Effect of Alcalase modification of yellow lupin (Lupinus luteus L.) protein isolate on some functional properties and antioxidant activity

Iwona TESAROWICZ (iwona.tesarowicz@urk.edu.pl)
University of Agriculture in Krakow
Ireneusz MACIEJASZEK
University of Agriculture in Krakow
Krzysztof SURÓWKA
University of Agriculture in Krakow
Agnieszka ZAWIŚLAK
University of Agriculture in Krakow

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Abstract

Protein isolate obtained from sweet yellow lupin (*Lupinus luteus L.*) and its Alcalase hydrolysates were examined for their functional and antioxidant properties in relation to surface hydrophobicity of proteins and peptides, and molecular weight distribution. Enzymatic hydrolysis improved the foaming characteristics of lupin proteins, while the emulsifying properties deteriorated. It means that good foaming properties of preparations are determined by the presence of low-molecular δ conglutin and small subunits of γ conglutins. In turn, larger proteins such as α and β conglutin are responsible for maintaining good emulsifying properties. The measured surface hydrophobicity was consistent with the results of emulsifying properties. It has also been shown that the scope of changes in antioxidant properties due to hydrolysis, measured by DPPH method and as reducing power, is more pronounced than with the use of ABTS and FRAP methods.

1. Introduction

The use of legume seeds as a source of food protein is of growing interest to consumers, especially in view of the increasing trend towards vegetarian diet. From a global perspective, soybeans are traditionally the most popular protein raw material from this group of plants, although consumers also appreciate legumes such as chickpea, pea, lentils etc.

Relatively less attention is paid to lupin, which is after all a valuable legume with high protein content, comparable with that in soy. The protein quality of its seeds is equally good, and rated by some authors as better than soybeans. Although it is poor in sulfur-containing amino acids, it also has less antinutritional factors than soybeans – trypsin inhibitors and hemagglutinins are practically absent [1, 2].

Lupin is also easy to grow, because it tolerates frost, drought, and poor soils quite well. However, although known as human food since early Roman times, it is utilized to a small extent so far, primarily because of alkaloid content and low agronomic yield. Commonly found in lupins are quinolizidine alkaloids such as lupanine, sparteine or lupinine, which have been shown to be reversible inhibitors of acetylcholinesterase. Alkaloid level depends on cultivar, soil type and growing season. Some cultivars have been developed, however, which are sweet and contain alkaloids at the level safe for human health [1, 3, 4]. Moreover, alkaloid content can be substantially decreased by soaking and rinsing lupin beans as well as by technological processing.

It is believed that lupin can be widely used as a good source of protein, fiber and as a functional supplement in many food products. Lupin flour, containing almost 40% of protein, is a good raw material for obtaining protein preparations. In the available literature only limited number of reports could be found about the use of enzyme proteolysis to modify functional and antioxidant properties of lupin proteins [5, 6, 7]. Functional properties of lupin protein are considered as relatively good [1, 2]. However, they can be modified in order to change, for example, their foaming and emulsifying ability or other features affecting their technological suitability. An important and widely approved method of modifying

vegetable proteins is enzymatic hydrolysis. Due to this method Lquari et al. [5] improved some functional properties of *Lupinus angustifolius* protein. In turn, Surówka et al. [8] showed that limited enzyme proteolysis enables the improvement of the emulsifying and foaming properties of extruded soy flour.

It was assumed that limited hydrolysis of proteins included in the lupin protein isolate (LPI) by Alcalase will not only allow for favourable modification of their technological properties, especially foaming and emulsifying properties, but will also affect antioxidant properties. As a result, the hydrolysates obtained may potentially be used as functional ingredients protecting food products from oxidative deterioration.

2. Materials And Methods

2.1. Materials

The raw material was seeds of sweet yellow lupin (*Lupinus luteus L.* var. Mister). They were obtained from Poznańska Hodowla Roślin Ltd, Plant Breeding Station Wiatrowo (52°45’ N; 17°08’ E). Lupin beans were ground in Thermomix (Thermomix 31 − 1, Vorwerk, Germany) and sieved to collect the fraction 250 μm of lupin flour, which was then used in further experiments.

Food grade alkaline bacterial protease Alcalase of *Bacillus licheniformis* (Novo Nordisk A/S) with activity equal to 2.4 Anson Units/g was used for hydrolysis.

