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Impact of Microwave Thermal Processing on Major Grain Quality Traits of Linseed (*Linum usitatissium* L.)

Nikola Puvača [1,*], Dragana Ljubojević Pelić [2], Milica Živkov Baloš [2], Jovanka Lević [3], Radivoj Prodanović [1], Vidosava Puvača Čović [1], Sanja Popović [3] and Olivera Đuragić [3]

1 Department of Engineering Management in Biotechnology, Faculty of Economics and Engineering Management in Novi Sad, University Business Academy in Novi Sad, 21000 Novi Sad, Serbia; rprodanovic@fimek.edu.rs (R.P.); puvacav@gmail.com (V.P.Č.)

2 Scientific Veterinary Institute "Novi Sad", 21000 Novi Sad, Serbia; dragana@niv.ns.ac.rs (D.L.P.); milica@niv.ns.ac.rs (M.Ž.B.)

3 Scientific Institute of Food Technology, University of Novi Sad, 21000 Novi Sad, Serbia; jovanka.levic@fins.uns.ac.rs (J.L.); sanja.popovic@fins.uns.ac.rs (S.P.); olivera.djuragic@fins.uns.ac.rs (O.Đ.)

* Correspondence: nikola.puvaca@fimek.edu.rs; Tel.: +381-65-219-1284

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Abstract: The current study investigated the effects of thermal processing of the microwave technology on nutritive value, crude protein solubility, urease activity and amino acid profile on linseed grains. Samples were treated in a SAMSUNG GE82N-B microwave oven at 450W for 0 (L1), 60 (L2), 180 (L3), 300 (L4), and 420 (L5) seconds, respectively. Microwave treatment for 300 seconds showed a significant ($p \leq 0.05$) decrease in activity urease comparing to raw linseed. The raw and treated linseed protein solubility index (PDI) show statistical differences ($p \leq 0.05$) between all the treatments compared. High performance liquid chromatography (HPLC) analyses of samples differences in the amino acid composition between controls and experimental treatments showed that amino acids were not significantly affected ($p \geq 0.05$), except isoleucine and leucine amino acid ($p \leq 0.05$). From the results of the present study, it is possible to identify that the best method for improving linseed quality for animal feed is the application of microwave for 60 second (treatment L2). Our results indicate that microwave thermal processing or micronizing dry thermal processing of grains could be successfully used in large industrial feed production with a short period of time and the improved nutritional parameters of grains, increased shelf-life and the unchanged amino acid profile of treated grains.

Keywords: linseed; thermal processing; proteins; HPLC; amino acids

1. Introduction

Linum usitatissium L., commonly referred to as linseed, or flaxseed, is thought to be one of the oldest agricultural plants on the earth, with evidence of thousands of years of cultivation. According to FAO records, the cumulative production of linseed in the period from 1994 to 2018 was 43.8% in the USA, 33.6% in Asia, 17.5% in Europe, 4.7% in Africa, and 0.4% in Oceania, respectively. During that period, the largest producers of linseed in the world, with 779,264.68 tons, were Canada, followed by the USA, with a total production of 189,836.92 tons, and Russia, with 186,536.08 tons; while the lowest recorded total production was in Germany with 37,767.2 tons, respectively. It is known for its high amount of protein (20.3 to 27.9%) and oil (31.2–43.6%) [1,2]. The remaining cake after oil extraction is a high residual source of protein for animal nutrition after oil extraction known as linseed or flaxseed meal [3,4]. The interest in using linseed meal and linseed oil in daily animal nutrition was revived recently, as it can be used to change the composition of fatty acids in eggs and meat, thereby giving the consumer to choose between functional or conventional products, respectively [5–7]. Thanks to the

growing issues about humans safety in recent years, the requirement for linseed and other nutrients with functional properties in foods and beverages has significantly increased [1,8,9]. Having all of this in mind, the urgency of the current research to investigate the microwave thermal processing on the major grain quality traits of linseed is very necessary.

The fatty acid profile for linseed oil is very balanced, with low saturated fat levels, moderate monounsaturated fat levels and higher concentrations of polyunsaturated fatty acids (PUFAs) [2]. The PUFA content consists of about 16% n-6 acids, mainly linoleic acid (LA), and 57% α-linolenic acid (ALA), an n-3 acid [10].

