

EFFECTS OF HEAT PROCESSING ON SOYA BEAN FATTY ACIDS  
CONTENT AND THE LIPOXYGENASE ACTIVITY

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**Abstract:** Effects of increased temperatures on the lipoxygenase activity and changes of soya bean fatty acids were observed in the present study. The kernels of soya bean cultivars Bosa and ZPS 015 were subjected to the treatments of extrusion, autoclaving, micronisation and microwave roasting. Depending on the technological processing procedure, the kernels were exposed to temperatures ranging from 60 to 150°C for 25 to 30 seconds during extrusion and for 30 minutes during autoclaving. The temperature that developed in the course of the microwave radiation and autoclaving did not cause statistically significant differences between oil content in heat treated and fresh kernels of soya bean. However, the oil content was higher in soya bean flakes (micronized kernels) and lower in grits than in fresh kernels. The heat treatments resulted in the significant decrease of the linolenic fatty acid content. Depending on the temperature and applied heat treatments, the content of linoleic and oleic fatty acid oscillated. High temperatures caused changes in unsaturated fatty acids with 18 carbon atoms resulting in relative increase of the stearic acid content. The lipoxygenase activity decreased in correlation with increased temperatures and the time of heating. The maximum drop of the activity was observed after kernel exposure to the extrusion and micronisation processes at the temperature of 100°C. However, a significant lipoxygenase activity increase was recorded in both studied cultivars after one-minute microwave heating, i.e. at the temperature about 60°C. A further temperature increase led to a gradual denaturation of the enzyme and therefore to its decreased activity.

**Key words:** soya bean, oil, fatty acid, lipoxygenase, heat treatments.

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## Introduction

Among the edible legumes, soya bean, with the oil content of 20%, ranks the second. Beside triglycerides that are dominant components of soya bean oil, this oil contains a low amount of phospholipids (1.5 to 2.5%), non-saponified substances, free fatty acids (0.3 to 0.7%) and metals in traces (2 do 3ppm) (Liu, 1997). According to Hammond and Glatz (1989) and Kitamura et al. (1983), the highest percentage of fatty acids in soya bean oil belongs to linoleic acid (25% to 60%, 53.2% on the average), then to oleic acid (25% to 60%, 23% on the average), palmitic acid (8% to 17%, 11% on the average), linolenic acid (2% to 15%, 7% on the average) and stearic acid (3% to 30%, 4% on the average). Soya bean oil even contains some less presented fatty acids such as arachidic acid (approximately 0.3%), behenic acid (approximately 0.1%), palmitoleic acid (approximately 0.1%) and myristic acid (approximately 0.1). As a great source of essential fatty acids, especially linoleic (18:2n), that belongs to  $\omega$ -6 series of fatty acids, and linolenic (18:3n), that belongs to  $\omega$ -3 series of fatty acids, soya bean has a beneficial effects on nutrition and health (Simopoulos, 1991; Friedman and Brandon, 2001; Wathes et al., 2007). However, a high content of essential polyunsaturated fatty acids makes soya bean oil less stable. The unsaturated bonds in soya bean oil represent active sites that can react with oxygen even at a low temperature. This reaction, lipid peroxidation, leads to formation of primary, secondary and tertiary oxidation products that impart undesirable odour and flavour known as rancid. As the reaction progresses, such flavour becomes intense and unpleasant. The resulting product eventually not only becomes unsuitable for consumption but also possesses health potential (Haumann, 1993).

Lipoxygenase (LOX) EC 1.13.11.12 linoleate: oxidoreductase, is an important enzyme in processes of lipid peroxidation. This enzyme catalyses the oxidation of polyunsaturated fatty acids, producing conjugated unsaturated fatty acid hydroperoxides. The enzyme also has an ability to form free radicals, which can then attack other constituents. Lipoxygenases from soya bean are of a particular interest because they have been implicated as the principal cause of undesirable flavours of soya kernels, commonly known as “greeny” or “beany”. Also, LOX catalyses cooxidation of such pigments as carotenoids and chlorophyll.

Because of a high content of anti-nutritional components, soya bean kernels must be heated before being used in feed and food. But this process can also indiscriminately destroy essential nutrients. 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 degradation of fatty acids. Also, the effect of the heating on the LOX inactivation was investigated.

## Materials and Methods

Kernels of a standard chemical composition of commercially used soya bean cultivars, Bosa and ZPS 015, developed at the Maize Research Institute, Zemun Polje, were used in these experiments.