2.2. Protein isolation

In order to obtain lupin protein isolate 10% suspension of lupin flour (LF) in deionized water with the addition of $NaHSO_3$ (0.77 mM) was brought to pH 8.6 with 1.0 M NaOH and stirred for 40 min at 30°C [9]. Subsequently, suspension was centrifuged at 2000·g for 20 min and protein was precipitated from supernatant by acidification to pH 4.5 with 1.0 M HCl. Whole was stirred for 15 min, then centrifuged for 20 min (900·g). Protein precipitate was rinsed twice with water, decanted, suspended in deionized water in a ratio of 1:9, neutralized with 1.0 M NaOH and lyophilized.

2.3. Hydrolysis

Lyophilized LPI was subjected to enzymatic hydrolysis. For this purpose, 25 g sample was suspended in 225 mL of deionized water and Alcalase solution was added to obtain enzyme:substrate ratio 18 mAU/g protein. Hydrolysis was carried out at 55°C and pH 8.5 was kept constant with 1.0 M NaOH. As a result, three hydrolysates were obtained, which differed in processing time (0.5 h, 1 h and 2 h; for hydrolysates H-30, H-60 and H-120, respectively).

The process was terminated by heating reaction environment for 15 min in 90°C. Subsequently, samples were cooled and centrifuged (15 min, 4000·g). Supernatants were frozen and lyophilized.

2.4. Proximate composition

The water content (g H_2O/g) was determined by standard oven drying at 105°C for 3h. The amount of protein (N×6.25) was detected by means of the Kjeldahl method using K-435 and B-324 combustion and distillation units, respectively (Büchi, Flawil, Switzerland) [10].

2.5. Foaming and emulsifying properties

Foaming properties were studied according to the method described by Surówka et al. [8]. Foam was created by passing argon at a rate of 10.4 mL/s through 100 mL of analysed hydrolysate solution (2% total protein). Following parameters were determined: foaming capacity (FC), foam overrun (FO) and foam stability as a liquid drainage (LD_5). Foaming capacity was calculated as the ratio of foam volume to the volume of pressed gas necessary to its production. Foam overrun was constituted by ratio of the gas volume in the foam to the volume of the liquid involved in its formation. In turn, liquid drainage representing foam stability was the ratio of the volume of foam liquid released during 5 min to the volume of liquid in the foam at the moment of finishing the aeration [11–13].

Emulsifying activity (EAI) and emulsion stability (ESI) indexes were analysed turbidimetrically [8, 14, 15]. EAI informs about the oil/water interface area (m^2), which can be formed by 1 g protein of the analysed hydrolysate in the experimental conditions, whereas ESI was calculated from the formula:

$$ESI=(A_0 \times 5\ min)/(A_0 - A_5)$$

where A_0 – turbidance measured at 500 nm immediately after homogenization and A_5 – turbidance measured at 500 nm after 5 min after homogenization.

2.6. Surface hydrophobicity

Surface hydrophobicity was determined by the spectrofluorimetric method of Hayakawa and Nakai [16] with the use of 1-anilino-8-naphtalene-sulfonate (ANS) as the fluorescence probe. Fluorescence intensity (FI) was measured for two dilution series of the examined sample solutions (0.03–0.8 mg protein/mL), one was without ANS and the other with ANS (8 mM ANS). Measurements were performed using a Cary-Eclipse spectrofluorimeter, at wavelengths λ_{ex} and λ_{em} 390 and 470 nm, respectively. The netto value of fluorescence intensity (FI_{NETTO}) was calculated by subtracting FI of each solution without probe from that with probe. The plot of FI_{NETTO} versus protein concentration led to determination of slope, which was established as an index of the surface hydrophobicity.

2.7. Electrophoretic separations

To analyse molecular weight distribution of protein, lyophilized samples were homogenized in distilled water to obtain 1% protein suspensions. The aliquots of homogenate were diluted (1:1) with a denaturing buffer (0.125 M Tris, 4% SDS, 20% glycerol, 2% 2-mercaptoethanol, pH 6.8) and heated for 90 s in a boiling water bath. The extracts were centrifuged at 5000 g for 15 min and clear supernatants were collected. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out according to the Laemmli [17] method using a 12.5% w/v gel concentration. The molecular weights of the

bands were estimated using MW-SDS-200 and MW-SDS-70L marker kits (Sigma Chemical Co., St. Louis, MO, USA). For peptide separations Schägger and von Jagow [18] procedure was applied. The separation was carried out in a discontinuous system in the presence of a buffer containing Tricine. A Hoefer Mighty Small SE 260 unit coupled with an EPS 301 power supply unit (Amersham Pharmacia Biotech, Uppsala, Sweden) was employed in all electrophoretical analyses. Stained gels were scanned and analysed using Image Master TotalLab (Amersham Pharmacia Biotech, Uppsala, Sweden) software.