Linseed presents a great supply of oil and protein for both humans and animals nutrition [11,12]. The aim of development in the case of patients with malnutrition-related to cancer, burns, wounds and liver disease, as well as for nutritional assistance in children with chronic illnesses, is to identify proteins, which, in their structure contain high branched chain amino acids (AA) levels [13] (valine, leucine, isoleucine commonly called BCAA), low levels of aromatic amino acids (AAA) and high Fisheries (BCAA/AAA) ratio [14]. Besides linseed and other plant sources of amino acids, a very rich source of amino acids is eukaryotes [15]. The BCAA and Fischer ratio of linseed protein fractions is equivalent to those that one finds in soybean [16,17]. It is considered that three AA present in proteins of linseed, such as arginine, glutamine and histidine, have a significant effect on the protective functions of the body [18]. The high content of cysteine and methionine in linseed proteins may increase body antioxidant levels, theoretically regulate the cell division of DNA and minimize the risk of some types of colon cancer [19,20].

The linseed includes, however, certain specific antinutritional factors which have to be considered before this feed is used in the daily animal nutrition [21]. Linseed in animal nutrition is often associated with growth depression when used in an amount greater than 5 or 10 percent, particularly in poultry production [2,22]. The occurrence and composition of antinutritional factors constitute primary practical limitations. The major antinutritional elements in raw linseed are cyanogenic glycosides, linatine, non-starch soluble polysaccharides, trypsin inhibitor and urease [23]. A trypsin inhibitor that inhibits the function of pancreatic proteases is one of the most powerful antinutritive factors in the raw linseed [24]. Growth inhibition, reduction of feed efficiency, goitrogenic reaction, pancreas hypertrophy, and hypoglycemia can be caused by the use of antinutritive factors in linseed, including inhibitors of urease or trypsin, especially in poultry [17]. The microwave thermal processing of linseed improves the utilization of this high quality nutrients and reduces the harmful effects of antinutrients, which is the part of linseed itself [25].

Ahmad Khan et al. [26] evaluated the effect of heating methods on the alteration of the protein molecular structure in linseed, in relation to changes in the protein subfraction profile and its digestibility in animals. Linseed samples were either maintained in their raw state or heated in an air-draft oven, autoclaved or microwaved. Obtained results from their study have shown that microwaved heat treatment increases crude protein digestibility without altering the amide I to amide II ratio. Overall, Ahmad Khan et al. [26] concluded that heat-induced changes in protein nutritive value and digestibility were strongly associated with heat-induced alteration in protein molecular structures. Compared to the microwave heat treatment influence on the nutrient changes of linseed, Kaur et al. [27] investigated the effect of sand roasting and microwave heating on the phenolic acid composition of linseed and came to a conclusion that both roasting and microwave heating affected the antioxidant properties of linseed, where microwaving was shown to be a less invasive heat treatment. The results show that the optimal toasting time and output power are 2 min and 640 W, respectively, for the microwave toasting process when used for detoxification of linseed [23].

Microwave as dry thermal processing, when compared to the mechanical treatments of extrusion or pelleting process, which vary in their use, types of heat source, system configuration and implementation of process parameters, could be one between the most important thermal processing treatments for improving linseed which already has high nutrition [25,28,29].

Therefore, the aim of the research was to investigate the effects of microwave thermal processing on major grain quality traits of linseed.

2. Materials and Methods

The linseed used in the current study was a feed grade product of unknown cultivar and was provided by a commercial company Infinity feed from Novi Sad, Serbia, region of Vojvodina in year 2019. The current study with the microwave processing of linseed samples was performed at the laboratory of the Department of Engineering Management in Biotechnology, while all further analytical procedures were conducted at the laboratory of Scientific Institute of Food Technology in Novi Sad, Serbia. An experimental plan with linseed is given in Table 1.

Table 1. Experimental design with the linseed.

Treatment	Microwave Processing (450 W)				
	L1	**L2**	**L3**	**L4**	**L5**
Processing time (s)	0	60	180	300	420

2.1. Sample Preparation Procedure

Linseed grown in the Vojvodina region in the north part of Serbia in 2019 was purchased from local feed supplier and used for analyses. Analyses were performed on homogenized linseed from the different fields of the same region. Raw linseed was used for microwave thermal processing. A Samsung microwave oven, model GE82N-B, was used for the linseed samples treatment. Raw samples of linseed were uniformly distributed in a thin layer on an oven plate in diameter size of 150 mm. The frequency of the microwave was adjusted to 2450 MHz, as well as the processing power to 450 W. The timeframe for samples treatments was equidistantly divided as follows, L1: 0; L2: 60; L3: 180; L4: 300, and L5: 420 seconds for adjusted processing power. After microwave treatment, samples were ground to pass 1 mm sieve and stored at room temperature in a dark place. After 24 h, samples were taken, and the chemical analysis was performed.