These parameters were analysed also in products obtained after the application of heat treatments: (a) dry extrusion at 100, 125, 140 and 150°C for 25-30 seconds; (b) micronisation at 100, 125, 140 and 150°C for 50-60 seconds; (c) kernel roasting in the microwave oven of 800W and 2450 MHz in the intervals of 1, 2, 3, 4 and 5 minutes; (d) autoclaving 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 in the microwave oven with other results, the sample temperature was checked after kernel roasting by the "thermos bottle test": Bosa (1 min. = 57°C, 2 mins = 88°C, 3 mins = 108°C, 4mins = 121°C, 5 mins = 132°C) and ZPS 015 (1 min. = 60°C, 2 mins = 90°C, 3 mins = 108°C, 4 mins = 118°C, 5 mins = 137°C).

The LOX (EC 1.13.11.12) activity was determined in the crude flour homogenate prepared by shaking the sample with five volumes of 0.2 M sodium phosphate buffer (pH 6.5) at 4°C for 120 mins. The supernatant obtained by centrifugation at 20,000 g for 15 mins, was used to measure the Lx 1 activity. The assay mixture consisted of 50 mM linoleic acid in 0.2 M borate buffer, pH 9.0, and the aliquot of the sample. The initial rate of the absorbance changes at 234 nm ( $\epsilon = 2.5 \times 10^4 \text{ M}^{-1}\text{cm}^{-1}$ ) was recorded. The cuvette contained 2.5 ml of substrate, 10  $\mu\text{l}$  of the extract was added with rapid mixing for 5 secs, and the change in absorbance was recorded for 5 mins. The activity of Lx 1 was expressed in  $\mu\text{mol}$  of conjugated diene formed per minute and g d.m. (Axelrod et al., 1981). The total oil content was determined by the diethyl ether-based Soxhlet extraction procedure (Službeni list SFRJ, 1987). Total soya oil fatty acid composition was determined by gas chromatography (GC; VARIAN chromatograph, model 1400; Varian Associates, Walnut Creek, CA), equipped with a flame ionization detector and a 3.0 m  $\times$  0.32 cm steel column, packed with LAC-3R-728 (20%; Cambridge Ind. Co., Cambridge, UK) on ChromosorbW/AW(80-100 mesh; Merck, Darmstadt, Germany). Nitrogen was used as a carrier gas (flow rate, 24 mL min<sup>-1</sup>) (ISO 5509-1978 (E); ISO 5508-1978 (E)). All chemical analyses were performed in four replicates and obtained 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.

## Results and Discussion

In general, any kind of heat processing is widely believed to reduce the nutritional value of foods. On the other hand, thermal processing in general increases digestibility and bioavailability of nutrients and phytochemicals (Slavin et al., 2000).

The content of oil and fatty acid composition of the fresh and thermally treated soya bean kernels is presented in Table 1 and 2. The content of oil in fresh kernels of cultivars Bosa and ZPS 015 was high and amounted to 24.5% and 23.5%, respectively. The oil content was higher in soya bean flakes than in fresh kernels. This content was increased by 8% in flakes of the cultivar ZP 015 after micronisation at 140°C and by 6% in flakes of the cultivar Bosa after micronisation at 150°C. Collins and Beaty (1980) state that the temperatures affect the protein denaturation in soya kernels leading to an easier lipid extraction. The temperature that developed in the course of the microwave radiation and autoclaving did not cause statistically significant differences between oil contents in heat treated and fresh kernels of soya bean (Table 1 and 2). Results presented in Table 1 show that the oil content in soya bean grits obtained by wet and dry extrusion was lower than in the fresh kernel. The drop of the oil content was approximately 15% in grits obtained by dry extrusion at 150°C. The reason for this could be the specificity of the extrusion process in which the kernel becomes a special hot mash due to the friction caused by the increased pressure and temperature; hence, the oil extraction already occurs in the extruder cylinder.

Blumenthal and Stier (1991) point out that chemical changes occurring during the oil heating are based on two types of reactions - thermolytic and oxidative. Thermolytic reactions proceed without the oxygen presence, and during these reactions polymerisation, isomerisation, cyclisation and hydrolysis of soya bean oil components occur. Hydrogen peroxides and free peroxide radicals of unsaturated fatty acids are formed in the process of oxidation, causing a decrease of a nutritive value, and unpleasant odour and flavour of soya bean oil.