2.8. Antioxidant properties

Water solutions of the isolate and hydrolysates (5% m/v) were prepared to determine antioxidant properties. Antioxidant activity was measured using four different spectrophotometric techniques. Two radical-scavenging methods were employed: one with the use of 2,2-diphenyl-1-picryl hydrazyl (DPPH) according to Brand-William's method [19] and the other applying 2,2'-azino-bis (3-ethylobenzotiazolino-6-sulfonate) (ABTS) in accordance with Re et al. [20]. Two remaining analyses based on the reduction of Fe^{3+} ions to Fe^{2+} ions, resulting in the formation of intense blue colour. In the Benzie and Strain method [21] the ions are complexed by 2,4,6-tris (2-pyridyl) -1,3,5-triazine (TPTZ). In the Yen and Chen method [22] the solution of prussian blue forming during reduction reaction is responsible for sample colour.

3. Results And Discussion

3.1. Protein content, molecular weight distribution and surface hydrophobicity

In vegetable protein products protein concentration, molecular weight distribution and surface hydrophobicity determine their functional properties.

In this study, lupin protein isolate, with 87.9 ± 0,2% protein content, was produced from yellow lupin flour containing 39.3 ± 2.4% protein, by means of alkaline leaching and isoelectric precipitation. ElAdawy et al. [2] reported higher protein content in the isolate (about 91%); however, process parameters applied by the Authors were slightly different. In turn, Lampart-Szczapa and Mossor [23] received an isolate containing approx. 4% less of protein. The enzymatic hydrolysis had a limited effect on the content of protein substances. In the H-30, H-60 and H-120 hydrolysates they were found at the level of 89,4 ± 2,0, 90,1 ± 1,1, and 87,4 ± 0,3%, respectively. Also, Schlegel et al. [7] did not observe a significant change in protein content due to enzymatic proteolysis of lupin protein isolate.

The quantitative results of SDS-PAGE separation of proteins in LPI and hydrolysates are shown in Fig. 1 and Table 1.

Table 1. Relative content (%) of protein fractions in lupin protein preparations (results of SDS-PAGE).

Molecular weight (kDa)	Protein content [%]				
	LPI	H-30	H-60	H-120	
> 200	3.1	10.3	0.3	2.0	
74–100	1.6	0.0	0.0	0.0	
42–66	30.7	13.3	17.8	10.0	acidic subunits of α conglutin, HMW subunits of β conglutin
34–40	16.0	0.0	0.0	0.0	IMW subunits of β conglutin
26–34	8.8	0.3	1.1	0.0	IMW subunits of β conglutin, large subunits of γ-conglutin
20–22	16.3	0.5	6.8	4.7	basic subunits of α conglutin
18–20	6.4	1.3	0.0	0.0	LMW subunits of β conglutin
< 15	17.0	74.4	74.0	83.3	subunits of δ conglutin and products of hydrolysis

According to Capraro et al. [24] and Duranti et al. [25], 7S-β- and 11S-α-globulins are predominant in lupin flour proteins. This corresponds to the SDS-PAGE results, since the proportion of protein fractions of 42–66 kDa was the highest (30.7%) in the analysed non-hydrolyzed LPI. It is assumed that within this range there are subunits of one of the two main globulin fractions: legumin-like α conglutins (11S-α-globulins with above 330 kDa molecular weight) and vicilin-like βconglutins (7S-β-globulins with molecular weight of 143–260 kDa). Legumins are hexamers composed of heterogeneous acidic subunits (42–52 kDa) and basic subunits (20–22 kDa), while vicilins are trimers consisting of three subunits, which molecular weights range between 17 and 64 kDa (HMW: 53–64 kDa, IMW: 25–46 kDa and LMW: 17–20 kDa) [25]. Since these fractions contain polypeptides with similar molecular weights, it is not possible to distinguish these subunits clearly on the polyacrylamide gel used.