2.2. Nutritive Value Analyses

Linseed nutritive value characteristics were determined according to the ISO recommended standards for moisture, protein, fat, fiber, ash, Ca and P contents, as previously described in the research of Puvača et al. [30] for food samples. Data presented are means of five measurements.

2.3. Crude Protein Solubility Analyses

The protein solubility was analyzed according to the methodology previously described by Licitra et al. [31]; the protein insoluble in pepsin was analyzed using the Kjeldahl method after 48 h of incubation in a pepsin hydrochloric acid solution, previously prepared according to the instruction of Bachmann et al. [32]. The nitrogen-ammonia concentrations in the substrates were analyzed according to the official AOAC method.

2.4. Urease Activity Analyses

The activity of the urease was analyzed by the production of ammonia nitrogen according to McCullough [33], while the extraction was performed according to the methodology given by Watson and Miller [34]. A total of 0.2 g of ground sample was first placed in 8 mL of Na_3PO_4 buffer with added urea (pH 7.4), which was subsequently incubated at a temperature of 30 °C with constant stirring for three hours. Immediately after the finished first phase, the 0.5 mL aliquot of the extracted 2.5 mL of reagent I: C_6H_5OH in the amount of 0.1 mol/L, and $C_5FeN_6Na_2O$ in the amount of 170 µmol/L was added, followed by stirring. The following procedure was the further addition of 2.5 mL of reagent II:

NaOH, in the amount of 0.125 mol/L, Na_2HPO_4 in the amount of 0.15 mol/L, and NaClO in 3% Cl_2. After incubation and the addition of reagents I and II, samples were incubated again at 37 °C in a total of 35 min. Further quantifications were performed in a spectrophotometer at 625 nm and the results were expressed as mgN/g/min.

2.5. Amino Acid Profile Analyses

A total of 10 g of linseed representative samples were dissolved in 200 mL of 80% C_2H_5OH and stored for 12 h, before further analyses. The prepared solution was afterward filtered through the Whatman filter paper and concentrated on an evaporator (Eyela Rotary evaporator N-1100) at a temperature of 45 °C and dissolved in 40 mL of distilled water. Infranatant separated by funnel after adding 20 mL of $(C_2H_5)_2O$ was evaporated. The residue was dissolved in 20 mL of 0.2 M citrate buffer and filtered through a 0.45 µm syringe filter, after which AA was analyzed using high performance liquid chromatography (HPLC) (L-8900, Hitachi, Tokyo, Japan). Cation exchange resin (4.6 mm × 60 mm) was used as a column, $Li_3C_6H_5O_7$ buffer solution in the amount of 0.35 mL/min was used as a mobile phase A, and $C_9H_6O_4$ reagents in the amount of 0.3 mL/min solution was used as a mobile phase B. The standard solution of AA mixture was used as derivatives. Procedures were conducted by the methodology previously described by Wahid [35].

2.6. Statistical Analyses

The data obtained in the experiment were analyzed by one-way analysis of variance (ANOVA) within statistical software STATISTICA 13. When the ANOVA showed statistical significance, Tukey's HSD post-hoc test was performed and $p < 0.05$ indicated a statistically significant difference.

3. Results and Discussion

Examinations of nutritive value, crude protein solubility, and urease activity levels of raw and microwaved heat-processed linseed can be seen in Table 2, and the AA profile is shown in Table 3.

Table 2. Nutritive value, crude protein solubility and urease activity levels of linseed.