Linolenic acid (18:3 $\omega$ -3) is the most unstable fatty acid in soya bean oil due to the presence of three double bonds (Frankel, 1998). This result is in accordance with our study. The maximal drop of linoleic acid content (8.5%) was observed after the kernel of soya bean Bosa exposure to the micronisation at the temperature of 140°C. The content of oleic acid was increased by 14% in flakes of the cultivar ZP 015 after micronisation at 140°C. However, changes of linolenic acid content affected by heat treatments were higher. In our study it was noted that high temperature during the processes of micronisation and microwave roasting had a far than the temperature caused by a vapour pressure during treatments of autoclaving and extrusion. The content of linolenic acid in the micronised kernel of the soya bean cultivar ZPS 015 dropped by 14 to 38%, while this content in grits made in the processes of dry extrusion dropped by 6 to 12%. The explanation could be that the kernel moisture level provides the protection from losses of unsaturated fatty acids, linoleic and linolenic, during heating, and that is in accordance with the findings of Yoshida and Kajimoto (1988). Based on gained results it can be concluded that linolenic acid degradation already occurred at the temperature as low as 57°C - 60°C during one minute of kernel roasting in the

Table 1. Content of oil and fatty acids after treatments of autoclaving, dry and wet extrusion.

Cultivars	Time/ temperature	Oil (%)	16:0 (%)	18:0 (%)	18:1 (%)	18:2 (%)	18:3 (%)
Autoclaving							
Bosa	Fresh kernel	24.18 <sup>b</sup>	10.7 <sup>a</sup>	5.2 <sup>bc</sup>	27.5 <sup>a</sup>	51.3 <sup>a</sup>	6.2 <sup>c</sup>
	10 mins	23.77 <sup>b</sup>	10.2 <sup>bc</sup>	4.7 <sup>cd</sup>	26.6 <sup>bc</sup>	51.3 <sup>a</sup>	7.3 <sup>ab</sup>
	20 mins	24.14 <sup>b</sup>	10.1 <sup>bc</sup>	4.9 <sup>cd</sup>	27.1 <sup>bc</sup>	51.1 <sup>ab</sup>	6.8 <sup>bc</sup>
	30 mins	24.75 <sup>a</sup>	10.2 <sup>bc</sup>	4.6 <sup>d</sup>	27.4 <sup>a</sup>	50.5 <sup>bc</sup>	6.3 <sup>c</sup>
ZPS 015	Fresh kernel	23.66 <sup>bc</sup>	10.2 <sup>b</sup>	6.8 <sup>a</sup>	26.8 <sup>bc</sup>	49.9 <sup>d</sup>	7.1 <sup>a</sup>
	10 mins	23.22 <sup>c</sup>	10.0 <sup>bc</sup>	5.7 <sup>b</sup>	26.5 <sup>c</sup>	50.1 <sup>cd</sup>	7.7 <sup>a</sup>
	20 mins	23.11 <sup>c</sup>	10.4 <sup>ab</sup>	5.6 <sup>b</sup>	26.9 <sup>abc</sup>	50.2 <sup>cd</sup>	7.5 <sup>ab</sup>
	30 mins	23.38 <sup>c</sup>	9.8 <sup>c</sup>	5.6 <sup>b</sup>	27.2 <sup>ab</sup>	51.7 <sup>a</sup>	6.9 <sup>bc</sup>
LSD 0.05	0.525	0.429	0.539	0.660	0.648	0.729	
Dry extrusion							
Bosa	Fresh kernel	24.40 <sup>a</sup>	11.4 <sup>a</sup>	4.2 <sup>abcd</sup>	24.1 <sup>bcd</sup>	52.4 <sup>b</sup>	7.6 <sup>b</sup>
	100°C	23.73 <sup>ab</sup>	11.1 <sup>bc</sup>	4.5 <sup>ab</sup>	25.2 <sup>a</sup>	52.6 <sup>ab</sup>	6.8 <sup>c</sup>
	125°C	22.97 <sup>c</sup>	11.4 <sup>ab</sup>	4.2 <sup>bcd</sup>	25.2 <sup>abc</sup>	53.0 <sup>ab</sup>	6.4 <sup>d</sup>
	140°C	22.72 <sup>c</sup>	11.4 <sup>a</sup>	4.7 <sup>a</sup>	23.8 <sup>d</sup>	53.0 <sup>ab</sup>	7.0 <sup>c</sup>