The next important protein fraction of the LPI (17%) were low-molecular-weight proteins below 15 kDa. These proteins are probably part of the δ conglutins (2S globulins) with molecular weight of about 13 kDa, composed of subunits: small (4 kDa) and large (9 kDa) [25]. In this work, the fraction of proteins in the molecular weight range of 34–40 kDa was represented by a fairly distinct band, representing 16% of all proteins in LPI. It is, most probably, a protein identified in the literature as an IMW subunit of β conglutins [24, 26]. A band with a MW in the range of 20–22 kDa had a similar relative share in LPI proteins representing basic subunits of legumins.

Analysis of the obtained electrophoretic data showed that some fractions were occurring only in individual lines and there were others, which repeated in all samples – namely in LPI and hydrolysates. The repetitive fractions visible on the gel scan (Fig. 1) were mainly proteins with molecular weights 42–66 kDa, partly 26–34 kDa, 20–22 kDa as well as low-molecular proteins (< 15 kDa).

In hydrolysates the main fractions (above 70%) were proteins with a molecular weight below 15 kDa. The relative content of these low-molecular-weight fractions in enzyme hydrolysates was 4–5 times higher than their level in the raw material (LPI). When comparing the LPI and products of its modification with Alcalase, a significant decrease in the content of the: high-molecular-weight (HMW) β conglutin (53–66 kDa) and acidic α conglutin (42–52 kDa) subunits as well as in other polypeptide chains of molecular weight above 15 kDa was observed. This is due to the breakdown of proteins into low molecular weight peptides and amino acids.

Analysis of protein band percentages showed differences between individual hydrolysates, which may result from differences in the examined functional properties. The product obtained as a result of two-hour proteolysis (H-120) had significantly higher content of low-molecular-weight protein fractions (below 15 kDa) than H-30 and H-60 hydrolysates.

In order to identify the products of proteolysis more accurately, the separation of peptides was performed according to the Schägger and von Jagow procedure [18], using a tricine cathode buffer. The results of the peptide separation are given in Fig. 2 and Table 2.

Table 2.Relative content (%) of protein fractions in lupin protein preparations (Schägger and von Jagow [18] **procedure).**

Molecular weight (kDa)	Protein content [%]			
	LPI	H-30	H-60	H-120
>100	2.5			
74–100	2.8			
43–65	26.9	31.2	27.1	28.8
34–40	8.0	14.7	6.0	0.0
24–33	20.3	9.2	27.7	25.5
20–23	4.9	8.9	11.8	11.1
18–20	7.7	0.6	0.0	0.0
12–16	16.8	9.0	5.2	3.1
<10	10.1	26.4	22.2	31.5

The electrophoretic analysis by means of the Schägger and von Jagow procedure revealed that proteins with molecular weights in the range of 43–65 kDa (more than 26%) were predominant in LPI and this result confirmed the findings obtained by the standard SDS-PAGE electrophoresis. With regard to hydrolysates, however, the predominant protein fraction changed with a prolongation of the proteolysis

length. It was found that in hydrolysates H-30, H-60 and H-120, the dominant compounds were those with molecular weight of 43–65 kDa, 24–33 kDa, and below 10 kDa, respectively.

The bands that represented the low molecular weight fractions in these preparations were wide and fuzzy, especially lines 6 and 7 in the gel area corresponding to the molecular weight below 6.2 kDa. The observed phenomenon is a result of polydispersity, i.e. an approximately continuous molecular weight distribution, with no dominant bands corresponding to a higher content of the molecular weight fraction.

The results obtained for the surface hydrophobicity of lupin protein preparations, presented as a plot and the simple equations fitted to them, are shown in Fig. 3. The highest value, expressed as a slope in a straight line equation representing changes in fluorescence intensity with increasing protein concentration, was observed for the LPI. In turn, the hydrophobicity of hydrolysates was distinctly lower and assumed similar values each other, showing no significant differences. This indicates that the initial hydrolysis of lupin proteins is already enough to reduce this parameter significantly; the prolongation of the process does not change it substantially.

Similar results of reducing surface hydrophobicity were obtained by Surówka et al. [27], who hydrolyzed soy protein concentrate and its extrudate using both Alcalase and Esperase.

3.2. Functional properties

Table 3 shows the results of the functional properties analysis of investigated preparations. The foam formed due to aeration of the LPI solution was quite difficult to obtain and was characterized by a coarse structure and very thin walls of the film. It was, however, relatively stable. The values of FC, which when higher reflect better foaming efficiency, increased noticeably as a result of hydrolysis. This index in all tested hydrolysates was about 10% higher than in the LPI. This means that the process of proteolysis significantly improves the foaming properties.

Table 3. Functional properties of LPI and its hydrolysates (H-30, H-60 i H-120).

