh}	4.173^{abc}
	125	6.57	6.53	3.924^{gh}	4.213^{a}
	140	6.78	5.71	3.896^{h}	4.109^{bcde}
	150	6.44	5.46	3.886^{h}	3.767^{i}
Autoclaving	10	6.84	6.77	4.069^{ef}	3.989^{fg}
	20	5.99	6.68	4.058^{ef}	3.950^{gh}
	30	7.98	6.89	4.098^{cde}	3.777^{i}
LSD 0.05		0.156		0.091	
CV (%)		1.42		1.32	

T/T-temperature/time (°C/min); LSD-Least significant difference; CV-coeffcient of variation
a-o significance of difference among the means at $P < 0.05$.

Table 2 Contents of available lysine and moisture after grain technological processing treatments of the soybean cultivars Bosa and ZPS 015.

Yeo and Shibamoto, (1991), heated the mixture of cystine and glucose in the microwave oven of 700 W for one to four minutes using samples with 14%, 22% and 26% moisture [20]. These authors observed absorbance of solutions at 420 nm and determined that browning developed in all three samples for up to 2.5 minutes when it practically stopped. Browning was the most intensive in the sample with the lowest moisture. Although browning was much less pronounced in samples with higher moisture, considering that solution absorbance of these samples was greater than of samples with 14% moisture, it is believed that moisture of the initial stages of microwave toasting had a key role in the browning development. Moran and Summers, (1968), studying the effect of the moisture content on the intensity of the Maillard reaction, determined that the moisture content had a significant effect not only on protein denaturation, but also on the Maillard reaction process [21]. However, although the presence of moisture accelerates the reaction between lysine and carbohydrates, because water molecules have a certain role as a carrier of free sugars, on the one hand, a high moisture content affects the reduction of amino acids destruction by blocking access of air oxygen necessary for proceeding the Maillard reaction. Results obtained by these authors accounts for the high content of available lysine in soybean grits and autoclaved kernels and explains why they were unchanged in relation to the content in fresh kernels obtained in our studies. Based on our results obtained in the process of autoclaving–which involved adding water–the moisture content in autoclaved kernel was higher than in the initial material; i.e., the fresh soybean kernel. The moisture content gradually increased with the duration of the autoclaving process, hence soybean kernels of the cultivar Bosa, i.e. ZPS 015 autoclaved for 30 minutes, had moisture higher by 31.2% and 23%, respectively, than fresh soybean kernels. Although a maximum drop of the moisture content (36.2% in the cultivar Bosa and 28.6% in the cultivar ZPS 015) already occurred at 100°C during the process of dry extrusion, this process proceeds within the closed system in which vapour pressure and temperature have a crucial effect on quality of this technological process and maintenance of lysine (Table 2).

According to the results obtained, changes in the moisture content in all applied treatments were statistically very significantly positively correlated with changes in the content of available lysine. Tables 3 and 4 present a correlation dependent on changes in the parameters studied in nutritive quality, after the processes of grain micronisation and dry extrusion of the soybean cultivar ZPS 015 and after processes of grain microwave toasting and autoclaving of the soybean cultivar Bosa.

	Micronisation			Dry extrusion		
	Salt soluble proteins	Available lysine	Moisture	Salt soluble proteins	Available lysine	Moisture
Water soluble proteins	0.99**	0.96**	0.98**	0.99**	0.38	0.35
Salt soluble proteins		0.97**	0.95*		0.37	0.35
Available lysine			0.93*			0.86*

* $P < 0.01$, **$P < 0.05$ statistical significance

Table 3 Correlation dependence of changes of studied parameters of nutritive quality after the process of grain micronisation and dry extrusion of the soybean cultivar ZPS 015.

It is important to emphasise that our results confirm that the efficiency of amino acids does not have to be correlated with the content of soluble proteins. The correlation dependence of changes in the available lysine content on water and salt soluble proteins was very low (r = 0.38, r = 0.37) after the process of grain dry extrusion of the soybean cultivar ZPS 015, and even negative, although statistically insignificant, after grain autoclaving of the soybean cultivar Bosa (r = -0.29). However, this dependence was statistically significant after the process of micronisation and microwave toasting (Tables 3 and 4) - that is, the temperature caused by infrared rays and microwaves had the same effect on both, protein solubility and lysine availability.

	Microwave toasting			Autoclaving		
	Salt soluble proteins	Available lysine	Moisture	Salt soluble proteins	Available lysine	Moisture
Water soluble proteins	0.99**	0.80*	0.98**	0.99**	-0.29	-0.33
Salt soluble proteins		0.81*	0.98**		-0.29	-0.37
Available lysine			0.90*			0.98**

* P < 0.01, **P < 0.05 statistical significance

Table 4 Correlation dependence of changes of studied parameters of nutritive quality after the process of grain microwave toasting and autoclaving of the soybean cultivar Bosa.

4 Conclusion

The content of water and salt soluble proteins declined in both cultivars after the application of all technological processing procedures under impacts of higher temperatures.

According to the results obtained, it can be concluded that the available lysine content primarily depends on the moisture content in the system, as well as on the applied heat processing treatment. As the Maillard reaction is a type of redox reactions, dry extrusion and autoclaving proceeding within the closed systems within which the higher relative humidity has a special effect by blocking the access of air oxygen, caused a significantly lesser changes in the content of available lysine. Higher temperatures during processes of micronisation and microwave toasting caused a very pronounced reduction of lysine availability.

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