	150°C	20.90 <sup>e</sup>	11.4 <sup>a</sup>	4.0 <sup>bcd</sup>	25.2 <sup>ab</sup>	53.1 <sup>a</sup>	6.2 <sup>d</sup>
ZPS 015	Fresh kernel	24.32 <sup>a</sup>	11.2 <sup>abc</sup>	4.4 <sup>abc</sup>	23.6 <sup>d</sup>	52.7 <sup>ab</sup>	8.2 <sup>a</sup>
	100°C	21.02 <sup>d</sup>	11.3 <sup>ab</sup>	3.7 <sup>d</sup>	24.0 <sup>cd</sup>	53.0 <sup>a</sup>	7.2 <sup>c</sup>
	125°C	21.19 <sup>d</sup>	11.3 <sup>abc</sup>	3.9 <sup>cd</sup>	24.4 <sup>d</sup>	52.9 <sup>ab</sup>	7.5 <sup>b</sup>
	140°C	21.45 <sup>d</sup>	11.4 <sup>a</sup>	4.2 <sup>abcd</sup>	23.9 <sup>d</sup>	52.8 <sup>ab</sup>	7.7 <sup>b</sup>
	150°C	20.82 <sup>d</sup>	11.1 <sup>c</sup>	3.7 <sup>d</sup>	23.1 <sup>d</sup>	52.6 <sup>ab</sup>	7.4 <sup>b</sup>
LSD 0.05	0.703	0.277	0.485	1.147	0.663	0.452	
Wet extrusion							
Bosa	Fresh kernel	24.69 <sup>a</sup>	10.0 <sup>a</sup>	4.2 <sup>c</sup>	26.4 <sup>c</sup>	53.6 <sup>a</sup>	7.2 <sup>b</sup>
	100°C	23.24 <sup>b</sup>	10.1 <sup>a</sup>	4.9 <sup>b</sup>	28.1 <sup>a</sup>	50.8 <sup>c</sup>	6.9 <sup>cd</sup>
	125°C	22.56 <sup>c</sup>	10.1 <sup>a</sup>	4.5 <sup>bc</sup>	27.2 <sup>b</sup>	51.1 <sup>c</sup>	6.7 <sup>d</sup>
	140°C	22.09 <sup>cd</sup>	10.0 <sup>a</sup>	4.4 <sup>bc</sup>	26.7 <sup>bc</sup>	50.7 <sup>cd</sup>	7.1 <sup>bc</sup>
ZPS 015	Fresh kernel	23.34 <sup>b</sup>	10.1 <sup>a</sup>	4.9 <sup>b</sup>	26.3 <sup>c</sup>	51.9 <sup>b</sup>	8.0 <sup>a</sup>
	100°C	23.47 <sup>b</sup>	9.9 <sup>a</sup>	4.2 <sup>c</sup>	26.7 <sup>bc</sup>	52.2 <sup>b</sup>	6.7 <sup>d</sup>
	125°C	22.38 <sup>c</sup>	10.2 <sup>a</sup>	5.5 <sup>a</sup>	26.7 <sup>bc</sup>	50.6 <sup>cd</sup>	6.7 <sup>d</sup>
	140°C	21.56 <sup>de</sup>	10.3 <sup>a</sup>	5.5 <sup>a</sup>	27.2 <sup>b</sup>	50.2 <sup>d</sup>	6.8 <sup>cd</sup>
LSD 0.05	0.607	0.588	0.560	0.736	0.564	0.343	

<sup>a-b</sup> Means followed by the same letter within the same column are not significantly different ( $P < 0.05$ ); LSD-Least significant difference; C 16:0–palmitic acid, C 18:0–stearic acid, C 18:1–oleic acid, C 18:2–linoleic acid, C 18:3–linolenic acid.

microwave oven and that the great losses also happened during initial steps of micronisation and dry extrusion processes. Further increases of the initial temperature did not affect the gradual decrease of linolenic acid content in soya bean products during microwave, micronisation and extrusion processes (Table 1

and 2). The linolenic acid increased stability during prolonged heating could be a result of either the increased content of the products of Maillard reaction that are produced under impacts of the temperature and that are known to have antioxidant properties or the position of linolenic acid in the triglyceride molecule. Bolton and Sanders (2002) in their study connected the oxidative stability of prolonged roasted peanut oil with the antioxidative activity of products that were made in the process of the Maillard reaction.

Table 2. Content of oil and fatty acids after treatments of micronisation and microwave roasting.