	LPI	H-30	H-60	H-120
FC (%)	62.5 ± 0.9^{a}	72.7 ± 2.0^{b}	70.6 ± 1.3^{b}	74.1 ± 2.0^{b}
FO (mL)	153.4 ± 6.8^{a}	46.0 ± 5.0^{b}	33.4 ± 3.9^{c}	28.5 ± 2.4^{c}
LD_5 (%)	66.2 ± 7.2^{a}	79.9 ± 2.9^{b}	81.0 ± 4.7^{b}	81.8 ± 1.3^{b}
EAI (m^2/g)	10.9 ± 0.6^{a}	8.5 ± 0.3^{b}	7.3 ± 0.3^{c}	7.2 ± 0.6^{c}
ESI (min)	59.1 ± 7.0^{a}	13.3 ± 2.2^{b}	9.4 ± 1.1^{b}	11.6 ± 2.2^{b}
The presented values are means ± standard deviations (n = 3). Values with different superscripts (a–c) in rows differ significantly at $p \leq 0.05$.				

In foams characterized by high FO values, gas bubbles are large and/or the foam walls are relatively thin. Thus, high FO values indicate a technologically unfavourable structure of the foam. As for hydrolysate preparations, the FO values decreased significantly compared to the FO values determined for the LPI. As a result, the foam obtained from the hydrolysate solutions had a desirable, more finely porous structure with strong walls. As in the case of FC, an improvement of this aeration property was noted due to enzymatic hydrolysis. However, according to the data in Table 3, the LD5 value increased, which means that foam stability decreased as compared to the LPI. Moreover, this decrease seems to be to some extent related to the length of the process. Therefore, in order to improve the foam stability of hydrolysate solutions, they should be used in preparations with stabilizing substances, e.g. polysaccharides increasing the viscosity.

According to Surówka et al. [27], who investigated soy protein hydrolysates, and Lqari et al. [5], who examined narrow-leafed lupin, partial enzymatic hydrolysis improves the foaming capacity compared to the raw material, from which the hydrolysate was prepared. However, the foams from these hydrolysates were not very stable. This agrees with our results obtained for the lupin hydrolysates, according to which peptides and δ conglutins, the LMW subunits and small subunits of β and γ conglutins had better adsorption capacity on the surface of air bubbles, providing good foaming properties to these products. Peptides present in hydrolysates can reach the interfacial surface much faster than proteins and create films that form the foam structure. The above authors concluded that degree of hydrolysis determines the foaming capacity of hydrolysates - with an increase in the degree of hydrolysis and a decrease in the average molecular weight, the ability to form foams increases. This is due to the fact that proteins with lower molecular weight have a greater ability to adsorb on the surface of air bubbles. Polar moieties located on the surface of the protein molecule more easily turn towards liquid and non-polar ones towards the air. As a result, a coherent, flexible film forms around the air bubbles.

Emulsion with the use of LPI is formed relatively efficiently as measured by EAI (Table 3). This index can be slightly improved by applying mild hydrolysis conditions, as was the case with limited enzymic hydrolysis of soy flour extrudates [8]. In this study, however, such improvement was not observed. The hydrolysate obtained after the half-hour process (H-30) produced emulsion with EAI lower by 22% compared to the isolate. Prolongation of the hydrolysis resulted in hydrolysates having even lower EAI values.

Alcalase hydrolysis had an even greater effect on ESI. While the emulsions obtained from LPI showed good stability, as a result of the hydrolysis, the ESI index decreased several times (Table 3).

It is believed that the surface hydrophobicity of proteins affects the emulsifying properties [28, 29]. When comparing the results of the analysis of emulsifying properties and surface hydrophobicity of LPI and its hydrolysates, it can be approximated that the results of hydrophobicity analysis and EAI and ESI are convergent, i.e. the hydrolysis leads to a deterioration of these indexes in line with the decrease of hydrophobicity.

In this study, as a result of α and β conglutins fragmentation, the number of peptides increased, but the number of both hydrophobic and hydrophilic moieties in their molecules was reduced. Even when the peptides are positioned on the surface of the dispersed lipids, interactions with the aqueous phase, which stabilize emulsions, will not be formed. In consequence, a loss is observed of emulsifying properties and decreased stabilization of the emulsion. Therefore, among the analysed preparations, LPI showed better emulsifying properties, than hydrolysates. Similar observations were reported in studies on rape protein hydrolyzed with Alcalase, where a decrease in emulsifying capacity as a result of hydrolysis was observed [30].