Parameter	Microwave Processing (450 W)				
	L1	L2	L3	L4	L5
Moister (%)	5.82 ^{a} ± 0.06	5.90 ^{a} ± 0.01	3.31 ^{b} ± 0.01	1.71 ^{c} ± 0.01	1.78 ^{c} ± 0.02
Crude protein (%)	23.26 ^{b} ± 0.49	23.24 ^{b} ± 0.61	23.57 ^{b} ± 0.39	23.99 ^{b} ± 0.07	24.76 ^{a} ± 0.43
Crude fat (%)	39.52 ^{a} ± 0.34	35.42 ^{b} ± 0.83	39.22 ^{a} ± 0.20	38.42 ^{a} ± 0.23	39.25 ^{a} ± 0.31
Crude fiber (%)	10.09 ^{a} ± 0.13	10.35 ^{a} ± 0.54	10.80 ^{a} ± 0.39	10.50 ^{a} ± 0.24	8.58 ^{b} ± 0.51
Ash (%)	3.49 ^{a} ± 0.17	3.48 ^{a} ± 0.37	3.75 ^{a} ± 0.15	3.69 ^{a} ± 0.29	3.63 ^{a} ± 0.16
Ca (%)	0.26 ^{a} ± 0.01	0.28 ^{a} ± 0.07	0.29 ^{a} ± 0.01	0.28 ^{a} ± 0.01	0.27 ^{a} ± 0.12
P (%)	0.52 ^{a} ± 0.01	0.54 ^{a} ± 0.04	0.55 ^{a} ± 0.01	0.61 ^{a} ± 0.01	0.55 ^{a} ± 0.04
PDI (%)	27.06 ^{b} ± 0.03	36.62 ^{a} ± 0.56	12.19 ^{d} ± 0.22	9.24 ^{e} ± 0.15	15.58 ^{c} ± 0.39
Urease activity (mgN/g/min)	0.12 ^{a} ± 0.01	0.09 ^{b} ± 0.02	0.09 ^{b} ± 0.01	0.07 ^{c} ± 0.01	0.10 ^{b} ± 0.39
Urease activity index (%)	100.00	75.00	75.00	58.33	83.33

$^{a–e}$ indicated the difference within a row was significant ($p \leq 0.05$); PDI—protein solubility index; Results are presented as mean ± SD, n = 5.

Table 3. Amino acid profile of microwave untreated and treated linseed.

Amino Acids	Microwave Processing (450 W)				
	L1	**L2**	**L3**	**L4**	**L5**
Serine (%)	0.721 ^{a} ± 0.26	0.648 ^{a} ± 0.18	0.657 ^{a} ± 0.12	0.636 ^{a} ± 0.26	0.644 ^{a} ± 0.14
Histidine (%)	0.239 ^{a} ± 0.32	0.203 ^{a} ± 0.41	0.205 ^{a} ± 0.47	0.207 ^{a} ± 0.14	0.194 ^{a} ± 0.22
Glycine (%)	0.475 ^{a} ± 0.45	0.423 ^{a} ± 0.31	0.439 ^{a} ± 0.22	0.470 ^{a} ± 0.47	0.428 ^{a} ± 0.10
Threonine (%)	0.254 ^{a} ± 0.12	0.219 ^{a} ± 0.12	0.237 ^{a} ± 0.87	0.220 ^{a} ± 0.64	0.218 ^{a} ± 0.45
Arginine (%)	1.960 ^{a} ± 0.22	1.855 ^{a} ± 0.17	2.201 ^{a} ± 0.31	1.843 ^{a} ± 0.37	1.836 ^{a} ± 0.15
Alanine (%)	0.467 ^{a} ± 0.63	0.428 ^{a} ± 0.54	0.498 ^{a} ± 0.14	0.472 ^{a} ± 0.49	0.440 ^{a} ± 0.08
Tyrosine (%)	0.480 ^{a} ± 0.48	0.469 ^{a} ± 0.47	0.454 ^{a} ± 0.25	0.477 ^{a} ± 0.33	0.480 ^{a} ± 0.14
Valine (%)	1.035 ^{a} ± 0.15	1.009 ^{a} ± 0.19	1.102 ^{a} ± 0.41	1.083 ^{a} ± 0.21	1.012 ^{a} ± 0.12
Methionine (%)	0.447 ^{a} ± 0.55	0.453 ^{a} ± 0.43	0.432 ^{a} ± 0.65	0.486 ^{a} ± 0.09	0.442 ^{a} ± 0.54
Phenylalanine (%)	0.642 ^{a} ± 0.14	0.623 ^{a} ± 0.17	0.609 ^{a} ± 0.31	0.654 ^{a} ± 0.42	0.651 ^{a} ± 0.41
Isoleucine (%)	1.410 ^{a} ± 0.32	1.240 ^{b} ± 0.29	1.241 ^{b} ± 0.47	1.391 ^{a} ± 0.60	1.354 ^{a} ± 0.39
Leucine (%)	1.500 ^{a} ± 0.74	1.395 ^{b} ± 0.86	1.400 ^{a} ± 0.36	1.485 ^{a} ± 0.47	1.449 ^{a} ± 0.23
Lysine (%)	1.078 ^{a} ± 0.25	1.099 ^{a} ± 0.44	0.940 ^{a} ± 0.34	0.982 ^{a} ± 0.87	0.938 ^{a} ± 0.12