Cultivars	Time/ Temperature	Oil (%)	16:0 (%)	18:0 (%)	18:1 (%)	18:2 (%)	18:3 (%)
Micronisation							
Bosa	Fresh kernel	24.69 <sup>b</sup>	10.0 <sup>a</sup>	4.2 <sup>de</sup>	26.4 <sup>c</sup>	53.6 <sup>a</sup>	7.2 <sup>b</sup>
	100°C	24.33 <sup>b</sup>	10.0 <sup>a</sup>	4.2 <sup>d</sup>	28.4 <sup>b</sup>	50.8 <sup>c</sup>	7.0 <sup>bc</sup>
	125°C	24.32 <sup>b</sup>	10.0 <sup>a</sup>	4.5 <sup>cd</sup>	29.0 <sup>b</sup>	49.8 <sup>de</sup>	6.4 <sup>de</sup>
	140°C	25.05 <sup>b</sup>	10.3 <sup>a</sup>	5.2 <sup>a</sup>	28.8 <sup>b</sup>	49.0 <sup>f</sup>	6.9 <sup>bc</sup>
	150°C	26.19 <sup>a</sup>	9.8 <sup>a</sup>	4.9 <sup>abc</sup>	28.8 <sup>b</sup>	49.8 <sup>de</sup>	6.7 <sup>cd</sup>
ZPS 015	Fresh kernel	23.34 <sup>c</sup>	10.1 <sup>a</sup>	4.9 <sup>abc</sup>	26.3 <sup>c</sup>	51.9 <sup>b</sup>	8.0 <sup>a</sup>
	100°C	24.51 <sup>b</sup>	10.0 <sup>a</sup>	5.0 <sup>ab</sup>	28.1 <sup>b</sup>	51.0 <sup>c</sup>	6.1 <sup>ef</sup>
	125°C	24.55 <sup>b</sup>	9.7 <sup>a</sup>	3.7 <sup>e</sup>	30.5 <sup>a</sup>	51.1 <sup>c</sup>	5.7 <sup>f</sup>
	140°C	25.46 <sup>a</sup>	10.0 <sup>a</sup>	4.2 <sup>de</sup>	30.6 <sup>a</sup>	50.4 <sup>cf</sup>	5.0 <sup>g</sup>
	150°C	25.00 <sup>b</sup>	10.2 <sup>a</sup>	4.7 <sup>bc</sup>	30.3 <sup>a</sup>	49.3 <sup>ef</sup>	5.7 <sup>f</sup>
LSD 0.05		0.930	0.690	0.458	1.00	0.780	0.423
Microwave roasting							
Bosa	Fresh kernel	24.69 <sup>ab</sup>	10.0 <sup>de</sup>	4.2 <sup>d</sup>	26.4 <sup>c</sup>	53.6 <sup>a</sup>	7.2 <sup>b</sup>
	1 min.	24.35 <sup>ab</sup>	9.9 <sup>e</sup>	3.8 <sup>de</sup>	27.3 <sup>a</sup>	53.2 <sup>ab</sup>	5.9 <sup>ef</sup>
	2 mins	24.31 <sup>abc</sup>	9.8 <sup>e</sup>	3.5 <sup>e</sup>	29.9 <sup>abc</sup>	53.0 <sup>bc</sup>	5.7 <sup>f</sup>
	3 mins	24.61 <sup>a</sup>	10.0 <sup>de</sup>	3.8 <sup>de</sup>	26.7 <sup>abc</sup>	53.3 <sup>ab</sup>	6.3 <sup>de</sup>
	4 mins	24.43 <sup>ab</sup>	10.6 <sup>ab</sup>	3.9 <sup>de</sup>	25.5 <sup>d</sup>	52.5 <sup>c</sup>	6.4 <sup>de</sup>
	5 mins	23.52 <sup>bcd</sup>	10.5 <sup>abc</sup>	5.0 <sup>c</sup>	27.1 <sup>ab</sup>	51.1 <sup>e</sup>	6.4 <sup>de</sup>
ZPS 015	Fresh kernel	3.34 <sup>bcd</sup>	10.1 <sup>cde</sup>	4.9 <sup>c</sup>	26.3 <sup>c</sup>	51.9 <sup>d</sup>	8.0 <sup>a</sup>
	1 min.	22.13 <sup>ef</sup>	10.1 <sup>cde</sup>	6.0 <sup>a</sup>	25.3 <sup>d</sup>	51.6 <sup>de</sup>	7.2 <sup>bc</sup>
	2 mins	22.80 <sup>ef</sup>	10.4 <sup>abcd</sup>	5.4 <sup>bc</sup>	26.5 <sup>bc</sup>	51.4 <sup>de</sup>	6.6 <sup>cd</sup>
	3 mins	22.33 <sup>def</sup>	10.2 <sup>bcde</sup>	6.1 <sup>a</sup>	26.3 <sup>c</sup>	50.1 <sup>f</sup>	7.2 <sup>b</sup>
	4 mins	22.95 <sup>cde</sup>	10.4 <sup>abcd</sup>	5.9 <sup>ab</sup>	26.9 <sup>abc</sup>	50.0 <sup>f</sup>	7.2 <sup>b</sup>
	5 mins	22.38 <sup>def</sup>	10.7 <sup>a</sup>	5.3 <sup>c</sup>	26.8 <sup>abc</sup>	50.5 <sup>f</sup>	6.7 <sup>bcd</sup>
LSD 0.05		1.37	0.497	0.582	0.634	0.599	0.578