3.3. Antioxidant properties

Analyses of antioxidant activity performed by four various methods showed that the results obtained were strongly dependent on the measurement methods (Fig. 4 and Fig. 5). Large differences in antioxidant activity between the isolate and hydrolysates were noted for DPPH free radical scavenging and reducing power assays. The half-hour hydrolysis caused higher reduction in antioxidant activity of the product, than extending the length of this process to 2 hours. With regard to the remaining two methods, i.e. using ABTS free radicals and FRAP, there were no such big differences. In these cases, a statistically significant slight reduction in antioxidant activity was noticed only in the H-60 hydrolysates. Guo et al. [31] reported that lupin protein isolate had lower antioxidant activity determined with DPPH and FRAP methods than hydrolysate obtained with Alcalase after 15 minutes of the process. Then, the antioxidant activity decreased with the subsequent slight increase at the end of the process. When compared to our study, differences may be due to the method of preparing the protein isolate; in our work the flour was not defatted before the procedure of protein isolation. It could have caused migration of some amounts of fat together with antioxidant compounds into the isolate. Pena-Ramos et al. [32], who studied the effect of enzymatic hydrolysis of soy protein isolate on the antioxidant activity, found that hydrolysates obtained after an one-hour process with the use of Alcalase showed a little higher antioxidant activity than protein isolate and the remaining products of hydrolysis.

4. Conclusions

In the lupin protein isolate, protein fractions of 42–66 kDa and high-molecular proteins, i.e. proteins from α and β conglutin, are predominant. In contrast, in the products of hydrolysis, proportion of the low-molecular fraction is the largest. The highest content of protein fractions below 20 kDa was recorded in the product obtained after two-hour proteolysis. Changes in the length of polypeptide chains caused by hydrolysis and the accompanying significant decrease in surface hydrophobicity affected substantially the examined functional properties. This was manifested by the reduced emulsification capacity of hydrolysates and much poorer emulsion stability compared to LPI. The peptide chains formed as a result of proteolysis were too short and hydrophobic domains were exposed insufficiently to create the film on the interface stable enough to limit coalescence. Only the proteins still present in the half-hour hydrolysate - α and β conglutins, cause the preservation of emulsifying properties and emulsion stabilization.

On the other hand, it was observed that along with the prolongation of the hydrolysis time and the decrease in the protein average molecular weight, the foam capacity of hydrolysates was improved, although stability of the foam decreased.

The best aeration properties were noted for the H-120 hydrolysate. In comparison with the hydrolysis products, LPI was characterized by much lower foam formation capabilities. The improvement of foaming properties due to two-hour proteolysis was connected with the presence of low-molecular δ conglutin, LMW subunits of vicilin-like β conglutins and small subunits of γ conglutins.

When considering antioxidant properties, LPI was characterized by a distinctly better antioxidant activity than hydrolysates, as measured by the DPPH method and as reducing power, whereas the results obtained by the ABTS and FRAP methods were comparable.

Declarations

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Author Contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Iwona Tesarowicz and Agnieszka Zawiślak. The first draft of the manuscript was written by Iwona Tesarowicz and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data Availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Human and Animal Rights This article does not contain any studies with human or animal subjects.

Conflict of Interest All authors declare that they have no conflict of interest.