^{a,b} indicated the difference within a row was significant ($p \leq 0.05$); Results are presented as mean ± SD, n = 5.

From the presented results, it can be seen that the different linseed processing time has no adverse influence on nutritive value. Statistically significant ($p \leq 0.05$) differences can be seen regarding the moister, crude protein and crude fat content, while the significant differences ($p \geq 0.05$) were not noticed regarding the treated or untreated mineral content of linseed. Regarding the crude protein solubility index (PDI), the application of heat treatments had a significant influence ($p \leq 0.05$) compared between experimental treatments and control treatment. The obtained values of crude protein solubility index were 27.06; 36.62; 12.19; 9.24 and 15.58% ($p \leq 0.05$), respectively. Shen et al. [36] examined the effects of processing, including pelleting, autoclaving, and microwave processing on nutrient utilization in leghorn roosters. The results gained in our study show that the application of microwave thermal processing during 300 s, with the processing power of 450 W, showed a significant ($p \leq 0.05$) reduction (41.67%) of urease activity compared to raw linseed. Recorded decreases of urease activity after applied heat treatments were 25.00%; 25.00%; 41.67% and 16.67%, respectively.

Seeds pelleting three times improved the preservation of fatty acid by 29%. Microwave increased fatty acid use by 39% for four minutes, and autoclave increased fat usage by 20%, which demonstrated the positive effects of thermal technology on linseed use. The heat and physical treatment of nitrogen accumulation has also greatly increased. Feng et al. [37] found that the maximum degree of HCN linseed reduction between the pelleting, dry oven heating and autoclaving was obtained with microwave treatment. They found that the main nutrient and fatty acid profile were not substantially modified due to microwave processing. Wu et al. [29] investigated the detoxification of flaxseed by extrusion. Extrusions can be used as a highly successful mechanism to eliminate cyanogenic glycosides and trypsin inhibitors according to their findings.

The mycotoxin detoxification of feedstuffs and compound feed for animal nutrition using different mechanical and biotechnological procedures was described in detail by Čolović et al. [38] Ivanov et al. [23] investigated and concluded that conditions of 400 W of the microwave power and 4 min 50s of the process were ideal to reduce the HCN level under the allowed limits in the flaxseed. Shen et al. [36] fed broiler chickens 12% flaxseed from 1–21 days and 15% from 22–40 days. Feeding whole flaxseed reduced body weight, feed intake and feed efficiency, but feeding flax seed that had been previously pelleted and mashed significantly improved body weight gain, feed conversion efficiency, and feed intake. The results of amino acids analyses show no statistically significant ($p \geq 0.05$) differences between treated samples, and what is telling is that applied heat microwaved treatment with one working power of 450 W in different time frames, with also different exit temperatures of treated materials, did not affect the amino acids profile and can be usefully used as possible thermal treatment. A statistically significant difference ($p \leq 0.05$) was noticed only regarding the isoleucine

(L1, L4, L5: L2, L3), which was slightly higher. A similar significant ($p \leq 0.05$) tendency was noticed regarding the leucin (L1, L3, L4, L5: L2), respectively.

4. Conclusions

At the end of the experiment, and having in mind the obtained results, it can be concluded that microwave thermal treatment led to a significant inactivation of urease activity and to increased crude protein solubility index, without changes in amino acids profile of treated linseed grain. The gained results show that the best solution, in practice, for the enrichment of the nutritive quality of linseed aimed for animal nutrition is microwave thermal treatment, with a working power of 450 W during the time of 60 s. Our results indicate that microwave thermal processing or micronizing dry thermal processing of grains could be successfully used in large industrial feed production with a short period of time and improved nutritional parameters of grains, increased shelf-life and unchanged amino acid profile of treated grains.

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