<sup>a-b</sup> Means followed by the same letter within the same column are not significantly different ( $P < 0.05$ ); LSD-Least significant difference; C 16:0–palmitic acid, C 18:0–stearic acid, C 18:1–oleic acid, C 18:2–linoleic acid, C 18:3–linolenic acid.

The content of linoleic acid (18:6 $\omega$ -2) oscillated in soya bean products depending on the temperature and the applied treatment. In our study high temperature during autoclaving and dry extrusion did not affect the gradual decrease of linoleic acid content in soya bean products. However, this content was decreased by 8.5% in flakes of the cultivar ZP 015 after micronisation at 140°C and by 5% in kernels of the same cultivar Bosa after roasting in microwave oven for five minutes. Oleic acid, with only one double bond in its structure, was more thermostable, which is indicated by the statistically significantly increased content of oleic acid in soya bean products made mainly of the cultivar Bosa. The transition of polyunsaturated fatty acids in the more saturated state can cause the increase of the content of fatty acids with a smaller number of double bonds. According to the obtained results it can be concluded that the increased temperature did not affect the content of saturated palmitic acid (Table 1 and 2). Results of the stearic acid changes under the impacts of increased temperatures cannot be observed separately from the results of the analysis of unsaturated fatty acids. Stearic acid, as a saturated fatty acid, is very stable. However, the changes in unsaturated fatty acids due to thermal oxidation lead to the relative increase of stearic acid in the soya bean products made by the heat treatments. This phenomenon was observed in our study mainly in the kernel of the soya bean cultivar ZPS 015 that was roasted in a microwave oven and also in grits made by the wet extrusion of kernels of the same cultivar (Table 1 and 2).

The destruction of fatty acids was simultaneously observed with changes in lipoxygenase activities. The genotypes Bosa and ZPS 015 had a very similar initial activity of lipoxygenase 1 (5.63 and 5.34  $\mu\text{mol ml}^{-1} \text{min}^{-1}$ , respectively). According to the obtained results it can be concluded that activities of lipoxygenase 1 in both soya bean cultivars decreased after the application of all five technological processing procedures (Table 3). The maximal drop of the activity was observed after the kernel exposure to the extrusion and micronisation at the temperature of 100°C. The activity of lipoxygenase in flakes of cultivars Bosa and ZP 015 made by micronisation at 100°C was 2.74 and 2.80  $\mu\text{mol ml}^{-1} \text{min}^{-1}$ , respectively and the process of dry extrusion was even more effective when at 100°C the activity of lipoxygenase in grits of cultivars Bosa and ZP 015 dropped to 0.043 and 0.034  $\mu\text{mol ml}^{-1} \text{min}^{-1}$ , respectively. These results are in accordance with those of Zhu et al. (1996). These authors reported that the lipoxygenase 1 enzyme activity was decreased to 0 when soya beans were extruded at 107°C. The significant increase of the lipoxygenase activity was recorded in both studied cultivars only after one minute microwave heating, i.e. at the temperature of about 60°C. This increase is probably a result of denaturation of antioxidants (Esaka et al., 1987) or some inhibitors of the lipoxygenase 1 activity, whereby their complex with the enzyme is disrupted and the active site of the enzyme in the fresh grain is released for the reaction with fatty acids. A further temperature increase leads to a gradual denaturation of the enzyme and therefore to the final decrease of its activity.