Ethics approval Not applicable

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Figures

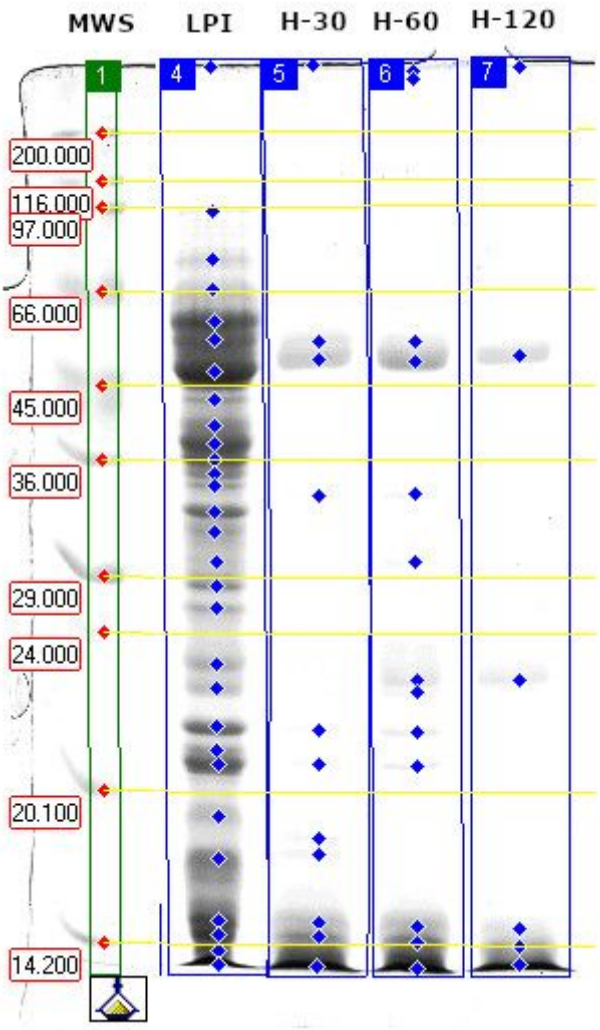

Figure 1

The results of SDS-PAGE of lupin protein preparations. Lanes: MWS – molecular weight standards (Da), LPI – lupin protein isolate, H-30, H-60, H-120 - LPI hydrolysates.

Figure 2

Results of the peptide separation (Schägger and von Jagow [18] method) of lupin protein preparations. Lanes: PLWS - peptide and low molecular weight standards, LPI - lupin protein isolate, H-30, H-60, H-120 – LPI hydrolysates.