Table 3. Activity of lipoxygenase ( $\mu\text{mol ml}^{-1}\text{min}^{-1}$ ) after different thermal treatments.

Thermal treatments	Time/Temperature	Bosa	ZPS 015
	Fresh kernel	5.630 <sup>ab</sup>	5.341 <sup>c</sup>
Autoclaving	10 mins	0.236 <sup>h</sup>	0.226 <sup>h</sup>
	20 mins	0.014 <sup>i</sup>	0.011 <sup>i</sup>
	30 mins	0.006 <sup>i</sup>	0.003 <sup>i</sup>
Dry extrusion	100°C	0.042 <sup>i</sup>	0.035 <sup>i</sup>
	125°C	0.043 <sup>i</sup>	0.035 <sup>i</sup>
	140°C	0.041 <sup>i</sup>	0.034 <sup>i</sup>
	150°C	0.019 <sup>i</sup>	0.035 <sup>i</sup>
Wet extrusion	100°C	1.527 <sup>c</sup>	2.615 <sup>d</sup>
	125°C	0.113 <sup>h</sup>	0.136 <sup>h</sup>
	140°C	0.070 <sup>hi</sup>	0.078 <sup>hi</sup>
Micronisation	100°C	2.742 <sup>d</sup>	2.803 <sup>d</sup>
	125°C	0.291 <sup>h</sup>	0.171 <sup>h</sup>
	140°C	0.097 <sup>hi</sup>	0.064 <sup>hi</sup>
	150°C	0.044 <sup>i</sup>	0.062 <sup>hi</sup>
Microwave roasting	1 min.	5.818 <sup>a</sup>	6.031 <sup>a</sup>
	2 mins	0.861 <sup>f</sup>	0.577 <sup>g</sup>
	3 mins	0.026 <sup>i</sup>	0.016 <sup>i</sup>
	4 mins	0.022 <sup>i</sup>	0.017 <sup>i</sup>
	5 mins	0.019 <sup>i</sup>	0.006 <sup>i</sup>
LSD 0.05		0.234	

<sup>a-b</sup> Means followed by the same letter within the same column are not significantly different ( $P < 0.05$ ); LSD-Least significant difference.

This study was carried out to a better understanding of, and ultimately a better controlling of the processing conditions which affect the nutritional properties of soya bean, mainly the fatty acid content and the activity of enzyme lipoxygenase 1.

### Conclusion

The content of polyunsaturated fatty acids was more stable after the application of high temperature caused by a vapour pressure during treatments of autoclaving and extrusion than after processes of micronisation and microwave roasting. At the same time, the lipoxygenase 1 enzyme activity was decreased to 0.043 and 0.034  $\mu\text{mol ml}^{-1}\text{min}^{-1}$ , respectively when soya beans Bosa and ZP 015 were extruded at 100°C. Among investigated fatty acids, linolenic acid was the most unstable in soya bean. The content of linolenic acid in the micronised kernel of the soya bean cultivar ZPS 015 dropped by 14 to 38%, while this content in grits made in the processes of dry extrusion dropped by 6 to 12%. Also, we can conclude that the changes in polyunsaturated fatty acids due to thermal oxidation lead to the relative increase of stearic and oleic fatty acids in the soya bean products made by the heat treatments.



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## References

- Axelrod, B., Cheesbrough, T.M., Laakso, S. (1981): Lipoxygenase from soybeans. *Method in Enzymology* 71:441-451.
- Blumenthal, M.M., Stier, R.F. (1991): Optimatization of deep-fat frying operations. *Trends Food Sci. Technol.* 2:144-148.
- Bolton, G.E., Sanders, T.H. (2002): Effect of roasting oil composition on the stability of roast high-oleic peanuts. *J. Amer. Oil Chem. Soc.* 79:129-131.
- Collins, J.B., Beaty, B.F. (1980): Heat inactivation of trypsin inhibitor in fresh green soybean and physiological responses of rats fed the beans. *J. Food Sci.* 45:542-549.
- Esaka, M., Suzuki, K., Kubota, K. (1987): Effect of microwave heating on lipoxygenase and trypsin inhibitor activities and water absorption of widgeenbeens seeds. *J. Food Sci.* 52:1738-1739.
- Frankel, E.N. (1998): *Lipid oxidation*. The Oily Press Ltd., Glasgow, United Kingdom.
- Friedman, M., Brandon, D.L. (2001): Nutritional and health benefits of soy proteins. *J. Agric. Food Chem.* 49:1069-1086.
- Hammond, E.G., Glatz, B.A. (1989): Biotechnology applied of fats and oils. In: King, R., Cheetham, P.S.J (Eds.), *Developments in food biotechnology*. John Wiley and Sons, Inc., New York, pp. 173-217.
- Haumann, B.F. (1993): Health implications of lipid oxidation. *INFORM* 4:800.
- Kitamura, K., Devies, S.C., Kiazuma, N., Nielsen, N.C. (1983): Genetic analysis of null-allele for lipoxygenase-3 in soybean seeds. *Crop Sci.* 23:924-927.
- Liu, K. (1997): Chemistry and nutritional value of soybean components. In: Liu, K. (Ed.), *Soyabean chemistry, technology and utilization*. Chapman and Hall's International Resource Center, Dept. BC, New York, pp. 26-36.
- Simopoulos, A.P. (1991): Omega-3 fatty acids in health and disease and in growth and development. *American Journal of Clinical Nutrition* 54:438-463.
- Slavin, J.L. Jacobs, D., Marquart, L. (2000): Grain processing and nutrition *Crit. Rev. Food Sci. Nutr.* 40:309-326.
- Službeni list SFRJ (1987): The rule book of methods of sampling and of methods for performing physical, chemical and microbiological analyses of feeds. Institution for Standardisation of Serbia 15/1987.
- Wathes, D.C., Abayasekera, D.R., Aitken, R.J. (2007): Polyunsaturated fatty acids in male and female reproduction. *Biology of Reproduction* 77:190-201.
- Yoshida, H., Kajimoto, G. (1988): Effects of microwave treatment on the trypsin inhibitor and molecular species of triglycerides in soybeans. *J. Food Sci.* 53:1756-1760.
- Zhu, S., Riaz, M.N., Lusas, E.W. (1996): Effect of different extrusion temperatures and moisture content on lipoxygenase inactivation and protein solubility in soybeans. *J. Agric. Food Chem.* 44:3315-3318.

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UTICAJ TERMIČKIH TRETMANA PRERADE NA SADRŽAJ MASNIH  
KISELINA SOJINOG ZRNA I AKTIVNOST LIPOKSIGENAZE

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R e z i m e

U ovom radu prikazani su rezultati promene aktivnosti lipoksigenaze 1 i sadržaja masnih kiselina u sojinom zrnu pod uticajem povišene temperature. Zrno soje sorte Bosa i ZPS 015 bilo je podvrgnuto tretmanima suve i vlažne ekstruzije, autoklaviranju, mikronizaciji i prženju u mikrotalasnoj peći. U zavisnosti od tehnološkog tretmana prerade zrno je bilo izloženo dejstvu povišene temperature od 60 do 150°C u trajanju od svega 25 do 30 sekundi pri ekstruziji do 30 minuta pri autoklaviranju. Povišena temperatura tokom mikrotalasnog prženja i autoklaviranja nije uticala na sadržaj ulja u sojinom zrnu. Međutim, u sojinim flekicama sadržaj ulja bio je viši, a u grizu niži od sadržaja u sirovom sojinom zrnu. Primenjeni termički tretmani prerade uticali su na značajno smanjenje sadržaja linolenske kiseline u ulju sojinog zrna. U zavisnosti od temperature, kao i vrste termičkog tretmana sadržaj linolne i oleinske kiseline u prerađenom sojinom zrnu je varirao. Povišena temperatura uslovlila je promene nezasićenih masnih kiselina sa 18 ugljenikovih atoma rezultirajući povećanje sadržaja stearinske kiseline. Prema našim rezultatima aktivnost lipoksigenaze je opadala sa povećanjem temperature i produženjem tretmana prerade. Maksimalan pad aktivnosti lipoksigenaze utvrđen je već nakon izlaganja zrna temperaturi od 100°C tokom ekstruzije i mikronizacije. Međutim, nakon jednog minuta prženja zrna u mikrotalasnoj peći (60°C) aktivnost lipoksigenaze je bila viša u odnosu na aktivnost u sirovom zrnu. Daljim produženjem tretmana, a time i povećanjem temperature došlo je do postepene denaturacije enzima i smanjenja aktivnosti.

**Ključne reči:** soja, termički tretmani, ulje, masne kiseline, lipoksigenaza.

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