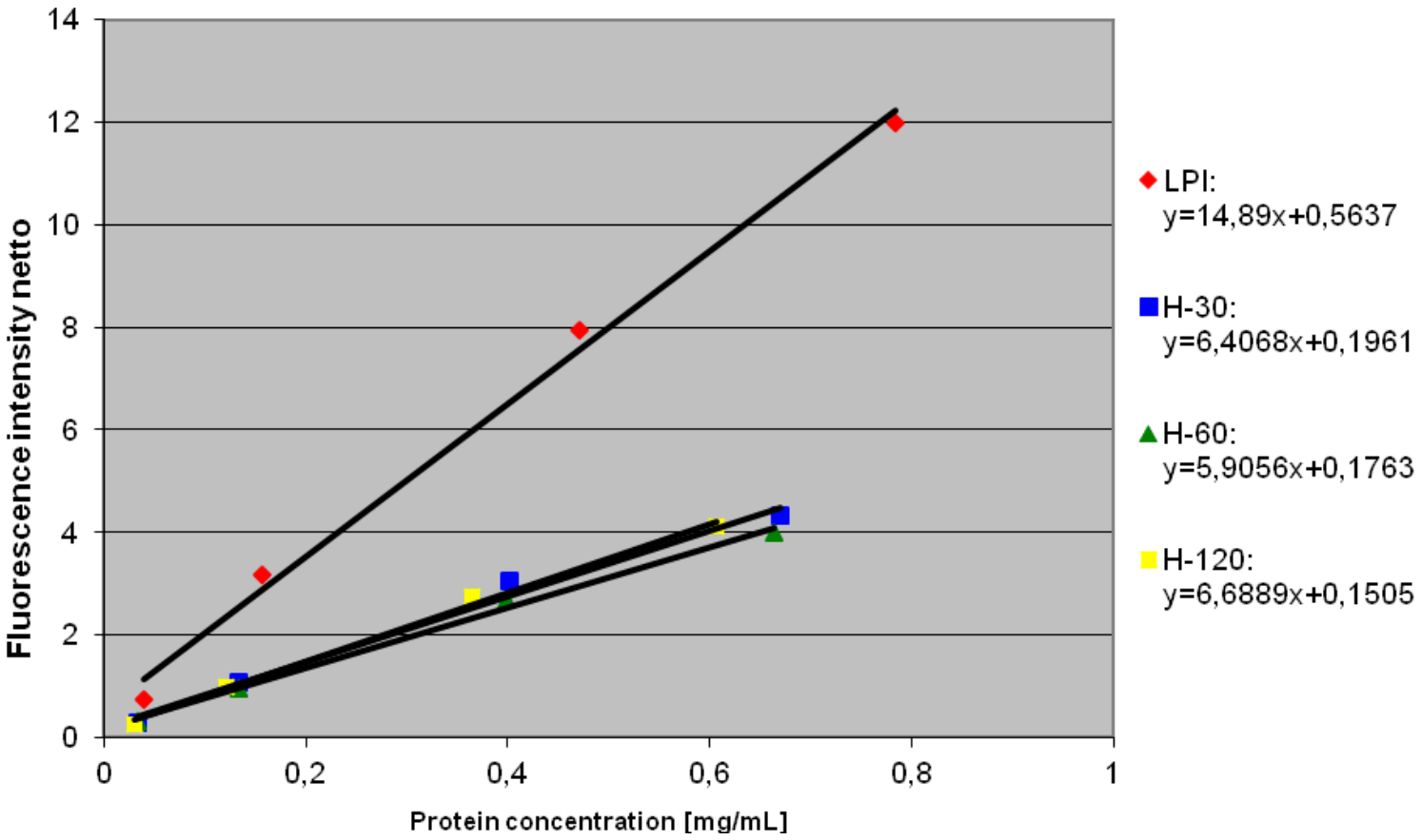

Figure 3

Surface hydrophobicity analysis results of LPI and its hydrolysates (H-30, H-60, H-120).

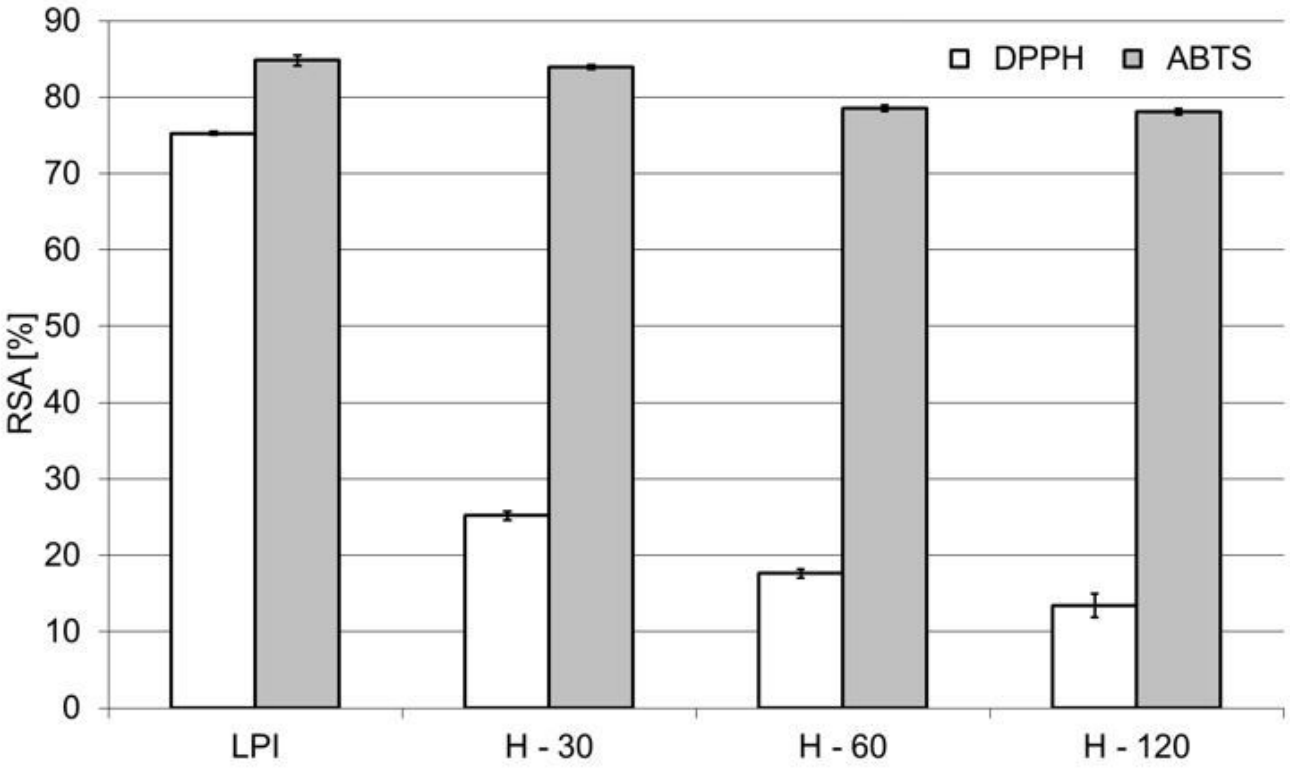

Figure 4

Antioxidant activity expressed as radical scavenging activity.

Figure 5

Antioxidant activity expressed as ability to reduce Fe^{3+}.